1 Title: Macrofaunal communities associated with chemosynthetic habitats from the U.S. Atlantic

2 margin: a comparison among depth and habitat types

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4 Authors: Jill R. Bourque[a]*, Craig M. Robertson[b], Sandra Brooke[c], Amanda W.J. Demopoulos[a],

5 Affiliations: [a] U.S. Geological Survey Wetland and Aquatic Research Center; [b] Bangor

6 University; [c] Florida State University

7

8 * Corresponding Author; 7920 NW 71st ST, Gainesville, FL, 32653, USA; jbourque@usgs.gov

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10 Abstract:

11   Hydrocarbon seeps support distinct benthic communities capable of tolerating extreme

12 environmental conditions and utilizing reduced chemical compounds for nutrition. In recent

13 years, several locations of methane seepage have been mapped along the U.S. Atlantic

14 continental slope. In 2012 and 2013, two newly discovered seeps were investigated in this

15 region: a shallow site near Baltimore Canyon (BCS, 366-412 m) and a deep site near Norfolk

16 Canyon (NCS, 1467-1602 m), with both sites containing extensive chemosynthetic mussel bed

17 and microbial mat habitats. Sediment push cores, suction samples, and Ekman box cores were

18 collected to quantify the abundance, diversity, and community structure of benthic macrofauna

19 (>300 μm) in mussel beds, mats, and slope habitats at both sites. Community data from the deep

20 site were also assessed in relation to the associated sediment environment (organic carbon and

21 nitrogen, stable carbon and nitrogen isotopes, grain size, and depth). Infaunal assemblages and

22 densities differed both between depths and among habitat types. Macrofaunal densities in

23 microbial mats were four times greater than those present in mussel beds and slope sediments

24 and were dominated by the annelid families Dorvilleidae, Capitellidae, and Tubificidae, while

25 mussel habitats had higher proportions of crustaceans. Diversity was lower in BCS microbial mat

26 habitats, but higher in mussel and slope sediments compared to NCS habitats. Multivariate

27 statistical analysis revealed specific sediment properties as important for distinguishing the

28 macrofaunal communities, including larger grain sizes present within NCS microbial mat

29 habitats and depleted stable carbon isotopes (δ^{13}C) in sediments present at mussel beds. These

30 results suggest that habitat differences in the quality and source of organic matter are driving the

31 observed patterns in the infaunal assemblages, including high β diversity and high variability in

32 the macrofaunal community composition. This study is the first investigation of seep infauna

33 along the U.S. Atlantic slope north of the Blake Ridge Diapir and provides a baseline for future

34 regional comparisons to other seep habitats along the Atlantic margin.

35

36 **Highlights:**

37 - First investigation of seep infaunal communities in U.S. mid-Atlantic margin north of

38   Blake Ridge at multiple depths

39 - Microbial mats and mussel bed habitats support locally high densities of infauna

40 - High β diversity over small and large spatial scales

41 - Stable carbon isotopic composition (δ^{13}C) and mud content explained the most variation

42   among NCS seep and non-seep habitats

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1. Introduction:

Cold seeps occur worldwide, often where methane or sulfide is forced upward through the sediment by pressure gradients (Levin, 2005). Anaerobic oxidation of methane and sulfate reduction results in the formation of carbonates and often high concentrations of hydrogen sulfide in sediments, which is toxic to most fauna (Vetter et al., 1991). The flow of seep products through sediments often results in recognizable biogenic habitats, including mussel and clam beds, microbial mats, and tube worm aggregations (Bernardino et al., 2012), where the dominant megafauna are dependent on chemoautotrophic endosymbiotic bacteria for nutrition (Kochevar et al., 1992). In addition, the physical structure created by chemosynthetic organisms provides heterogeneous habitat for diverse communities (Bergquist et al., 2003; Van Dover and Trask, 2000); thus these organisms serve as ecosystem engineers (e.g., Jones et al., 1996).

Sediment fauna associated with seep communities, including microbial mats and clam beds, have been studied in many locations worldwide (Cordes et al., 2010b; Levin, 2005; Sibuet and Olu, 1998); however, sediments associated with mussel habitats have only been examined at a few locations, including the Blake Ridge Diapir (Robinson et al., 2004) and the Gulf of Guinea (Menot et al., 2010). Densities of macrofauna in seep sediments are often higher than in background non-seep sediments, particularly at increasing water depth (Levin, 2005) where food is often a limited resource and seep-derived carbon provides an additional food source (Levin and Michener, 2002). Globally, however, density differences among seep habitat types has been variable (Bernardino et al., 2012), with microbial mat, clam beds, or mussel beds exhibiting

65   similar (Levin et al., 2010) or differing densities in comparison to each another (Levin et al.,

66   2015; Menot et al., 2010; Robinson et al., 2004; Sahling et al., 2002). At the Blake Ridge Diapir,

67   macrofaunal densities in sediments near mussels were higher than in microbial mat sediments,

68   although macrofaunal densities were low for all sampled habitats (0-6,400 ind. m^{-2}; Robinson et

69   al., 2004). High densities found in microbial mat habitats have been attributed to the exploitation

70   of the chemosynthetically derived food source by seep tolerant taxa, and has been compared to

71   similar faunal responses from disturbance and sediment organic enrichment events (Bernardino

72   et al., 2012; Sahling et al., 2002).

73   Macrofaunal diversity patterns among seep and non-seep habitats have also been

74   variable. Microbial mat habitats often exhibit low diversity and high dominance of a few tolerant

75   taxa compared to other seep and non-seep habitats due to high sediment sulfide concentrations

76   (Levin et al., 2003; Sahling et al., 2002). However, low sulfide concentrations in clam beds on

77   the California slope led to increased macrofaunal diversities by supporting populations of both

78   ambient and sulfophilic taxa (Levin et al., 2003). In other locations, macrofaunal diversity in

79   sediments associated with clam beds has been similar (Hydrate Ridge, Sahling et al., 2002) or

80   lower (Gulf of Guinea, Menot et al., 2010) than non-seep habitats. Differences in the diversity of

81   mussel-bed sediment macrofauna have also been mixed, and possibly related to site-specific

82   factors. Higher diversities were found in mussel-associated sediments compared to microbial

83   mats and non-seep sediments at Blake Ridge (Robinson et al., 2004); in contrast, diversity was

84   lower in sediments adjacent to mussels than non-seeps in the Gulf of Guinea (Menot et al.,

85   2010). High β diversity has also been reported for seep habitats, supported by the habitat

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86   heterogeneity of foundation species and the varying geochemical environments (Cunha et al.,

87   2013; Cordes et al., 2010b).

88   Infaunal community assemblages associated with different seep habitats are distinct from

89   one another (Bernardino et al., 2012; Levin, 2005; Menot et al., 2010) and differ from

90   background non-seep sediments. Dorvilleid polychaetes are common in seep habitats (Levin,

91   2005) and are particularly abundant in microbial mat habitats, which is attributed to their broad

92   environmental tolerance to sulfide concentrations and opportunistic lifestyle (Levin et al., 2013;

93   Levin et al., 2006; Levin et al., 2003; Robinson et al., 2004; Sahling et al., 2002). Other

94   characteristic seep macrofauna include the polychaete families Siboglinidae, Capitellidae, and

95   Ampharetidae, oligochaetes, and thyasirid bivalves (Bernardino et al., 2012; Dando et al., 1991;

96   Levin et al., 2000; Levin et al., 2003), some of which can benefit from reducing habitats (Levin

97   et al., 2000). At Blake Ridge, mussel sediment communities were more similar to non-seep

98   communities (60% similar) than to microbial mat communities (11-54%), suggesting that

99   mussels help maintain low concentrations of methane and sulfide in sediments, facilitating

100   communities more similar to non-seep sediments (Robinson et al., 2004). The specific mussel

101   species present in the sediments has been found to influence the associated faunal community

102   assemblage (Cordes et al., 2010a). Mussels in the *Bathymodiolus childressi*-complex contain

103   only methanotrophic symbionts, while those of the *Bathymodiolus boomerang*-complex,

104   including *Bathymodiolus heckerae* present at Blake Ridge, contain both methanotrophs and

105   thiotrophs (Olu-LeRoy et al., 2007b). The effect of mussel species composition on epifaunal

106   communities has been suggested to act as a proxy for habitat chemistry (Cordes et al., 2010a),

5

107 since the resources (i.e., methane or sulfide) required by the endosymbionts of the different

108 mussel types are likely indicative of the chemical environment surrounding the mussels. The

109 extent of endemic infauna in seep habitats globally is still unresolved (Bernardino et al., 2012),

110 but may be a function of depth (Cordes et al., 2010a; Levin, 2005; Sahling et al., 2003), with

111 many species occupying seep sediments comprised of the regionally available taxon pool (e.g.,

112 Levin, 2005).

113   Depth-related patterns have been observed among seep sites worldwide, with

114 communities at upper bathyal depths (200-1500m) distinct from those at deeper depths (>1500m;

115 Bernardino et al., 2012). However, there are few comparisons of seeps with depths ranging

116 >1000m within a geographic region (Cunha et al., 2013; Rodrigues et al., 2013; Cordes et al.,

117 2010a; Sahling et al., 2003), where other factors structuring deep-sea communities (e.g., food

118 availability, bottom water oxygen concentrations) are more directly comparable. In the Gulf of

119 Cadiz, shallow (353-732m) mud volcanoes exhibited higher diversity, lower heterogeneity, and

120 different community assemblages from deep (1318-3860m) mud volcanoes (Cunha et al., 2013).

121 Cordes et al. (2010a) observed distinct depth-related patterns in both mussel and tube worm

122 communities in the Gulf of Mexico (1005-2746m), with mussel beds exhibiting a mid-slope

123 (~1000m) diversity maximum, consistent with similar trends for soft-sediment communities.

124 However, the relationship between depth and the sediment communities found within microbial

125 mats and along the fringe of mussel beds within a particular region is unknown.

