Community composition of mussel associates at deep-sea methane seeps in the Gulf of Mexico and the US Atlantic Margin

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Abstract

Efforts to understand and preserve methane seep communities of the Gulf of Mexico and the US western Atlantic margin begin with an understanding of biodiversity and community composition. In this study, 10143 individuals representing 63 different taxa were sampled from mussel-beds surrounding methane seeps at various depths within two different ocean basins. Diversity in mussel beds was highest at Baltimore Canyon, the shallowest site sampled in the Atlantic. Although only four species were sampled at more than one site, species composition was most alike among sites found at similar depths. The two deepest sites sampled, Florida Escarpment and Blake Ridge showed a 9.6% similarity. Baltimore Canyon and Chincoteague are both located in the Atlantic Ocean at different depths but had a 5.3% similarity. The high diversity of individuals sampled among these sites emphasizes the importance of preserving seep communities, which provide essential nursery habitats that further support more trophic levels within the deep-sea ecosystem.

1. Introduction

Cold seeps are defined by the upward convection of methane and other hydrocarbons from the subsurface seabed to the seafloor and are typically found on continental margins worldwide (Ruff et al., 2015). The exploration of cold-seep communities first began in 1984 at depths ranging from 500 to 1000 m (Kennicutt et al., 1985). With the growing expansion of deep-sea exploration by commercial interests such as oil drilling, sea-floor mining, and deep-water fisheries, it is imperative to understand the community compositions surrounding these seeps to determine how they might be affected by anthropogenic factors. Much of the deep sea is food limited, and methane seeps offer essential niches that provide habitat structure and primary production, supporting multi-trophic communities (Turner et al., 2020). Oxygen availability is often limited to the immediate surrounding area, resulting in a thin suboxic layer below the emitted hydrocarbon. It is here that partial pressure causes methane to diffuse out of bubbles into the water column where microbial methane-oxidizing archaea (ANME) and sulfate-reducing bacteria (SRB) symbionts can create a habitable environment. The conversion of inorganic energy sources to fuel communities, known as chemosynthesis, is responsible for the primary production of communities of microorganisms and marine invertebrates in the deep sea (Ruff et al., 2015). Chemosynthetic bacteria and archaea occur worldwide at cold seeps but are locally selected by the environment (Ruff et al., 2015).

Cold seeps are often characterized by one or more foundation species, often symbiontbearing megafauna such as tubeworms or mussels (Turner et al., 2020). Bathymodiolin mussels act as habitat engineers by modifying the physical and chemical environment, forming biogenic habitats that support a variety of additional species (Govenar 2010). Methane seeps are initially dominated by resident species, which use the foundation species for attachment, shelter, and access to food through grazing or currents (Cordes et al., 2010; Levin et al., 2016). The compositions of invertebrates living on the foundation species help characterize methane seeps. The ecological patterns surrounding seeps are typically influenced by bathymetric changes in regards to interspecific interactions such as predation and competition (Cordes et al., 2010). Methane seeps with mussel beds as the foundation species are typically dominated by resident grazing gastropods, smaller decapod crustaceans, and worms of various phyla (MacAvoy et al., 2002). Many seep communities have been found to support both resident and vagrant species. The resident species are integrated into the phytoplankton detritus-based food web of the surrounding ecosystem. In contrast, vagrant species are characterized by a high degree of movement into and out of seep communities. This movement is essential for the export of seep production into the vast ecosystem of the deep sea (MacAvoy et al., 2002).

Variations in species compositions at seep communities may be attributed to abiotic variables such as depth, food availability, latitude, and substrate type (Rex et al., 2000). Biogenic habitats are also sensitive to changes in fluid flux and chemical composition, which determine the distribution of symbiont-bearing megafauna and community composition (MacDonald et al., 1989). As depth and distance from shore increase, fewer nutrients are transported by currents to seep communities (Turner et al., 2020). In the Atlantic Ocean, variation in seep community patterns has been attributed to the faunal boundary between the upper-bathyal (200-1500 m) and lower bathyal/abyssal (>1500 m) seeps (Bernardino et al., 2012). A similar transition zone is thought to be at 1000 m in the Gulf of Mexico (Cordes et al., 2010). This study aims to determine the differences in species compositions between methane seeps in the Gulf of Mexico and the US western Atlantic margin, and among methane seeps at different depths.

