1 **INTERANNUAL TEMPORAL PATTERNS OF DESOTO CANYON**
2 **MACROFAUNA AND EVALUATION OF INFLUENCE FROM THE**
3 **DEEPWATER HORIZON**

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22   **Abstract**

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24   Submarine canyons are highly dynamic and productive ecosystems, but time-series

25   studies of metazoan benthic communities in canyons are scarce. Deep-sea macrofauna from the

26   DeSoto Canyon in the northern Gulf of Mexico were sampled annually from 2012 through 2014

27   from five stations within the Canyon and from two stations in 2013 and 2014 on the adjacent

28   open slope, for analysis of interannual dynamics, temporal variability, and potential influence of

29   the Deepwater Horizon oil spill (DwH), which occurred nearby in 2010. At a few sites, elevated

30   abundance was observed at the start of the time-series for overall macrofauna and for deposit

31   feeder abundance. However, diversity metrics showed no difference within stations among time

32   points. Community and feeding guild structure varied by station, as expected, but showed no

33   statistical difference among time points within a station. Some temporal variability was visible

34   in temporal trajectory overlays. Cluster analyses showed grouping more by station than by time

35   point. Metrics utilized for measuring potential oil contamination impact and overall community

36   stress including the benthic polychaete/amphipod ratio, feeding guild abundance, macrofaunal

37   indicators designed from the DwH, and community dispersion, generally exhibited a paucity of

38   evidence of impact, both yearly and with site-to-site comparisons. This suggests low levels of

39   impact in the canyon consistent with the low deposition of hydrocarbons, the timing of sampling,

40   and quick recovery of canyon foraminifera. Taken together these results suggest relatively low

41   levels of temporal variability within the DeSoto Canyon macrofauna with little evidence of oil

42   influence on these sites within the studied time range.

I.   Introduction

Despite its seemingly quiescent nature, closer inspection of the deep-sea indicates natural temporal variability that can be linked to seasonal and stochastic events, e.g. surface primary productivity changes, climate, sediment slumping, and sediment resuspension from benthic storms (reviewed by Smith et al. 2009; Glover et al. 2010). Not surprisingly, natural variations in deep-sea fauna through time have also been tied to this environmental variability, with fluctuations in abundance and diversity as well as species composition (Glover et al., 2010 and references therein; Laguionie-Marchais et al., 2013; Ramalho et al., 2014; Rogers 2015). Ecological processes such as succession, disturbance, and recovery can also contribute to fluctuations in abundance and community structure in deep-sea benthic communities over time (Smith 1986; Kukert and Smith 1992; Young and Richardson 1998; Smith et al. 2002; Bernardino et al. 2010).

From a basic science perspective, since the deep sea varies through time in both environmental and faunal patterns, characterizing the range of these natural variations is critical to our understanding of deep-sea ecology. In a more applied context, quantifying the natural range of variation in deep benthic populations through time also provides a baseline for assessing natural and anthropogenic disturbances. Once the normal variation of a system has been quantified, this data can act as a baseline to assess whether any observed changes can be attributed to a disturbance event or to natural variability. However, in the most recent review of deep-sea time-series studies, Glover et al. (2010) found there were only 11 deep-sea sedimented ecosystems with multi-year time-series studies, of which only 2 were longer than 5 years. Thus, the paucity of time-series studies in the deep ocean makes it hard to determine whether changes observed after a human impact are caused by the impact, or a part of the natural variation of the system. The lack of data also leads to an inability to predict whether the effects from disturbances will be prolonged or ephemeral.

67   Increasing human-induced, large-scale disturbances have accentuated the imperative for

68   more time-series studies to allow comparisons and prediction of impact outcomes (Glover and

69   Smith 2003; Ramirez-Llodra et al. 2011; Mengerink et al. 2014). Highly dynamic, ecologically

70   important environments such as submarine canyons in particular, need more focused studies as

71   they are noted to be hotspots of diversity, with large spatial and biogeochemical heterogeneity. In

72   the last decade, canyons have increasingly become targets for bottom-trawling fisheries, are

73   exploited for oil and gas exploration, are sinks for litter and chemical pollution, and the biota

74   inhabiting them may be sensitive to the effects of climate change over time (Fernandez-Arcaya et

75   al. 2017; De Leo and Puig 2018).

76   The DeSoto Canyon is a major topographic feature of the northern Gulf of Mexico

77   (NGOM). Environmentally, it stands as a noted sedimentary transition zone (Antoine and Bryant

78   1968), which can accumulate high amounts of organic matter (Morse and Beazley 2008; Silva

79   2017; Wei and Rowe 2019), and potentially has a strong hydrodynamic regime given its inherent

80   topography (Clarke and Van Gorder 2016). Ecologically, it is recognized as a hotspot of

81   meiofaunal and macrofaunal benthic abundance (Baguley et al. 2006; Wei et al. 2010, Shantharam

82   et al in revision), benthic diversity (Wicksten and Packard, 2005), and biomass (Wei et al. 2012).

83   To date, however, the temporal dynamics of resident macrofauna in this canyon have not been

84   characterized, leaving a gap in baseline knowledge on how biota of this important bathymetric

85   feature behaves ecologically through time. It has been shown that the larger NGOM macrobenthos

86   seems subject to some interannual variability in abundance (Montagna et al. 2020) and community

87   structure (Salcedo et al. 2016). Thus, the primary goal of this study was to test the null hypothesis

88   that macrofauna in more localized ecosystems like the DeSoto Canyon exhibit interannual

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89   variability similar to non-canyon sites and that individual locations exhibit similar inter-year

90   patterns.

91   Given the proximity of the DeSoto Canyon to the Deepwater Horizon oil spill (DwH), it is

92   necessary to also consider whether any of the study sites were influenced by the spill. DwH was

93   the largest oil spill in United States history and the first in the deep sea (wellhead depth 1500m).

94   The infaunal impact in the area immediately around the DwH wellhead was extensive and impacts

95   have been long-lasting (Montagna et al. 2013; Baguley et al. 2015; Montagna et al. 2016; Reuscher

96   et al. 2017; Washburn et al. 2017). Further away, the picture is less clear. In the DeSoto Canyon,

97   40 – 185 km from the wellhead, rapidly deposited labile and non-labile hydrocarbon sedimentation

98   was confirmed in sites throughout the canyon (Brooks et al. 2015). Impacts to sediment ecosystems

99   included changes to the redoxcline, similar to an influx of enriched organic matter, from 2010 –

100 2013 (Brooks et al. 2015; Hastings et al. 2015). Oil-degrading bacteria in sediments near the deeper

101 plumes spiked in September/October 2010 and in the summer seasons of 2012 – 2014 (Mason et

102 al. 2014; Overholt 2018). Impacts to local benthic meiofauna were inferred from the drop in

103 foraminiferan density and diversity, and overall sediment bioturbation (Schwing et al. 2013;

104 Brooks et al. 2015; Schwing et al. 2015; Schwing et al. 2017).

105   By 2014 – 2016, the impacts to DeSoto Canyon ecosystems may have subsided.

106 Perturbations to phytoplankton productivity at the initiation of the DwH mostly abated by 2014

107 and 2015 (Li et al. 2019). Signs of the mass deposition event in the canyon had ended by 2013 -

108 2016 (Larson et al. 2018). Bacterial communities in canyon sediment may have returned to

109 baseline conditions by 2015 (Yang et al. 2016; Liu et al. 2017; Overholt et al. 2019) as bioturbation

110 recommenced between 2013 – 2016 (Larson et al. 2018) and the redoxcline resumed steady-state

111 conditions (Hastings et al. 2020). Additionally, canyon foraminiferan diversity rose and steadied

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112   by 2012 (Schwing et al. 2017; Schwing et al. 2018; Schwing and Machain-Castillo 2020).

113   However, foraminiferans only comprise a small portion of the benthic fauna occupying this soft

114   sediment ecosystem, leaving many facets of metazoan abundance, diversity and structure

115   unexplored. Thus, with macrofauna sampled during the summer of 2012 – 2014 in the DeSoto

116   Canyon, and adjacent continental slope, a further goal of this study was to test whether any

117   observed temporal variability might be attributable to influence from the oil spill and to compare

118   observations between communities in the canyon to those on the non-canyon slope.

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120   **II.   Materials and methods**

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122   *2.1 Study location*

123   The DeSoto Canyon is an S-shaped canyon located on the edge of the NW Florida outer

124   continental shelf, cutting from the shelf to the abyssal region of the Gulf of Mexico (GOM). It is

125   a sedimentary ecotone with siliclastic clays to the west and biogenic carbonates to the east

126   (Gould and Stewart, 1955; Doyle and Sparks, 1980). Sediment accumulates at a rate of ~17

127   cm/ky (Emiliani et al., 1975) in the northwest and ~10 cm/ky in the southeast (Emiliani et al.,

128   1975; Nürnberg et al., 2008) of the canyon. In the summer season, the head of the canyon is

129   occupied by low salinity, biologically productive waters, driven there by cyclonic and

130   anticyclonic eddies (Müller-Karger et al., 1991; Belabbassi et al., 2005; Walker et al., 2005;

131   Biggs et al., 2008; Jochens and DiMarco, 2008). Strong thermohaline stratification prevents

132   further intrusion, leaving highly oxygenated water typical of the North Atlantic Deepwater

133   (NADW) to be the predominant water mass in the deeper (>1000 m) reaches of the canyon

134   (Rivas et al., 2005; Morse and Beazley, 2008). Further detail on habitat characteristics are

135   reviewed in Shantharam et al (in revision).

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137   *2.2 Relation to the Deepwater Horizon deposition*

138   The DeSoto Canyon is located ~40 – 185 km east/southeast of the DwH wellhead (Fig 1)

139   and towards the eastern edge of the total estimated 24,000 km^2 depositional footprint of the oil

140   spill (Chanton et al. 2014). The extent of the surface slick in relation to the canyon is depicted in

141   Figure 1A. Plumes from the DwH spill were detected within the DeSoto Canyon at ~400, ~1000,

142   and 1400 m depths in 2010 (Hollander et al. 2012). Within a year after the spill, rapid deposition

143   of soluble and insoluble hydrocarbons was observed. Sediment hydrocarbon concentrations

144   increased five times more than pre-spill levels (Romero et al. 2015; Romero et al. 2017). The

145   distribution of radiocarbon (^{14}C) of DeSoto Canyon seafloor sediments is presented in Fig 1B,

146   more depleted values are indicative of petrocarbon from the DwH. These mostly comprised

147   degraded, high molecular weight compounds n-alkanes (67%), low molecular weight n-alkanes

148   (9%) and low weight PAHs (6%). This composition remained relatively unchanged for 3 years

149   though large reductions in concentrations did occur for homohopanes (~67%) and low weight

150   compounds (n-alkanes and PAHs, ~65% and ~66% respectively) and to a lesser degree high

151   molecular weight n-alkanes (~43%) and PAHs (~12%) (Romero et al. 2020). Most of the

152   deposition of this material seems to be concentrated west of the Canyon, but a few patches are

153   observed in Fig 1B within the canyon.