126   The distinct epifaunal and infaunal assemblages present in seep habitats are a function of

127 their proximal sediment geochemical environment (Levin et al., 2003; Sibuet and Olu, 1998),

128   including seepage rates, sulfide concentrations, and biological activity (Cordes et al., 2010a; Olu

129   et al., 2009; Levin, 2005; Sahling et al., 2002). Microbial mats often form in habitats with high

130   methane flux rates, with corresponding high sulfide concentrations and low oxygen penetration

131   into the sediment (Sahling et al., 2002). In contrast, habitats that support clam beds exhibit lower

132   but variable methane flow through sediments, lower sulfide concentrations, and higher oxygen

133   penetration through bioturbation (Levin et al., 2003). Comparable data from mussel beds is

134   limited; however, while these habitats can have similar oxygen penetration profiles, their

135   associated sediment organic carbon concentrations and methane concentrations in the overlying

136   water (Menot et al., 2010; Olu-Le Roy et al., 2007a) are often higher than clam beds. Due to

137   variations in seep activity and fluid flux, the sediment geochemical properties (e.g., organic

138   carbon and nitrogen, stable carbon and nitrogen isotopes, grain size) often differ between seep

139   and non-seep habitats (Levin et al., 2000; Levin et al., 2010; Menot et al., 2010; Valentine et al.,

140   2005). Microbial mats have been documented to contain higher percent carbon content, high

141   carbon to nitrogen (C:N) ratios, and lower percent nitrogen content than clam beds and non-seep

142   sediments (Levin et al., 2010). Clam and mussel beds also contain higher organic carbon content

143   than non-seep sediments at multiple depths (Levin et al., 2000; Levin et al., 2010; Menot et al.,

144   2010; Valentine et al., 2005).

145   Stable carbon isotopic (δ^{13}C) composition of sediments and fauna from seep habitats

146   often reflects the primary nutritional sources available in the environment, where phytoplankton-

147   derived organic matter typically produce δ^{13}C values ranging from -25‰ to -15‰ (Fry and

148   Sherr, 1984), very low δ^{13}C values derived from biogenic methane ($\leq$-50‰; Van Dover, 2007;

149   Whiticar, 1999), and carbon derived from sulfide oxidation with $\delta^{13}C$ ranging from -37‰ to -

150   27‰ (Brooks et al., 1987; Fisher, 1990; Robinson and Cavanaugh, 1995). In the Gulf of Mexico,

151   sediments near seeps containing bacterial filaments were depleted in both ^{13}C and ^{15}N compared

152   to those with no bacterial filaments present (Demopoulos et al., 2010). Stable isotope values of

153   seep sediments can vary with seep activity, where higher methane fluxes near mytilid beds were

154   associated with lower $\delta^{13}C$ and $\delta^{15}N$ as compared to clam beds and the sediments may contain

155   different microorganism communities (Cambon-Bonavita et al., 2009; Olu-Le Roy et al., 2007a;

156   Olu et al., 2009). Thus, light $\delta^{13}C$ values can be a useful indicator of seep habitats where

157   biogenic methane is present. While methane flux and sulfide concentrations are important

158   mechanistic factors structuring seep faunal communities (Bernardino et al., 2012), stable isotopes

159   and other sediment parameters also can serve as proxies for and provide insight into the

160   mechanisms of seep activity occurring within sediments.

161   While originally thought to be unusual on the western Atlantic margin (Van Dover,

162   2000), increasing numbers of seep areas have been documented since 2011 (Skarke et al.,

163   2014). Prior to 2011, only two chemosynthetic seep areas were known, the Blake Ridge Diapir

164   (Paull et al., 1995; Van Dover et al., 2003) and the Cape Fear Diapir (Brothers et al., 2013), both

165   in deep water (2100-2600m) off of South Carolina, US. However, recent large-scale projects

166   using high resolution multibeam sonar and backscatter data now document 570 seep areas

167   between Cape Hatteras and Georges Bank (Skarke et al., 2014), and suggest that tens to

168   thousands more may be present along the passive Atlantic margin. During this study, two

169   recently identified chemosynthetic seep areas were examined near Baltimore Canyon (BCS) and

170   Norfolk Canyon (NCS) separated by 90 km. This study addresses the role of geographic setting,

171   seep habitat type, and sediment geochemistry in determining infaunal densities, community

172   composition, and diversity of sediment macrofauna (>300μm). We hypothesized that (i)

173   communities found at seep and non-seep habitats will differ within sites (BCS and NCS) and

174   between sites; (ii) similar seep habitats at BCS and NCS will exhibit similar community

175   composition, and (iii) community patterns in seep and non-seep habitats will be related to

176   sediment geochemical properties. To support our hypotheses, we expect higher macrofaunal

177   density but lower diversity at shallower BCS than at deeper NCS, similar taxonomic composition

178   between seep habitat types at BCS and NCS, and distinct sediment geochemical parameters

179   associated with community assemblages in each habitat type.

180

181   **2. Methods:**

182   *2.1 Study Area*

183   Two large cold-seep communities were explored on the U.S. Mid-Atlantic margin in

184   2012 and 2013 (Figure 1). The first seep, BCS, was located on the slope south of Baltimore

185   Canyon at depths ranging 366 to 402m. First documented by Hecker et al. (1983) during towed

186   camera surveys, the exact location was re-discovered in 2012 during this study. The second

187   seep, NCS, was located south of Norfolk Canyon at depths ranging 1457 to 1602m. The NCS

188   was identified by the Okeanos Explorer during multibeam mapping activities which detected

189   active bubble plumes (Skarke et al., 2014). The BCS seep contained large, but patchy,

190   communities of the bathymodiolid deep-sea mussels (Figure 2a), along with discrete white

191   microbial mats (Figure 2b) and large areas of shell debris. The NCS seep contained extensive

192   mussel communities (Figure 2c), with areas of filamentous white (Figure 2d) and yellow

193   microbial mats and shell debris. While the species has yet to be confirmed, initial examination

194   of the specimens indicate that the mussel species at BCS and NCS are within the *B. childressi*-

195   complex (Olu-Le Roy et al., 2007b) and will be referred to as *B. childressi*-complex throughout.

196

197   *2.2 Sampling Procedures*

198   Sediment samples were collected from seep habitats on two cruises (Table 1); one in

199   2012 aboard the NOAA Ship *Nancy Foster* (17 Aug-14 Sep) and one in 2013 aboard the NOAA

200   Ship *Ronald H. Brown* (2-18 May). Push cores (6.35-cm diameter) were collected in microbial

201   mats, mussel habitats, and background soft-sediment habitats using the ROV *Kraken* (2012) and

202   ROV *Jason II* (2013).   Background soft-sediments were collected at NCS in the main axis of

203   Norfolk Canyon using a NIOZ box core, which was sub-sampled with push core tubes (6.35-cm

204   diameter). Bow wave effects on the box core were minimized by reducing the speed of descent

205   of the box core as it approached the seafloor. Additionally, the NIOZ box corer completely seals

206   upon triggering, preventing the loss of surface sediment layers, and only cores that had

207   undisturbed surface layers were processed in this study. In addition, the sub-coring with push

208   core tubes provides direct sample-size effort comparisons for our study, which are directly

209   comparable to other seep studies (Levin and Mendoza, 2007; Levin et al., 2010; Robinson et al.,

210   2004). Additional cores and non-quantitative suction samples were collected via ROV in 2013

211   in microbial mats and mussel beds (Table 1). An Ekman corer (0.063 m^2) was used to collect

212   mussel bed material at both BCS and NCS. Push cores were sectioned vertically (0-2, 2-5 cm)

213   after recovery for either faunal or sediment geochemistry analysis. Due to time constraints and

214   the limited number of possible core collections on the ROV, sediments from BCS were only

215   processed for faunal analysis. Faunal core sections, Ekman samples, and suction samples were

216   preserved whole in 10% buffered formalin solution until they were returned to the laboratory

217   where they were stained with rose bengal and washed through a 300-µm mesh sieve to retain the

218   macrofauna portion. Macrofauna were sorted under a dissecting microscope and identified to the

219   lowest practical taxonomic level, including family level for polychaetes, oligochaetes, peracarid

220   crustaceans, and molluscs. Sediment geochemistry core fractions were frozen whole at -20°C

221   until returned to the lab. Subsamples of geochemistry cores were analyzed for the stable isotopes

222    $\delta^{13}C$ and $\delta^{15}N$, and percent carbon and nitrogen. Sediment samples were homogenized prior to

223   drying and acidified with 1.0 N phosphoric acid before weighing into tin boats. Samples were

224   analyzed for $\delta^{13}C$ and $\delta^{15}N$ referenced to Vienna PeeDee Belemnite and atmospheric nitrogen

225   gas, respectively. Analyses were conducted at Washington State University using a Costech

226   (Valencia, USA) elemental analyzer interfaced with a GV instruments (Manchester, UK)

227   Isoprime isotope ratio mass spectrometer. Isotope ratios were expressed in standard delta

228   notation, $\delta^{13}C$ and $\delta^{15}N$, as per mil (‰). Grain size analysis was performed on fractions of the

229   sediment geochemistry cores using the Folk method (Folk, 1974).

230

231   *2.3 Data Analysis*

232   Abundance of individuals and univariate measures of biodiversity were analyzed using

233 one-way (within sites) and two-way (among sites) analysis of variance (ANOVA) with habitat

234 type (microbial mat, mussels, background) and site as factors and individual cores as replicates,

235 followed by post-hoc test Tukey's HSD for multiple comparisons. All data were tested for

236 normality and heteroscedasticity using Shapiro-Wilk and Levene's tests (Zar, 1999) and $\log_e$-

237 transformed when necessary. If transformation did not achieve normality, a non-parametric

238 Kruskal-Wallis test was used on univariate measures with a post-hoc pairwise Wilcoxon test

239 using a Holm correction for multiple comparisons. Depth relationships with abundance and

240 diversity measures were tested using Spearman's rank correlation. A significance level of $p <$

241 0.05 was used in all tests. Univariate statistics were computed with the program R (R

242 Development Core Team, 2011). Diversity was examined using Pielou's evenness (J'), Shannon

243 diversity (H'$\log_e$), and ES(n) rarefaction based on untransformed abundance data using

244 DIVERSE in PRIMER Statistical Software version 7 (Clarke and Gorley, 2015).

245   Community structure was assessed by examining the overall contribution of higher level

246 taxa, composition of polychaete feeding guilds, and multivariate community analysis.