2. Methods

2.1. Sample Collection

Mussel-bed communities were sampled from a total of six methane seeps, three sites along the western Atlantic margin (WAM), and three sites in the Gulf of Mexico (GOM) (Fig. 1). Scoops of mussels and their associates were collected from the seafloor in February and March of 2019 using HOV Alvin deployed from RV *Atlantis*. Collections were also made in May and June of 2021 using ROV Jason deployed from RV *Thomas G. Thompson*. Both vehicles recovered the samples inside closed bioboxes, and the associated invertebrates were then sorted from the foundation species (*Gigantidas childressi* and *Bathymodiolus heckerae*). The species were then identified and sorted based on morphology on board the ship. Samples were preserved in 10% buffered formalin and later transferred to 70% ethanol for storage. Preserved samples were more carefully sorted by differences in morphology and identified to the lowest possible taxonomic level before being photographed. Some samples were too degraded to photograph but were still included in the statistical analysis.

2.2. Data Analysis

Samples collected in 2019 were organized into presence or absence at each site as there was no overlap in species. Diversity indices and the Bray-Curtis similarity index were not run on these data, which resulted in one site, Bush Hill, being excluded from the statistical analysis. However, this site was still used in this study by comparing the taxa sampled. Samples from 2021 were organized into counts of species present as there was some overlap of species found at each site. Diversity among these sites was estimated using a combination of diversity indices, including Shannon-Weiner diversity (H'), Pielou's index of evenness(J'), Margalef's richness (D), and individual-based rarefaction curves calculated using Primer v6 (Clarke and Gorley 2006). A Bray-Curtis similarity index assessed similarity among sites following a fourth-root transformation of species densities. The data were transformed to balance species with high individual counts. The Bray-Curtis similarity matrix was then visualized using a non-metric multi-dimensional scaling plot.

3. Results

A total of 10143 individuals representing 63 different taxa across six phyla were sampled from the mussel beds surrounding the studied methane seeps (Fig. 2). The six phyla present were Cnidaria, Nemertea, Annelida, Arthropoda, Mollusca, and Echinodermata. The majority of the samples represented morphologically distinct species; however, the taxonomy remains largely unresolved. Of the six different phyla represented in this study, not all groups were present at each site (Table 1). The annelid subclass Oligochaeta was only sampled from the Atlantic sites: Blake Ridge (2167 m), Baltimore Canyon (388 m), and Chincoteague (1028 m). The molluscan class Polyplacophora was only found at the Brine Pool (651 m) in the Gulf of Mexico. The shallowest site sampled, Baltimore Canyon, was the only site that contained representatives from all six phyla present in this study. Blake Ridge, the deepest site sampled in the Atlantic, contained five out of the six phyla, only missing Nemertea. Chincoteague, an Atlantic site, contained species from the phyla Annelida, Mollusca, Arthropoda, and Echinodermata. Florida Escarpment (3287 m) and Brine Pool, sampled in the Gulf of Mexico, had Cnidaria, Annelida, Mollusca, and Echinodermata representatives. Bush Hill (562 m), the shallowest site represented from the Gulf of Mexico, only contained species from two phyla: Annelida and Arthropoda.

Despite the large number of individuals represented in this study, very few species were found at multiple sampling sites. A small brittle star, *Ophioctenella acies*, was found at both Blake Ridge and Florida Escarpment. These sites are located in different ocean basins but were the deepest sites sampled (Table 2). The Brine Pool and Bush Hill sites are located close by one another and at similar depths within the Gulf of Mexico, but only one species was found at both sites, the galatheid crab, *Munidopsis sp.1* (Table 3). Bush Hill and Chincoteague are also located in different ocean basins, and Chincoteague is almost twice as deep, but the shrimp, *Alvinocaris stactophila*, was present at both sites (Table 3). The unidentified morphotype of "juvenile ophiuroid" was the only species present at more than two sites: Florida Escarpment, Blake Ridge, Baltimore Canyon, and Chincoteague (Table 2). These four sites represent both oceanic basins sampled in this study as well as the shallowest and deepest depths sampled.