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155   *2.2 Sampling*

156   Samples were collected during the *R/V Weatherbird II* cruise numbers WB1305,

157   WB1306, WB1405, and WB1411. Sampling in 2012 and 2013 took place in late August to early

158 September and sampling in 2014 from late June to early July. Five sites within the DeSoto

159 Canyon, PCB06, XC2, XC3, S36, and XC4 were sampled (Figure 1). Three sites, PCB06, S36,

160 and XC4, were sampled annually within the study period and XC2 and XC3 were only sampled

161 in 2012 and 2014 (due to weather in 2013 and equipment malfunctions) (Table 1). Two sites on

162 the adjacent eastern non-canyon slope, expected to be fully outside the oil influence, were also

163 sampled in 2013 and 2014: S42 and NT800 (Table 1, Fig 1). Only site XC4 shows potential spill

164 influence based on the Chanton et al. (2014) data (Fig 1B). However, all canyon sites fall under

165 the surface footprint of the oil slick. XC2, PCB06, and XC3 of this study were also shown to

166 have had direct deposition in 2010 (XC2 and PCB06) and 2011 (XC3) (Romero et al. 2015).

167 Sediment at each site was collected using an Ocean Instruments MC-800 multicorer

168 capable of collecting up to eight, 10-cm diameter by 70-cm long cores per deployment. Four

169 cores from three replicate deployments at each site were processed for resident macrofauna

170 (sensu stricto), except for NT800 in 2013 where equipment malfunctions allowed only two

171 successful deployments. The top 10 cm of each core was sectioned into 0 – 1, 1 – 5, and 5 – 10

172 cm depth fractions and fixed in buffered 10% formalin. Once returned to the laboratory,

173 macrofauna were sieved through a 300 µm sieve and stained with Rose Bengal, then preserved in

174 70% ethanol. Once extracted, macrofaunal specimens were identified (usually to family), and

175 enumerated for subsequent analyses. Family level classification is generally regarded as

176 sufficient to discern multivariate faunal patterns in marine ecosystems (Warwick 1988,

177 Narayanaswamy et al. 2003) and in oil impact assessments (Gomez Gesteira et al., 2003).

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180 *2.3 Statistical analyses*

181   For all statistical analyses, the four cores from each deployment were combined and

182   treated as one sample, with each deployment considered a replicate for that site in that year. This

183   study focuses on temporal variation within sites while Shantharam et al (in revision) focuses on

184   spatial variation among sites with a larger set of sites sampled throughout the canyon and

185   adjacent slope in 2014. Thus all statistical analyses are designed specifically to test for temporal

186   change within a site. Standard abundance and diversity metrics (species richness and Pielou's

187   evenness (J')) were computed for each sample using the DIVERSE routine in Primer v 7.0.13

188   (Clarke and Gorley 2015). To test for changes in trophic structure, macrofaunal taxa were

189   assigned to feeding guilds adapted from Demopoulos et al. (2014), which included carnivore,

190   omnivore, deposit feeder, suspension feeder, and species harboring chemosymbiotic bacteria.

191   Abundance values were square root transformed and Bray-Curtis similarity was calculated to

192   measure macrofaunal community and feeding guild structure using group-average clustering.

193   These were portrayed with non-metric multidimensional scaling (NMDS) and cluster diagrams.

194   Samples were then summed across replicates within years and ordinated with a trajectory

195   through time overlaid to depict temporal change in community structure.

196   For tests of macrofaunal abundance, diversity, and community structure, a two-way

197   Permutational Analysis of Variance (PERMANOVA) (Anderson et al. 2008) was performed

198   with time point as a fixed factor and station as a random factor followed by pairwise

199   comparisons.

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201   *2.4 Bioindicators of oil influence*

202   Additional oil-impact faunal metrics that might provide insights into any effects of the oil

203   spill were incorporated into the statistical analyses. The first of these is a bioindicator known as

204   the benthic polychaete/amphipod ratio (BPA) (Andrade and Renaud 2011), which was designed

205   for gauging benthic impact of offshore oil and gas production in shallow water. There are no

206   thresholds or definitive measures of impact, rather it contrasts the ratio in contaminated areas to

207   non-contaminated areas to indicate community differences due to pollution. Higher values

208   indicate a greater impact due to the increased presence of opportunistic polychaetes and

209   reduction of sensitive amphipods.

210   The second set of indicators used were a set of designations of macrofauna taxa in terms

211   of their tolerance to or sensitivity to oil, that were developed in the context of the DwH closer to

212   the wellhead (Washburn et al. 2016). From a known oil spill impact zone, Washburn et al.

213   (2016), using taxa identified to the family level, designated families as "pollution tolerant" if

214   they were significantly higher in abundance and "pollution sensitive" if significantly lower in

215   abundance in the oil impact zone. If taxa were close to being significantly different between

216   impact and non-impact zones and abundances were at least 50% higher in one zone versus the

217   other, they were deemed "possibly tolerant" or "possibly sensitive". Taxa that did not

218   demonstrate a significant difference from background were termed "cosmopolitan". Collectively,

219   these groups are hereafter termed DwH macrofaunal indicators. In the setup of the current study,

220   these were assigned to the abundance data and the ratio of these groups to the total macrofaunal

221   abundance of a sample were determined. The results are interpreted as a measure of impact to a

222   site within a given year.

223   Finally, to get a sense of the community-level impact, two measures of community

224   variability, the relative dispersion and the Index of Multivariate Dispersion (IMD), were

225   computed by the MVDISP routine in Primer v 7.0.13 (Clarke and Gorley 2015). Relative

226   dispersion was computed as the average for each station within a year. Ranging from 0 to 2,

227   lower values generally indicate lower stress (Warwick and Clarke, 1995). IMD produces a

228   singular value that reflects the contrast of the rank of Bray-Curtis similarities in one group

229   against the ranked similarities of another. A value of +1 signifies all similarities among samples

230   of a group are higher than any among the contrasting group. A value of 0 and -1 infers little to no

231   difference between groups (Warwick and Clarke, 1995). For either dispersion metric, individual

232   sample values cannot be computed thus statistical testing cannot be applied, and inferences are

233   instead made by empirical contrasts.

234   Tests of oil-impact based on macrofauna (feeding guild abundance, BPA, and DwH

235   macrofauna indicators) were tested in a two-way PERMANOVA with site and time as fixed

236   factors. This was followed by pairwise comparisons to identify differences between time points

237   and between sites within time points. Significance was determined by Monte Carlo simulation

238   when low permutations (< 100) occurred. In order to run PERMANOVA tests of abundance,

239   BPA, and DwH indicators, values from these metrics were root-transformed and Bray-Curtis

240   similarities were calculated. Pairwise Euclidean similarities were calculated for untransformed

241   species richness and Pielou's evenness for the PERMANOVA. Choice of similarity index was

242   made as prescribed by Anderson et al. (2008).

243   To lower the risk of a type I error for inter-site pairwise comparisons, a Bonferroni

244   correction of α/k where k represents the number of comparisons was applied (see Cabin and

245   Mitchell (2000)), thus based on a critical α of < 0.05, significance was detected if α fell below

246   0.005 for 2012 and 2013, and 0.0024 for 2014 for pairwise comparisons. All abundance and

247   diversity metrics were computed and PERMANOVA tests were conducted in PRIMER v 7.0.13

248   (Clarke and Gorley 2015). Plots of univariate metrics were made in R 3.5.3 (R Core Team 2019).

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250   ### III.   Results

251   *3.1 Analysis of abundance, community and feeding guild composition and analysis of structure*
252
253   A total of 2455 macrofaunal individuals across 119 families and higher taxa were

254   identified and counted. Macrofaunal proportional abundances remained relatively stable through

255   time for each of the sites in the canyon and on the adjacent slope (Table 2). Polychaetes

256   dominated in each community through time, with $51 - 66\%$ of the community composition, and

257   the highest proportions at XC3 and XC4. Tanaids were the next most abundant, with $5.3 -$

258   15.35% of the total abundance. Tanaids had rather high proportions for NT800 in both years, and

259   at S36 and XC4 in 2012. Aplacophorans exhibited high abundances at XC2 in both years and

260   bivalves exhibited high abundances in both years at XC3. In the three-year sites, amphipods and

261   the non-tanaid crustaceans generally exhibited slight increases through time. Tanaids dropped by

262   almost 8.5% at the deepest site of XC4 between 2012 and 2013. Bivalves, aplacophorans, and

263   other molluscs displayed more disparate patterns with sporadic increases and decreases through

264   time with no group showing large changes. All crustacean and molluscan groups exhibited

265   somewhat marginal decreases in proportions in 2014 compared to 2012 in XC2. Similar trends

266   were observed at XC3 though tanaids and bivalves increased. Polychaetes increased almost 10%

267   in 2014 versus 2012 for XC2 but annelids otherwise did not change between years for other sites.

268   PERMANOVA showed time point was not significant overall for within site abundance,

269   species richness, or evenness (Figure 2). However, abundance was higher in 2012 for PCB06 (vs

270   2013, t = 3.4619, **p = 0.032**; vs 2014, t = 3.0148, **p = 0.04**) and S36 (vs 2013, t = 3.5856, **p =**

271   **0.019**; vs 2014, t = 2.9593, **p = 0.038**) (Fig 2A). Mean species richness showed no significant

272   pairwise yearly change at any sites (Fig 2B). Average evenness for XC4 and PCB06 showed a

273 general increase across years, while XC2 stayed about level and S42 and NT800 decreased (Fig

274 2C).

275   Table 3 summarizes the feeding guild composition of the DeSoto Canyon macrofauna in

276 2012-2014. Deposit feeders dominated through time and across sites, representing 60% or more

277 of all individuals, with XC4 having higher proportional abundances than any other site.

278 Omnivores and carnivores followed in dominance and varied across stations but maintained

279 relatively similar proportions through time. Suspension feeders and chemosymbiotic species

280 were the least abundant, holding proportions of 9% or less across time and stations. XC3 had

281 chemosymbiotic taxa rise almost 7% between 2012 and 2014. No consistent changes in feeding

282 guild proportions were observed across sites except for carnivores who consistently increased

283 through time for all canyon stations. No drastic difference in proportions were observed between

284 slope and canyon stations.

