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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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

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 distinguishing the macrofaunal communities, including larger grain sizes present within NCS microbial mat habitats and depleted stable carbon isotopes ($\delta^{13}\text{C}$) 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.

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

similar (Levin et al., 2010) or differing densities in comparison to each another (Levin et al., 2015; Menot et al., 2010; Robinson et al., 2004; 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 also 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. Differences in the diversity of mussel-bed sediment macrofauna have also been mixed, and possibly related to site-specific factors. Higher diversities were found in mussel-associated sediments compared to microbial mats and non-seep sediments at Blake Ridge (Robinson et al., 2004); in contrast, diversity was lower in sediments adjacent to mussels than non-seeps in the Gulf of Guinea (Menot et al., 2010). High β diversity has also been reported for seep habitats, supported by the habitat

heterogeneity of foundation species and the varying geochemical environments (Cunha et al., 2013; Cordes et al., 2010b).

Infaunal community assemblages associated with different seep habitats are distinct from one another (Bernardino et al., 2012; Levin, 2005; Menot et al., 2010) 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 tolerance to sulfide concentrations and opportunistic lifestyle (Levin et al., 2013; 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 (Bernardino et al., 2012; 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 in sediments, facilitating communities more similar to non-seep sediments (Robinson et al., 2004). The specific mussel species present in the sediments has been found to influence the associated faunal community assemblage (Cordes et al., 2010a). Mussels in the *Bathymodiolus childressi*-complex contain only methanotrophic symbionts, while those of the *Bathymodiolus boomerang*-complex, including *Bathymodiolus heckerae* present at Blake Ridge, contain both methanotrophs and thiotrophs (Olu-LeRoy et al., 2007b). The effect of mussel species composition on epifaunal communities has been suggested to act as a proxy for habitat chemistry (Cordes et al., 2010a),

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),

including seepage rates, sulfide concentrations, and biological activity (Cordes et al., 2010a; Olu et al., 2009; Levin, 2005; Sahling et al., 2002). Microbial mats often form in habitats with high methane flux rates, with corresponding high sulfide concentrations and low oxygen penetration into the sediment (Sahling et al., 2002). In contrast, habitats that support clam beds exhibit lower but variable methane flow through sediments, lower sulfide concentrations, and higher oxygen penetration through bioturbation (Levin et al., 2003). Comparable data from mussel beds is limited; however, while these habitats can have similar oxygen penetration profiles, their associated sediment organic carbon concentrations and methane concentrations in the overlying water (Menot et al., 2010; Olu-Le Roy et al., 2007a) are often higher than clam beds. Due to variations in seep activity and fluid flux, the sediment geochemical properties (e.g., organic carbon and nitrogen, stable carbon and nitrogen isotopes, grain size) often differ between seep and non-seep habitats (Levin et al., 2000; Levin et al., 2010; Menot et al., 2010; Valentine et al., 2005). Microbial mats have been documented to contain higher percent carbon content, high carbon to nitrogen (C:N) ratios, and 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 ($\delta^{13}\text{C}$) 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 $\delta^{13}\text{C}$ values ranging from -25‰ to -15‰ (Fry and Sherr, 1984), very low $\delta^{13}\text{C}$ values derived from biogenic methane (≤ -50 ‰; Van Dover, 2007;

Whiticar, 1999), and carbon derived from sulfide oxidation with $\delta^{13}\text{C}$ 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ as compared to clam beds and the sediments may contain different microorganism communities (Cambon-Bonavita et al., 2009; Olu-Le Roy et al., 2007a; Olu et al., 2009). Thus, light $\delta^{13}\text{C}$ values can be a useful indicator of seep habitats where biogenic methane is present. While methane flux and sulfide concentrations are important mechanistic factors structuring seep faunal communities (Bernardino et al., 2012), stable isotopes and other sediment parameters also can serve as proxies 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 in deep water (2100-2600m) off of South Carolina, US. However, recent large-scale projects using high resolution multibeam sonar and backscatter data now document 570 seep areas between Cape Hatteras and Georges Bank (Skarke et al., 2014), and suggest that tens to thousands more may be present along the passive Atlantic margin. During this study, two recently identified chemosynthetic seep areas were examined near Baltimore Canyon (BCS) and Norfolk Canyon