247 Multivariate analysis of community structure across cores for sites and habitats was performed

248 on square-root transformed abundance data using Bray-Curtis similarities in PRIMER version 7

249 (Clarke and Gorley, 2015) with the PERMANOVA+ add on (Anderson et al., 2008).   Samples

250 collected via Ekman cores and suction were analyzed as presence/absence transformed

251 abundance data within the multivariate analyses, with multiple suction samples from mussel

252 habitats pooled. Communities were examined using one-way, two-way, and pairwise analysis of

253 variance by permutation (PERMANOVA) with distance-based tests for homogeneity of

254 multivariate dispersions (PERMDISP). Similarity of percentages (SIMPER) was used to identify

255 the taxa responsible for discriminating between sites and habitats, and to assess the variability of

256 the communities within habitats. Variability among Bray-Curtis similarities within site-habitat

257 combinations was also assessed using multivariate dispersion (MVDISP).

258   To address the relationship of the environmental variables to the multivariate community

259 data, distance-based linear modeling (DistLM) and distance-based redundancy analysis (dbRDA)

260 were performed using the PERMANOVA+ add on package to PRIMER 7. DistLM performs

261 nominal tests of each variables explanatory power on community structure and builds a

262 multivariate statistical model of explanatory power of a suite of variables when considered

263 together. Environmental data was only collected at NCS, thus analysis was limited to only the

264 deep site. Variables included were depth, mud content, stable isotopic composition (δ^{13}C and

265 δ^{15}N), and organic carbon content. Organic nitrogen content was excluded from the analysis due

266 to high correlation (>0.95) with organic carbon content to reduce redundancy.

267

268 **3. Results:**

269 *3.1 Density*

270   A total of 2,609 individuals were collected from cores in our study, encompassing 86

271 taxa, including 35 polychaete families, 22 crustacean families, 20 mollusca families, and 9 other

272 taxa (see Supplemental Table 1 for full list). A further 2,518 individuals were collected from

273 suction and Ekman grab samples, including 21 additional taxa. Macrofaunal density was

274   significantly higher at BCS than at NCS for all habitat types (Figure 3; Two-way ANOVA,

275     $F_{1,19}$=11.34, p=0.003), with the highest densities occurring in microbial mats (137,756 ind. m^{-2}).

276   At both sites, the highest densities occurred in microbial mat habitats, followed by mussel

277   habitats and background habitats. At BCS, macrofaunal density differed among habitats (One-

278   way ANOVA, $F_{2,9}$=7.58, p=0.011), with significantly higher densities in bacterial mats (83,649 ±

279   28,466 ind. m^{-2}) than in background soft-sediments (15,719 ± 1,582 ind. $m^{-2,}$ Tukey HSD; p =

280   0.009). Likewise, at NCS macrofaunal density also differed among habitats (One-way ANOVA,

281     $F_{2,10}$ = 10.87, p = 0.003), with densities in microbial mats (47,962 ± 13,547 individuals m^{-2})

282   significantly higher than both mussel (Tukey HSD, p = 0.007) and background soft-sediments

283   (Tukey HSD, p=0.007). The upper 2 cm of sediments at BCS contained slightly higher

284   proportions of macrofauna in bacterial mat sediments (79%) as compared to mussel sediments

285   (76%) and soft sediments (76%). The proportion of macrofauna found in the upper 2cm at NCS

286   was higher in bacterial mat sediments (84%) as compared to mussel sediments (66%) and soft

287   sediments (55%).

288

289   *3.2 Diversity*

290   Macrofaunal diversity patterns among habitat types differed between BCS and NCS. At

291   BCS, diversity (H'log$_e$; Table 2) was significantly lower in bacterial mat sediments than in both

292   mussel (Tukey HSD, p < 0.0001) and background sediments (Tukey HSD, p < 0.0001).

293   Similarly, taxa evenness (J'; Table 3) was significantly lower in bacterial mat sediments than in

294   both mussel (Tukey HSD, p = 0.0001) and background sediments (Tukey HSD, p < 0.0001). In

14

295 contrast at NCS, there was no significant difference in diversity (One-way ANOVA, $F_{2,10}=0.96$,

296 p=0.42) or evenness (One-way ANOVA, $F_{2,10}=4.05$, p=0.051) among habitat types (Table 2).

297 Rarefaction analysis within BCS (Figure 4a) and NCS (Figure 4b) indicated similar within-site

298 patterns as given using Shannon diversity; however, overall diversity of all habitats combined

299 (Figure 4c) indicated higher diversity at NCS than at BCS.

300   There was high β diversity among habitats. At BCS, 15% of the observed taxa were

301 shared across all sediment habitats, 23-47% of the taxa were shared between any two habitats,

302 and 42% of the taxa were unique to a single habitat. Approximately 42% of the taxa in BCS

303 sediments only occurred in seep habitats. Mussel bed samples (Ekman core) at BCS shared more

304 taxa with mussel sediment habitats (60%) than with microbial mat (20%) or background

305 sediments (40%); however, the low number of taxa present in the single mussel bed sample

306 resulted in low overall diversity compared to mussel sediments (Figure 4a). At NCS, there was

307 overall greater β diversity than at BCS, with only 13% of taxa shared among all three sediment

308 habitats and 21-35% occurring in two or more habitats. A high percentage of taxa, 59%,

309 occurred only in a single habitat at NCS, and 59% of the taxa were only observed in seep

310 sediments. Similar to BCS, the mussel bed samples at NCS (Ekman core) shared the most taxa

311 with the mussel cores (56%). The non-quantitative suction samples also shared the most taxa

312 with their analogous sediment communities; the mat suction sample shared 51% of its taxa with

313 mat sediments, and the mussel suction samples shared 30% with mussel sediment. Overall, the

314 mussel bed and mussel suction samples had similar diversity to the mussel sediments, while the

315 microbial mat suction had higher diversity than microbial mat sediments (Figure 4b). Pooled

316 rarefaction (Figure 3c) for seep habitat push cores combined with Ekman cores and suction

317 samples resulted in an increase in diversity with each additional habitat at both sites. The high

318 difference in taxa between the mussel bed samples (Ekmans), compared to cores collected

319 adjacent to the mussel bed suggests high taxonomic turnover on a small (<1m) spatial scale with

320 minimal taxon overlap.

321

322 *3.3 Community composition*

323 Overall taxonomic composition was similar among habitat types between BCS and NCS

324 based on push core collections (Figure 5). Polychaetes dominated microbial mat and background

325 habitats, comprising 63-67% of the communities at BCS and 73-77% at NCS. The polychaete

326 families Dorvilleidae and Capitellidae composed a large proportion of microbial mat

327 communities at BCS (66%) and NCS (57%), with the addition of Spionidae and other

328 polychaetes at NCS. The proportion of oligochaetes was higher in microbial mats at BCS (31%)

329 than at NCS (4%), while NCS contained higher proportions of Crustacea, Mollusca, and other

330 taxa. In mussel habitats at both sites, polychaete composition was low (39-47%), with high

331 proportions of crustaceans (23-50%), specifically amphipods and tanaids. Background

332 sediments contained the highest proportion of molluscs (BCS: 18%, NCS: 13%). The overall

333 taxonomic composition of the Ekman cores and suction samples did not resemble the

334 macrofaunal composition in sediment cores collected from adjacent mussel or mat habitats

335 (Figure 4). The BCS Ekman core contained a high proportion of isopods (65%), while the NCS

336 Ekman core contained a lower proportion of amphipods (7%) relative to sediment communities

16

337 adjacent to mussel beds. The NCS mussel suction contained the highest proportion of

338 gastropods (46%) while the NCS mat suction contained high proportions of other polychaetes

339 (33%) and other taxa (31%), specifically Sipuncula (28%), in comparison to mussel and mat

340 sediment communities. In addition, the Ekman and suction samples were better able to collect

341 more highly mobile taxa, as indicated by the presence of Nebaliidae and Caridean shrimp.

342   Macrofaunal communities differed both between sites (Figure 6; Two-way

343 PERMANOVA, Pseudo-F=5.78, p=0.0001) and among habitat types (Two-way PERMANOVA,

344 Pseudo-F=7.30, p=0.0001) but with a significant interaction of site and habitat (Two-way

345 PERMANOVA, Pseudo-F=5.04, p=0.0001). Estimates of the source of variation in communities

346 indicate that differences among habitat types (Estimate=1056) were greater than differences

347 between sites (Estimate=539). Within each site, community variability among cores was highest

348 within microbial mat sediments (Table 2, MVDISP). Pairwise analysis of site and habitat

349 combinations showed significant differences in macrofaunal communities between all

350 site/habitat combinations (Table 3) except between BCS mussel and background habitats.

351 Microbial mat communities at BCS and NCS were more similar to each other than they were to

352 other habitats at their respective sites (Table 3). At BCS, bacterial mats had higher densities of

353 Capitellidae (Polychaeta), Dorvilleidae (Polychaeta), and Tubificidae (Oligochaeta) than the

354 background and mussel habitats, contributing 33% of the dissimilarity with mussel habitats and

355 42% with background habitats. Mussel habitats had higher densities of Tubificidae

356 (Oligochaeta), Leptocheliidae (Tanaidacea), and Typhlotanaidae (Tanaidacea) but lower

357 densities of Opheliidae (Polychaeta) and Yoldiidae (Bivalvia) compared to background soft

358   sediments, contributing 22% of the overall dissimilarity. SIMPER analysis using

359   presence/absence data (Table 3) indicated the Ekman core collected within the mussel bed at

360   BCS were more similar to the sediment communities associated with mussels, than to

361   background sediments, and mat habitats at BCS. However, the taxonomic composition of the

362   BCS Ekman core was more similar to NCS Ekman and suction samples than to sediment

363   communities at BCS (Table 3).

364   At NCS, bacterial mats differed from both mussel and background habitats by high

365   densities of Capitellidae (Polychaeta), Dorvilleidae (Polychaeta), and Spionidae (Polychaeta)

366   contributing 26% of the dissimilarity with mussel habitats and 27% with background habitats.

367   Mussel habitats differed from background soft-sediment habitats, with higher densities of

368   Oedicerotidae (Amphipoda) and Spionidae (Polychaeta), but low densities of Cossuridae

369   (Polychaeta) and Paraonidae (Polychaeta) contributing 31% of the dissimilarity. At NCS, the

370   highest community similarities were observed between the NCS Ekman core and mussel

371   sediment communities (44%, Table 3) and among the Ekman and suction samples (43-54%).