285   Cluster analysis of macrofaunal community structure showed samples clustered mostly

286 by depth and by station and somewhat by year within station (Fig 3A). Samples from XC4, the

287 deepest station at 2200 m, branched off as the most disparate cluster. Open slope stations S42

288 and NT800 grouped together in another single, separate cluster with a high degree of overlap in

289 structure between the sites, regardless of time point. Among the remaining samples, two of the

290 XC3 samples from 2012 cluster outside any other group. All time points for S36 form a cluster,

291 and all time points from PCB06 and XC2 cluster together with the remaining XC3 samples. A

292 NMDS of community structure (Fig 3B) largely reflected groups delineated by the cluster

293 analysis. Tests of community structure mainly indicated a significant difference by station (**p <**

294 **0.001**, Table 4) rather than by time point (p = 0.17, Table 4). The interaction of time point and

295 station also produced a low p-value (**p = 0.004**, Table 4). Despite these results, the trajectory

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296   plots of community structure for each station suggests some interannual variability in community

297   structure at each site (Fig 3C). Two-year time-series sites, XC2 and XC3, showed relatively little

298   change between 2012 and 2014. All three-year sites, PCB06, S36, and XC4, seemed to

299   experience a larger change in structure going from 2012 to 2013, compared to 2013 to 2014.

300   Feeding guild structure cluster analysis showed a higher percent similarity among

301   samples but did not group as distinctly by station as the data based on family identification, or by

302   time point, but some depth-based clustering was observed (Fig 4A). XC4 again formed its own

303   cluster. All other stations formed a second, larger cluster that split into 2 groups, one containing

304   2012 and 2014 samples from PCB06, XC2 and three of the XC3 samples, the other with the

305   remaining stations and time points. Feeding guild structure depicted by NMDS, affirmed the

306   cluster analysis (Fig 4B). Community structure did not show a strong grouping by time point (p

307   = 0.729, Table 4) nor by the interaction (p = 0.208, Table 4). Trajectories of structure across time

308   illustrate stronger changes from the beginning of time-series (compared to the same graph based

309   on families Fig 3B) for all stations except S42 (Fig 4C) that suggest modest interannual

310   variability. Three-year time-series sites in the canyon suggest less differentiation between 2013

311   and 2014 (which are also the only years S42 was sampled) than compared to the differentiation

312   of either year to the 2012 time point.

313

314   *3.2 Tests of Deepwater Horizon indicators*

315   The various indicators employed to gauge the impact of the Deepwater Horizon indicated

316   minimal differences through time. The BPA index exhibited a high variance in 2012 across

317   almost all sites sampled in that year (Fig 5). S36 show a gradual decline from 2012 - 2014 while

318   XC2, NT800 and S42 seemed to show a slight increase. Due to the absence of amphipods in two

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319   of the three replicates for XC3 in 2014, the BPA could not be computed thus the whole station

320   was excluded from analysis. Though the BPA could not be computed for XC3 in 2014,

321   polychaete abundance for that year is plotted to contrast the BPA computed in 2012 and shows

322   the high number of polychaetes in that year. Through time, average BPA ratios for open slope

323   stations in 2013 (10.46 – 15.16) were generally lower than canyon stations in the same year

324   (19.88 – 37.83) while 2014 open slope (15.00 – 15.89) and canyon (12.33 – 17.48) values were

325   more equivalent except for XC3 where only polychaetes were recorded at 122 individuals and

326   for XC4 with a mean BPA of 26.06. Time point was not significant for the comparison of BPA

327   but site (**p < 0.001**, Table 5) and the interaction of site and time point were (**p = 0.021**, Table 5).

328   The only pairwise comparisons where effects were observed were in 2013 when average BPA

329   for S36 (20.6) (**p = 0.016**, Table 5) and XC4 (37.8) (**p = 0.013**, Table 5) were greater than S42

330   (10.16).

331   Among feeding guild abundances, two-way PERMANOVA did not indicate differences

332   for time points but did among stations for most feeding guilds (Pseudo- $F_{6,49}$ = 8.4258 – 25.322,

333    $p < 0.05$, Table A1) except for suspension feeders (Pseudo-$F_{2,49}$ = 1.4517, p = 0.15) (Table A1).

334   The interaction term was only significant for chemosymbiotic species (Pseudo-$F_{8,49}$ = 2.3414, **p

335   = 0.03**). A couple of pairwise site comparisons for chemosymbiotic feeders resulted in negative

336   test statistics (Table A1), an artifact of PERMANOVA whose meaning remains elusive

337   (Anderson et al., 2008), and thus were not interpreted. Time point pairwise tests only resulted in

338   differences for deposit feeders at only PCB06, with larger abundances in 2012 (vs 2013, t =

339   2.7542, **p = 0.039**; vs 2014, t = 3.4156, **p = 0.022**) (Fig 6B) and chemosymbiotic taxa showed a

340   significantly higher abundance at XC3 in 2014 versus 2012 (t = 2.7919, **p = 0.014**) (Fig 6E).

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341 XC2 had higher proportional abundances of carnivores in 2014, but the difference was not

342 significant (t = 2.5478, p = 0.074).

343   Site-to-site carnivores in 2012, XC4 (mean 6.7 ind) were significantly less abundant than

344 PCB06 (mean 29.7 ind, **p = 0.004**), XC2 (30.3 mean ind, **p = 0.001**) and S36 (mean 18.7 ind, **p =**

345 **0.004**). No differences were observed in 2013 but by 2014, the average number of carnivores at

346 S42 (16.7 ind) was lower than PCB06 (32.3 ind, **p = 0.001**) and XC2 (46 ind, **p = 0.001**), while

347 XC2 was greater than XC3 (20 ind, **p = 0.002**). Deposit feeders were more abundant at PCB06

348 than S36 (average 154.7 ind against 104 ind, **p = 0.003**) in 2012. In 2013 at S42 (80.7 mean ind)

349 and NT800 (85.5 mean ind) (**p = 0.002**) deposit feeders were greater than XC4 (48 mean ind, **p =**

350 **0.001**). XC4 was less abundant in deposit feeders than S36 (76.7 mean ind, **p = 0.003**) in 2013.

351 Non-canyon stations in 2013, S42 (80.7 ind) and NT800 (85.5) also showed higher abundance

352 than XC4 (48 ind) (**p = 0.001** and **0.002** respectively). Average abundance for deposit feeders in

353 2014 for S42 (76.7 ind) was less than XC2 (159 ind) (**p = 0.002**). No inter-site comparisons were

354 significant for omnivores and suspension feeders. Chemosymbiotic feeders were only significant

355 in 2014 where XC3 (mean 18.7 ind) was higher than S36 (mean 1.3 ind) and XC4 (mean 1.3 ind)

356 (**p=0.002** respectively). Test results of site pairwise comparisons are summarized in Table A2 of

357 the supplementary materials.

358   DwH macrofaunal indicator species proportions occupied a large range of values across

359 time points. Cosmopolitan taxa (Fig 7A) generally held high proportions, averaging about 18.5 –

360 35% across all sites. Tolerant taxa (Fig 7B) exhibited average proportions of 10 – 16% for non-

361 canyon stations and 16 – 40% for sites within the canyon. Possibly tolerant taxa (Fig 7C) held

362 low proportions, 0 – 5.4%, across all sites. Possibly sensitive proportions averaged 4.4 to 18.7%

363 (Fig 9D) and sensitive groups averaged 17.6 – 47.5% in proportion (Fig 7E). Two-way

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364   PERMANOVA indicated a significant difference among sites (Pseudo-$F_{8,49}$ = 5.1431 – 23.471, **p

365   < 0.05**, Table A2), but not among time points (Table A2). The interaction term was only

366   significant for sensitive taxa (Pseudo-$F_{8,49}$ = 2.2515, **p = 0.027**, Table A2). Pairwise tests,

367   however, yielded differences where global tests did not find them. XC2 and XC3 demonstrated

368   some of the sharpest differences between years. Possibly tolerant groups were observed to be

369   more abundant in 2014 compared to 2012 at XC2 (t = 3.5601, **p = 0.024**) and fewer in XC3 (t =

370   3.0391, **p = 0.045**) for the same time period. Further, S36 exhibited greater possibly sensitive

371   group proportions in 2012 compared to 2013 (t = 3.4796, **p = 0.032**) but not versus 2014.

372   Site-by-site test results are summarized in Table A2. By site, cosmopolitan taxa

373   constituted larger proportions for XC2 (mean 35.2%) than XC3 (mean 21.8%) **(p = 0.002)** in

374   2012. In 2013, cosmopolitan taxa proportions were higher in S42 (32.6%) than NT800 (24.0%)

375   **(p = 0.004)**. S42 was also higher than XC4 (average 18.5%) **(p = 0.001)**. No differences were

376   observed by 2014. Tolerant taxa proportions were only higher for XC3 (mean 37.5%) compared

377   to S36 (24.9%) **(p = 0.001)** in 2012. In 2013 average tolerant taxa proportions at XC4 (26.4%)

378   were lower than non-canyon stations S42 (0.5%) **(p = 0.004)** and NT800 (9.8%) **(p = 0.003)**, and

379   by 2014 no differences were observed. Average proportions of possibly tolerant taxa were only

380   different in 2012 where XC3 (3.5%) was greater than XC4 (1.3%) **(p = 0.003)**, and 2014 where

381   XC2 (5.4%) was greater than XC4 (0.0%) **(p = 0.001)**. No pairwise differences were observed

382   between sites for possibly sensitive taxa. Sensitive taxa community proportions in 2012 were

383   higher at S36 (40.2%) and XC4 (47.5%) **(p = 0.001)** compared to XC3 (17.6%) **(p = 0.001)**. No

384   differences were observed in 2013, but by 2014 proportions of sensitive taxa were higher at

385   NT800 (average 39.2%) **(p = 0.001)** and S36 (40.1%) **(p = 0.002)** than XC2 (21.0%).

17

386   Community dispersion ranged from 0.12 – 1.693 among stations and years (Table 6).

387   Non-canyon slope sites had dispersion values ranging from 0.87-1.24. PCB06 and XC2 had

388   some of the lowest values of any station, ranging from 0.12 – 0.733, indicating generally low

389   community stress. XC3 exhibited a decrease with a dispersion value of 1.28 in 2012 and 0.413 in

390   2014. S36 had relatively high values (1.24 – 1.52), but XC4 exhibited the highest dispersion,

391   ranging from 1.693 to 1.8.