(NCS) separated by 90 km. This study addresses the role of geographic setting, seep habitat type, and sediment geochemistry in determining infaunal densities, community composition, and diversity of sediment macrofauna ($>300\mu\text{m}$). We hypothesized that (i) communities found at seep and non-seep habitats will differ within sites (BCS and NCS) and between sites; (ii) similar seep habitats at BCS and NCS will exhibit similar community composition, and (iii) community patterns in seep and non-seep habitats will be related to sediment geochemical properties. To support our hypotheses, we expect higher macrofaunal density but lower diversity at shallower BCS than at deeper NCS, similar taxonomic composition between seep habitat types at BCS and NCS, and distinct sediment geochemical parameters associated with community assemblages in each habitat type.

2. Methods:

2.1 Study Area

Two large cold-seep communities were explored on the U.S. Mid-Atlantic margin in 2012 and 2013 (Figure 1). The first seep, BCS, was located on the slope south of Baltimore Canyon at 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 bathymodiolid deep-sea mussels (Figure 2a), along with discrete white

microbial mats (Figure 2b) and large areas of shell debris. The NCS seep contained extensive mussel communities (Figure 2c), with areas of filamentous white (Figure 2d) and yellow microbial mats and shell debris. While the species has yet to be confirmed, initial examination of the specimens indicate that the mussel species at BCS and NCS are within the *B. childressi*-complex (Olu-Le Roy et al., 2007b) and will be referred to as *B. childressi*-complex throughout.

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 core tubes (6.35-cm diameter). 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 (0.063 m²) 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- μ m 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, 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 $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ 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, $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$, as per mil (‰). 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 abundance data using Bray-Curtis similarities in PRIMER version 7 (Clarke and Gorley, 2015) with the PERMANOVA+ add on (Anderson et al., 2008). Samples collected via Ekman cores and suction were analyzed as presence/absence transformed abundance data within the multivariate analyses, with multiple suction samples from mussel habitats pooled. 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 ($\delta^{13}\text{C}$ and $\delta^{15}\text{N}$), 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 86 taxa, including 35 polychaete families, 22 crustacean families, 20 mollusca families, and 9 other taxa (see Supplemental Table 1 for full list). A further 2,518 individuals were collected from suction and Ekman grab samples, including 21 additional taxa. Macrofaunal density was

significantly higher at BCS than at NCS for all habitat types (Figure 3; Two-way ANOVA, $F_{1,19}=11.34$, $p=0.003$), with the highest densities occurring in microbial mats ($137,756 \text{ ind. m}^{-2}$). 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 ($83,649 \pm 28,466 \text{ ind. m}^{-2}$) than in background soft-sediments ($15,719 \pm 1,582 \text{ ind. m}^{-2}$; 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 ($47,962 \pm 13,547 \text{ individuals m}^{-2}$) 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 2) 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, $p < 0.0001$). In

contrast at NCS, there was no significant difference in diversity (One-way ANOVA, $F_{2,10}=0.96$, $p=0.42$) or evenness (One-way ANOVA, $F_{2,10}=4.05$, $p=0.051$) among habitat types (Table 2). Rarefaction analysis within BCS (Figure 4a) and NCS (Figure 4b) indicated similar within-site patterns as given using Shannon diversity; however, overall diversity of all habitats combined (Figure 4c) indicated higher diversity at NCS than at BCS.

