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Title: Macrofaunal communities associated with chemosynthetic habitats from the U.S. Atlantic margin: a comparison among depth and habitat types

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Abstract:

Hydrocarbon seeps support distinct benthic communities capable of tolerating extreme environmental conditions and utilizing reduced chemical compounds for nutrition. In recent years, several locations of methane seepage have been mapped along the U.S. Atlantic continental slope. In 2012 and 2013, two newly discovered seeps were investigated in this region: a shallow site near Baltimore Canyon (BCS, 366-412 m) and a deep site near Norfolk Canyon (NCS, 1467-1602 m), with both sites containing extensive chemosynthetic mussel bed and microbial mat habitats. Sediment push cores, suction samples, and Ekman box cores were

collected to quantify the abundance, diversity, and community structure of benthic macrofauna (>300 μm) in mussel beds, mats, and slope habitats at both sites. Community data from the deep site were also assessed in relation to the associated sediment environment (organic carbon and nitrogen, stable carbon and nitrogen isotopes, grain size, and depth). Infaunal assemblages and densities differed both between depths and among habitat types. Macrofaunal densities in microbial mats were four times greater than those present in mussel beds and slope sediments and were dominated by the annelid families Dorvilleidae, Capitellidae, and Tubificidae, while mussel habitats had higher proportions of crustaceans. Diversity was lower in BCS microbial mat habitats, but higher in mussel and slope sediments compared to NCS habitats. Multivariate statistical analysis revealed specific sediment properties as important for structuring the macrofaunal communities, including larger grain sizes present within NCS microbial mat habitats and depleted stable carbon isotopes ($\delta^{13}C$) in sediments present at mussel beds. These results suggest that habitat differences in the quality and source of organic matter are driving the observed patterns in the infaunal assemblages, including high β diversity and high variability in the macrofaunal community composition. This study is the first investigation of seep infauna along the U.S. Atlantic slope north of the Blake Ridge Diapir and provides a baseline for future regional comparisons to other seep habitats along the Atlantic margin.

Highlights:

- First investigation of seep infaunal communities in U.S. mid-Atlantic margin north of Blake Ridge at multiple depths

- Microbial mats and mussel bed habitats support locally high densities of infauna
- High taxonomic turnover over small and large spatial scales
- Stable carbon isotopic composition ($\delta^{13}C$) and mud content explained the most variation among NCS seep and non-seep habitats

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 (see Levin, 2005 for review); 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).

Macrofauna often exhibit distinct assemblages associated with the biogenic seep habitat types (Cordes et al., 2010). Macrofaunal communities at clam beds in the Gulf of Guinea were similar to those in sediments adjacent to mussel beds (Menot et al., 2010), suggesting similar community function and sediment geochemical parameters of sediments occupied by these two molluscs. 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 similar (Levin et al., 2010) or higher densities in comparison to each another (Levin et al., 2015; Menot et al., 2010; Sahling et al., 2002). At the Blake Ridge Diapir, macrofaunal densities in sediments near mussels were higher than in microbial mat sediments, although macrofaunal densities were low for all sampled habitats (0-6,400 ind. m^{-2}; Robinson et al., 2004). High densities found in microbial mat habitats have been attributed to the exploitation of the chemosynthetically derived food source by seep tolerant taxa, and has been compared to similar faunal responses from disturbance and sediment organic enrichment events (Bernardino et al., 2012; Sahling et al., 2002).

Macrofaunal diversity patterns among seep and non-seep habitats have been variable. Microbial mat habitats often exhibit low diversity and high dominance of a few tolerant taxa compared to other seep and non-seep habitats due to high sediment sulfide concentrations (Levin et al., 2003; Sahling et al., 2002). However, low sulfide concentrations in clam beds on the

California slope led to increased macrofaunal diversities by supporting populations of both ambient and sulfophilic taxa (Levin et al., 2003). In other locations, macrofaunal diversity in sediments associated with clam beds has been similar (Hydrate Ridge, Sahling et al., 2002) or lower (Gulf of Guinea, Menot et al., 2010) than non-seep habitats. At Blake Ridge, mussel-associated habitats had higher diversity than microbial mats and non-seep sediments (Robinson et al., 2004).

Infaunal community assemblages associated with different seep habitats are distinct (Bernardino et al., 2012; Levin, 2005; Menot et al., 2010) from one another and differ from background non-seep sediments. Dorvilleid polychaetes are common in seep habitats (Levin, 2005) and are particularly abundant in microbial mat habitats, which is attributed to their broad environmental tolerances and opportunistic lifestyle (Levin et al., 2006; Levin et al., 2003; Robinson et al., 2004; Sahling et al., 2002). Other characteristic seep macrofauna include the polychaete families Siboglinidae, Capitellidae, and Ampharetidae, oligochaetes, and thyasirid bivalves (Dando et al., 1991; Levin et al., 2000; Levin et al., 2003), some of which can benefit from reducing habitats (Levin et al., 2000). At Blake Ridge, mussel sediment communities were more similar to non-seep communities (60% similar) than to microbial mat communities (11-54%), suggesting that mussels help maintain low concentrations of methane and sulfide, facilitating communities more similar to non-seep sediments (Robinson et al., 2004). The extent of endemic species in seep habitats globally is still unresolved (Bernardino et al., 2012), but may be a function of depth (Levin, 2005; Sahling et al., 2003), with many species occupying seep sediments comprised of the regionally available taxa pool (e.g. Levin, 2005). In addition, depth-

107 related patterns have been observed among seep sites world-wide, with communities at upper
108 bathyal depths (200-1500m) distinct from those at deeper depths (>1500m; Bernardino et al.,
109 2012). However, there are few comparisons of seeps with depths ranging >1000m (Sahling et al.,
110 2003) within a geographic region, where other factors structuring deep-sea communities (e.g.
111 food availability, bottom water oxygen concentrations) are more directly comparable.
112 The distinct epifaunal and infaunal assemblages present in seep habitats are a function of
113 their proximal sediment geochemical environment (Levin et al., 2003; Sibuet and Olu, 1998),
114 including seepage rates, sulfide concentrations, and biological activity (Cordes et al., 2010a; Olu
115 et al., 2009; Levin, 2005; Sahling et al., 2002). Microbial mats often form in habitats with high
116 methane flux rates, with corresponding high sulfide concentrations and low oxygen penetration
117 into the sediment (Sahling et al., 2002). In contrast, habitats that support clam bed exhibit lower
118 but variable methane flow through sediments, lower sulfide concentrations, and higher oxygen
119 penetration through bioturbation (Levin et al., 2003). Comparable data in mussel beds is limited,
120 but they have been documented to have similar oxygen penetration profiles and higher organic
121 carbon concentrations than clam beds (Menot et al., 2010). Due to variations in seep activity and
122 fluid flux, the sediment geochemical properties (e.g. organic carbon and nitrogen, stable carbon
123 and nitrogen isotopes, grain size) often differ between seep and non-seep habitats (Levin et al.,
124 2000; Levin et al., 2010; Menot et al., 2010; Valentine et al., 2005). Microbial mats have been
125 documented to contain higher percent carbon content, high carbon to nitrogen (C:N) ratios, and
126 lower percent nitrogen content than clam beds and non-seep sediments (Levin et al., 2010). Clam

and mussel beds also contain higher organic carbon content than non-seep sediments at multiple depths (Levin et al., 2000; Levin et al., 2010; Menot et al., 2010; Valentine et al., 2005).

Stable carbon isotopic ($\delta^{13}C$) composition of sediments and fauna from seep habitats often reflects the primary nutritional sources available in the environment, where phytoplankton-derived organic matter typically produce $\delta^{13}C$ values ranging from -25‰ to -15‰ (Fry and Sherr, 1984), very low $\delta^{13}C$ values derived from methane (≤-50‰; Van Dover, 2007; Whiticar, 1999), and carbon derived from sulfide oxidation with $\delta^{13}C$ ranging from -37‰ to -27‰ (Brooks et al., 1987; Fisher, 1990; Robinson and Cavanaugh, 1995). In the Gulf of Mexico, sediments near seeps containing bacterial filaments were depleted in both ^{13}C and ^{15}N compared to those with no bacterial filaments present (Demopoulos et al., 2010). Stable isotope values of seep sediments can vary with seep activity, where higher methane fluxes near mytilid beds were associated with lower $\delta^{13}C$ and $\delta^{15}N$ as compared to clam beds and may contain different microorganism communities (Cambon-Bonavita et al., 2009; Olu-Le Roy et al., 2007a; Olu et al., 2009). Thus, light $\delta^{13}C$ values can be a useful indicator of seep habitats. While methane flux and sulfide concentrations are important mechanistic factors structuring seep faunal communities (Bernardino et al., 2012), stable isotopes and sediment parameters also can serve as a proxy for and provide insight into the mechanisms of seep activity occurring within sediments.

While originally thought to be unusual on the western Atlantic margin (Van Dover, 2000), increasing numbers of seep areas have been documented since 2011 (Skarke et al., 2014). Prior to 2011, only two chemosynthetic seep areas were known, the Blake Ridge Diapir (Paull et al., 1995; Van Dover et al., 2003) and the Cape Fear Diapir (Brothers et al., 2013), both

148 in deep water (2100-2600m) off of South Carolina, US. However, recent large-scale projects
149 using high resolution multibeam sonar and backscatter data now document 570 seep areas
150 between Cape Hatteras and Georges Bank (Skarke et al., 2014), and suggest that tens to
151 thousands more may be present along the passive Atlantic margin. During this study, two
152 recently identified chemosynthetic seep areas were examined near Baltimore Canyon (BCS) and
153 Norfolk Canyon (NCS) separated by 90 km. This study addresses the role of geographic setting,
154 seep habitat type, and sediment geochemistry in determining infaunal densities, community
155 composition, and diversity of sediment macrofauna (>300μm). We hypothesized that (i)
156 communities found at seep and non-seep habitats will differ within sites and between BCS and
157 NCS; (ii) similar seep habitats at BCS and NCS will exhibit similar community composition, and
158 (iii) seep and non-seep habitats will exhibit community differences based on sediment
159 geochemical properties. To support our hypotheses, we expect higher macrofaunal density but
160 lower diversity at shallower BCS than at deeper NCS, similar taxonomic composition between
161 seep habitat types at BCS and NCS, and distinct sediment geochemical parameters associated
162 with community assemblages in each habitat type.
163
164 **2. Methods:**
165 2.1 Study Area
166 Two large cold-seep communities were explored on the U.S. Mid-Atlantic margin in
167 2012 and 2013. The first seep, BCS, was located on the slope south of Baltimore Canyon at
168 depths ranging 366 to 402m. First documented by Hecker et al. (1983) during towed camera

surveys, the exact location was re-discovered in 2012 during this study. The second seep, NCS, was located south of Norfolk Canyon at depths ranging 1457 to 1602m. The NCS was identified by the Okeanos Explorer during multibeam mapping activities which detected active bubble plumes (Skarke et al., 2014). The BCS seep contained large, but patchy, communities of the deep-sea mussel *Bathymodiolus childressi*, along with white microbial mats and large areas of shell debris. The NCS seep contained extensive *B. childressi* communities, with areas of white and yellow microbial mats and shell debris.