372

373   *3.4 Relationship to sediment geochemistry*

374   Sediment geochemical properties differed among microbial mat, mussel, and background

375   soft-sediment habitats at NCS (Table 4). Sediment $\delta^{13}C$ and $\delta^{15}N$ values were significantly lower

376   in mussel habitats than both microbial mats and background soft-sediments (Tukey HSD, $\delta^{13}C$,

377   $p<0.001$; $\delta^{15}N$, $p<0.033$). Microbial mat sediments also contained lower $\delta^{13}C$ and $\delta^{15}N$ values

378   compared to background soft-sediments (Tukey HSD, $\delta^{13}C$, $p<0.001$; $\delta^{15}N$, $p=0.001$). In

18

379   contrast, mussel habitats contained higher percent organic carbon and nitrogen content than both

380   microbial mat and background soft-sediments (Tukey HSD, %C, $p<0.006$; %N, $p<0.001$). There

381   was no difference in the C:N among habitat types (One-way ANOVA, $F_{2,7}=2.37$, $p=0.16$).

382   Background soft-sediments had the highest mud content, followed by mussel and microbial mat

383   sediments. It is notable that deeper fractions (2-5 cm) of the microbial mat cores contained

384   authigenic carbonate rubble that contributed to the higher grain size in those samples.

385   Principal coordinate analysis of macrofaunal communities at NCS (Figure 7) indicates

386   that two orthogonal axes are capable of explaining 63% of the natural variation among cores.

387   PCO1 separates mussel from microbial mat and background communities, while PCO2 separates

388   microbial mat from background communities. Variable correlation with PCO axes indicated that

389   PCO1 was positively correlated with δ^{13}C, δ^{15}N, and the C:N ratio, and negatively correlated

390   with percent organic carbon (%C). Mud content and depth were positively correlated with

391   PCO2. DISTLM analysis (Table 5) indicated that all environmental variables except C:N can

392   individually explain a significant portion (24-31%, $p<0.019$) of the variation in NCS

393   communities. A combination of δ^{13}C and mud content provide the best explanation of variation

394   in NCS macrofaunal communities, accounting for 58% of the variation among samples in two

395   axes.

396

397   **4. Discussion:**

398   *4.1 Depth-related patterns*

399   Differences between the depths represented by BCS and NCS habitats were apparent for

400   most of the community parameters measured (e.g., density, diversity, community composition).

401   Macrofaunal densities along non-seep slope ecosystems generally decrease with depth and

402   distance from shore, related to decreases in food availability from surface productivity and

403   transport of organic matter from shelf areas (Rex and Etter, 2010). We observed lower densities

404   with depth in background sediments, a trend that continues regionally with even lower non-seep

405   macrofaunal densities at Blake Ridge (Table 6; Robinson et al., 2004). A trend of decreasing

406   density with depth was also present for seep habitats; however, given the additional nutrition

407   source provided by the seep this trend cannot be attributed to depth-related patterns alone. Each

408   habitat at NCS exhibited higher variability in macrofaunal communities (MVDISP) as compared

409   to BCS habitats, suggesting increased patchiness with depth, consistent with observations from

410   deep mud volcanoes (Cunha et al., 2013) and general deep-sea ecological paradigms (Rex and

411   Etter, 2010). The higher variability within seep communities at NCS could be due to the larger

412   physical distance between the collected individual mussel and microbial mat sediments than at

413   BCS. However, background sediments at NCS were collected at a finer spatial scale than at

414   BCS, and we would have expected lower variability at NCS if spatial separation alone was the

415   controlling factor. Higher community patchiness with depth was also supported by the differing

416   results from the two diversity analyses (Shannon diversity vs. rarefaction). The higher Shannon

417   diversity at BCS indicates that diversity was high within cores, but rarefaction suggests there was

418   a lower overall taxonomic pool present at BCS compared to NCS, although under sampling was

419   evident for both sites. Overall diversity followed the expected trend and increased with depth

420   (e.g., Rex, 1981), although the opposite pattern was observed for background habitats. Given the

421   low abundance and limited sampling in background habitats at both sites, our results likely

422   provide an underrepresentation of background soft-sediment diversity.

423   Community assemblage differences between BCS and NCS may also be depth driven,

424   consistent with the separation of macrofaunal communities between upper bathyal (200-1500m)

425   and lower bathyal/abyssal (>1500m) depths worldwide documented by Bernardino et al. (2012).

426   Similar depth differences have been observed for seep megafaunal communities (Olu et al.,

427   2010), supporting the interconnection between infaunal and epifaunal communities. Differences

428   among seep and non-seep sediment communities have been observed to increase with depth

429   (Levin, 2005), suggesting the greater importance of the additional nutrition source provided by

430   the seep at increasing depths (Levin and Michener, 2002). Within a geographic region,

431   comparisons among seeps at different depths have been limited (Cordes et al., 2010a; Cunha et

432   al., 2013), but consistent with the overall results of Bernardino et al. (2012). In the Gulf of

433   Mexico, depth was the main driver of communities within seep habitats, with observed

434   community differences between shallow and deep seeps, but no observed difference across the

435   large geographic range (~650 km) of the study (Cordes et al., 2010a). Similar depth separation

436   of communities was observed on a smaller geographic scale (~200 km) for mud volcanoes in the

437   Gulf of Cadiz (Cunha et al., 2013). Other studies with multiple depths over large geographic

438   ranges (>425 km) have also found significant community differences, including seeps along the

439   Pacific margin (525 and 770m; Levin et al., 2010) and the Aleutian margin (3300m and 4400m;

440   Levin and Mendoza, 2007); however, the distance between seeps in these studies may be

441   confounding depth patterns. The closer proximity between BCS and NCS (90km) than in

442   previous studies reduces the geographic location effect and community differences likely

443   highlight depth-related patterns.

444   The depth-related patterns observed at BCS and NCS suggest that depth is a large driving

445   mechanism that affects both seep and non-seep communities in the mid-Atlantic region. The

446   complex interaction of continental shelf and slope topography, circulation patterns in the South

447   Atlantic Bight, and high organic input from enhanced surface productivity (Csanady and

448   Hamilton, 1988; DeMaster et al., 1994; Rex and Etter, 2010; Schaff et al., 1992; Yoder et al.,

449   1985) is known to result in regionally high macrofaunal abundance and biomass (Blake and

450   Grassle, 1994; Blake and Hilbig, 1994; Schaff et al., 1992), with regional patterns of declining

451   macrofaunal density but increasing diversity with depth (Boesch, 1979). The high macrofaunal

452   abundance observed at BCS is consistent with regional patterns, suggesting all habitats at this

453   site are benefiting from the high organic input. Regional patterns are likely to affect seeps

454   globally, as suggested by similar regional patterns for soft-sediment environments in the Gulf of

455   Mexico observed in seep communities (Cordes et al., 2010a), providing a framework from which

456   to assess seep influence on benthic communities.

457

458   *4.2 Among habitat comparisons*

459   Macrofaunal densities observed in BCS microbial mat sediments (Table 6) were among

460   the highest recorded for any seep environment worldwide. Locally high densities in seep

461   habitats have been reported from multiple locations, with the highest densities recorded from

462   microbial mats in the Gulf of Mexico (Table 6; Robinson et al., 2004). High densities have been

463   recorded in frenulate fields on the Norwegian margin (Decker et al., 2012), microbial mats on the

464   northern California margin (Levin et al., 2006), and an ampharetid bed in New Zealand (Thurber

465   et al., 2013), all of which were at deeper depths (Table 6). Macrofaunal density in microbial

466   mats was also high at NCS compared to microbial mat habitats at similar depths in other

467   locations (Table 6; Ritt et al., 2011; Robinson et al., 2004). Macrofaunal densities in microbial

468   mat and mussel sediments at BCS and NCS were greater than those measured at the nearest

469   previously known seep located 802 km to the southeast at Blake Ridge (Robinson et al., 2004).

470   Regionally, both seep sites represent localized areas of high densities, as indicated by the lower

471   densities in background sediments, similar to results for other seep communities worldwide

472   (Menot et al., 2010). Background sediments at both BCS and NCS also exhibited higher

473   densities than from other regional and historical sampling efforts north of Cape Hatteras (Table

474   6; Maciolek et al., 1987; Robertson et al., 2015; Sanders et al., 1965). Cape Hatteras represents a

475   known zoogeographic barrier (e.g., Cutler, 1975; Hilbig, 1994) due to the convergence of the

476   northward flowing Gulf Stream and the southward flowing Western Boundary Undercurrent, and

477   slope densities in this region were both higher (Cape Hatteras, Table 6; Blake and Hilbig, 1994)

478   and lower (Cape Lookout, Table 6; Blake and Grassle, 1994) than similar background depths at

479   NCS.

480   The habitats characterized by their dominant faunal component (e.g., microbial mats,

481   mussel beds) are known to be distinct from one another in other seep locations (Bernardino et al.,

482   2012; Cordes et al., 2010a; Levin, 2005). While macrofaunal abundances in seep habitats are

483   commonly higher than background soft-sediments (Levin and Mendoza, 2007), differences

484   between seep habitats (i.e., microbial mats, clam beds, mussel beds) have been variable

485   (Bernardino et al., 2012). Microbial mat sediments near Costa Rica had macrofaunal densities

486   two times higher than in clam beds (400-1796m; Levin et al., 2015) while microbial mats on the

487   Pacific margin (252-770m) had similar (Levin et al., 2010; Levin et al., 2003) or higher densities

488   than in clam beds (Sahling et al., 2002). The high densities observed in microbial mat habitats at

489   both BCS and NCS differs from the regional pattern observed at Blake Ridge, where mussel bed

490   habitats contained higher macrofaunal densities than microbial mats (Robinson et al., 2004).

491   Low densities in microbial mats at Blake Ridge may also be the result of high sediment sulfide

492   and methane concentrations that exceed the tolerance limit for most macrofaunal taxa (e.g.,

493   Bernardino et al., 2012). In addition, the mussel species at Blake Ridge, *B. heckerae*, known to

494   support both methanotrophs and sulfide oxidizers, differed from the dominant mussels present at

495   BCS and NCS, *B. childressi*-complex, which are known to support only methanotrophic bacteria

496   (Olu-Le Roy et al., 2007b). While *B. heckerae* has only been reported from deeper depths (2150-

497   3300m; Cordes et al., 2010a; Levin and Mendoza, 2007; Robinson et al., 2003), potentially

498   preventing establishment at either BCS or NCS, the depth range of *B. childressi* (500-2284m;

499   Cordes et al., 2010a) encompasses that of Blake Ridge. However, the two species were never

500   collected together in the Gulf of Mexico despite overlapping depth ranges (Cordes et al., 2010a).