There was high β diversity among habitats. At BCS, 15% of the observed taxa were shared across all sediment habitats, 23-47% of the taxa were shared between any two habitats, and 42% of the taxa were unique to a single habitat. Approximately 42% 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 (20%) or background sediments (40%); however, the low number of taxa present in the single mussel bed sample resulted in low overall diversity compared to mussel sediments (Figure 4a). At NCS, there was overall greater β diversity than at BCS, with only 13% of taxa shared among all three sediment habitats and 21-35% occurring in two or more habitats. A high percentage of taxa, 59%, occurred only in a single habitat at NCS, and 59% 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 (56%). The non-quantitative suction samples also shared the most taxa with their analogous sediment communities; the mat suction sample shared 51% of its taxa with mat sediments, and the mussel suction samples shared 30% 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 than microbial mat sediments (Figure 4b). Pooled

rarefaction (Figure 3c) for seep habitat push cores combined with Ekman cores and suction samples resulted in an increase in diversity with each additional habitat at both sites. The high difference in taxa between the mussel bed samples (Ekman), compared to cores collected adjacent to the mussel bed suggests high taxonomic turnover on a small (<1m) spatial scale with minimal taxon overlap.

3.3 Community composition

Overall taxonomic composition was similar among habitat types between BCS and NCS based on push core collections (Figure 5). 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 in microbial mats at BCS (31%) than at NCS (4%), 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 sediment cores collected from adjacent mussel or mat habitats (Figure 4). The BCS Ekman core contained a high proportion of isopods (65%), while the NCS Ekman core contained a lower proportion of amphipods (7%) relative to sediment communities

adjacent to mussel beds. The NCS mussel suction contained the highest proportion of gastropods (46%) while the NCS mat suction contained high proportions of other polychaetes (33%) and other taxa (31%), specifically Sipuncula (28%), in comparison to mussel and mat sediment communities. In addition, the Ekman and suction samples were better able to collect more highly mobile taxa, as indicated by the presence of Nebaliidae and Caridean shrimp.

Macrofaunal communities differed both between sites (Figure 6; Two-way PERMANOVA, Pseudo-F=5.78, $p=0.0001$) and among habitat types (Two-way PERMANOVA, Pseudo-F=7.30, $p=0.0001$) but with a significant interaction of site and habitat (Two-way PERMANOVA, Pseudo-F=5.04, $p=0.0001$). Estimates of the source of variation in communities indicate that differences among habitat types (Estimate=1056) were greater than differences between sites (Estimate=539). Within each site, community variability among cores was highest within microbial mat sediments (Table 2, MVDISP). Pairwise analysis of site and habitat combinations showed significant differences in macrofaunal communities between all site/habitat combinations (Table 3) except between BCS mussel and background habitats. Microbial mat communities at BCS and NCS were more similar to each other than they were to other habitats at their respective sites (Table 3). At BCS, bacterial mats had higher densities of Capitellidae (Polychaeta), Dorvilleidae (Polychaeta), and Tubificidae (Oligochaeta) than the background and mussel habitats, contributing 33% of the dissimilarity with mussel habitats and 42% with background habitats. Mussel habitats had higher densities of Tubificidae (Oligochaeta), Leptocheliidae (Tanaidacea), and Typhlotanaiidae (Tanaidacea) but lower densities of Opheliidae (Polychaeta) and Yoldiidae (Bivalvia) compared to background soft

sediments, contributing 22% of the overall dissimilarity. SIMPER analysis using presence/absence data (Table 3) indicated the Ekman core collected within the mussel bed at BCS were more similar to the sediment communities associated with mussels, than to background sediments, and mat habitats at BCS. However, the taxonomic composition of the BCS Ekman core was more similar to NCS Ekman and suction samples than to sediment communities at BCS (Table 3).

At NCS, bacterial mats differed from both mussel and background habitats by high densities of Capitellidae (Polychaeta), Dorvilleidae (Polychaeta), and Spionidae (Polychaeta) contributing 26% of the dissimilarity with mussel habitats and 27% with background habitats. Mussel habitats differed from background soft-sediment habitats, with higher densities of Oedicerotidae (Amphipoda) and Spionidae (Polychaeta), but low densities of Cossuridae (Polychaeta) and Paraonidae (Polychaeta) contributing 31% of the dissimilarity. At NCS, the highest community similarities were observed between the NCS Ekman core and mussel sediment communities (44%, Table 3) and among the Ekman and suction samples (43-54%).