2.2 Sampling Procedures

Sediment samples were collected from seep habitats on two cruises (Table 1); one in 2012 aboard the NOAA Ship *Nancy Foster* (17 Aug-14 Sep) and one in 2013 aboard the NOAA Ship *Ronald H. Brown* (2-18 May). Push cores (6.35-cm diameter) were collected in microbial mats, mussel habitats, and background soft-sediment habitats using the ROV *Kraken* (2012) and ROV *Jason II* (2013). Background soft-sediments were collected at NCS in the main axis of Norfolk Canyon using a NIOZ box core, which was sub-sampled with push cores. Bow wave effects on the box core were minimized by reducing the speed of descent of the box core as it approached the seafloor. Additionally, the NIOZ box corer completely seals upon triggering, preventing the loss of surface sediment layers, and only cores that had undisturbed surface layers were processed in this study. In addition, the sub-coring with push core tubes provides direct sample-size effort comparisons for our study, which are directly comparable to other seep studies (Levin and Mendoza, 2007; Levin et al., 2010; Robinson et al., 2004). Additional cores and non-

quantitative suction samples were collected via ROV in 2013 in microbial mats and mussel beds (Table 1). An Ekman corer was used to collect mussel bed material at both BCS and NCS. Push cores were sectioned vertically (0-2, 2-5 cm) after recovery for either faunal or sediment geochemistry analysis. Due to time constraints and the limited number of possible core collections on the ROV, sediments from BCS were only processed for faunal analysis. Faunal core sections, Ekman samples, and suction samples were preserved whole in 10% buffered formalin solution until they were returned to the laboratory where they were stained with rose bengal and washed through a 300-µm mesh sieve to retain the macrofauna portion. Macrofauna were sorted under a dissecting microscope and identified to the lowest practical taxonomic level, including family level for polychaetes, oligochaetes, peracarid crustaceans, and molluscs. Sediment geochemistry core fractions were frozen whole at -20°C until returned to the lab. Subsamples of geochemistry cores were analyzed for the stable isotopes $\delta^{13}C$ and $\delta^{15}N$, and percent carbon and nitrogen. Sediment samples were homogenized prior to drying and acidified with 1.0 N phosphoric acid before weighing into tin boats. Samples were analyzed for $\delta^{13}C$ and $\delta^{15}N$ referenced to Vienna PeeDee Belemnite and atmospheric nitrogen gas, respectively. Analyses were conducted at Washington State University using a Costech (Valencia, USA) elemental analyzer interfaced with a GV instruments (Manchester, UK) Isoprime isotope ratio mass spectrometer. Isotope ratios were expressed in standard delta notation, $\delta^{13}C$ and $\delta^{15}N$, as parts per thousand (‰). Grain size analysis was performed on fractions of the sediment geochemistry cores using the Folk method (Folk, 1974).

2.3 Data Analysis

Abundance of individuals and univariate measures of biodiversity were analyzed using one-way (within sites) and two-way (among sites) analysis of variance (ANOVA) with habitat type (microbial mat, mussels, background) and site as factors and individual cores as replicates, followed by post-hoc test Tukey's HSD for multiple comparisons. All data were tested for normality and heteroscedasticity using Shapiro-Wilk and Levene's tests (Zar, 1999) and $\log_e$-transformed when necessary. If transformation did not achieve normality, a non-parametric Kruskal-Wallis test was used on univariate measures with a post-hoc pairwise Wilcoxon test using a Holm correction for multiple comparisons. Depth relationships with abundance and diversity measures were tested using Spearman's rank correlation. A significance level of $p < 0.05$ was used in all tests. Univariate statistics were computed with the program R (R Development Core Team, 2011). Diversity was examined using Pielou's evenness (J'), Shannon diversity (H'$\log_e$), and ES(n) rarefaction based on untransformed abundance data using DIVERSE in PRIMER Statistical Software version 7 (Clarke and Gorley, 2015).

Community structure was assessed by examining the overall contribution of higher level taxa, composition of polychaete feeding guilds, and multivariate community analysis. Multivariate analysis of community structure across cores for sites and habitats was performed on square-root transformed data using Bray-Curtis similarities in PRIMER version 7 (Clarke and Gorley, 2015) with the PERMANOVA+ add on (Anderson et al., 2008). Multivariate analyses including Ekman and suction samples were performed on presence/absence transformed abundance data. Communities were examined using one-way, two-way, and pairwise analysis of

variance by permutation (PERMANOVA) with distance-based tests for homogeneity of multivariate dispersions (PERMDISP). Similarity of percentages (SIMPER) was used to identify the taxa responsible for discriminating between sites and habitats, and to assess the variability of the communities within habitats. Variability among Bray-Curtis similarities within site-habitat combinations was also assessed using multivariate dispersion (MVDISP).

To address the relationship of the environmental variables to the multivariate community data, distance-based linear modeling (DistLM) and distance-based redundancy analysis (dbRDA) were performed using the PERMANOVA+ add on package to PRIMER 7. DistLM performs nominal tests of each variables explanatory power on community structure and builds a multivariate statistical model of explanatory power of a suite of variables when considered together. Environmental data was only collected at NCS, thus analysis was limited to only the deep site. Variables included were depth, mud content, stable isotopic composition $\delta^{13}C$ and $\delta^{15}N$, and organic carbon content. Organic nitrogen content was excluded from the analysis due to high correlation (>0.95) with organic carbon content to reduce redundancy.

3. Results:

3.1 Density

A total of 2,609 individuals were collected from cores in our study, encompassing 84 taxa, including 34 polychaete families, 22 crustacean families, 20 mollusca families, and 7 other taxa (Table 2). At both sites, the majority of individuals were collected in microbial mat habitats (BCS: 66%; NCS: 76%), followed by mussel habitats (BCS: 22%; NCS: 16%) and background

soft-sediment habitats (BCS: 12%; NCS: 8%). Macrofaunal density was significantly higher at BCS than at NCS for all habitat types (Figure 2; Two-way ANOVA, F=11.34, p=0.003), with the highest densities occurring in microbial mats (137,756 ind. m^{-2}). At both sites, the highest densities occurred in microbial mat habitats, followed by mussel habitats and background habitats. At BCS, macrofaunal density differed among habitats (One-way ANOVA, $F_{2,9}$=7.58, p=0.011), with significantly higher densities in bacterial mats (83,649 ± 28,466 ind. m^{-2}) than in background soft-sediments (15,719 ± 1,582 ind. $m^{-2,}$ Tukey HSD; p = 0.009). Likewise, at NCS macrofaunal density also differed among habitats (One-way ANOVA, $F_{2,10}$ = 10.87, p = 0.003), with densities in microbial mats (47,962 ± 13,547 individuals m^{-2}) significantly higher than both mussel (Tukey HSD, p = 0.007) and background soft-sediments (Tukey HSD, p=0.007). The upper 2 cm of sediments at BCS contained slightly higher proportions of macrofauna in bacterial mat sediments (79%) as compared to mussel sediments (76%) and soft sediments (76%). The proportion of macrofauna found in the upper 2cm at NCS was higher in bacterial mat sediments (84%) as compared to mussel sediments (66%) and soft sediments (55%).

3.2 Diversity

Macrofaunal diversity patterns among habitat types differed between BCS and NCS. At BCS, diversity (H'$\log_e$; Table 3) was significantly lower in bacterial mat sediments than in both mussel (Tukey HSD, p < 0.0001) and background sediments (Tukey HSD, p < 0.0001). Similarly, taxa evenness (J'; Table 3) was significantly lower in bacterial mat sediments than in both mussel (Tukey HSD, p = 0.0001) and background sediments (Tukey HSD, p < 0.0001). At

NCS, there was no significant difference in diversity among habitat types (One-way ANOVA, $F_{2,10}$=1.11, p=0.37) although diversity in background habitats was slightly higher than microbial mat and mussel habitats (Table 3). Similarly, there was no significant difference in taxa evenness among habitat types (One-way ANOVA, $F_{2,10}$=1.11, p=0.51); however, taxa evenness was slightly higher in background soft-sediments compared to microbial mats, although this pattern was not significant (Tukey HSD, p = 0.055). Rarefaction analysis within BCS (Figure 3a) and NCS (Figure 3b) indicated similar within-site patterns as given using Shannon diversity; however, overall diversity of all habitats combined (Figure 3c) indicated higher diversity at NCS than at BCS.

There was a high amount of taxa turnover (β diversity) among habitats. At BCS, 16% of the observed taxa were shared across all sediment habitats, 24-48% of the taxa were shared between any two habitats, and 49% of the taxa were unique to a single habitat. Approximately 40% of the taxa in BCS sediments only occurred in seep habitats. Mussel bed samples (Ekman core) at BCS shared more taxa with mussel sediment habitats (60%) than with microbial mat (40%) or background sediments (20%); however, the low number of taxa present in the single mussel bed sample resulted in low overall diversity compared to mussel sediments (Figure 3a). At NCS, there was overall greater β diversity than at BCS, with only 13% of taxa shared among all three sediment habitats and 22-29% occurring in two or more habitats. A higher percentage of taxa, 58%, occurred only in a single habitat at NCS, and 58% of the taxa were only observed in seep sediments. Similar to BCS, the mussel bed samples at NCS (Ekman core) shared the most taxa with the mussel cores (58%). The non-quantitative suction samples also shared the

most taxa with their analogous sediment communities, the mat suction sharing 53% of its taxa with mat sediments, and the mussel suction sharing 29% with mussel sediment. Overall, the mussel bed and mussel suction samples had similar diversity to the mussel sediments, while the microbial mat suction had higher diversity (Figure 3b). Pooled rarefaction (Figure 3c) for seep habitat push cores combined with Ekman cores and suction samples indicated an increase in diversity with each inclusion of habitats at both sites. The high difference in taxa between the mussel bed samples (Ekmans), compared to cores collected adjacent to the mussel bed suggests high taxonomic turnover on a small (<1m) spatial scale with minimal taxa overlap.