501   As suggested by Cordes et al., (2010a), the specific mussel species present in these seep habitats

502   may serve as a proxy for the surrounding habitat chemistry, and by extension, the sediment

503   geochemistry. The different species complexes present at Blake Ridge and our sites suggests an

504   underlying difference in concentrations of methane and sulfide which may help explain the

505   differing infaunal community patterns observed between sites.

506

507   *4.3 Patterns in macrofaunal diversity*

508   The low (α) diversity observed in microbial mat habitats, particularly at BCS, is

509   consistent with previous studies which observed lower diversity within microbial mat habitats

510   compared to nearby clam beds (Bernardino et al., 2012; Levin and Mendoza, 2007; Levin et al.,

511   2003). Microbial mat sediments at both BCS and NCS were dominated by the annelid families

512   Capitellidae, Dorvilleidae, and Tubificidae, all of which have been previously observed in seep

513   habitats (Levin, 2005; Levin et al., 2010; Levin et al., 2003). Dorvilleids are a common

514   component of seep communities (Levin, 2005), often occurring in high densities in microbial

515   mat sediments (Robinson et al., 2004; Sahling et al., 2002) where they are likely consuming mat-

516   forming sulfur bacteria, methanotrophs (Levin and Michener, 2002), and/or archaea (Thurber et

517   al., 2012). Multiple species of dorvilleids are also known co-occur in seep habitats, with species

518   exhibiting high trophic and niche partitioning (Levin et al., 2013). Capitellids are known to be a

519   stress tolerant, opportunistic taxa, with a strong preference for sulfidic environments (Levin et

520   al., 2000; Levin et al., 2003) and are known to consume methanotrophs at other seeps (Carlier et

521   al., 2010; Decker and Olu, 2010, 2012). Only the polychaete families Dorvilleidae, Cirratulidae,

522   and Hesionidae were documented in microbial mat sediments at Blake Ridge (Robinson et al.,

523   2004), all of which were present in microbial mat sediments at NCS, while Hesionidae were

524   absent in mat sediments at BCS. In contrast to microbial mats, sediments adjacent to mussels at

525 BCS and NCS contained high proportions of crustaceans, particularly amphipods and tanaids.

526 Amphipods are known to be sensitive to organic enrichment, increased hydrocarbon

527 concentrations (Peterson et al., 1996), and oxygen stress (Thurber et al. 2013), and their

528 distribution at BCS may indicate high methane flux, high sulfide concentrations and low oxygen

529 concentrations at our microbial mat habitats. For the seeps at Blake Ridge, crustaceans were

530 only documented in mussel sediments (Robinson et al., 2004), suggesting similarities among

531 mussel communities across depth regimes. In addition, increased variability in communities has

532 been used as an indicator of stressed and/or disturbed environments (Fisher et al., 2014; Warwick

533 and Clarke, 1993). Although fluid flux and sulfide concentrations were not measured, the high

534 variability (MVDISP) and low diversity in microbial mat sediments, compared to the greater

535 similarity within mussel and background sediment communities, also suggest a higher stress

536 environment in microbial mat sediments.

537   High β diversity at seep sites was present over both small (<1 m) and large spatial scales.

538 High β diversity among seep habitats (mussels and mats) has been documented at seep sites

539 worldwide (see Cordes et al., 2010b for review) and is suggested to be a result of small-scale

540 variation in the vertical distribution and concentration of sulfides in sediments (Levin et al.,

541 2003) and habitat heterogeneity provided by seep megafauna (Cordes et al., 2010b). Hints at

542 these small-scale variations were observed both in sediment cores collected in mat and

543 background habitats at BCS and between the Ekman cores collected within the mussel habitat

544 and cores collected directly adjacent to mussel habitats at both sites. Similar to results observed

545 in Pacific seeps (Levin et al., 2010), the seep habitats contribute significantly to the regional

546   biodiversity for their specific depth, providing 37-49% of infaunal taxa and high taxon turnover

547   between seeps and background soft-sediment communities. In addition, while the taxonomic

548   level applied in this study (family-level) was sufficient to ascertain differences among habitat-

549   specific communities, further identification (e.g., genus and/or species level) will likely provide

550   increased separation of habitat-specific communities, biodiversity estimates, identification of

551   biogeographic boundaries, and insight into seep endemism at these sites. High β diversity among

552   the mussel habitat, adjacent sediments, and background sediments highlights that habitat

553   provision of dense mussel communities influences not only the *in situ* macrofaunal communities

554   found within the beds, but also the communities that occur in the sediments beyond the perimeter

555   of the mussel bed itself. This 'reef' effect has also been, observed for deep-sea coral

556   communities (Demopoulos et al., 2014). While the effect of seep habitats on sediment

557   macrofaunal communities has not been detected at distances greater than 250 m from seep

558   megafauna (Menot et al., 2010), discrete transects from mussel beds to adjacent sediments and

559   beyond would help quantify the sphere of influence of seep activity and biogenic structures on

560   adjacent habitats.

561

562   *4.4 Relationship to sediment geochemistry*

563   The higher proportion of taxa found in the upper 2 cm of sediments in microbial mats

564   versus deeper sediments, particularly at NCS, may reflect different geochemical settings present

565   within each habitat. Seeps, along with other reducing environments such as areas of organic

566   enrichment, large organic falls, and oxygen minimum zones, are often characterized by low

567   oxygen, sulfidic sediments (Levin et al., 2010; Tunnicliffe et al., 2003). The vertical distribution

568   of taxa in sediments is regulated partly by oxygen and sulfide concentrations (Levin, 2005),

569   resulting in a trade-off between sulfide tolerance and food availability (Menot et al., 2010). Few

570   taxa tolerate sulfide concentrations >1 mM, while Dorvilleidae polychaetes can occur in high

571   densities at concentrations ranging 1 to 6 mM (Levin et al., 2003). The higher proportion of taxa

572   present in the upper 2 cm of microbial mat sediments suggests these habitats have low oxygen

573   and potentially high sulfide concentrations that restrict fauna to the surface sediments (Levin et

574   al., 2003). Whereas, the higher proportion of taxa present in sub-surface sediments (>2cm) in

575   mussel and background habitats suggests deeper oxygen penetration and lower sulfide

576   concentrations, allowing more individuals to survive at greater depth within the sediments (Levin

577   et al., 2001; Levin, 2005). Bioturbation by deeper dwelling taxa in turn facilitates oxygen

578   penetration and the transfer of organic material, thus also increasing the food availability for

579   other organisms residing deeper in the sediments. Similar faunal sediment-depth patterns were

580   reported for microbial mat (Levin et al., 2003) and mussel-associated sediments (Menot et al.,

581   2010) at other seeps, suggesting that in the absence of specific oxygen and sulfide concentration

582   measurements, inferences about the geochemical setting based on the faunal composition may be

583   possible.

584   The high variation observed within NCS microbial mat communities suggests a gradient

585   among sampling locations in the underlying seep fluid flow, sediment geochemistry, and depth.

586   Sediments supporting microbial mats are known to sustain high rates of methane emissions, high

587   concentrations of sulfide, and low oxygen penetration (Bernardino et al., 2012). In contrast,

588   mollusc-dominated habitats (e.g. clam beds) often have lower methane emission rates and lower

589   sulfide concentrations near the sediment surface (Boetius and Suess, 2004; Levin, 2005; Levin et

590   al., 2013; Levin et al., 2003; Sahling et al., 2002), while mussel beds are known to have

591   increased methane concentrations in overlying water (Olu-Le Roy et al., 2007a). The large

592   continuous fields of mussels present at BCS and NCS suggest regular and diffuse fluid flow

593   (Olu-Le Roy et al., 2007a), although the patchiness and large areas of shell debris at BCS also

594   suggest spatially or temporally intermittent flow. Animals occupying sediments below microbial

595   mats must be tolerant to high levels of sulfide, while those near mussel habitats may not require a

596   high tolerance, but fall within a tolerance gradient. The high methane flux expected in microbial

597   mat sediments should contribute to higher sulfate reduction and anaerobic methane oxidation,

598   while low methane emission rates in mussel sediments may concentrate isotopically depleted

599   carbon, due to the physical presence of mussels possibly limiting the release of methane. Both of

600   these processes would yield light carbon isotope values in sediments, and sediment $\delta^{13}C$ values

601   in microbial mats were higher than in mussel bed habitats. Isotopic composition of mussels

602   collected within these seeps yielded isotopically light $\delta^{13}C$ (-64‰ to -61‰; Prouty et al., 2014)

603   and $\delta^{15}N$ values (-2‰ to 6‰; Prouty et al., 2014). The contribution of mussel tissues and/or

604   waste products to the organic matter pool is indicated by the enriched percent organic carbon

605   content and depleted ^{13}C values, and similar organic carbon concentrations were observed in

606   Gulf of Guinea mussel bed sediments (Menot et al, 2010). Microbial composition may also

607   influence the stable isotope composition of the microbial mat sediments. Filamentous sulfide

608   oxidizing bacteria (e.g., *Beggiatoa*, *Thioplaca*) differ from amorphous forms (e.g. *Arcobacter*)

609 and iron-oxidizers, and sediment $\delta^{13}C$ values reported here may reflect the microbial

610 communities supporting the food chain, as well as organic matter contribution from mussels

611 (Levin and Mendoza, 2007). The presence of mussels from the *B. childressi*-complex, which

612 contain only methanotrophic symbionts, further suggests methanotrophs are playing a large role

613 in the transfer of energy at our seeps (e.g., Levin et al., 2015; Levin and Michener, 2002;

614 Thurber et al., 2012; Thurber et al., 2013). The portion of variation in microbial mat

615 communities at NCS characterized by mud content, and by extension, the amount of authigenic

616 carbonate rubble within cores, may reflect the role and duration of anaerobic oxidation of

617 methane within the sediment, which can influence the macrofaunal community structure.

618   Although we did not measure any sediment pore-water geochemistry at BCS, given the

619 similar patterns exhibited among microbial mat, mussel, and background sediment communities

620 in relation to those at NCS, similar sediment geochemical patterns may be structuring infaunal

621 communities at BCS. Sediment geochemistry for sites within 2 km at shallower (282m) and

622 deeper (515m) depths on the Baltimore slope indicate lower sediment organic carbon (0.31-

623 0.43%) and nitrogen (0.1%), C:N ratios (3.1-4.3), $\delta^{15}N$ (4.6-4.8‰) values, and mud content (12-

624 38%, 515m only), but comparable $\delta^{13}C$ (-22.3 to -21.9) compared to background sediments

625 collected at NCS (Mienis et al., 2014). These data suggest a food-limited environment with

626 increased hydrodynamic flow, as indicated by water column turbidity patterns over the slope

627 (Mienis et al., 2014). Additional sampling of sediment geochemistry at BCS would allow

628 regional comparisons between these two discrete seep habitats, and provide further insight into

629 the mechanisms supporting seep communities in the mid-Atlantic region.