3.4 Relationship to sediment geochemistry

Sediment geochemical properties differed among microbial mat, mussel, and background soft-sediment habitats at NCS (Table 4). Sediment $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values were significantly lower in mussel habitats than both microbial mats and background soft-sediments (Tukey HSD, $\delta^{13}\text{C}$, $p<0.001$; $\delta^{15}\text{N}$, $p<0.033$). Microbial mat sediments also contained lower $\delta^{13}\text{C}$ and $\delta^{15}\text{N}$ values compared to background soft-sediments (Tukey HSD, $\delta^{13}\text{C}$, $p<0.001$; $\delta^{15}\text{N}$, $p=0.001$). 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 7) 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 $\delta^{13}\text{C}$, $\delta^{15}\text{N}$, 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 5) indicated that all environmental variables except C:N can individually explain a significant portion (24-31%, $p < 0.019$) of the variation in NCS communities. A combination of $\delta^{13}\text{C}$ and mud content provide the best explanation of variation in NCS macrofaunal communities, accounting for 58% of the variation among samples in two axes.

4. Discussion:

4.1 Depth-related patterns

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 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 6; Robinson et al., 2004). A trend of decreasing density with depth was also present for seep habitats; however, given the additional nutrition source provided by the seep this trend 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 observations from deep mud volcanoes (Cunha et al., 2013) and general deep-sea ecological paradigms (Rex and Etter, 2010). The higher variability within seep communities at NCS could be due to the larger physical distance 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 was 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 was a lower overall taxonomic pool present at BCS compared to NCS, although under sampling was 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 abundance and limited sampling in background habitats at both sites, our results likely provide an underrepresentation of background soft-sediment diversity.

Community assemblage differences between BCS and NCS may also be depth driven, consistent with the separation of macrofaunal communities between upper bathyal (200-1500m) and lower bathyal/abyssal (>1500m) depths worldwide documented by Bernardino et al. (2012). Similar depth differences have been observed for seep megafaunal communities (Olu et al., 2010), supporting the interconnection between infaunal and epifaunal communities. Differences among seep and non-seep sediment communities have been observed to increase with depth (Levin, 2005), suggesting the greater importance of the additional nutrition source provided by the seep at increasing depths (Levin and Michener, 2002). Within a geographic region, comparisons among seeps at different depths have been limited (Cordes et al., 2010a; Cunha et al., 2013), but consistent with the overall results of Bernardino et al. (2012). In the Gulf of Mexico, depth was the main driver of communities within seep habitats, with observed community differences between shallow and deep seeps, but no observed difference across the large geographic range (~650 km) of the study (Cordes et al., 2010a). Similar depth separation of communities was observed on a smaller geographic scale (~200 km) for mud volcanoes in the Gulf of Cadiz (Cunha et al., 2013). Other studies with multiple depths over large geographic ranges (>425 km) have also found significant community differences, including seeps along the Pacific margin (525 and 770m; Levin et al., 2010) and the Aleutian margin (3300m and 4400m; 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.

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

microbial mats in the Gulf of Mexico (Table 6; Robinson et al., 2004). High densities have been recorded in frenulate fields on the Norwegian margin (Decker et al., 2012), microbial mats on the northern California margin (Levin et al., 2006), and an ampharetid bed in New Zealand (Thurber et al., 2013), all of which were at deeper depths (Table 6). Macrofaunal density in microbial mats was also high at NCS compared to microbial mat habitats at similar depths in other locations (Table 6; 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 6; Maciolek et al., 1987; Robertson et al., 2015; Sanders et al., 1965). Cape Hatteras represents a known zoogeographic barrier (e.g., Cutler, 1975; Hilbig, 1994) due to the convergence of the northward flowing Gulf Stream and the southward flowing Western Boundary Undercurrent, and slope densities in this region were both higher (Cape Hatteras, Table 6; Blake and Hilbig, 1994) and lower (Cape Lookout, Table 6; Blake and Grassle, 1994) than similar background depths at NCS.