3.3 Community composition

Overall taxonomic composition was similar among habitat types between BCS and NCS based on push core collections (Figure 4). Polychaetes dominated microbial mat and background habitats, comprising 63-67% of the communities at BCS and 73-77% at NCS. The polychaete families Dorvilleidae and Capitellidae composed a large proportion of microbial mat communities at BCS (66%) and NCS (57%), with the addition of Spionidae and other polychaetes at NCS. The proportion of oligochaetes was higher at BCS (31%) than at NCS (13%), while NCS contained higher proportions of crustacea, mollusca, and other taxa. In mussel habitats at both sites, polychaete composition was low (39-47%), with high proportions of crustaceans (23-50%), specifically amphipods and tanaids. Background sediments contained the highest proportion of molluscs (BCS: 18%, NCS: 13%). The overall taxonomic composition of the Ekman cores and suction samples did not resemble the macrofaunal composition in

316 sediment cores collected from adjacent mussel or mat habitats (Figure 4). The BCS Ekman core
317 contained a higher proportion of isopods (65%), while the NCS Ekman core contained a lower
318 proportion of amphipods (7%) relative to sediment communities adjacent to mussel beds. The
319 NCS mussel suction contained the highest proportion of gastropods (54%) while the NCS mat
320 suction contained high proportions of other polychaetes and other taxa, specifically Sipuncula (0-
321 12%) in comparison to mussel and mat sediment communities. In addition, the Ekman and
322 suction samples were better able to collect more highly mobile taxa, as indicated by the numbers
323 of Nebaliidae and Euphausiacea (Table 2).
324 Macrofaunal communities differed both between sites (Figure 5; Two-way
325 PERMANOVA, Pseudo-F=5.82, p=0.0001) and among habitat types (Two-way PERMANOVA,
326 Pseudo-F=7.23, p=0.0001). Estimates of the source of variation in communities indicate that
327 differences among habitat types (Estimate=1051) were greater than differences between sites
328 (Estimate=547). Within each site, community variability among cores was highest within
329 microbial mat sediments (Table 3, MVDISP). Pairwise analysis of site and habitat combinations
330 showed significant differences in macrofaunal communities between all site/habitat
331 combinations (Table 4) except between BCS mussel and background habitats (Table 4).
332 Microbial mat communities at BCS and NCS were more similar to each other than they were to
333 other habitats at their respective sites (Table 4). At BCS, bacterial mats had higher densities of
334 Capitellidae (Polychaeta), Dorvilleidae (Polychaeta), and Tubificidae (Oligochaeta) than the
335 background and mussel habitats, contributing 33% of the dissimilarity with mussel habitats and
336 42% with background habitats. Mussel habitats had higher densities of Tubificidae

337 (Oligochaeta), Leptocheliidae (Tanaidacea), and Typhlotanaidae (Tanaidacea) but lower
338 densities of Opheliidae (Polychaeta) and Yoldiidae (Bivalvia) compared to background soft
339 sediments, contributing 23% of the overall dissimilarity. SIMPER analysis using
340 presence/absence data (Table 4) indicated the Ekman core collected within the mussel bed at
341 BCS were more similar to the sediment communities associated with mussels, than to
342 background sediments, and mat habitats at BCS. However, the taxonomic composition of the
343 BCS Ekman core was more similar to NCS Ekman and suction samples than to sediment
344 communities at BCS (Table 4).
345 At NCS, bacterial mats differed from both mussel and background habitats by high
346 densities of Capitellidae (Polychaeta), Dorvilleidae (Polychaeta), and Spionidae (Polychaeta)
347 contributing 26% of the dissimilarity with mussel habitats and 27% with background habitats.
348 Mussel habitats differed from background soft-sediment habitats, with higher densities of
349 Oedicerotidae (Amphipoda) and Spionidae (Polychaeta), but low densities of Cossuridae
350 (Polychaeta) and Paraonidae (Polychaeta) contributing 31% of the dissimilarity. At NCS, the
351 highest community similarities were observed between the NCS Ekman core and mussel
352 sediment communities (45%, Table 4) and between the Ekman and suction samples (41-49%).
353
354 3.4 Relationship to sediment geochemistry
355 Sediment geochemical properties differed among microbial mat, mussel, and background
356 soft-sediment habitats at NCS (Table 5). Sediment $\delta^{13}C$ and $\delta^{15}N$ values were significantly lower
357 in mussel habitats than both microbial mats and background soft-sediments (Tukey HSD, $\delta^{13}C$,

$p<0.001$; $\delta^{15}N$, $p<0.033$). Microbial mat habitats also contained lower $\delta^{13}C$ and $\delta^{15}N$ values compared to background soft-sediments (Tukey HSD, $\delta^{13}C$, $p<0.001$; $\delta^{15}N$, $p=0.001$). In contrast, mussel habitats contained higher percent organic carbon and nitrogen content than both microbial mat and background soft-sediments (Tukey HSD, %C, $p<0.006$; %N, $p<0.001$). There was no difference in the C:N among habitat types (One-way ANOVA, $F_{2,7}=2.37$, $p=0.16$). Background soft-sediments had the highest mud content, followed by mussel and microbial mat sediments. It is notable that deeper fractions (2-5 cm) of the microbial mat cores contained authigenic carbonate rubble that contributed to the higher grain size in those samples.

Principal coordinate analysis of macrofaunal communities at NCS (Figure 6) indicates that two orthogonal axes are capable of explaining 63% of the natural variation among cores. PCO1 separates mussel from microbial mat and background communities, while PCO2 separates microbial mat from background communities. Variable correlation with PCO axes indicated that PCO1 was positively correlated with $\delta^{13}C$, $\delta^{15}N$, and the C:N ratio, and negatively correlated with percent organic carbon (%C). Mud content and depth were positively correlated with PCO2. DISTLM analysis (Table 6) indicated that all environmental variables except C:N can individually explain a significant portion (24-31%, $p<0.017$) of the variation in NCS communities. A combination of $\delta^{13}C$ and mud content provide the best explanation of variation in NCS macrofaunal communities, accounting for 60% of the variation among samples in two axes.

4. Discussion:

Differences between the depths represented by BCS and NCS habitats were apparent for most of the community parameters measured (e.g. density, diversity, community composition). Macrofaunal densities along non-seep slope ecosystems generally decrease with depth and distance from shore, related to decreases in in food availability from surface productivity and transport of organic matter from shelf areas (Rex and Etter, 2010). We observed lower densities with depth in background sediments, a trend that continues regionally with even lower non-seep macrofaunal densities at Blake Ridge (Table 7; Robinson et al., 2004). This trend was also present for seep habitats; however, given the additional nutrition source provided by the seep it cannot be attributed to depth-related patterns alone. Each habitat at NCS exhibited higher variability in macrofaunal communities (MVDISP) as compared to BCS habitats, suggesting increased patchiness with depth consistent with deep-sea community ecology (Rex and Etter, 2010). The higher variability within seep communities at NCS could be due to the larger separation between the collected individual mussel and microbial mat sediments than at BCS. However, background sediments at NCS were collected at a finer spatial scale than at BCS, and we would have expected lower variability at NCS if spatial separation alone was the controlling factor. Higher community patchiness with depth is also supported by the differing results from the two diversity analyses (Shannon diversity vs. rarefaction). The higher Shannon diversity at BCS indicates that diversity was high within cores, but rarefaction suggests there is a lower overall taxonomic pool present at BCS compared to NCS, although undersampling is evident for both sites. Overall diversity followed the expected trend and increased with depth (e.g., Rex, 1981), although the opposite pattern was observed for background habitats. Given the low

400 abundance and limited sampling in background habitats at both sites, our results likely provide
401 an underrepresentation of background soft-sediment diversity.
402 Community assemblage differences between BCS and NCS may also be depth driven,
403 consistent with the separation of macrofaunal communities between upper bathyal (200-1500m)
404 and lower bathyal/abyssal (>1500m) depths worldwide documented by Bernardino et al. (2012).
405 Differences among seep and non-seep sediment communities have been observed to increase
406 with depth (Levin, 2005), suggesting the greater importance of the additional nutrition source
407 provided by the seep at increasing depths (Levin and Michener, 2002). Within a geographic
408 region, comparisons among seeps at different depths have been limited. Significant community
409 differences have been observed at seeps along the Pacific margin (525 and 770m; Levin et al.,
410 2010) and the Aleutian margin (3300m and 4400m; Levin and Mendoza, 2007). However, the
411 depth sampling locations in both Levin et al. (2010) and Levin and Mendoza (2007) were
412 separated by >425 km, potentially confounding the effect of geographic and depth patterns. The
413 higher proximity between BCS and NCS (90km) than in previous studies should reduce the
414 geographic location effect and community differences likely highlight depth-related patterns.
415 Macrofaunal densities observed in BCS microbial mat sediments (Table 7) were among
416 the highest recorded for any seep environment worldwide. Locally high densities in seep
417 habitats have been reported from multiple locations, with the highest densities recorded from
418 microbial mats in the Gulf of Mexico (Table 7; Robinson et al., 2004). High densities have been
419 recorded in frenulate fields on the Norwegian margin (Decker et al., 2012), microbial mats on the
420 northern California margin (Levin et al., 2006), and an ampharetid bed in New Zealand (Thurber,

2010), all of which were at deeper depths (Table 7). Macrofaunal density in microbial mats was also high at NCS compared to microbial mat habitats at similar depths in other locations (Table 7; Ritt et al., 2011; Robinson et al., 2004). Macrofaunal densities in microbial mat and mussel sediments at BCS and NCS were greater than those measured at the nearest previously known seep located 802 km to the southeast at Blake Ridge (Robinson et al., 2004). Regionally, both seep sites represent localized areas of high densities, as indicated by the lower densities in background sediments, similar to results for other seep communities worldwide (Menot et al., 2010). Background sediments at both BCS and NCS also exhibited higher densities than from other regional and historical sampling efforts north of Cape Hatteras (Table 7; Maciolek et al., 1987; Robertson et al., 2015; Sanders et al., 1965).

The habitats characterized by their dominant faunal component (e.g. microbial mats, mussel beds) are known to be distinct from one another in other seep locations (Bernardino et al., 2012; Cordes et al., 2010a; Levin, 2005). While macrofaunal abundances in seep habitats are commonly higher than background soft-sediments (Levin and Mendoza, 2007), differences between seep habitats (i.e. microbial mats, clam beds, mussel beds) have been variable (Bernardino et al., 2012). Microbial mat sediments near Costa Rica had macrofaunal densities two times higher than in clam beds (400-1796m; Levin et al., 2015) while microbial mats on the Pacific margin (252-770m) had similar (Levin et al., 2010; Levin et al., 2003) or higher densities than in clam beds (Sahling et al., 2002). The high densities observed in microbial mat habitats at both BCS and NCS differs from the regional pattern observed at Blake Ridge, where mussel bed habitats contained higher macrofaunal densities than microbial mats (Robinson et al., 2004).