630   There are potential limitations to the comparisons made between seep and background

631   habitats at both BCS and NCS in our study, including seasonality and inter-annual variation,

632   location, and sampling methods. At BCS, all of the background sediments were collected in

633   August 2012, while all but one core from seep habitats were collected in May 2013. Seasonality

634   in surface productivity and hydrodynamic regimes, as well as disturbance events, promotes shifts

635   in community assemblages. However, there was no observed difference in the abundance of taxa

636   in the upper 2 cm of sediments between 2012 and 2013 samples collected at BCS, which might

637   have been expected if there had been an organic enrichment event during this time period. In

638   addition, previous temporal studies within the mid-Atlantic region found little inter-annual

639   variation in macrofaunal communities (Boesch, 1979). Proximity of background, soft-sediment

640   cores to seep habitats may also the affect their observed similarity to seep habitats. Three of the

641   four background cores were collected within the axis of Baltimore Canyon, while the fourth was

642   in close proximity (<1m) to microbial mat habitats at the seep on the adjacent slope. The high

643   similarity among BCS background cores (59%) with the inclusion of the near-mat core suggests

644   they are an adequate representation of nearby background communities. However, the close

645   proximity of (<1m) of the 2012 microbial mat core with At NCS, the box cores collected for

646   background sediments were 18-19 km north from the seep habitats and were located at the base

647   of the Norfolk Canyon channel. While macrofaunal communities are known to differ between

648   canyon axis and slope habitats for Norfolk Canyon (Robertson et al., 2015), the samples

649   examined in this study represent the best comparative information available.

650   Seep habitat-specific communities on the western Atlantic margin exhibit many

651   similarities to other microbial mat and mollusc-dominated communities worldwide, suggesting

652   similar environmental controls within these settings. This study is the first to examine seep-

653   associated infaunal communities at depths <2000 m and in the context of their geochemical

654   environment in this region of the Atlantic. Discrete differences among seep habitats and sites

655   indicate that seep community patterns may be driven, in part, by variation in the intricate sub-

656   seafloor structures supplying methane to the upper sediment/water interface. The potential

657   ephemeral nature of these seeps and their associated fluid flux (Condon et al., 2015) may

658   represent a strong driver influencing infaunal communities. Enhanced understanding of the seep

659   plumbing, methane flux, and associated sediment geochemistry (e.g., pore water sulfide and

660   methane concentrations) coupled with infaunal community metrics could benefit the

661   development of generalizations relating to seep environmental controls on infaunal structure and

662   function.

663

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907 **Figure Captions:**

908 **Figure 1.** Maps showing locations of sampling sites and closest known seeps (a) with detailed

909 sampling at b) BCS and c) NCS. ■ = Microbial mat habitat push cores; ▲ = Mussel habitat push

910 cores; ● = Background, soft-sediment habitats; ▲ = Mussel Ekman cores; △ = Mussel suction

911 samples; □ = Microbial mat suction sample.

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Figure 2. Habitats and sample collection at BCS mussel habitats (a), BCS microbial mat habitats

(b), NCS mussel habitats (c), and NCS microbial mat habitats (d). Black lines represent 10-cm

width of lasers on ROV. Images courtesy of Deepwater Canyons 2013 - Pathways to the Abyss,

NOAA-OER/BOEM/USGS.

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Figure 3. Mean macrofaunal density (ind. m^{-2}) (± 1 S.E.) of seep and background soft-sediment

habitats from push core samples collected at BCS and NCS. Letters indicate statistical groupings

(p>0.05) for each site tested separately.

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Figure 4. Rarefaction via estimated number of taxa for a) BCS samples; b) NCS samples and c)

pooled by sample type based on per sample untransformed data. For a and b, Mat, Mussel, and

Background include push cores only. Mat = pooled microbial mat cores; Seep Cores = pooled

mussel and microbial mat cores; Seep All = pooled mussel and microbial sediment cores, Ekman

cores, and suctions; All = pooled all samples.

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Figure 5. Taxonomic composition of dominant macrofauna at BCS and NCS seep and

background habitats collected from a) quantitative push cores b) Ekman cores and suction

samples. Other Taxa includes Halacaridae, Cnidaria, Echinodermata, Nemertea, Sipuncula, and

Turbellaria.

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933 **Figure 6.** Non-metric multidimensional scaling of Bray-Curtis similarities of square-root

934 transformed macrofaunal abundance data from push cores collected in BCS and NCS habitats.

935 Circles and percentages indicate average similarity among cores for each habitat from SIMPER

936 analysis. Stress value of 0.15 indicates a good to potentially useful confidence in the 2-

937 dimensional representation (Clarke and Gorley, 2015).

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939 **Figure 7.** Principal coordinate ordination of Bray-Curtis similarities of square-root transformed

940 abundance data from sediment push cores collected at NCS habitats with environmental

941 parameter vectors overlaid.

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954 **Table 1.** Number of samples collected at Baltimore and Norfolk seep and background sites, including

955 push cores collected for infaunal analysis (Fauna) and sediment geochemistry analysis (SC), Ekman

956 cores, and suction samples.

Site	Habitat	Year	Latitude	Longitude	Depth (m)	Fauna	SC	Ekman	Suction
Baltimore BCS	Mat	2012	38.04369	-73.82568	412	1			
		2013	38.04799	-73.82915	366	2			
		2013	38.04995	-73.82191	402	1			
	Mussel	2013	38.04820	-73.82534	372	2			
		2013	38.04848	-73.82193	400	2			
		2013	38.05026	-73.82194	400			1	
	Background	2012	38.09171	-73.80519	446	3			
		2012	38.04369	-73.82568	412	1			
Norfolk NCS	Mat	2013	36.86584	-74.49083	1602	1	1		
		2013	36.86664	-74.49026	1590	1	1		
		2013	36.87140	-74.47758	1487	1	1		
		2013	36.87087	-74.47290	1457	1	1		
		2013	36.87123	-74.47396	1467	1			
		2013	36.86586	-74.49082	1612				1
	Mussel	2013	36.86786	-74.48871	1576	1	1		
		2013	36.86827	-74.48822	1565	1			
		2013	36.86711	-74.48936	1585	1	1		
		2013	36.87139	-74.47633	1482	1	1		
		2013	36.87168	-74.47813	1487	1			
		2013	36.87148	-74.47626	1483			1	
		2013	36.86928	-74.48736	1612				1
		2013	36.87029	-74.48748	1612				1
		2013	36.86934	-74.48698	1612				1
	Background	2013	37.03347	-74.45039	1622	1			
		2013	37.03343	-74.45032	1619	1			
		2013	37.03343	-74.45030	1620	1	1		

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960 **Table 2.** Diversity (H'$\log_e$), evenness (J'), and multivariate dispersion (MVDISP) of

961 macrofaunal communities collected from cores at Baltimore and Norfolk seep and background

962 habitats.

Site	Habitat	N	J'		H'($\log_e$)		MVDISP
Baltimore BCS	Mat	4	0.49	(0.06)	0.96	(0.11)	0.97
	Mussel	4	0.87	(0.03)	2.82	(0.07)	0.75
	Background	4	0.92	(0.02)	2.80	(0.07)	0.52
Norfolk NCS	Mat	5	0.70	(0.07)	1.96	(0.26)	1.56
	Mussel	5	0.85	(0.03)	1.95	(0.12)	0.89
	Background	3	0.92	(0.03)	2.37	(0.19)	1.03

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Table 3. Similarity among habitats (above diagonal), within-habitat similarity (diagonal, bold), and PERMANOVA probabilities (below diagonal) based on Bray-Curtis similarities of square-root transformed abundance data for the push cores. Comparisons with suction and Ekman grab samples were based on Bray-Curtis similarities of presence/absence transformed abundance data.

Site		BCS				NCS					
Habitat		Mats	Mussels	Background	Ekman Mussel	Mats	Mussels	Background	Ekman Mussel	Suction Mussel	Suction Mat
BCS	Mats	**54.3**	19.9	14.2	14.5	29.9	11.0	16.2	26.1	12.2	16.5
	Mussels	0.002	**55.0**	49.5	20.1	25.7	16.9	33.1	41.3	41.4	35.3
	Background	0.001	0.056	**58.7**	17.9	25.4	17.6	33.6	37.1	34.6	36.0
	Ekman Mussel	-	-	-	**-**	21.0	17.9	20.4	34.3	26.7	26.7
NCS	Mats	0.049	0.021	0.015	-	**32.1**	15.5	20.1	31.2	30.4	31.8
	Mussels	0.001	0.001	0.001	-	0.002	**52.3**	20.0	43.9	27.1	14.8
	Background	0.006	0.014	0.013	-	0.031	0.004	**50.6**	31.5	24.2	31.6
	Ekman Mussel	-	-	-	-	-	-	-	**-**	53.3	43.3
	Suction Mussel	-	-	-	-	-	-	-	-	**-**	54.12
	Suction Mat	-	-	-	-	-	-	-	-	-	**-**

987   **Table 4.** Mean (±1 S.E.) sediment geochemical properties for cores collected at Norfolk seep and background habitats.

Habitat	N	$\delta^{13}C$		%C		$\delta^{15}N$		%N		C:N		%Mud	
Mat	4	-25.41	(0.28)	2.22	(0.32)	5.32	(0.23)	0.30	(0.03)	8.53	(0.29)	61.74	(3.91)
Mussel	3	-39.97	(0.61)	4.41	(0.20)	2.78	(0.22)	0.73	(0.01)	7.01	(0.20)	76.21	(2.39)
Background	3	-21.15	(0.05)	2.36	(0.30)	7.74	(0.97)	0.36	(0.04)	7.62	(0.89)	95.46	(0.52)

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1000 **Table 5.** Results from the distance-based linear modeling (DISTLM) of environmental variables

1001 with Norfolk microbial mat, mussel, and background soft-sediment communities using the AICc

1002 criteria and "best" model selection.