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). Low densities in microbial mats at Blake Ridge may also be the result of high sediment sulfide and methane concentrations that exceed the tolerance limit for most macrofaunal taxa (e.g., Bernardino et al., 2012). In addition, the mussel species at Blake Ridge, *B. heckerae*, known to support both methanotrophs and sulfide oxidizers, differed from the dominant mussels present at BCS and NCS, *B. childressi*-complex, which are known to support only methanotrophic bacteria (Olu-Le Roy et al., 2007b). While *B. heckerae* has only been reported from deeper depths (2150-3300m; Cordes et al., 2010a; Levin and Mendoza, 2007; Robinson et al., 2003), potentially preventing establishment at either BCS or NCS, the depth range of *B. childressi* (500-2284m; Cordes et al., 2010a) encompasses that of Blake Ridge. However, the two species were never collected together in the Gulf of Mexico despite overlapping depth ranges (Cordes et al., 2010a). As suggested by Cordes et al., (2010a), the specific mussel species present in these seep habitats may serve as a proxy for the surrounding habitat chemistry, and by extension, the sediment geochemistry. The different species complexes present at Blake Ridge and our sites suggests an

underlying difference in concentrations of methane and sulfide which may help explain the differing infaunal community patterns observed between sites.

4.3 Patterns in macrofaunal diversity

The low (α) diversity observed in microbial mat habitats, particularly at BCS, is consistent with previous studies which observed lower diversity within microbial mat habitats compared to nearby clam beds (Bernardino et al., 2012; Levin and Mendoza, 2007; Levin et al., 2003). Microbial mat sediments at both BCS and NCS were dominated by the annelid families Capitellidae, Dorvilleidae, and Tubificidae, all of which have been previously observed in seep habitats (Levin, 2005; Levin et al., 2010; Levin et al., 2003). Dorvilleids are a common component of seep communities (Levin, 2005), often occurring in high densities in microbial mat sediments (Robinson et al., 2004; Sahling et al., 2002) where they are likely consuming mat-forming sulfur bacteria, methanotrophs (Levin and Michener, 2002), and/or archaea (Thurber et al., 2012). Multiple species of dorvilleids are also known co-occur in seep habitats, with species exhibiting high trophic and niche partitioning (Levin et al., 2013). Capitellids are known to be a stress tolerant, opportunistic taxa, with a strong preference for sulfidic environments (Levin et al., 2000; Levin et al., 2003) and are known to consume methanotrophs at other seeps (Carlier et al., 2010; Decker and Olu, 2010, 2012). Only the polychaete families Dorvilleidae, Cirratulidae, and Hesionidae were documented in microbial mat sediments at Blake Ridge (Robinson et al., 2004), all of which were present in microbial mat sediments at NCS, while Hesionidae were absent in mat sediments at BCS. In contrast to microbial mats, sediments adjacent to mussels at

BCS and NCS contained high proportions of crustaceans, particularly amphipods and tanaids. Amphipods are known to be sensitive to organic enrichment, increased hydrocarbon concentrations (Peterson et al., 1996), and oxygen stress (Thurber et al. 2013), and their distribution at BCS may indicate high methane flux, high sulfide concentrations and low oxygen concentrations at our microbial mat habitats. For the seeps at Blake Ridge, crustaceans were only documented in mussel sediments (Robinson et al., 2004), suggesting similarities among mussel communities across depth regimes. In addition, increased variability in communities has been used as an indicator of stressed and/or disturbed environments (Fisher et al., 2014; Warwick and Clarke, 1993). Although fluid flux and sulfide concentrations were not measured, the high variability (MVDISP) and low diversity in microbial mat sediments, compared to the greater similarity within mussel and background sediment communities, also suggest a higher stress environment in microbial mat sediments.