442 However, the mussel species at Blake Ridge, *Bathymodiolus heckerae*, known to support both
443 methanotrophs and sulfide oxidizers, differed from the dominant mussel species present at BCS
444 and NCS, *Bathymodiolus childressi*, which is known to support only methanotrophic bacteria
445 (Olu-Le Roy et al., 2007b). The specific mussel species present in seep habitats may indicate
446 different sediment geochemical parameters, which may help explain the differing infaunal
447 community patterns observed between Blake Ridge and our sites.
448 The low (α) diversity observed in microbial mat habitats, particularly at BCS, is
449 consistent with previous studies which observed lower diversity within microbial mat habitats
450 compared to nearby clam beds (Bernardino et al., 2012; Levin and Mendoza, 2007; Levin et al.,
451 2003). Microbial mat sediments at both BCS and NCS were dominated by the annelid families
452 Capitellidae, Dorvilleidae, and Tubificidae, all of which have been previously observed in seep
453 habitats (Levin, 2005; Levin et al., 2010; Levin et al., 2003). Dorvilleids are a common
454 component of seep communities (Levin, 2005) and often occur in high densities in microbial mat
455 sediments (Robinson et al., 2004; Sahling et al., 2002) where they are likely consuming mat-
456 forming sulfur bacteria (Levin and Michener, 2002). Capitellids are known to be an opportunistic
457 taxa, tolerant to stress, and has shown a strong preference for sulfidic environments (Levin et al.,
458 2000; Levin et al., 2003). Only the polychaete families Dorvilleidae, Cirratulidae, and
459 Hesionidae were documented in microbial mat sediments at Blake Ridge (Robinson et al., 2004),
460 all of which were present in microbial mat sediments at NCS, while Hesionidae were missing in
461 mat sediments at BCS. In contrast to microbial mats, sediments adjacent to mussels at BCS and
462 NCS contained high proportions of crustaceans, particularly amphipods and tanaids. Amphipods

463 are known to be sensitive to organic enrichment and increased hydrocarbon concentrations
464 (Peterson et al., 1996), and their distribution at BCS may also be reflecting this intolerance to the
465 high methane flux and sulfide concentrations likely present at microbial mat habitats. For the
466 seeps at Blake Ridge, crustaceans were only documented in mussel sediments (Robinson et al.,
467 2004), suggesting similarities across depth regimes. In addition, increased variability in
468 communities has been used as an indicator of stressed and/or disturbed environments (Fisher et
469 al., 2014; Warwick and Clarke, 1993). Although fluid flux and sulfide concentrations were not
470 measured, the higher variability (MVDISP), lower diversity and greater similarity in mussel and
471 background sediment communities, also suggests a higher stress environment in microbial mat
472 sediments.
473 High taxonomic turnover (β diversity) at seep sites was present over both small (<1 m)
474 and large spatial scales. High turnover among seep habitats (mussels and mats) has been
475 documented at seep sites worldwide (see Cordes et al., 2010b for review) is suggested to be a
476 result of small-scale variation in the vertical distribution and concentration of sulfides in
477 sediments (Levin et al., 2003) and habitat heterogeneity (Cordes et al., 2010b). Hints at these
478 small-scale variations were observed both in sediment cores collected in mat and background
479 habitats at BCS and between the Ekman cores collected within the mussel habitat and cores
480 collected directly adjacent to mussel habitats at both sites. Similar to results observed in Pacific
481 seeps (Levin et al., 2010), the seep habitats contribute significantly to the regional biodiversity
482 for their specific depth, providing 37-49% of infaunal taxa and high turnover between seeps and
483 background soft-sediment communities. In addition, while the taxonomic level applied in this

484 study (family-level) was sufficient to ascertain differences among habitat-specific communities,
485 further identification (e.g. genus and/or species level) will likely provide increased separation of
486 habitat-specific communities, biodiversity estimates, identification of biogeographic boundaries,
487 and insight into seep endemism at these sites. High taxa turnover among the mussel habitat,
488 adjacent sediments, and background sediments highlights that habitat provision of dense mussel
489 communities influences not only the *in situ* macrofaunal communities found within the beds, but
490 also the communities that occur in the sediments beyond the perimeter of the mussel bed itself.
491 This 'reef' effect has also been, observed for deep-sea coral communities (Demopoulos et al.,
492 2014). While an effect of seep habitats on sediment macrofaunal communities has not been
493 detected at distances greater than 250 m from seep megafauna (Menot et al., 2010), discrete
494 transects from mussel beds to adjacent sediments and beyond would help quantify the sphere of
495 influence of seep activity and biogenic structures on adjacent habitats.
496 The higher proportion of taxa found in the upper 2 cm of sediments in microbial mats
497 versus deeper sediments, particularly at NCS, may reflect different geochemical settings present
498 within each habitat. Seeps, along with other reducing environments such as areas of organic
499 enrichment, organic falls, and oxygen minimum zones, are often characterized by low oxygen,
500 sulfidic sediments (Levin et al., 2010; Tunnicliffe et al., 2003). The vertical distribution of taxa
501 in sediments is regulated partly by oxygen and sulfide concentrations (Levin, 2005), resulting in
502 a trade-off between sulfide tolerance and food availability (Menot et al., 2010). Few taxa
503 tolerate sulfide concentrations >1 mM, while Dorvilleidae polychaetes can occur in high
504 densities at concentrations ranging 1 to 6 mM (Levin et al., 2003). The higher proportion of taxa

present in the upper 2 cm of microbial mat sediments suggests these habitats have low oxygen and potentially high sulfide concentrations that are restricting fauna to the surface sediments (Levin et al., 2003). Whereas, the higher proportion of taxa present in sub-surface sediments (>2cm) in mussel and background habitats suggests deeper oxygen penetration and lower sulfide concentrations, allowing more individuals to survive at greater depth within the sediments (Levin et al., 2001; Levin, 2005). Bioturbation by deeper dwelling taxa in turn facilitates oxygen penetration and the transfer of organic material, thus also increasing the food availability for other organisms residing deeper in the sediments. Similar faunal sediment-depth patterns were reported for microbial mat (Levin et al., 2003) and mussel-associated sediments (Menot et al., 2010) at other seeps, suggesting that in the absence of specific oxygen and sulfide concentration measurements, inferences about the geochemical setting based on the faunal composition may be possible.

The high variation observed in NCS microbial mat communities suggests a gradient among sampling locations in the underlying seep fluid flow and sediment geochemistry. Sediments supporting microbial mats are known to sustain high rates of methane emissions, high concentrations of sulfide, and low oxygen penetration (Bernardino et al., 2012). In contrast, mollusc-dominated habitats (e.g. clam beds) often have lower methane emission rates and lower sulfide concentrations near the sediment surface (Boetius and Suess, 2004; Levin, 2005; Sahling et al., 2002). Although the geochemical settings have been observed to differ between clam bed and mussel bed habitats (Menot et al., 2010), they contained similar macrofaunal communities suggesting similar habitat functioning. The large continuous fields of mussels present at BCS

526 and NCS suggest regular and diffuse fluid flow (Olu-Le Roy et al., 2007a), although the
527 patchiness and large areas of shell debris at BCS also suggest spatially or temporally intermittent
528 flow. Animals occupying sediments below microbial mats must be tolerant to high levels of
529 sulfide, while those near mussel habitats may not require a high tolerance, but fall within a
530 tolerance gradient. The high methane flux expected in microbial mat sediments should
531 contribute to higher sulfate reduction and anaerobic methane oxidation, while low methane
532 emission rates in mussel sediments may concentrate isotopically depleted methane, both
533 processes yielding light isotopic values in sediments. We observed higher $\delta^{13}C$ in microbial mats
534 than in mussel bed habitats. Isotopic composition of mussels collected within these seeps yielded
535 isotopically light $\delta^{13}C$ (-64‰ to -61‰; Prouty et al., 2014) and $\delta^{15}N$ values (-2‰ to 6‰; Prouty
536 et al., 2014). The contribution of mussel tissues to the organic matter pool is indicated by the
537 enriched percent organic carbon content and depleted ^{13}C values. Microbial composition may
538 also influence the stable isotope composition of the microbial mat sediments. Filamentous
539 sulfide oxidizing bacteria (e.g. *Beggiatoa, Thioplaca*) differ from amorphous forms (e.g.
540 *Arcobacter*) and iron-oxidizers and sediment $\delta^{13}C$ values reported here may reflect the very
541 different microbial communities supporting the food chain as well as organic matter contribution
542 from mussels (Levin and Mendoza, 2007). However, the variation within NCS microbial mat
543 communities was best characterized by mud content and depth. Mussel cores were collected
544 over similar depth range (~100m) as microbial mat cores without a corresponding variation in
545 community assemblages. The variation in mud content and authigenic carbonate rubble in

546 microbial mat cores may reflect the level of microbial activity occurring within the sediment,
547 which can influence the macrofaunal community structure.

548 Although we did not measure any sediment geochemistry at BCS, given the similar
549 patterns exhibited among microbial mat, mussel, and background sediment communities in
550 relation to those at NCS, similar sediment geochemical patterns may be structuring infaunal
551 communities at BCS. Sediment geochemistry for sites within 2 km (Mienis et al., 2014) at
552 shallower (282m) and deeper (515m) depths on the Baltimore slope indicate lower sediment
553 organic carbon (0.31-0.43%) and nitrogen (0.1%), C:N ratios (3.1-4.3), $\delta^{15}N$ (4.6-4.8‰) values,
554 and mud content (12-38%, 515m only), but comparable $\delta^{13}C$ (-22.3 to -21.9) compared to
555 background sediments collected at NCS (Mienis et al., 2014). These data suggest a food-limited
556 environment with increased hydrodynamic flow, as indicated by water column turbidity patterns
557 over the slope (Mienis et al., 2014). Additional sampling of sediment geochemistry at BCS
558 would allow regional comparisons between these two discrete seep habitats, and provide further
559 insight into the mechanisms supporting seep communities in the mid-Atlantic region.

560 There are potential limitations to the comparisons made between seep and background
561 habitats at both BCS and NCS in our study, including seasonality and interannual variation,
562 location, and sampling methods. At BCS, all of the background sediments were collected in
563 August 2012, while all but one core from seep habitats were collected in May 2013. Seasonality
564 in surface productivity and hydrodynamic regimes, as well as disturbance events, promotes shifts
565 in community assemblages. However, there was no observed difference in the abundance of taxa
566 in the upper 2 cm of sediments between 2012 and 2013 samples collected at BCS, which might

have been expected if there had been an organic enrichment event during this time period. In addition, previous temporal studies within the mid-Atlantic region found little interannual variation in macrofaunal communities (Boesch, 1979). Proximity of background, soft-sediment cores to seep habitats may also the affect their observed similarity to seep habitats. Three of the four background cores were collected within the axis of Baltimore Canyon, while the fourth was in close proximity (<1m) to microbial mat habitats at the seep on the adjacent slope. The high similarity among BCS background cores (59%) with the inclusion of the near-mat core suggests they are an adequate representation of nearby background communities. At NCS, the box cores collected for background sediments were 18-19 km north from the seep habitats and were located at the base of the Norfolk canyon channel. Macrofaunal communities are known to differ between canyon axis and slope habitats for Norfolk Canyon (Robertson et al., 2015). While the samples examined in this study represent the best information available, quantitative collections in near-field (~250 m) non-seep sediments would provide a better understanding of the localized effect of seep habitats on infaunal communities.