Variable	SS(trace)	Pseudo-F	P	Prop.
$\delta^{13}C$	8077.3	3.613	0.005	0.311
Percent Carbon	6423.8	2.630	0.014	0.247
$\delta^{15}N$	6493.7	2.668	0.010	0.250
C:N	4308.6	1.592	0.160	0.166
Mud Content	6815.3	2.847	0.010	0.262
Depth	6266	2.545	0.019	0.241

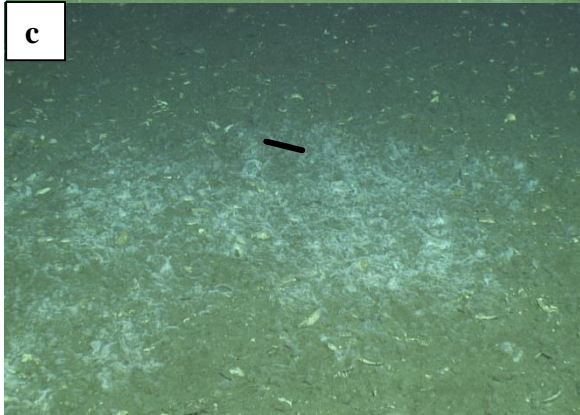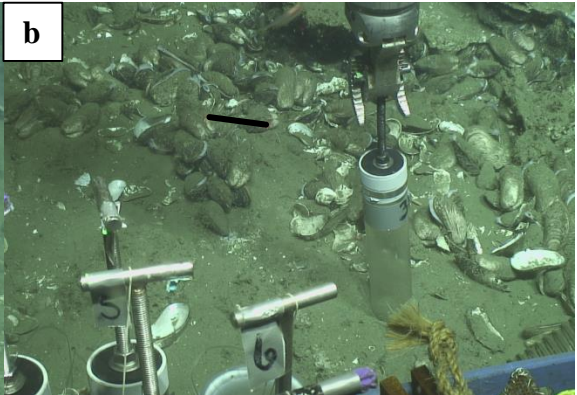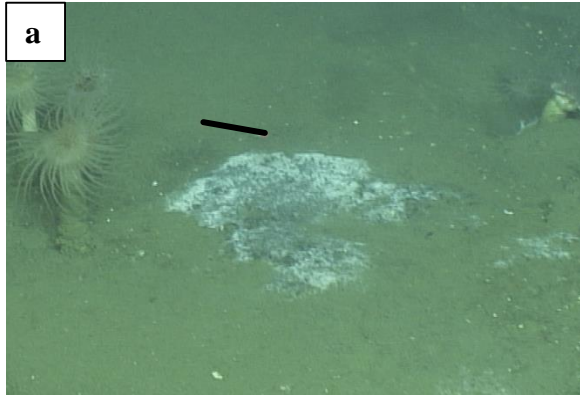| AICc	R^2	RSS	Selections
80.002	0.57755	10968	$\delta^{13}C$, Mud Content
80.606	0.3111	17886	$\delta^{13}C$
81.083	0.52932	12221	$\delta^{13}C$, Depth
81.288	0.26249	19149	Mud Content
81.298	0.51908	12487	$\delta^{15}N$, Mud Content
81.455	0.2501	19470	$\delta^{15}N$
81.491	0.24741	19540	Percent Carbon
81.552	0.50674	12807	Percent Carbon, Mud Content
81.571	0.24133	19698	Depth
81.779	0.49539	13102	Percent Carbon, Depth
Total SS(trace)		25964	

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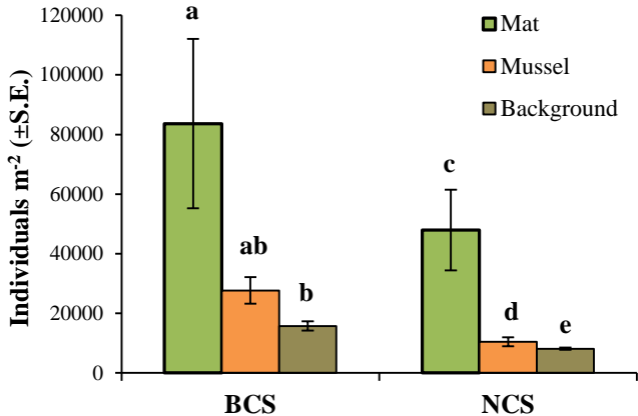
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Table 6. Summary of macrofaunal seep sediment and regional infaunal studies including closest geographic seeps, comparable depths,

1011 and observed high densities.

Study Location	Region	Seep Habitat	Depth (m)	Density individuals m^{-2}		Max Density individuals m^{-2}	Source
BCS	NW Atlantic	Microbial mat	366-412	83649	±28466	137757	This study
BCS	NW Atlantic	Mussel beds	372-400	27646	±4464	40758	This study
NCS	NW Atlantic	Microbial mat	1467-1602	47962	±13547	78357	This study
NCS	NW Atlantic	Mussel beds	1482-1585	10427	±1558	15482	This study
Blake Ridge Diapir	NW Atlantic	Microbial mat	2250	800	±506	2400	Robinson et al., 2004
Blake Ridge Diapir	NW Atlantic	Mussel beds	2250	5000	±1400	6400	Robinson et al., 2004
Håkon Mosby	NE Atlantic	Frenulate field	1256	92955	±21617	-	Decker et al., 2012
Gulf of Guinea	SE Atlantic	Mussel beds	3160	22306	-	-	Menot et al., 2010
Costa Rica	SW Atlantic	Microbial mat	376-1854	18060	±8190	-	Levin et al., 2015
Green Canyon	Gulf of Mexico	Microbial mat	700	198950	±78150	277100	Robinson et al., 2004
Atwater Canyon,	Gulf of Mexico	Microbial mat	1934	36400	-	-	Robinson et al., 2004
California Margin	E Pacific	Microbial mat	525	62160	-	-	Levin et al., 2006
New Zealand	W Pacific	Ampharetid bed	1057	56728	±4784	84000	Thurber et al., 2013
Nile Delta	Mediterranean	Microbial mat	1700	2783	±451	-	Ritt et al. 2011
BCS	NW Atlantic	Background	412-446	15719	±1582	17694	This study
NCS	NW Atlantic	Background	1619-1622	8110	±380	8847	This study
Gay Head-Bermuda	NW Atlantic	Background	400	6081	-	-	Sanders et al., 1965
Baltimore Slope	NW Atlantic	Background	550	6546	±2214	10934	Robertson et al., 2015
Gay Head-Bermuda	NW Atlantic	Background	1500	1719	-	-	Sanders et al., 1965
Mid-Atlantic Slope	NW Atlantic	Background	1613	4953	±754	6911	Maciolek et al., 1987
Cape Hatteras	NW Atlantic	Background	1410-1535	13219	±3942	17733	Blake and Hilbig, 1994

| | Cape Lookout | NW Atlantic | Background | 1380-1690 | 5017 | - | - | Blake and Grassle, 1994 |
| 1012 | | | | | | | | |

a — Composition bar charts for BCS and NCS sites: Mat, Mussel, Background.

b — Composition bar charts: Ekman Mussel (BCS); Ekman Mussel, Suction Mussel, Suction Mat (NCS).