392   Pairwise IMD for the 2012 inter-site comparisons generally indicated that dispersion was

393   only different for S36 compared to XC2 and XC3 vs XC4 (Table 6). In 2013, S36 and XC4

394   showed some slight differentiation in dispersion and stronger differentiation compared to non-

395   canyon stations S42 and NT800 and to each other. By 2014, XC2 and XC3 had moderate to high

396   dispersion differences with every other stations. S36 also had differences compared to S42 and

397   XC4, and XC4 was also different from both non-canyon stations.

398

399   **IV.   Discussion**

400   *4.1 Temporal variability in DeSoto Canyon macrofauna and the adjacent slope*

401   In this examination of temporal variability of Desoto Canyon macrofauna from 2012 –

402   2014, most community parameters showed no significant difference through time at a given

403   station including total macrofaunal abundance, species richness, evenness, and abundance of

404   most feeding guilds. Only a few parameters showed statistically significant changes at one or a

405   few specific sites these include a decrease in abundance of total macrofauna at PCB06 and S36,

406   and an increase in the abundance of deposit feeders decreased at PCB06, and in the abundance of

407   chemosymbiotic species at XC3 between 2012 and 2014. Community structure, though not

408   statistically significant, did shift over time within each station though, indicating some temporal

18

409   variability in community structure, with a consistently larger change within each site from 2012

410   to 2013 than there was from 2013 to 2014. These somewhat low levels of change suggest that

411   DeSoto Canyon communities were largely stable over the 3-year time period.

412   The lack of pronounced interannual variability in abundance at most stations is consistent

413   with studies of non-canyon central NGOM polychaete assemblages spanning the continental

414   shelf to the slope, from 1983 – 1984, in which Qu et al. (2017) and Reuscher and Shirley (2017)

415   found relatively minor interannual variability in abundance, present in only a subset of the total

416   sites investigated and often with the peak attributed to seasonal changes.

417   These results are inconsistent however, with other studies across the broader NGOM, that

418   indicated temporal changes in abundance. For example, Pequegnat et al (1990) were the first to

419   publish data to suggest interannual patterns in abundance among western, central, and eastern

420   regions of the NGOM. From a later broad survey of the NGOM, Montagna et al. (2020) showed

421   a significant 36% decrease in macrofaunal abundance from 2000 to 2001. Additionally,

422   northwestern GOM macrofauna in a timespan close to the present study, 2010 – 2012, increased

423   in abundance (Salcedo et al., 2017).

424   Inter-year investigations of diversity were not part of the focus of previous studies

425   examining NGOM benthic temporal variability but community structure has been. Central

426   NGOM continental slope polychaete assemblages showed large trajectory change in NMDS

427   plots in 1983-1984, but when statistically tested, differences were minor, especially for sites

428   deeper than 845 – 2540 m (Reuscher and Shirley, 2017). This is in keeping with the present

429   study as the sites sampled in the DeSoto Canyon exhibited trajectories indicative of change and

430   fall into the same depth range.

19

431   The short time period of the current study may have missed more gradual longer-term

432   changes that can occur in benthic communities. Studies across decadal time scales may be

433   needed to capture these patterns, but may also reveal long term stability in diversity in benthic

434   macrofauna. For example Qu et al. (2017) and Reuscher and Shirley (2017) found polychaete

435   assemblages variability between 1983 and 1984 though high, was not outside the range of

436   variation from 2000 – 2001 at the same sites. Similarly Qu et al. (2017) observed diversity was

437   similar for polychaetes between 1983 – 1985 and 2000 – 2002 on the western and central NGOM

438   slope. Wei and Rowe (2019) observed the same diversity pattern for NGOM macrofauna as a

439   whole across the same time period. Variability in community structure has less straightforward

440   patterns in the long term. Distinct decadal separation in community structure was observed for

441   upper continental slope polychaete assemblages (Qu et al., 2017) but not assemblages on the

442   lower slope (Reuscher and Shirley, 2017).

443   Another possibility for long-term patterns is that there may be minimal long-term

444   trajectories in communities that are punctuated by abrupt change brought on by stochastic events.

445   In longer term time-series, polychaete assemblages in the northeast Atlantic, for example,

446   displayed little change in density in the 5-6 years before the 'Amperima' event, wherein a

447   suspected food pulse caused a rise in opportunistic cirratulids, spionids, and opheliids and also

448   generated augmented numbers of deposit feeders and predators during and in the 2-3 years

449   afterwards (Soto et al. 2010). In two long-term time-series of northern Pacific (1991 – 2005) and

450   Atlantic (1991 – 1999) polychaetes, the greatest change in rank abundance of family and

451   functional group did not occur until 1998, seven years after monitoring had started (Laguionie-

452   Marchais et al. 2013). Temporal changes in community structure were also observed in that

453   study as the 1998 time point tended to form a singular cluster, indicating some perturbation that

20

454 year but no explanatory environmental factor could be identified (Laguionie-Marchais et al.

455 2013). Thus, greater temporal variability within the DeSoto Canyon cannot be ruled out as

456 further research on DeSoto Canyon macrobenthic temporal dynamics over longer time scales

457 may reveal patterns that are not apparent in a three-year temporal study.

458

459 *4.2 Is there any evidence of the influence of the DwH oil spill on DeSoto Canyon macrofauna?*
460
461   Patterns of macrobenthic oil spill response and recovery have primarily been investigated

462 in shallow, coastal environments where recovery is a product of time and distance from the

463 contamination source. After inundation by hydrocarbons, direct mortality can be observed, then

464 rapid recolonization (months to a year) of opportunistic taxa occurs followed by a stabilization in

465 abundance and richness (Berge 1990; Lu and Wu 2006). For macrofauna, this means initial

466 increases in abundance driven by a dominance of deposit feeders, like polychaetes, and a drop in

467 sensitive taxa such as crustaceans (especially amphipods) and molluscs (for an review of effects,

468 see Suchanek (1993) and Peterson et al. (1996)). Large shifts in community composition would

469 then result in a detectable alteration in community structure. Offshore GOM oil production

470 impact assessments and reviews have generally corroborated the same effect, concluding

471 continued industrial processes foster higher community dispersion, augmented abundances of

472 deposit feeding polychaetes (driven by increased organic loading or resource availability), and

473 are detrimental to sensitive crustaceans such as ostracods and amphipods (Montagna and Harper

474 1996; Peterson et al. 1996; Hernández-Arana et al. 2005). In terms of specific metrics of oil

475 impact, more impacted sites and time points would be expected to have higher BPA ratios and

476 higher dispersion (Andrade and Renaud, 2011). Among the DwH macrofaunal indicators, a

21

477 greater proportion of tolerant and possibly tolerant groups along with lower relative abundances

478 of sensitive and possibly sensitive taxa would be expected at impacted sites.

479　　　Schwing and Castillo (2020) posited oil spill recovery takes 3 – 5 years for GOM

480 infauna based on evidence from foraminiferan abundance and recovery rates from the Ixtoc I oil

481 spill in 1979 and the DwH. Sampling for the current study began two years after the initial spill,

482 thus based on the patterns outlined above, if oil reached the canyon, sampling likely missed the

483 initial phases of disturbance and the very start of the recovery process. It might be predicted that

484 any impacted sites would then be in the phase of decline and stabilization in abundance,

485 increasing diversity, and altered community structure through time. Year-to-year, only PCB06

486 and S36 exhibited any evidence of the expected impact patterns, showing peaks in abundance in

487 2012 decreasing into 2014. This lack of evidence in community patterns at most sites matches

488 the lack of evidence for spill influence based on the oil spill metrics. Functional group abundance

489 was only elevated for PCB06 in 2012 for deposit feeders and for chemosymbiotic taxa at XC3 in

490 2014. The increase in chemosymbiotic taxa was driven by an increase in thyasirid bivalves

491 which are tolerant to DwH contamination (Washburn et al., 2016), and may suggest an increased

492 influence at this site in 2014 compared to 2012. Deposit feeders increased at XC3 in 2014 also,

493 which could support this inference, however the increase was not significant. There was a

494 significant decrease in potentially tolerant species at XC3 in the same time period though, which

495 contradicts the potential for increased oil influence at this site through time. Also, the increase in

496 thyasirids at XC3 did not correspond with an increase in tolerant taxa at the same site over the

497 study period, but XC3 had a higher proportion of tolerant taxa than any other site. For other

498 stations, DwH indicator taxa proportions largely did not change through time except for a

499 significant increase in potentially tolerant groups at XC2. Community dispersion was

22

500   consistently high for XC4, but most other sites had dispersion comparable to or lower than non-

501   canyon control stations.

502   Site-to-site, the number of differences found between macrofaunal communities among

503   the oil-influence metrics were not high. Often, when differences were found, they were not

504   detected by the final year of the sampling program. Sites with confirmed hydrocarbon

505   deposition, PCB06, XC2, XC3, and XC4, most frequently exhibited greater potential indicators

506   of impact compared to other canyon sites or adjacent slope sites. XC3 contained the most

507   evidence for impact, but the evidence was stronger in 2014 rather than 2012. Chemosymbiotic

508   feeders there in 2014 had elevated numbers compared to all other canyon stations and time

509   points and, among the DwH macrofaunal indicators, contained greater proportions of tolerant

510   and possibly tolerant taxa and low amounts of sensitive taxa compared to other canyon sites,

511   especially in 2012. XC2 is the only other site to show low levels of sensitive taxa in both

512   sampling years. XC4 showed the highest levels of dispersion (i.e., community stress) in all years.

513   Finally, disparities among community dispersion were most frequent for XC2 and XC3 and

514   persisted until the final year of sampling.

515   Taken together these metrics of oil influence indicate that the evidence for impacts to any

516   of the stations was neither consistent among indicators nor overwhelming on a year-to-year or

517   site-to-site basis. If any stations were still being affected by 2014, stations XC2, XC3, and XC4

518   had the greatest potential effect followed by PCB06. The depths of PCB06, XC2, and XC3, are

519   consistent with the hydrocarbon plume depths (~1000 and 1400 m) reported by Hollander et al.

520   (2012). The increased effects at XC3 in 2014 (plus high dispersion in 2012) plus the high

521   dispersion at XC4 could potentially be explained by the turbidity-based downslope transport of

522   hydrocarbons that Romero et al. (2020) proposed.