Seep habitat-specific communities on the western Atlantic margin exhibit many similarities to other microbial mat and mollusc-dominated communities worldwide, suggesting similar environmental controls within these settings. This study is the first to examine seep-associated infaunal communities at depths <2000 m and in the context of their geochemical environment in this region of the Atlantic. Discrete differences among seep habitats and sites indicate that seep community patterns may be driven, in part, by the sub-seafloor seep plumbing supplying methane to the upper sediment/water interface. The potential ephemeral nature of

these seeps and their associated fluid flux (Condon et al., 2015) may represent a strong driver influencing infaunal communities. Enhanced understanding of the seep plumbing, methane flux, and associated sediment geochemistry (e.g., pore water sulfide and methane concentrations) coupled with infaunal community metrics are needed to develop generalizations relating seep environmental controls on infaunal structure and function.

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788 **Figure Captions:**

789 **Figure 1.** Maps showing locations of the sampling sites and closest known seeps (a) with
790 detailed sampling at b) BCS and c) NCS. ■ = Microbial mat habitats; ▲ = Mussel habitats; ● =
791 Background, soft-sediment habitats.

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Figure 2. 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.

Figure 3. 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 includes 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.

Figure 4. 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.

Figure 5. Non-metric multidimensional scaling of Bray-Curtis similarities of square-root transformed macrofaunal abundance data from push cores collected in BCS and NCS habitats. Circles and percentages indicate average similarity among cores for each habitat from SIMPER analysis.

Figure 6. Principal coordinate ordination of Bray-Curtis similarities of square-root transformed abundance data from sediment push cores collected at NCS habitats with environmental parameter vectors overlaid.

Table Titles

Table 1. Number of samples collected at Baltimore and Norfolk seep and background sites, including push cores collected for infaunal analysis (Fauna) and sediment geochemistry analysis (SC), Ekman cores, and suction samples.

Table 2. Mean (±1 S.E.) number of individuals per core ($32cm^2$) of macrofaunal taxa collected from push cores and total individuals collected in Ekman ($0.063m^2$) and suction samples in microbial mat, mussel, and background habitats.

Table 3. Diversity ($H'\log_e$), evenness (J'), and multivariate dispersion (MVDISP) of macrofaunal communities collected from cores at Baltimore and Norfolk seep and background habitats.

Table 4. 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 grab samples were based on Bray-Curtis similarities of presence/absence transformed abundance data.

834

835 **Table 5.** Mean (±1 S.E.) sediment geochemical properties for cores collected at Norfolk seep
836 and background habitats.

837

838 **Table 6.** Results from the distance-based linear modeling (DISTLM) of environmental variables
839 with Norfolk microbial mat, mussel, and background soft-sediment communities using the AICc
840 criteria.

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842 **Table 7.** Summary of macrofaunal seep sediment and regional infaunal studies including closest
843 geographic seeps, comparable depths, and observed high densities.

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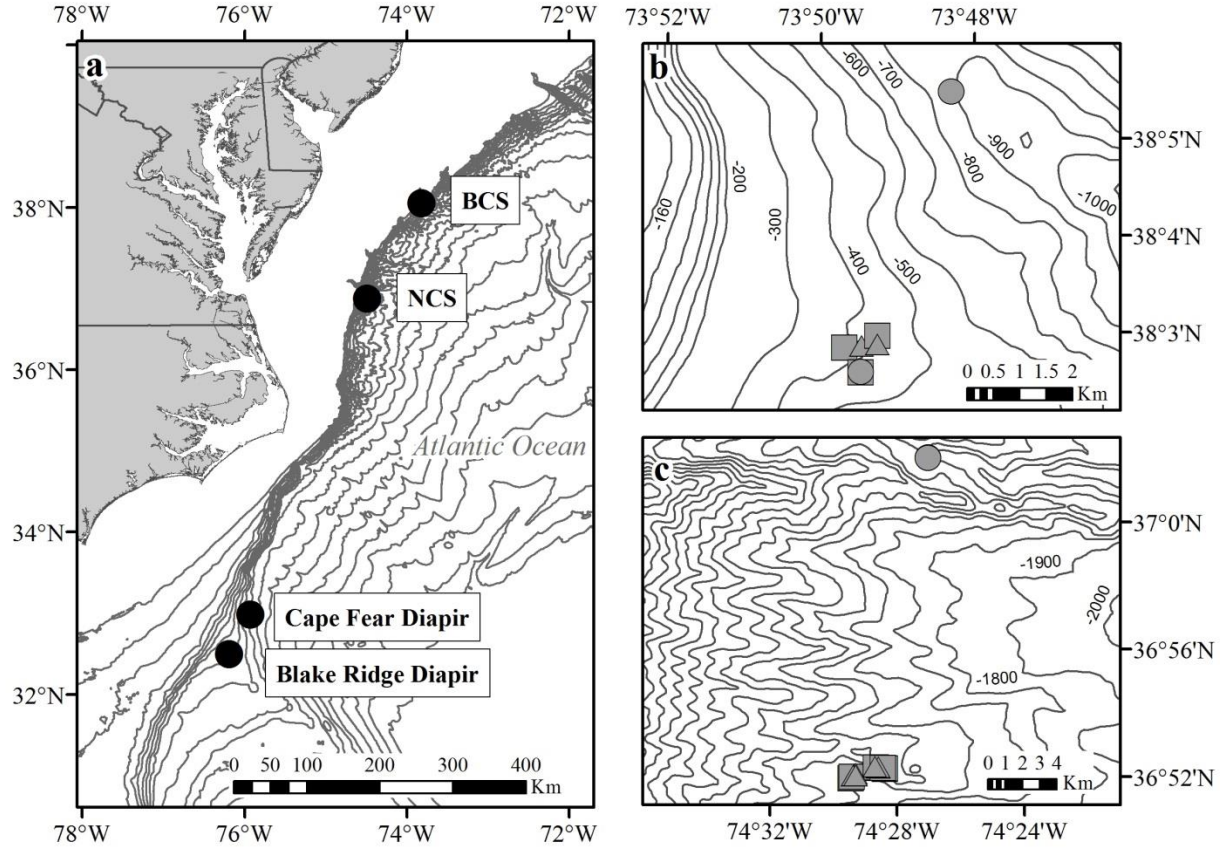
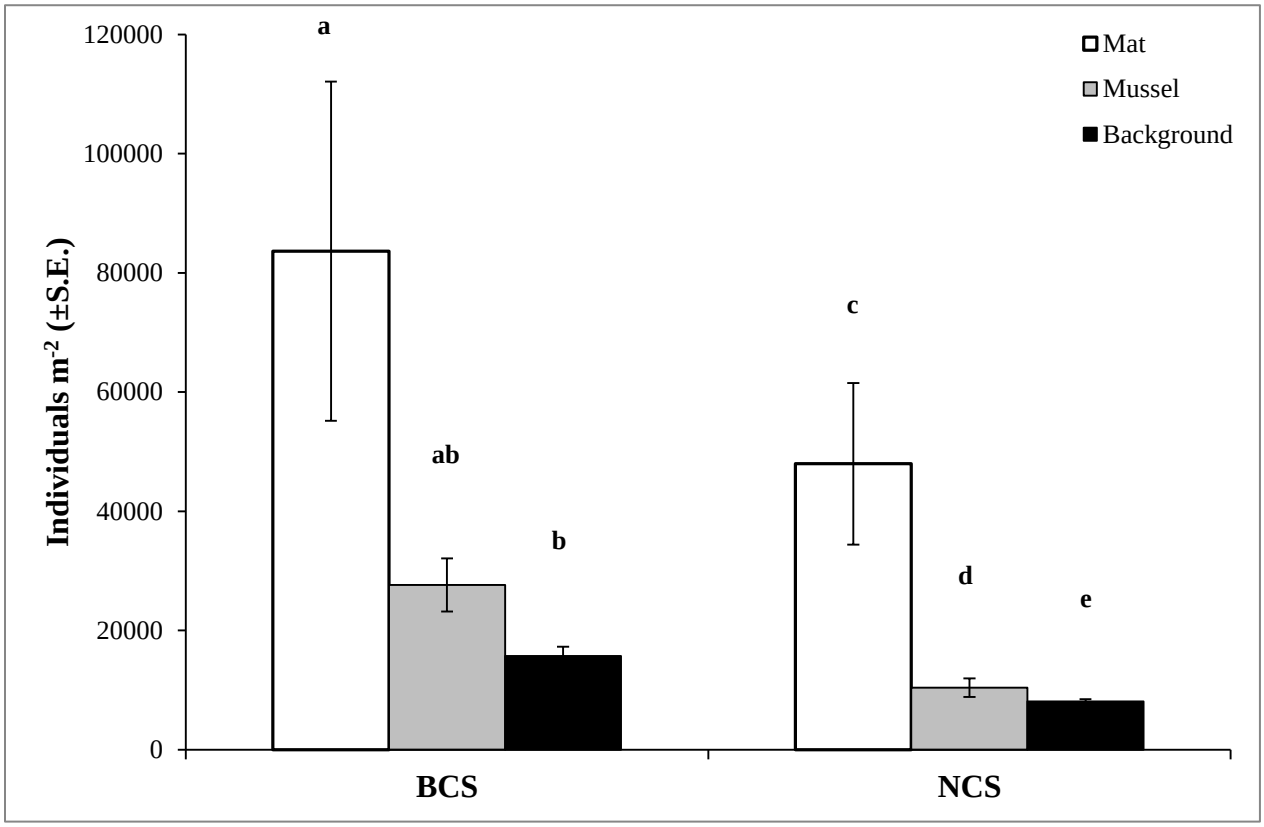

a
b
c
78°W
76°W
74°W
72°W
38°N
36°N
34°N
32°N
BCS
NCS
Cape Fear Diapir
Blake Ridge Diapir
Atlantic Ocean
0 50 100 200 300 400
Km
73°52'W
73°50'W
73°48'W
38°5'N
38°4'N
38°3'N
-160
-200
-300
-400
-500
-600
-700
-800
-900
-1000
0 0.5 1 1.5 2
Km
37°0'N
36°56'N
36°52'N
74°32'W
74°28'W
74°24'W
-1900
-2000
-1800
0 1 2 3 4
Km