Legend:
- Capitellidae
- Cossuridae
- Dorvilleidae
- Paraonidae
- Spionidae
- Other Polychaeta
- Tubificidae
- Amphipoda
- Isopoda
- Tanaidacea
- Other Crustacea
- Gastropoda
- Bivalvia
- Other Mollusca
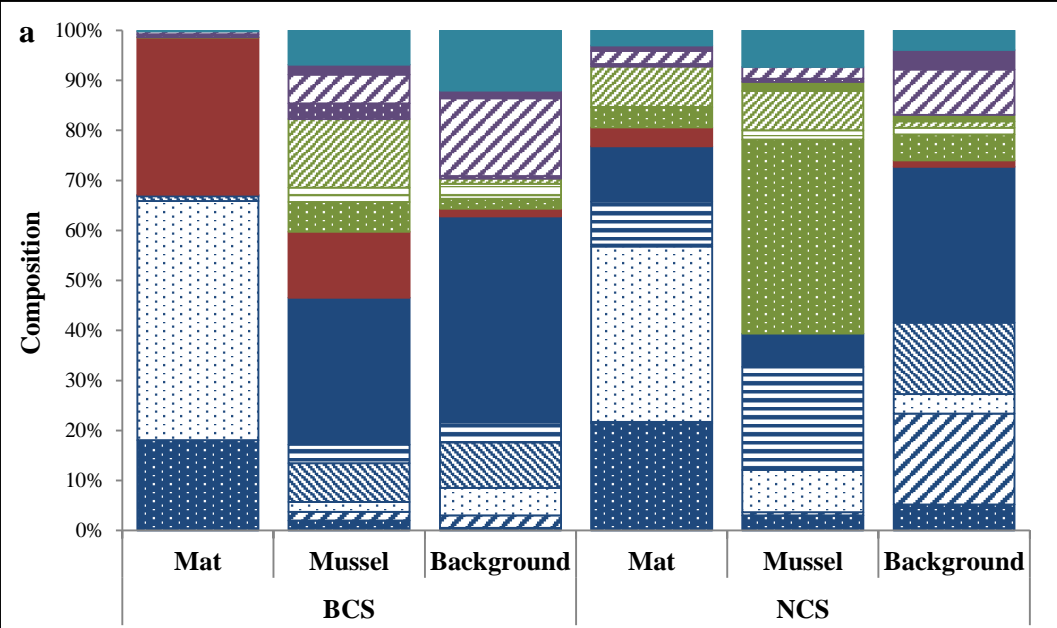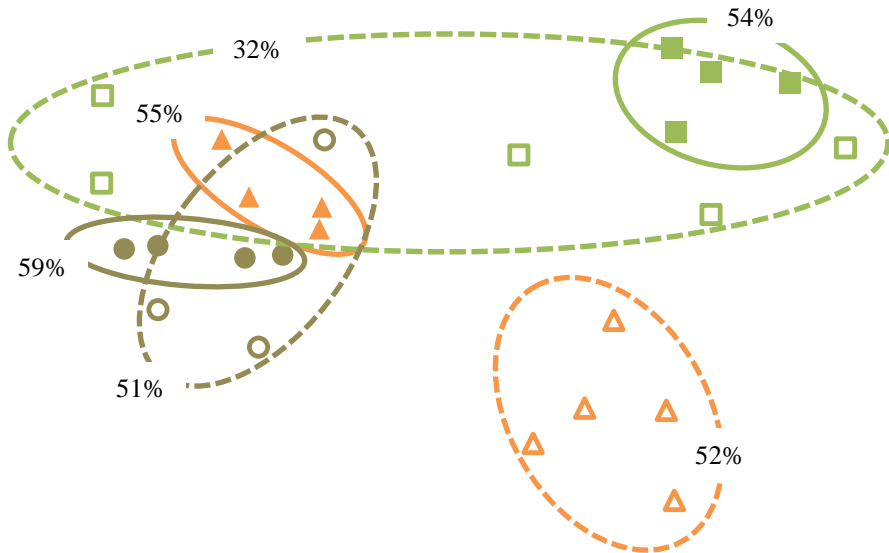- Other Taxa
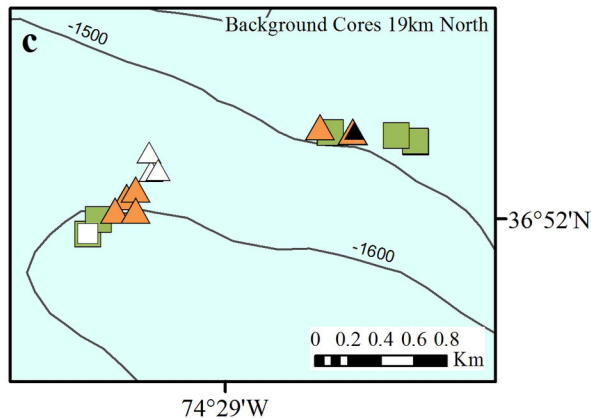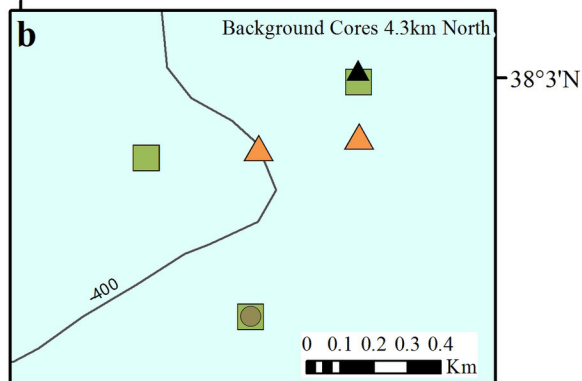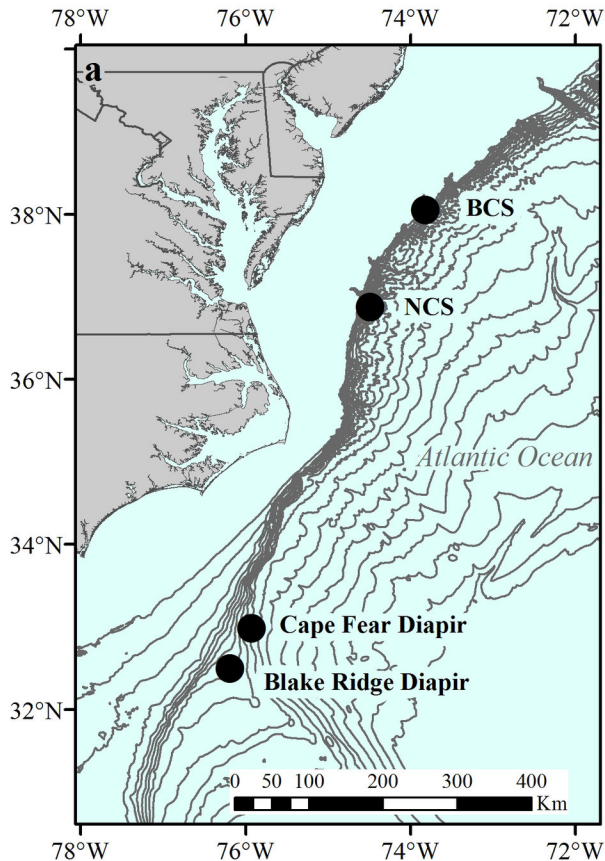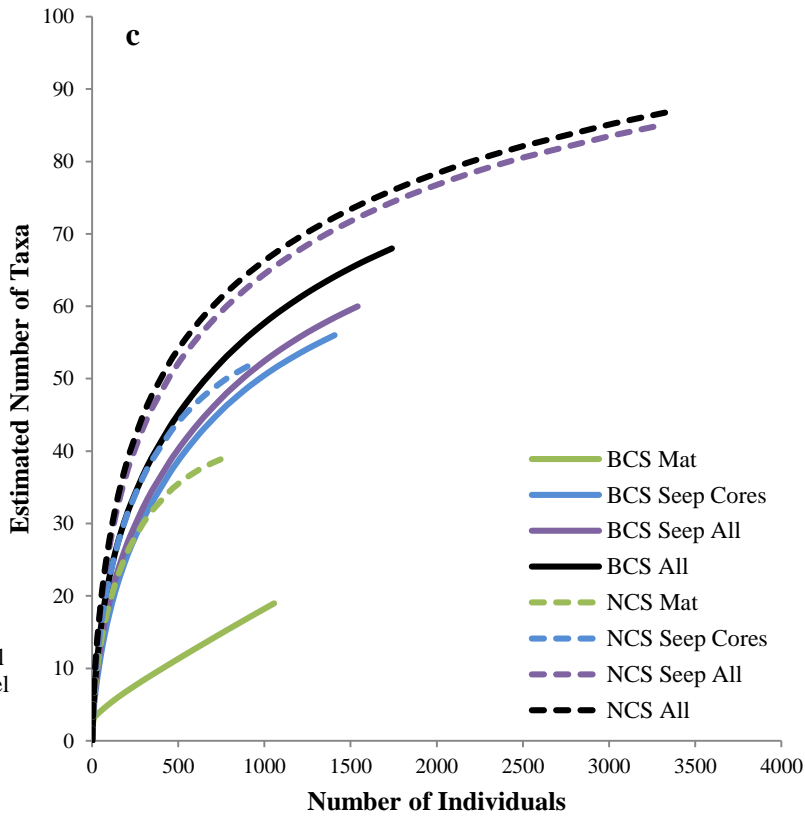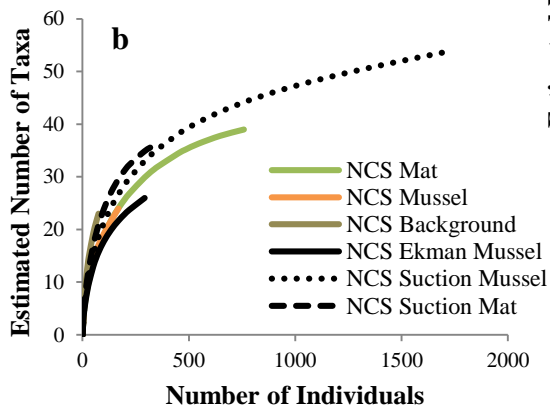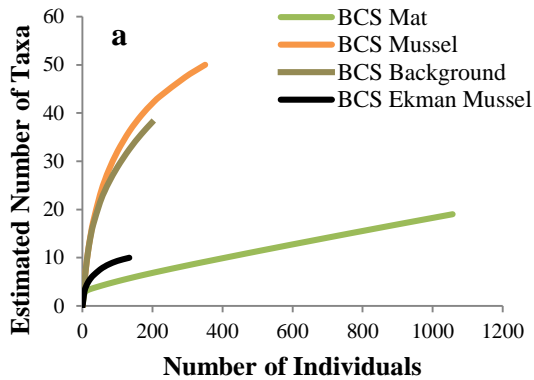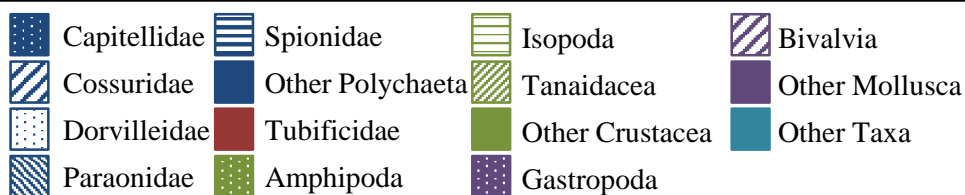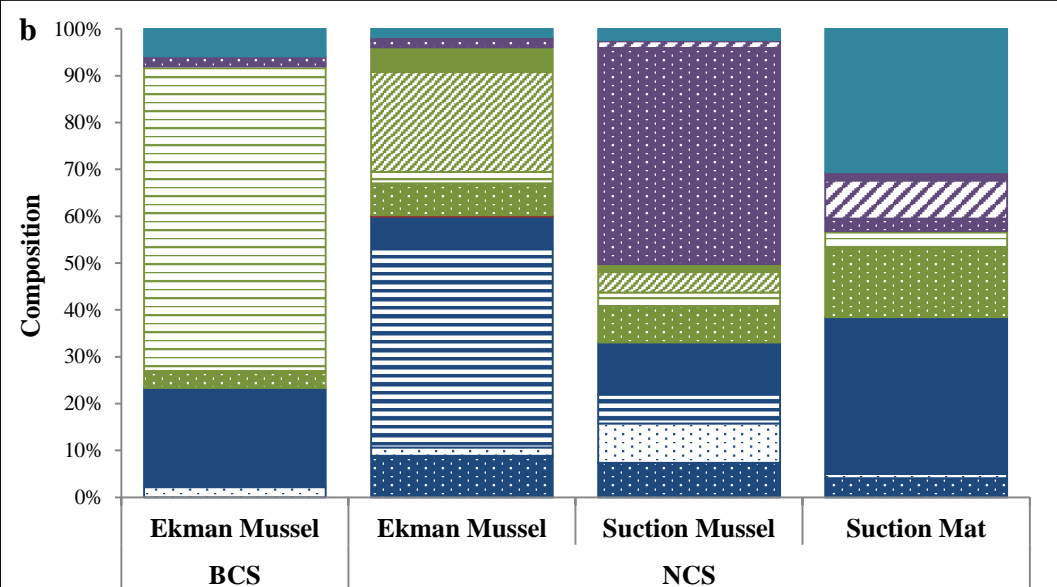
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Legend:
- ■ BCS Mat
- ▲ BCS Mussel
- ● BCS Background
- □ NCS Mat
- △ NCS Mussel
- ◯ NCS Background