23

523   Severe infaunal impact first documented in close proximity and through time for the

524   DwH (Qu et al. 2015; Montagna et al. 2016; Reuscher et al. 2017; Washburn et al. 2017)

525   indicates the distance, low level of deposition, and time of sampling likely mitigated the overall

526   effect observed for the DeSoto Canyon. Indeed, DeSoto Canyon stations are in the areas of the

527   least petrocarbon deposition (Fig 1). Canyon sites are also located in regions of low impact

528   according to predicted infaunal impact maps devised by Montagna et al (2013) and Reuscher et

529   al. (2020) (Fig A1A and B). Chemical evidence would also suggest that impact to DeSoto

530   Canyon macrofauna would not have been severe. For at least three of the sites in our target study

531   area, XC2, PCB06, and XC3; total PAHs were elevated in 2010 (up to 524 ng g^{-1}, 329 ng g^{-1},

532   and 190 ng g^{-1} respectively) and 2011 (up to 99 ng g^{-1}, 171 ng g^{-1}, 373 ng g^{-1}, respectively)

533   following the DwH (Romero et al. 2015). Deposition levels were not measured or reported for

534   XC4, but relatively heavy deposition can be inferred from the radiocarbon data presented in

535   Figure 1B. These levels are generally greater than previous surveys of the canyon where

536   background PAHs were observed to be 114 ± 47 ng g^{-1} (Rowe and Kennicutt 2009) but lower

537   than the typical range of urbanized and industrialized coastal regions worldwide which span

538   ~1200 – 11000 ng g^{-1} (Qiao et al. 2006; El Nemr et al. 2013). Further, concentrations reported by

539   Romero et al. (2015) are also considered low to moderate pollution levels based on the proposed

540   range of potential impact for aliphatics (10 – 100 μg g^{-1}) and PAHs (100 – 1100 ng g^{-1})

541   (Baumard et al. 1998; Commendatore et al. 2012). Observed PAH levels at these sites also fall

542   below the "low limit" of PAH concentrations thought to be biologically toxic in shallow

543   sediments (4402 ng g^{-1}) (Long et al. 1995). Further, in an effort to establish sediment quality

544   benchmarks for evaluating risks of oil-related impact to the deep-sea benthos from the DwH,

545   Balthis et al. (2017) derived a low probability of impact to macrofauna (< 20%) when total PAH

24

546   concentrations were lower than 4000 ng g^{-1} (ppm) and high (> 80%) when greater than 24000 ng

547   g^{-1}. Thus, it can be concluded the level of hydrocarbon influx into the DeSoto Canyon would not

548   have severely impacted resident biota and this conclusion is largely corroborated by the scant

549   effects observed in the macrofauna communities of the present study.

550

551   **IV.   Conclusions**

552   DeSoto Canyon macrofauna exhibited limited amounts of temporal change in abundance,

553   diversity, and community structure through the study period. Similarly, attempts to relate

554   observed trends to the DwH using established faunal oil-impact and disturbance metrics

555   indicated little to no evidence of impact. Sites with confirmed hydrocarbon deposition, XC2,

556   XC3, and XC4, had minor evidence of potential impacts, but results were not consistent across

557   metrics or years. The lack of temporal change and response to the DwH is likely a product of

558   distance from the wellhead (~40 – 185 km east/southeast), time of sampling, and the general low

559   deposition of DWH hydrocarbons within the canyon.

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Table 1. Summary of time-series sampling Sites in the DeSoto Canyon and the adjacent non-canyon continental slope. An "X" indicates samples were collected in that year at that station.

Site	Latitude	Longitude	Depth (m)	Time Points		
				2012	2013	2014
S42	28.2528	-86.4216	770	-	X	X
NT800	28.056	-85.9335	800	-	X	X
PCB06	28.99451	-87.4574	1100	X	X	X
XC2	29.1210	-87.8655	1137	X	-	X
XC3	28.9762	-87.8683	1510	X	-	X
S36	28.91851	-87.6722	1834	X	X	X
XC4	28.6365	-87.8685	2200	X	X	X

Table 2. Proportions of major macrofaunal taxa across pooled replicates of DeSoto Canyon sites (PCB06, XC2, XC3, S36, and XC4) and adjacent slope sites (S42 and NT800) by year.

	S42		NT800		PCB06			XC2		XC3		S36			XC4		
	2013	2014	2013	2014	2012	2013	2014	2012	2014	2012	2014	2012	2013	2014	2012	2013	2014
Amphipoda	4.84%	3.42%	3.53%	3.47%	1.64%	3.14%	3.28%	5.82%	3.87%	2.16%	0.16%	2.24%	2.45%	4.88%	0.79%	0.49%	1.46%
Tanaidacea	8.87%	7.12%	12.94%	10.92%	8.22%	8.45%	7.53%	7.15%	5.66%	5.30%	8.21%	10.37%	8.82%	7.20%	15.35%	7.80%	6.83%
Other Crustacea	5.91%	6.55%	6.27%	4.22%	5.98%	6.29%	4.05%	4.83%	2.62%	4.52%	2.09%	6.91%	8.58%	9.51%	7.48%	8.78%	11.71%
Aplacophora	4.30%	5.41%	3.53%	3.97%	5.53%	1.57%	3.47%	11.65%	8.29%	1.57%	1.29%	1.42%	1.96%	0.51%	0.00%	0.49%	0.98%
Bivalvia	5.65%	5.98%	7.45%	4.71%	8.37%	7.07%	8.88%	9.15%	7.60%	11.79%	13.53%	5.69%	4.66%	6.94%	5.51%	7.32%	6.34%
Other Mollusca	2.96%	0.85%	3.92%	1.24%	0.30%	1.96%	2.12%	0.83%	0.41%	0.00%	1.93%	0.61%	1.96%	2.83%	0.79%	2.44%	0.49%
Nemertea	4.57%	5.70%	4.31%	3.47%	2.39%	5.30%	4.83%	2.50%	3.73%	1.77%	4.51%	4.27%	2.94%	4.11%	3.54%	5.37%	4.39%
Polychaeta	55.91%	57.83%	51.37%	59.31%	60.69%	60.90%	59.85%	54.24%	63.81%	65.23%	65.54%	63.82%	64.71%	59.64%	65.75%	65.37%	65.37%
Other Annelida	1.34%	1.14%	0.78%	1.49%	2.24%	1.18%	0.77%	0.83%	0.83%	3.54%	1.29%	2.24%	1.23%	1.54%	0.39%	1.95%	0.49%
Miscellaneous	5.65%	5.98%	5.88%	7.20%	4.63%	4.13%	5.21%	3.00%	3.18%	4.13%	1.45%	2.44%	2.70%	2.83%	0.39%	0.00%	1.95%

Table 3. Feeding guild composition for all Sites and time points pooled across replicates.

	S42		NT800		PCB06			XC2		XC3		S36			XC4		
	2013	2014	2013	2014	2012	2013	2014	2012	2014	2012	2014	2012	2013	2014	2012	2013	2014
Carnivore	14.52%	14.25%	12.94%	12.16%	13.30%	16.70%	18.73%	15.14%	19.06%	9.23%	9.66%	11.38%	11.03%	12.08%	7.87%	11.22%	12.20%
Deposit	65.05%	65.53%	67.06%	66.00%	69.36%	64.24%	61.58%	68.22%	65.88%	65.62%	65.70%	63.41%	66.91%	62.47%	75.59%	70.24%	73.17%
Omnivore	14.25%	11.40%	11.37%	12.90%	7.92%	12.38%	9.85%	9.65%	8.43%	16.50%	11.59%	17.48%	14.71%	16.97%	7.87%	10.73%	7.32%
Suspension feeder	3.23%	5.13%	5.10%	7.69%	6.88%	5.11%	6.76%	5.32%	5.80%	6.29%	4.03%	7.32%	6.62%	7.46%	7.87%	7.32%	5.37%
Chemo-symbiotic	2.96%	3.70%	3.53%	1.24%	2.54%	1.57%	3.09%	1.66%	0.83%	2.36%	9.02%	0.41%	0.74%	1.03%	0.79%	0.49%	1.95%

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Table 4. PERMANOVA results for the test of macrofauna community structure among and between time points from 2012 – 2014 across all sites. Bolded values indicate significant differences.

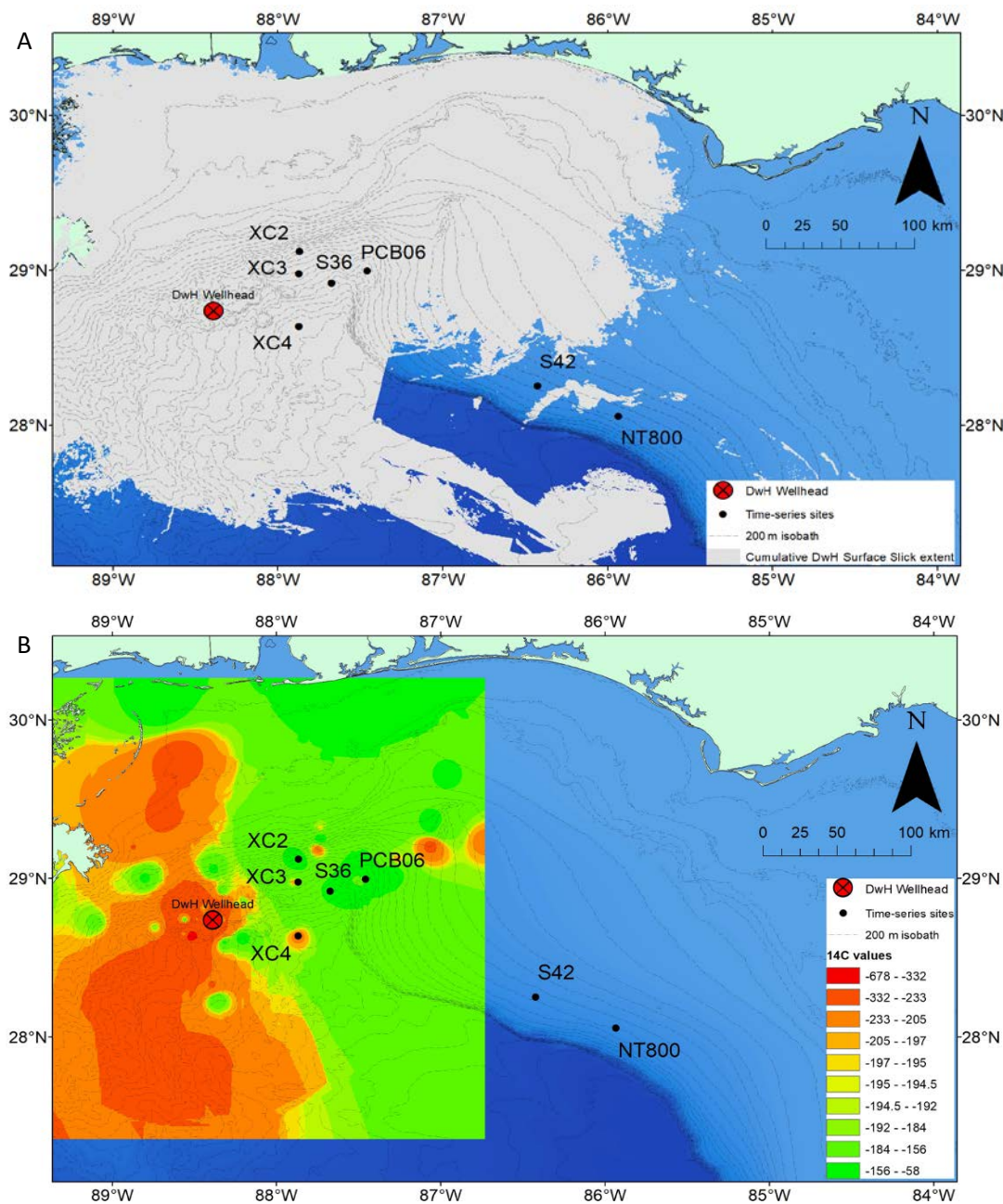| Source | df | Community Structure | | | | Feeding guild Structure | | | |
		SS	Pseudo-F	P(perm)	perms	SS	Pseudo-F	P(perm)	perms
Time point	2	1990.2	1.343	0.17	998	79.122	0.60557	0.729	999
Site	6	19489	5.9327	**0.001**	997	4697.8	15.077	**0.001**	999
Time point x Site**	8	5929.9	1.3539	0.004	999	522.77	1.2583	0.208	998
Res	33	18067				1713.7			
Total	49	46737				7330			