High β diversity at seep sites was present over both small (<1 m) and large spatial scales. High β diversity among seep habitats (mussels and mats) has been documented at seep sites worldwide (see Cordes et al., 2010b for review) and is suggested to be a result of small-scale variation in the vertical distribution and concentration of sulfides in sediments (Levin et al., 2003) and habitat heterogeneity provided by seep megafauna (Cordes et al., 2010b). Hints at these small-scale variations were observed both in sediment cores collected in mat and background habitats at BCS and between the Ekman cores collected within the mussel habitat and cores collected directly adjacent to mussel habitats at both sites. Similar to results observed in Pacific seeps (Levin et al., 2010), the seep habitats contribute significantly to the regional

biodiversity for their specific depth, providing 37-49% of infaunal taxa and high taxon turnover between seeps and background soft-sediment communities. In addition, while the taxonomic level applied in this study (family-level) was sufficient to ascertain differences among habitat-specific communities, further identification (e.g., genus and/or species level) will likely provide increased separation of habitat-specific communities, biodiversity estimates, identification of biogeographic boundaries, and insight into seep endemism at these sites. High β diversity among the mussel habitat, adjacent sediments, and background sediments highlights that habitat provision of dense mussel communities influences not only the *in situ* macrofaunal communities found within the beds, but also the communities that occur in the sediments beyond the perimeter of the mussel bed itself. This ‘reef’ effect has also been observed for deep-sea coral communities (Demopoulos et al., 2014). While the effect of seep habitats on sediment macrofaunal communities has not been detected at distances greater than 250 m from seep megafauna (Menot et al., 2010), discrete transects from mussel beds to adjacent sediments and beyond would help quantify the sphere of influence of seep activity and biogenic structures on adjacent habitats.

4.4 Relationship to sediment geochemistry

The higher proportion of taxa found in the upper 2 cm of sediments in microbial mats versus deeper sediments, particularly at NCS, may reflect different geochemical settings present within each habitat. Seeps, along with other reducing environments such as areas of organic enrichment, large organic falls, and oxygen minimum zones, are often characterized by low

oxygen, sulfidic sediments (Levin et al., 2010; Tunnicliffe et al., 2003). The vertical distribution of taxa in sediments is regulated partly by oxygen and sulfide concentrations (Levin, 2005), resulting in a trade-off between sulfide tolerance and food availability (Menot et al., 2010). Few taxa tolerate sulfide concentrations >1 mM, while Dorvilleidae polychaetes can occur in high 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 restrict 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 within NCS microbial mat communities suggests a gradient among sampling locations in the underlying seep fluid flow, sediment geochemistry, and depth. 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,

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 $\delta^{13}\text{C}$ values
601 in microbial mats were higher than in mussel bed habitats. Isotopic composition of mussels
602 collected within these seeps yielded isotopically light $\delta^{13}\text{C}$ (-64‰ to -61‰; Prouty et al., 2014)
603 and $\delta^{15}\text{N}$ 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*, *Thiopluca*) differ from amorphous forms (e.g. *Arcobacter*)

and iron-oxidizers, and sediment $\delta^{13}\text{C}$ values reported here may reflect the microbial communities supporting the food chain, as well as organic matter contribution from mussels (Levin and Mendoza, 2007). The presence of mussels from the *B. childressi*-complex, which contain only methanotrophic symbionts, further suggests methanotrophs are playing a large role in the transfer of energy at our seeps (e.g., Levin et al., 2015; Levin and Michener, 2002; Thurber et al., 2012; Thurber et al., 2013). The portion of variation in microbial mat communities at NCS characterized by mud content, and by extension, the amount of authigenic carbonate rubble within cores, may reflect the role and duration of anaerobic oxidation of methane within the sediment, which can influence the macrofaunal community structure.