Figure 1


120000
100000
80000
60000
40000
20000
0
Individuals m-2 (±S.E.)
a
ab
b
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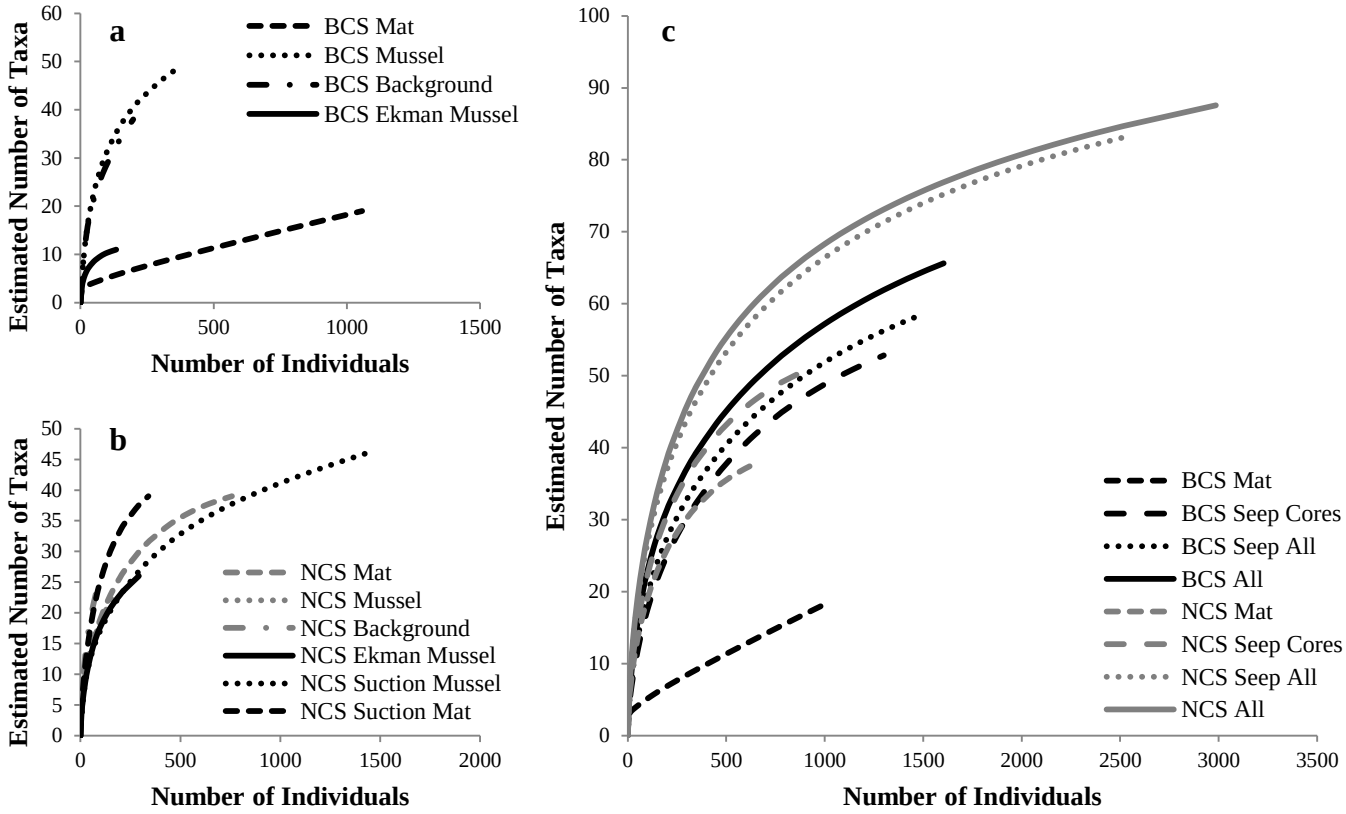
d
e
Mat
Mussel
Background
BCS
NCS

Figure 2



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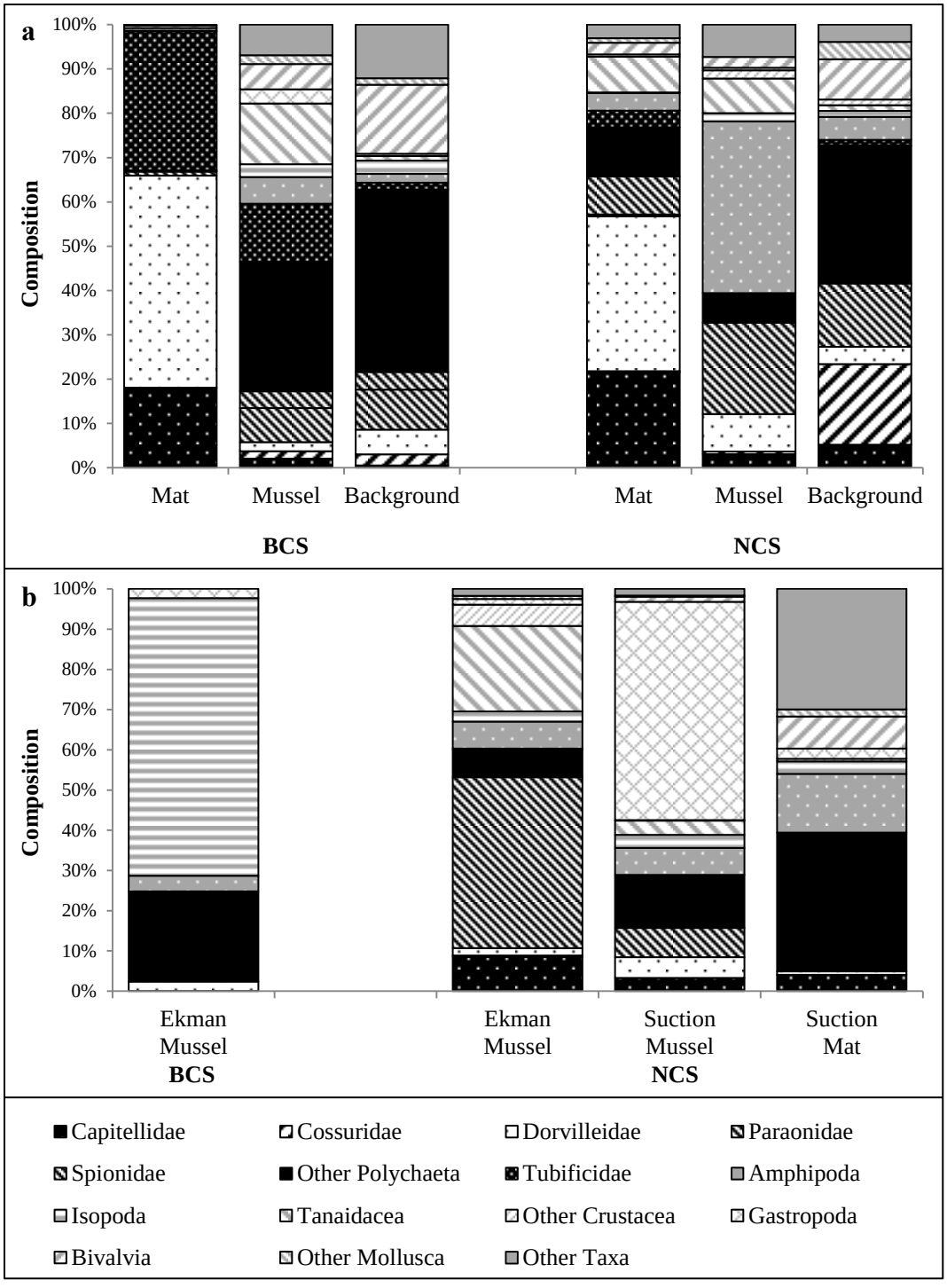
870 **Figure 3.**



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872 **Figure 4**

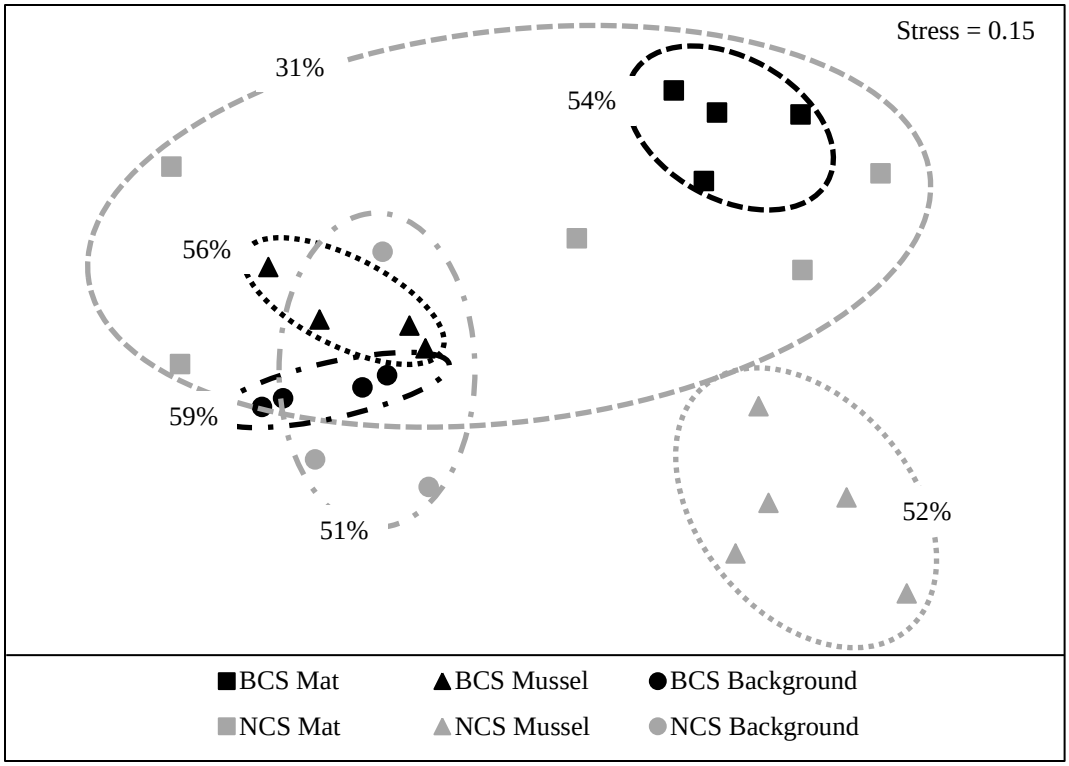
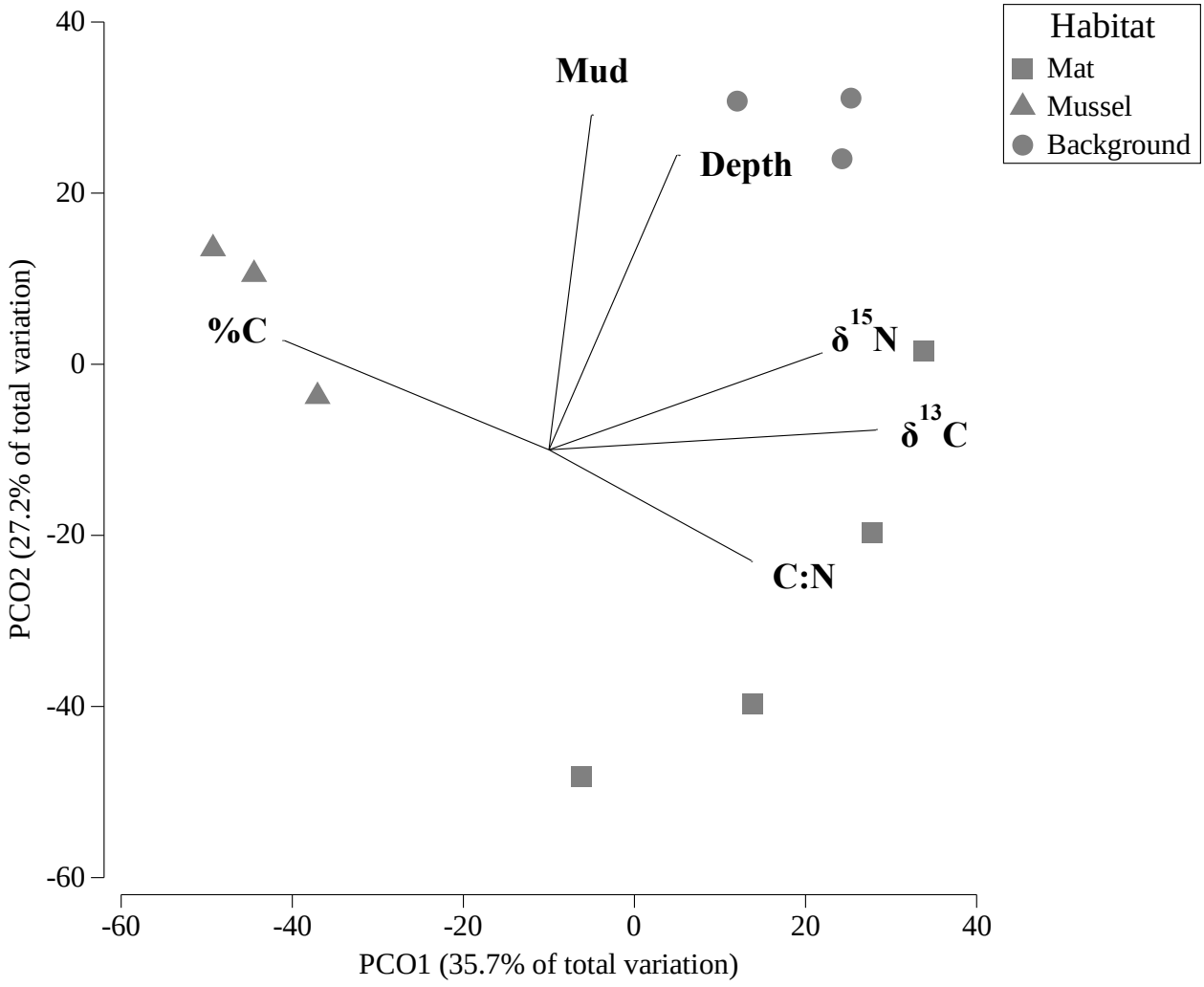