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Table 5. Summary of PERMANOVA results for the benthic polychaete/amphipod ratio. Sample size n = 3 for all groups in pairwise comparisons except for NT800 in 2012 where n = 2.
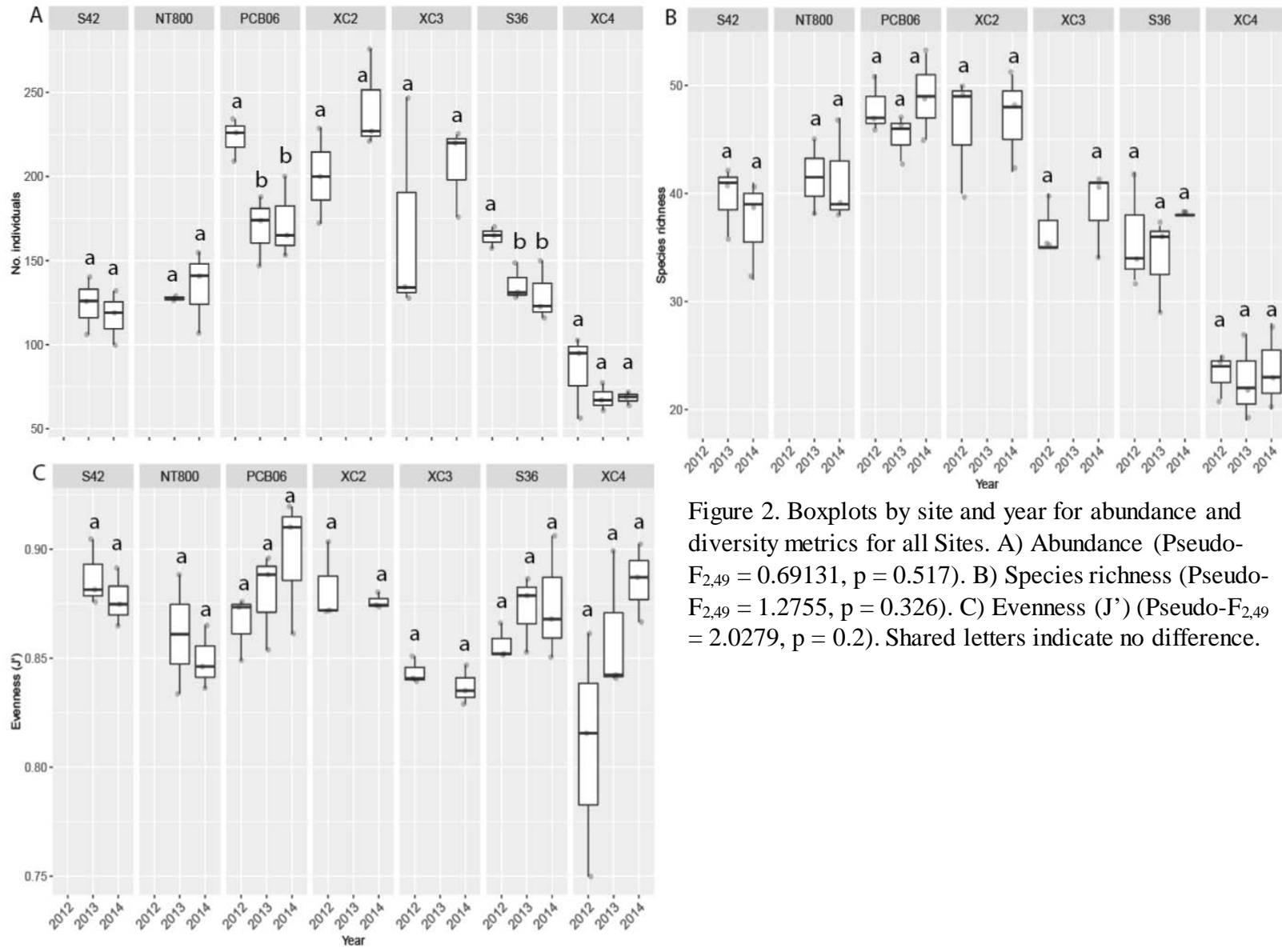
	df	SS	Pseudo-F	p-value		
Time Point	2	986.01	1.0892	0.381		
Station	6	8018.4	2.9524	**0.001**		
Time Point x Station	8	5924.6	1.6361	**0.021**		
Residual	33	14937				
Total	49	30137				
Site pairwise comparisons						
		2012		2013		2014
Groups	t	P(MC)	t	P(MC)	t	P(MC)
S42, NT800	-	-	0.65053	0.569	0.16321	0.914
S42, XC2	-	-	-	-	0.5	0.661
S42, XC3	-	-	-	-	1.511	0.155
NT800, XC2	-	-	-	-	0.65157	0.568
NT800, XC3	-	-	-	-	1.5468	0.144
XC2, XC3	2.2834	0.075	-	-	1.532	0.153
PCB06, S36	0.70725	0.523	0.42152	0.702	0.81779	0.448
PCB06, XC4	5.017E-2	0.993	1.4059	0.226	1.5176	0.192
PCB06, XC2	2.2968	0.062	-	-	0.45772	0.662
PCB06, XC3	0.11061	0.97	-	-	1.5417	0.131
PCB06, S42	-	-	1.2834	0.272	0.24232	0.869
PCB06, NT800	-	-	0.40902	0.709	0.18936	0.872
S36, XC4	0.70043	0.511	1.8634	0.125	1.7718	0.141
S36, XC2	2.3285	0.086	-	-	1.107	0.353
S36, XC3	0.61287	0.56	-	-	1.5353	0.137
S36, S42	-	-	4.0848	**0.016**	1.2571	0.677
S36, NT800	-	-	1.0185	0.37	1.6602	0.533
XC4, XC2	2.176	0.087	-	-	1.2071	0.303
XC4, XC3	0.14419	0.929	-	-	1.4755	0.172
XC4, S42	-	-	3.9943	**0.013**	1.2571	0.299
XC4, NT800	-	-	1.7854	0.159	1.6602	0.162

Table 6. Relative multivariate dispersion of macrofaunal communities at each site by year.

Site	2012	2013	2014
S42	-	0.867	1.053
NT800	-	1.24	1.2
PCB06	0.533	0.373	0.733
S36	1.173	1.52	1.24
XC2	0.627	-	0.12
XC3	1.28	-	0.413
XC4	1.8	1.293	1.693
Pairwise comparisons	2012 IMD	2013 IMD	2014 IMD
S42, NT800	-	-1	-0.111
S42, XC2	-	-	1
S42, XC3	-	-	0.778
NT800, XC2	-	-	1
NT800, XC3	-	-	0.556
XC2, XC3	-1		-0.556
PCB06, S36	-0.778	-1	-0.778
PCB06, XC4	-1	-1	-1
PCB06, XC2	-0.556	-	1
PCB06, XC3	-1	-	0.556
PCB06, S42	-	-0.778	-0.556
PCB06, NT800	-	-1	-0.556
S36, XC4	-1	0.111	-0.778
S36, XC2	0.778	-	1
S36, XC3	-0.111	-	0.778
S36, S42	-	1	0.333
S36, NT800	-	0.333	-0.111
XC4, XC2	1	-	1
XC4, XC3	0.778	-	1
XC4, S42	-	0.556	1
XC4, NT800	-	0.333	0.333