Although Baltimore Canyon had the fewest number of individuals present, it had the highest species richness, evenness, and diversity (Table 4). Chincoteague had the second greatest species richness, evenness, and diversity. Blake Ridge was the deepest site sampled in the Atlantic Ocean, and was less diverse, even, and species-rich than the shallower sites. However, the

opposite is true for the Gulf of Mexico sites. Florida Escarpment had a greater species richness, evenness, and diversity than Brine Pool (Table 4). Florida Escarpment also had substantially more individuals present than any other sites sampled. The individual-based rarefaction curves indicate that the species sampled represent the population well because the curves all plateau (Fig. 3). As indicated by the multidimensional scaling plot of communities, Florida Escarpment and Blake Ridge sites have the most similar species compositions (Fig. 4). The Bray-Curtis Resemblance matrix showed a 9.6% similarity between the two sites. They are both the deepest sites sampled in their respective oceanic basins. Baltimore Canyon and Chincoteague showed a 5.3% similarity within the Atlantic Ocean but at different depths (Fig. 4).

4. Discussion

In this study, Florida Escarpment (3287 m) and Blake Ridge (2167 m) are characterized by the same foundation species, Bathymodiolus heckarae, and were the two deepest sites sampled. However, they are located in different ocean basins. This suggests that the foundation species may be just as indicative of seep species compositions as depth. Baltimore Canyon (388 m) and Chincoteague (1028 m) are found close together along the western Atlantic margin and are characterized by the same foundation species, Gigantidas childressi, but have a large difference in depth. Throughout the Atlantic Equatorial belt, species richness is often highest at seeps of intermediate-depth between 1000-2000 m where deep and shallow species overlap, supporting the previously described bathymetric boundaries by Turner et al, (2020). The findings of this study show the site with the most species richness to be Baltimore Canyon (388 m). Runoff from Chesapeake Bay may be supporting increased productivity and, therefore, species richness at this site. This study suggests that depth plays an important role in the composition of seep communities while influencing nutrient availability, but that foundation species may be an additional dominant factor. Brine Pool (651 m) is most similar to Bush Hill (562 m) based on close proximity and the overlap of one decapod crustacean found at both sites. No formal statistical analysis was run on the data from Bush Hill, so the results are inconclusive.

When considering bathymetric boundaries responsible for community settlement, larval dispersal must be included as a potentially important factor. Variation in population connectivity can result from differences in timing and location of spawning, hydrodynamic processes, larval behavior, and post-settlement processes such as emigration and mortality (VanDover et al., 2002). The potential of larval dispersal is dependent on biological factors such as vertical

migration, buoyancy of embryos, predation, food availability, developmental rate, physical tolerances, and planktonic larval duration, (Cordes et al., 2007; Young et al., 2012) and is assumed to influence the habitat range of the adults (Thorson, 1950). Behaviors that determine larval depth may especially be important in the deep sea due to increased variability of current speeds and direction at different depths (McVeigh et al., 2017). However, short-lived larvae are less affected by depth, so this phenomenon may only relate to species with longer planktonic larvae duration (Young et al., 2012). Larval dispersal is highly dependent on currents present in the vicinity of spawning. Gulf of Mexico metapopulations are likely to be sources for larval dispersal, while western Atlantic margin populations are likely to be sinks, indicating a unidirectional exchange (Young et al., 2012). Planktonic larvae are subject to barriers such as seamounts, oceanic ridge axes, and other topography that may present an impediment to dispersal between basins (McClain and Hardy, 2007). The shallow straits of Florida create a biogeographical barrier to larval dispersal (McVeigh et al., 2017). This barrier, among other factors, may be responsible for the minimal overlap of invertebrates among the various sites sampled.