Stress = 0.15
31%
54%
56%
59%
51%
52%
BCS Mat
BCS Mussel
BCS Background
NCS Mat
NCS Mussel
NCS Background

Figure 5



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886 **Figure 6**

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Table 1. Number of samples collected at Baltimore and Norfolk seep and background sites, including push cores collected for infaunal analysis (Fauna) and sediment geochemistry analysis (SC), Ekman cores, and suction samples.

Site	Habitat	Year	Push cores Fauna	Push cores SC	Ekman core	Suction	Depth (m)
Baltimore BCS	Mat	2012	1	0	0	0	412
		2013	3	0	0	0	366-402
	Mussel	2013	4	0	1	0	372-400
	Background	2012	4	0	0	0	412-446
Norfolk NCS	Mat	2013	5	4	0	1	1467-1602
	Mussel	2013	5	3	1	1	1482-1585
	Background	2013	3	3	0	0	1619-1622

Table 2. Mean (±1 S.E.) number of individuals per core (32cm^2) of macrofaunal taxa collected from push cores and total individuals collected in Ekman (0.063m^2) and suction samples in microbial mat, mussel, and background habitats.

Taxa	Baltimore							Norfolk								
	Mat		Mussel		Background		Ekman Mussel	Mat		Mussel		Background		Ekman Mussel	Suction mat	Suction mussel
Annelida	261	(89.9)	52.3	(3.8)	32.0	(3.9)	32	122.4	(38.7)	13.0	(4.7)	19.0	(3.1)	170	132	415
Polychaeta	178	(60.5)	40.8	(3.7)	31.3	(3.5)	32	116.6	(39.2)	13.0	(4.7)	18.7	(2.7)	169	132	415
Aberrrantidae	-	-	0.8	(0.8)	-	-	-	-	-	-	-	-	-	-	-	-
Acrocirridae	-	-	-	-	-	-	-	-	-	-	-	0.3	(0.3)	-	-	3
Ampharetidae	-	-	5.3	(1.5)	2.8	(0.5)	-	-	-	-	-	-	-	-	-	1
Amphinomidae	-	-	-	-	-	-	-	0.4	(0.4)	-	-	-	-	-	6	-
Apistobranchidae	-	-	0.3	(0.3)	-	-	-	1.6	(1.4)	-	-	-	-	-	-	-
Capitellidae	48	(12.8)	1.8	(0.9)	0.3	(0.3)	-	32.6	(21.0)	1.0	(0.6)	1.3	(0.9)	24	14	47
Chaetopteridae	-	-	-	-	-	-	-	0.2	(0.2)	-	-	-	-	-	-	-
Cirratulidae	0	(0.3)	4.5	(1.2)	2.8	(1.4)	24	0.6	(0.4)	0.2	(0.2)	2.0	(0.6)	12	58	6
Cossuridae	-	-	1.5	(0.5)	1.3	(0.6)	-	0.4	(0.2)	0.2	(0.2)	4.7	(1.9)	1	-	-
Chyrsopetalidae	0	(0.3)	-	-	-	-	-	1.0	(0.4)	-	-	0.7	(0.7)	-	-	87
Dorvilleidae	127	(50.2)	1.8	(0.5)	2.8	(1.0)	3	53.2	(23.6)	2.8	(2.0)	1.0	(0.6)	5	3	74
Fabriciidae	-	-	1.8	(0.8)	2.8	(1.8)	-	2.2	(1.2)	-	-	-	-	-	-	-
Fauveliopsidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-
Flabelligeridae	-	-	-	-	-	-	-	-	-	1.4	(0.5)	-	-	6	-	1
Glyceridae	-	-	-	-	-	-	-	0.2	(0.2)	-	-	-	-	-	6	-
Hesionidae	-	-	1.0	(1.0)	-	-	1	0.2	(0.2)	0.2	(0.2)	-	-	-	-	4
Lumbrineridae	-	-	3.5	(0.6)	2.5	(0.3)	-	0.4	(0.2)	-	-	1.0	(0.6)	1	7	36
Maldanidae	-	-	4.5	(1.8)	3.5	(0.9)	-	0.8	(0.6)	-	-	0.3	(0.3)	-	3	3

Nephtyidae	-	-	-	-	0.5	(0.3)	-	0.6	(0.4)	-	-	-	-	-	-	-
Nereididae	-	-	-	-	0.5	(0.3)	-	-	-	0.2	(0.2)	1.3	(0.7)	-	-	-
Onuphidae	-	-	0.5	(0.5)	0.5	(0.3)	-	-	-	-	-	-	-	-	-	-
Opheliidae	-	-	0.8	(0.3)	3.0	(1.2)	-	-	-	0.2	(0.2)	0.3	(0.3)	-	-	-
Orbiniidae	-	-	0.3	(0.3)	-	-	-	-	-	-	-	0.3	(0.3)	-	-	-
Paraonidae	3	(1.5)	6.8	(1.4)	4.5	(1.7)	-	0.6	(0.4)	-	-	3.7	(1.8)	-	-	-
Pholoidae	-	-	-	-	-	-	-	0.4	(0.4)	-	-	-	-	-	-	-
Polynoidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	3	1
Sabellidae	-	-	-	-	-	-	-	1.6	(1.6)	-	-	-	-	-	20	6
Scalibregmatidae	-	-	-	-	0.3	(0.3)	-	-	-	-	-	-	-	-	2	-
Serpulidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1	1
Siboglinidae	-	-	0.5	(0.3)	0.8	(0.8)	-	4.2	(1.3)	-	-	-	-	-	-	-
Sigalionidae	-	-	-	-	-	-	-	0.6	(0.4)	-	-	1.7	(0.3)	-	5	-
Sphaerodoridae	-	-	0.3	(0.3)	-	-	-	-	-	-	-	-	-	-	-	-
Spionidae	-	-	3.3	(1.2)	2.0	(0.4)	-	13.0	(8.9)	6.8	(3.0)	-	-	120	-	105
Syllidae	-	-	-	-	0.5	(0.3)	4	0.4	(0.4)	-	-	-	-	-	2	36
Terebellidae	-	-	1.0	(0.6)	-	-	-	0.6	(0.6)	-	-	-	-	-	-	-
Trichobranchidae	-	-	0.8	(0.5)	0.3	(0.3)	-	0.2	(0.2)	-	-	-	-	-	-	4
Polychaeta A	-	-	-	-	-	-	-	0.6	(0.4)	-	-	-	-	-	-	-
Oligochaeta	83	(38.8)	11.5	(5.2)	0.8	(0.5)	-	5.8	(4.1)	-	-	0.3	(0.3)	1	-	-
Tubificidae	83	(38.8)	11.5	(5.2)	0.8	(0.5)	-	5.8	(4.1)	-	-	0.3	(0.3)	1	-	-
Crustacea	0	(0.3)	19.8	(13.4)	3.0	(0.7)	94	18.4	(14.3)	16.6	(1.2)	2.3	(0.9)	101	63	196
Amphipoda	0	(0.3)	5.3	(5.3)	1.0	(0.4)	5	6.0	(2.9)	12.8	(1.2)	1.3	(0.7)	19	51	96
Amphipoda Indet	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Ampeliscidae	-	-	-	-	0.3	(0.3)	-	-	-	-	-	-	-	-	-	-
Aristiidae	-	-	-	-	-	-	-	-	-	0.2	(0.2)	-	-	-	-	-
Caprellidae	-	-	-	-	0.3	(0.3)	-	-	-	-	-	-	-	2	-	7