Figure 1A) Map of DeSoto Canyon and open slope time-series sites. Sites were sampled from 2012 – 2014 in the DeSoto Canyon and 2013 – 2014 on the adjacent slope. The light gray overlay is the maximum extent of the surface petroleum slick (ERMA, 2019). B) Time-series sites in relation to sediment radiocarbon contoured with the Inverse Distance Weighting method with data from Chanton et al. (2014).
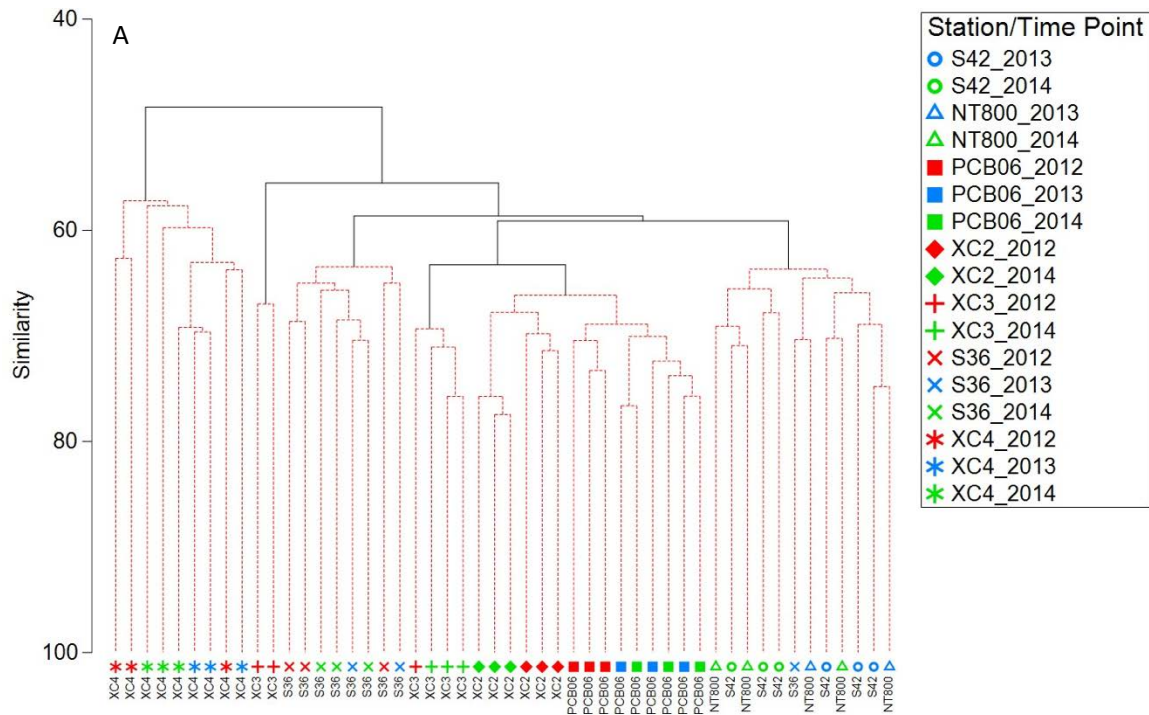
Figure 2. Boxplots by site and year for abundance and diversity metrics for all Sites. A) Abundance (Pseudo-$F_{2,49}$ = 0.69131, p = 0.517). B) Species richness (Pseudo-$F_{2,49}$ = 1.2755, p = 0.326). C) Evenness (J') (Pseudo-$F_{2,49}$ = 2.0279, p = 0.2). Shared letters indicate no difference.
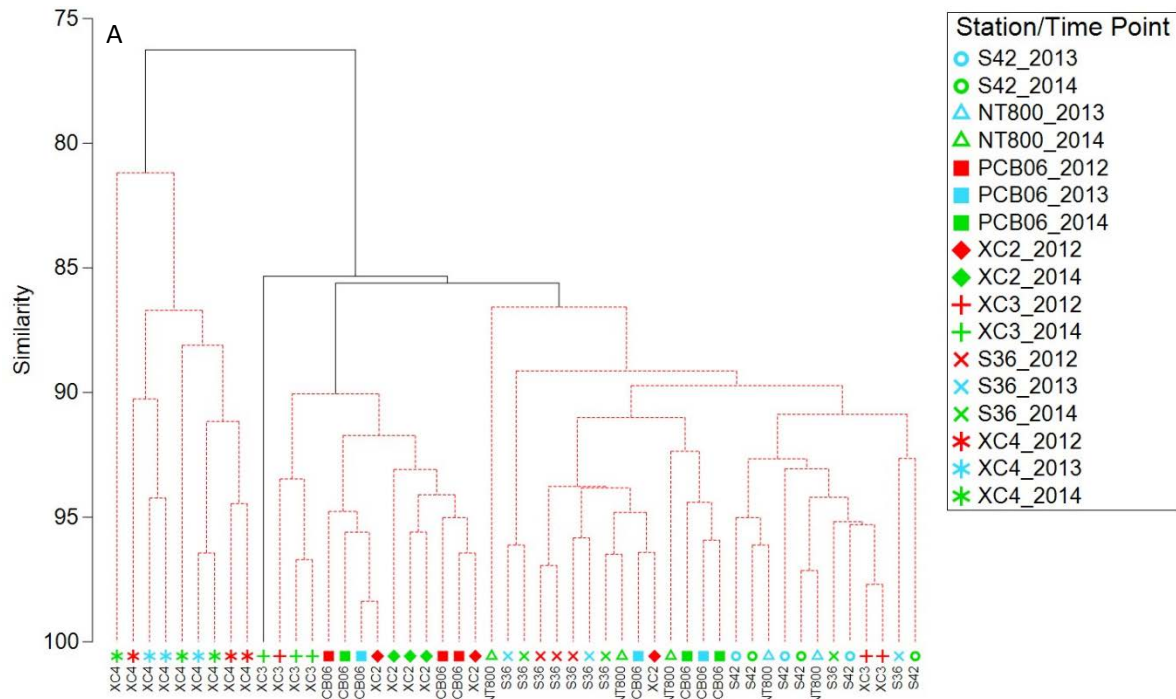
Figure 3A) Cluster analysis based on Bray-Curtis similarities of macrofauna abundance of DeSoto Canyon time-series sites and the open slope sites from 2012 – 2014. B) Non-metric multidimensional scaling of macrofauna community structure. C) Pooled samples for each time point at each Site with trajectory overlays from 2012-2014.
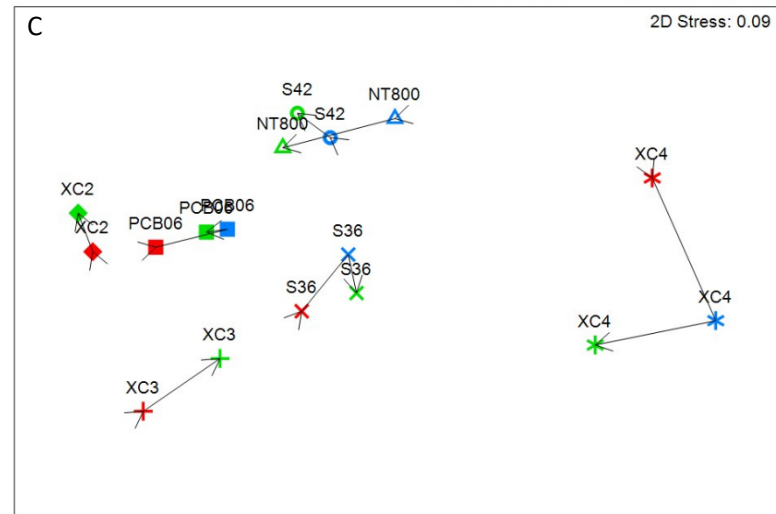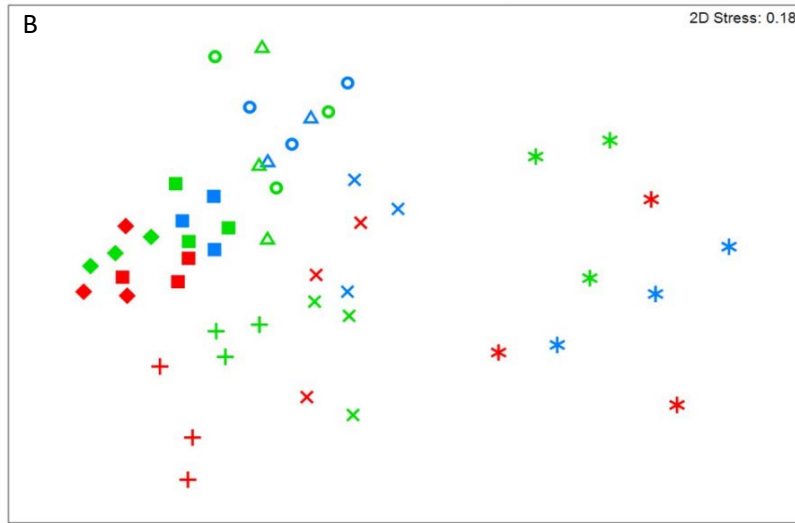
Figure 4A) Cluster analysis based on Bray Curtis similarities of macrofauna feeding guilds for DeSoto Canyon time-series sites and open slope sites from 2012 – 2014. B) Non-metric multidimensional scaling of feeding guild structure. C) Pooled samples for each time point at each Site with trajectory overlays from 2012-2014.
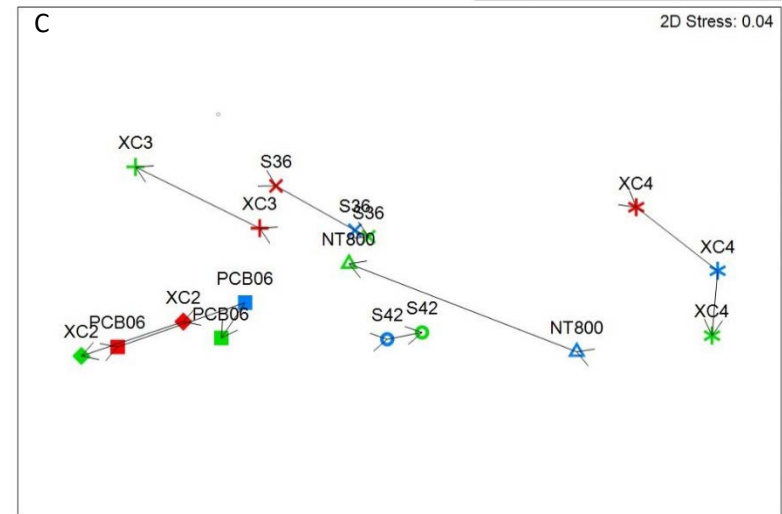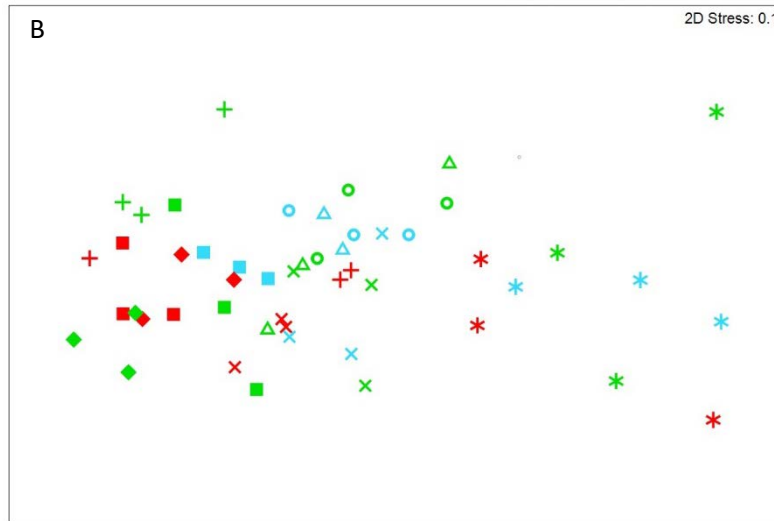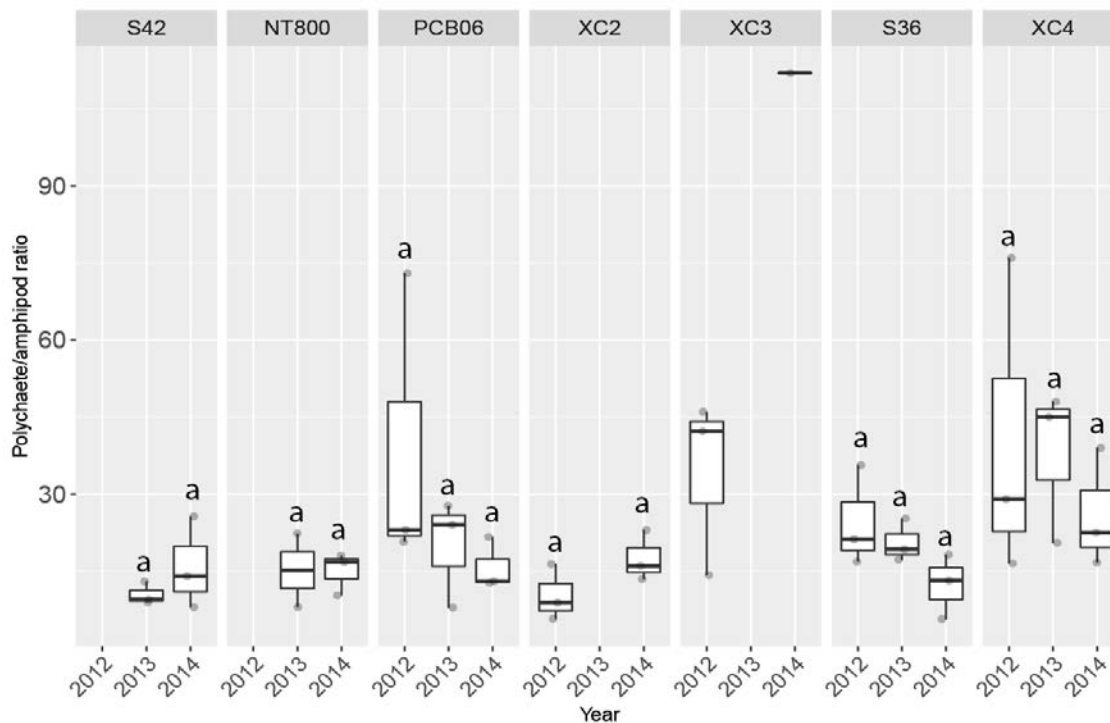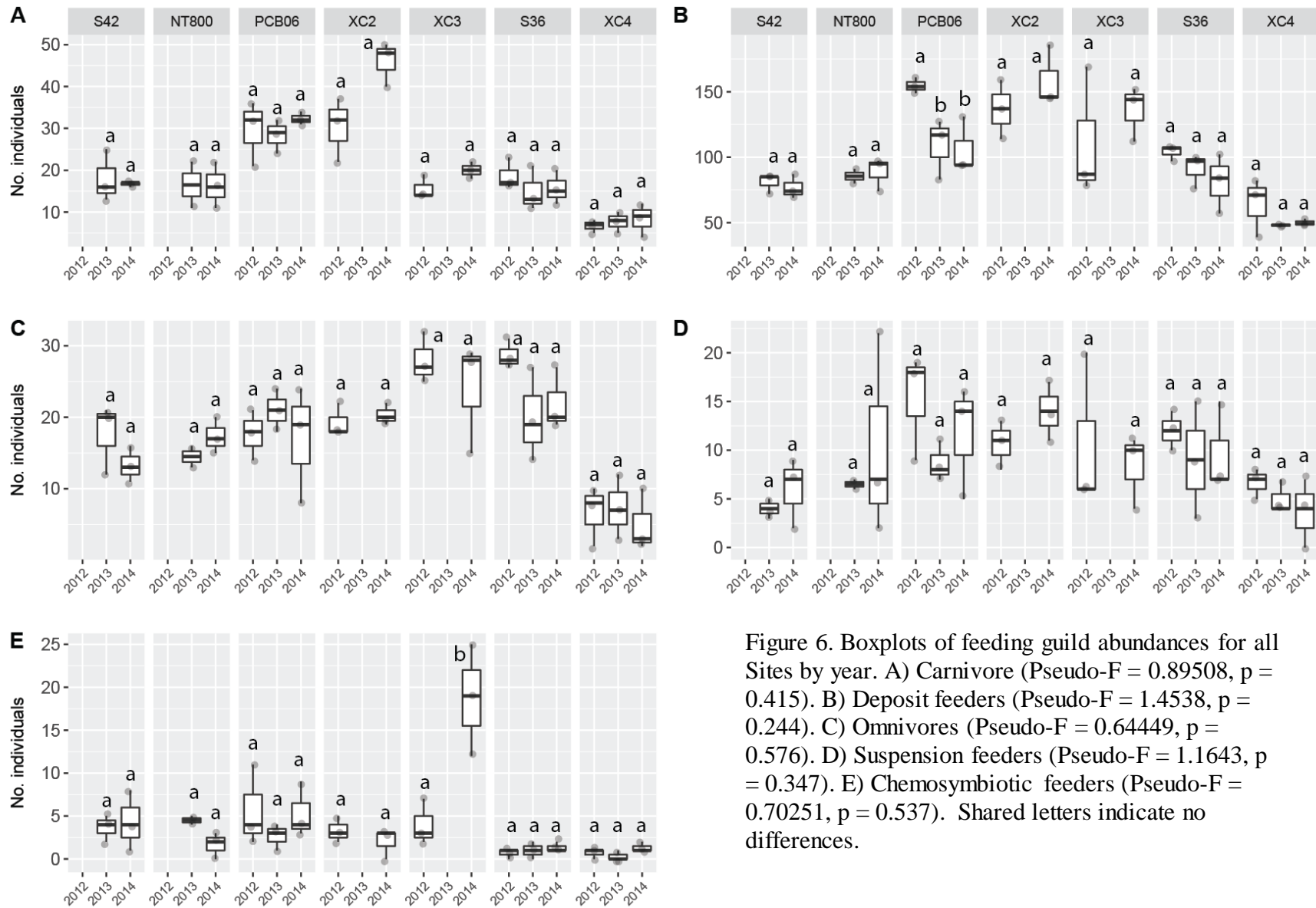
Figure 5. Benthic polychaete/amphipod ratio (BPA) across time for all sites (Pseudo-$F_{2,49}$ = 1.0892, p = 0.381). No amphipods occurred in two replicates for XC3 in 2014 and therefore it was excluded from analysis but the polychaete abundance for 2014 is plotted for comparison. Shared letters indicate no difference.