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

There are potential limitations to the comparisons made between seep and background habitats at both BCS and NCS in our study, including seasonality and inter-annual variation, location, and sampling methods. At BCS, all of the background sediments were collected in August 2012, while all but one core from seep habitats were collected in May 2013. Seasonality in surface productivity and hydrodynamic regimes, as well as disturbance events, promotes shifts in community assemblages. However, there was no observed difference in the abundance of taxa 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 inter-annual variation in macrofaunal communities (Boesch, 1979). Proximity of background, soft-sediment cores to seep habitats may also 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. However, the close proximity of (<1m) of the 2012 microbial mat core with 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. While macrofaunal communities are known to differ between canyon axis and slope habitats for Norfolk Canyon (Robertson et al., 2015), the samples examined in this study represent the best comparative information available.

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 variation in the intricate sub-seafloor structures 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 could benefit the development of generalizations relating to seep environmental controls on infaunal structure and function.

Acknowledgements:

The authors would like to thank the Bureau of Ocean Energy Management (BOEM), NOAA and USGS DISCOVRE Mid-Atlantic Canyons project teams, the crews of the NOAA Ships *Nancy Foster* and *Ron Brown*, ROV *Jason II* Group (WHOI), ROV *Kraken* group (UConn), S.W. Ross, J. Chaytor, N. Prouty, U. ten Brink, and C. Morrison. Special thanks go out to Jennifer McClain-Counts, Mike Rhode, Mark Lavaleye, and the USGS/WARC Benthic Ecology Group for assistance at sea and laboratory support. We thank Lisa Levin, Howard Jelks, and two

anonymous reviewers for their insightful and helpful comments that improved this manuscript. Funding was provided to A. Demopoulos from the USGS Environments Program through the Outer Continental shelf study DISCOVRE Mid-Atlantic Canyons, with ship time support provided by NOAA. Any use of trade, product, or firm names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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Figure Captions:

Figure 1. Maps showing locations of sampling sites and closest known seeps (a) with detailed sampling at b) BCS and c) NCS. ■ = Microbial mat habitat push cores; ▲ = Mussel habitat push cores; ● = Background, soft-sediment habitats; ▲ = Mussel Ekman cores; △ = Mussel suction samples; □ = Microbial mat suction sample.

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.

Figure 3. Mean macrofaunal density (ind. m⁻²) ($\pm$ 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 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 suction; All = pooled all samples.

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.

Figure 6. 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. Stress value of 0.15 indicates a good to potentially useful confidence in the 2-dimensional representation (Clarke and Gorley, 2015).

Figure 7. 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 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	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
		2013	37.03347	-74.45039	1622	1			
	Background	2013	37.03343	-74.45032	1619	1			
		2013	37.03343	-74.45030	1620	1	1		

Table 2. 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

979 **Table 3.** Similarity among habitats (above diagonal), within-habitat similarity (diagonal, bold), and PERMANOVA probabilities
980 (below diagonal) based on Bray-Curtis similarities of square-root transformed abundance data for the push cores. Comparisons with
981 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	-	-	-	-	-	-	-	-	-	-

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

Habitat	N	$\delta^{13}\text{C}$		%C		$\delta^{15}\text{N}$		%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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Table 5. 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 and “best” model selection.

Variable	SS(trace)	Pseudo-F	P	Prop.
$\delta^{13}\text{C}$	8077.3	3.613	0.005	0.311
Percent Carbon	6423.8	2.630	0.014	0.247
$\delta^{15}\text{N}$	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	$\delta^{13}\text{C}$, Mud Content
80.606	0.3111	17886	$\delta^{13}\text{C}$
81.083	0.52932	12221	$\delta^{13}\text{C}$, Depth
81.288	0.26249	19149	Mud Content
81.298	0.51908	12487	$\delta^{15}\text{N}$, Mud Content
81.455	0.2501	19470	$\delta^{15}\text{N}$
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	

1010 **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

1012	Cape Lookout	NW Atlantic	Background	1380-1690	5017	-	-	Blake and Grassle, 1994
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Figure 1

Figure 2

Figure 3

Figure 4

Figure 5

Figure 6

Stress = 0.15

■ BCS Mat	▲ BCS Mussel	● BCS Background
□ NCS Mat	△ NCS Mussel	○ NCS Background

Figure 7