Ischyroceridae	-	-	5.0	(5.0)	-	-	-	-	-	-	-	-	-	-	-	-
Liljeborgidae	-	-	0.3	(0.3)	-	-	-	-	-	-	-	-	-	-	-	-
Lysianassidae	-	-	-	-	-	-	2	-	-	1.4	(0.7)	-	-	3	-	9
Oedicerotidae	-	-	-	-	0.3	(0.3)	-	1.2	(0.6)	7.0	(1.4)	-	-	4	36	-
Phoxocephalidae	0	(0.3)	-	-	0.3	(0.3)	-	-	-	4.2	(1.2)	1.3	(0.7)	1	-	-
Pleustidae	-	-	-	-	-	-	2	4.8	(2.4)	-	-	-	-	-	-	-
Isopoda	-	-	2.5	(1.3)	1.5	(0.3)	89	0.2	(0.2)	0.6	(0.4)	0.3	(0.3)	7	11	47
Desmosomatidae	-	-	0.8	(0.5)	1.3	(0.3)	-	-	-	-	-	-	-	-	-	-
Gnathiidae	-	-	-	-	0.3	(0.3)	-	-	-	-	-	-	-	-	-	-
Janiridae	-	-	0.8	(0.8)	-	-	19	-	-	-	-	-	-	-	-	-
Leptanthuridae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-
Munnidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	38
Munnopsidae	-	-	0.8	(0.5)	-	-	61	-	-	0.2	(0.2)	-	-	5	4	8
Nannoniscidae	-	-	-	-	-	-	-	0.2	(0.2)	0.2	(0.2)	0.3	(0.3)	-	2	1
Paramunnidae	-	-	0.3	(0.3)	-	-	-	-	-	-	-	-	-	2	3	-
Paranthuridae	-	-	-	-	-	-	-	-	-	0.2	(0.2)	-	-	-	-	-
Cumacea	-	-	-	-	-	-	-	-	-	-	-	0.3	(0.3)	-	-	-
Leuconidae	-	-	-	-	-	-	-	-	-	-	-	0.3	(0.3)	-	-	-
Tanaidacea	-	-	12.0	(7.0)	0.5	(0.5)	-	12.2	(12.2)	2.6	(0.7)	0.3	(0.3)	60	-	51
Anarthruridae	-	-	0.3	(0.3)	0.3	(0.3)	-	-	-	-	-	-	-	-	-	-
Leptocheliidae	-	-	8.5	(7.2)	-	-	-	-	-	-	-	-	-	-	-	47
Pseudotanaidae	-	-	1.3	(0.3)	0.3	(0.3)	-	12.2	(12.2)	2.2	(0.7)	-	-	60	-	4
Typhlotanaidae	-	-	2.0	(0.7)	-	-	-	-	-	0.4	(0.4)	0.3	(0.3)	-	-	-
Mysidae	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Nebaliidae	-	-	-	-	-	-	-	-	-	0.6	(0.6)	-	-	10	1	-
Euphausiacea	-	-	-	-	-	-	-	-	-	-	-	-	-	5	1	1
Mollusca	3	(0.8)	9.5	(1.2)	8.8	(0.9)	3	6.4	(1.7)	1.0	(0.3)	3.3	(1.2)	6	43	806

Gastropoda	2	(0.6)	2.8	(1.3)	0.3	(0.3)	3	0.8	(0.5)	0.2	(0.2)	- -	4	9	784
Gastropoda Indet	1	(0.3)	-	-	0.3	(0.3)	3	0.2	(0.2)	-	-	- -	-	4	9
Buccindae	0	(0.3)	0.3	(0.3)	-	-	-	-	-	-	-	- -	-	3	-
Columbellidae	-	-	-	-	-	-	-	-	-	-	-	- -	-	-	3
Naticidae	-	-	-	-	-	-	-	-	-	0.2	(0.2)	- -	-	1	9
Opistobranchia	0	(0.3)	1.5	(1.2)	-	-	-	-	-	-	-	- -	1	-	-
Pyramidellidae	-	-	0.3	(0.3)	-	-	-	-	-	-	-	- -	-	-	-
Rissoellidae	-	-	-	-	-	-	-	0.6	(0.4)	-	-	- -	-	-	-
Rissoidae	-	-	0.8	(0.8)	-	-	-	-	-	-	-	- -	1	1	761
Skeneidae	-	-	-	-	-	-	-	-	-	-	-	- -	2	-	2
Skeneopsidae	1	(0.3)	-	-	-	-	-	-	-	-	-	- -	-	-	-
Scaphopoda	-	-	1.0	(0.7)	0.3	(0.3)	-	-	-	-	-	0.7 (0.3)	-	1	3
Bivalvia	2	(0.6)	5.0	(1.3)	7.8	(0.9)	-	4.0	(1.2)	0.8	(0.2)	2.3 (0.7)	-	28	18
Bivalvia Indet	-	-	-	-	-	-	-	-	-	-	-	0.3 (0.3)	-	-	-
Astartidae	-	-	0.3	(0.3)	-	-	-	-	-	-	-	- -	-	-	-
Limospidae			-	-	-	-	-	-	-	-	-	- -	-	1	-
Lucinidae	0	(0.3)	0.3	(0.3)	-	-	-	-	-	-	-	- -	-		-
Montocutidae	-	-	0.3	(0.3)	0.8	(0.5)	-	-	-	-	-	- -	-	-	-
Mytilidae	-	-	-	-	-	-	-	0.4	(0.2)	-	-	- -	-	-	8
Nuculidae	0	(0.3)	0.3	(0.3)	0.3	(0.3)	-	2.2	(1.2)	-	-	- -	-	7	1
Propeamussidae	-	-	-	-	-	-	-	-	-	-	-	- -	-	-	1
Solemyidae	0	(0.3)	-	-	-	-	-	-	-	-	-	- -	-	-	-
Thyasiridae	0	(0.3)	3.5	(0.9)	4.3	(1.0)	-	1.2	(0.5)	0.8	(0.2)	2.0 (0.6)	-	15	-
Veneridae	0	(0.3)	-	-	-	-	-	-	-	-	-	- -	-	-	-
Yoldiidae	0	(0.3)	0.5	(0.3)	2.5	(0.5)	-	0.2	(0.2)	-	-	- -	-	5	-
Aplacophora	-	-	0.8	(0.5)	0.5	(0.3)	-	1.6	(1.1)	-	-	0.3 (0.3)	2	5	1
Chaetodermatidae	-	-	-	-	0.5	(0.3)	-	0.2	(0.2)	-	-	- -	2	-	-

Limifossoridae	-	-	-	-	-	-	-	-	-	-	-	-	-	2	-
Prochaetodermatidae	-	-	-	-	-	-	-	1.4	(0.9)	-	-	0.3	(0.3)	-	3	-
Solenogastres	-	-	0.8	(0.5)	-	-	-	-	-	-	-	-	-	-	-	1
Other Taxa	1	(0.5)	6.0	(1.2)	6.0	(0.9)	-	4.6	(2.6)	2.4	(0.9)	1.0	(0.0)	5	105	24
Halacaridae	-	-	-	-	-	-	-	0.4	(0.2)	-	-	-	-	-	-	8
Cnidaria	0	(0.3)	0.3	(0.3)	0.3	(0.3)	-	-	-	2.2	(0.9)	0.3	(0.3)	1	7	1
Anthozoa	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Hydrozoa	0	(0.3)	0.3	(0.3)	0.3	(0.3)	-	-	-	2.2	(0.9)	0.3	(0.3)	1	7	-
Echinodermata	-	-	1.0	(0.4)	0.5	(0.5)	-	-	-	-	-	-	-	-	2	2
Holothuroidea	-	-	0.3	(0.3)	0.3	(0.3)	-	-	-	-	-	-	-	-	-	1
Ophiuroidea	-	-	0.8	(0.5)	0.3	(0.3)	-	-	-	-	-	-	-	-	2	1
Nemertea	-	-	1.0	(0.4)	0.8	(0.5)	-	1.0	(0.5)	0.2	(0.2)	0.7	(0.3)	1	2	6
Sipuncula	-	-	3.8	(1.3)	4.5	(1.3)	-	3.2	(2.5)	-	-	-	-	3	94	7
Turbellaria	0	(0.3)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Porifera	-	-	-	-	-	-	-	P		-	-	-	-	-	-	P
SampleTotal (individuals)	265	(90.1)	87.5	(14.1)	49.8	(5.0)	137.0	151.8	(42.9)	33.0	(4.9)	25.7	(1.2)	282	343	1441
Total (m^2)	83649	(28466)	27646	(4464)	15719	(1582)	2192	47962	(13547)	10427	(1558)	8110	(380)	4512	-	-

Table 3. Diversity (H'log$_e$), evenness (J'), and multivariate dispersion (MVDISP) of macrofaunal communities collected from cores at Baltimore and Norfolk seep and background 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

Table 4. 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 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**	20.1	14.2	14.5	30.0	11.0	16.2	27.0	12.6	16.9
	Mussels	0.001	**55.7**	50.6	20.4	25.5	17.1	33.5	43.7	40.6	35.5
	Background	0.001	0.074	**58.7**	17.9	25.0	17.6	33.6	37.9	32.1	34.7
	Ekman Mussel	-	-	-	-	21.3	17.9	20.4	29.4	32.0	22.7
NCS	Mats	0.050	0.022	0.017	-	**31.3**	15.5	20.2	28.5	27.1	29.7
	Mussels	0.001	0.001	0.001	-	0.002	**52.3**	20.0	45.2	20.6	20.6
	Background	0.006	0.013	0.011	-	0.032	0.004	**50.6**	32.3	21.2	32.3
	Ekman Mussel	-	-	-	-	-	-	-	-	46.9	41.4
	Suction Mussel	-	-	-	-	-	-	-	-	-	48.65
	Suction Mat	-	-	-	-	-	-	-	-	-	-

Table 5. 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)

Table 6. Results from the distance-based linear modeling (DISTLM) of environmental variables with Norfolk microbial mat, mussel, and background soft-sediment communities using the AICc criteria.

Variable	SS(trace)	Pseudo-F	P	Prop.
$\delta^{13}C$	8052.2	3.580	0.005	0.309
Percent Carbon	6384.6	2.598	0.014	0.245
$\delta^{15}N$	6491.7	2.656	0.009	0.249
C:N	4190.2	1.534	0.173	0.161
Mud Content	6696.8	2.769	0.011	0.257
Depth	6346.6	2.577	0.017	0.244

AICc	R^2	RSS	Selections
80.238	0.56883	11210	$\delta^{13}C$, Mud Content
80.666	0.30915	17994	$\delta^{13}C$
81.11	0.52952	12254	$\delta^{13}C$, Depth
81.392	0.25711	19327	Mud Content
81.491	0.51128	19554	$\delta^{15}N$, Mud Content
81.498	0.24924	12764	$\delta^{15}N$
81.553	0.24513	19661	Percent Carbon
81.572	0.24367	19699	Depth
81.726	0.49963	13017	Percent Carbon, Mud Content
81.811	0.49538	13143	Percent Carbon, Depth
Total SS(trace)		26046	

965 **Table 7.** Summary of macrofaunal seep sediment and regional infaunal studies including closest geographic seeps, comparable depths,

966 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, 2010
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

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