Figure 6. Boxplots of feeding guild abundances for all Sites by year. A) Carnivore (Pseudo-F = 0.89508, p = 0.415). B) Deposit feeders (Pseudo-F = 1.4538, p = 0.244). C) Omnivores (Pseudo-F = 0.64449, p = 0.576). D) Suspension feeders (Pseudo-F = 1.1643, p = 0.347). E) Chemosymbiotic feeders (Pseudo-F = 0.70251, p = 0.537). Shared letters indicate no differences.
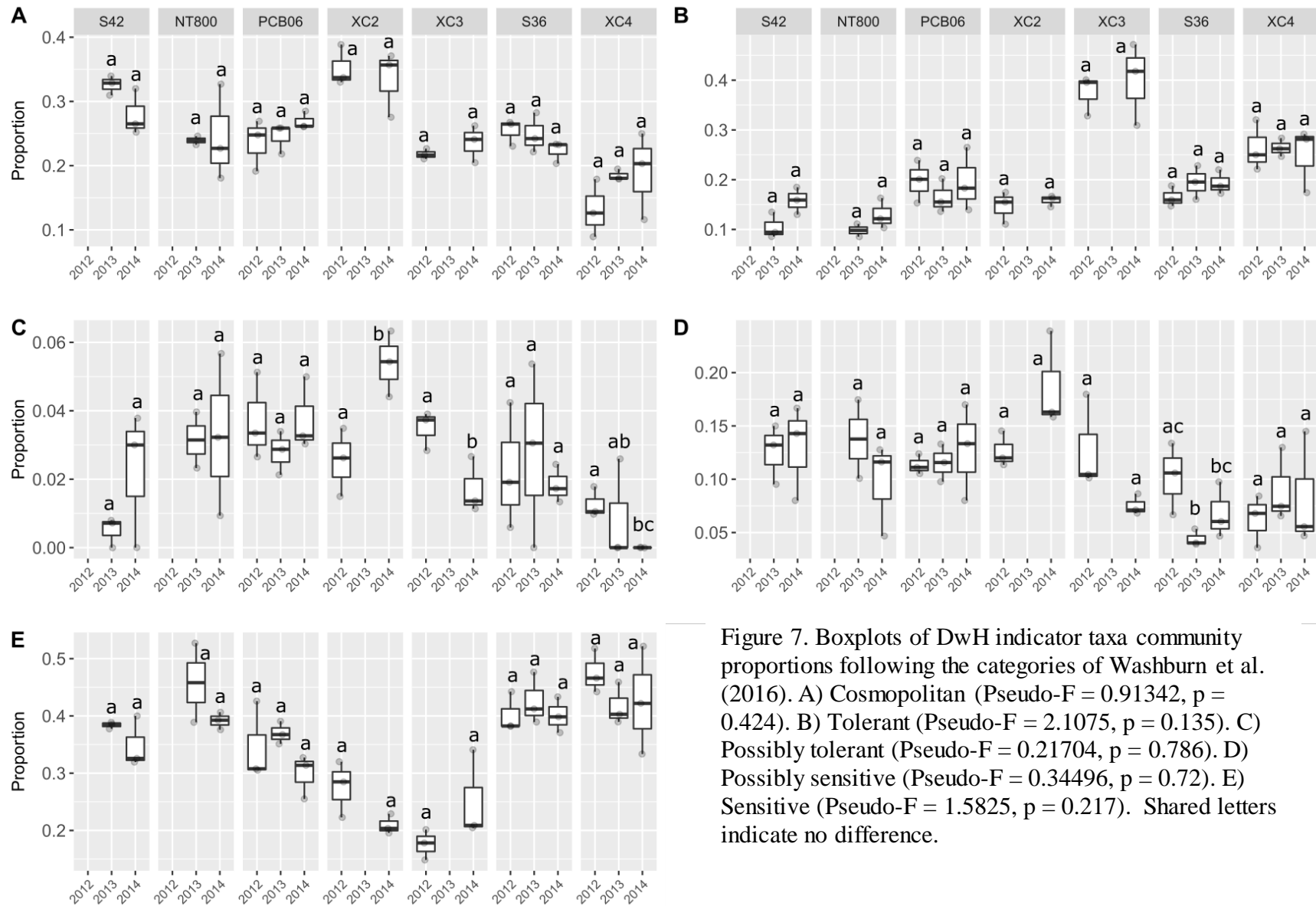
Figure 7. Boxplots of DwH indicator taxa community proportions following the categories of Washburn et al. (2016). A) Cosmopolitan (Pseudo-F = 0.91342, p = 0.424). B) Tolerant (Pseudo-F = 2.1075, p = 0.135). C) Possibly tolerant (Pseudo-F = 0.21704, p = 0.786). D) Possibly sensitive (Pseudo-F = 0.34496, p = 0.72). E) Sensitive (Pseudo-F = 1.5825, p = 0.217). Shared letters indicate no difference.