Foundation species patterns also account for variation among sites. The biogenic habitat of the two most similar sites in this study, Blake Ridge and Florida Escarpment, are composed of *B. heckerae. Bathymodiolus heckerae* may rely on Gulf Stream meanders to be deposited into shallower depths. In the absence of Gulf Stream meanders, larvae remain in deeper waters and are transported south along the western boundary current, explaining why the deeper site mussel beds are comprised of *B. heckerae* (Cordes et al., 2007). The biogenic habitat of the other sites included in this study are composed of *Gigantidas childressi*. Differences between these two species may be responsible for the variability among site species compositions, though foundation species composition is confounded with depth. Dissimilarity of the chemical environments among sites impacts foundation species' growth rates and reproductive output. The environmental factors within the two ocean basins sampled favor *G. childressi*, which outcompetes *B. heckerae* for space and resources (Turner et al., 2020). *Gigantidas childressi* contains only methanotrophic symbionts, possibly creating different chemical habitats than mussel beds composed of *B. heckerae*. *Gigantidas childressi* are also longer-lived and live closer to the surface where faster currents may facilitate dispersal (Arellano et al., 2014).

Communities found at deep-sea methane seeps are essential for the productivity of deep-sea ecosystems, climate change, fishery yields, and understanding how anthropogenic actions affect these ecosystems. Methane seeps provide habitat and food for various migratory deep-sea organisms by creating primary production and trophic levels that help structure the deep-sea food web (Turner et al., 2020). These ecosystems also provide breeding and nursery sites that help maintain species populations and reproductive success (Beck et al., 2001). Methane seeps are also paramount to the ecological succession of the deep sea. Mussel taxonomy suggests that the decomposition of large bone and wood deposits may serve as successional steps for the introduction of mussel taxa to seeps and, therefore, the support of chemoautotrophy-dependent invertebrates (Distel et al., 2000). Not only is the diversity of seep communities unique and important to the deep sea, but seeps may also be beneficial to the overall health of our planet's ecosystem. Macro- and microorganisms at methane seeps and sulfate methane transition zones consume 75% of the methane that reaches the seafloor from subsurface zones (Ruff et al., 2015). Since seep communities provide trophic support and reproductive success, they may be important factors for the success of fisheries (Levin et al., 2016). Furthermore, expanding exploration within the deep sea enhances the urgency to understand these interactions, as disturbances increase from gas extraction, seabed mining, and bottom trawling (Levin et al., 2016).

The results of this study highlight the uniqueness of seep communities in the deep sea. The differences in seep community compositions from the Gulf of Mexico and the western Atlantic margin include depth, foundation species, larval dispersal, and biogeographical barriers. Seep communities are relatively understudied due to the difficulties of accessing the deep sea, indicating undersampling as a limitation to the overall understanding of these ecosystems. Therefore, it is recommended that further studies be conducted to better understand the succession of seep communities, how they may influence fisheries, and finally, how they interact with other deep-sea organisms to better preserve and limit anthropogenic affects.

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Species	Brine Pool	Bush Hill	Fl. Escarpment	Blake Ridge	Baltimore Canyon	Chincoteague
Cnidaria			-		•	
Anemone sp.1	0	0	0	1	0	0
Anemone sp.2	0	0	1	0	0	0
Sponge cf. Sycon	0	0	0	0	1	0
Sponge sp.1	0	0	0	0	1	0
Sponge sp.2	0	0	0	0	1	0
Zoanthinaria	1	0	0	0	0	0
Nemertea						
Nemertean sp.1	0	0	0	0	1	0
Annelida						
Sipunculid						
Sipunculid sp.1	0	0	0	1	0	0
Sipunculid sp.2	0	0	0	0	1	0
Sipunculid sp.3	0	1	0	0	0	0
Polychaeta						
Cossura sp.1	0	0	0	1	0	0
Laubierus mucronatus	0	0	1	0	0	0
M. dendrobranchiata	1	0	0	0	0	0
Nereis sp.1	0	0	0	0	0	1
Nicomache sp.1	0	0	0	0	1	0
Nicomache sp.2	0	0	0	1	0	0
Nicomache sp.3	0	0	0	0	0	1
Polychaetae sp.1	0	0	0	0	1	0
Polychaeta sp.2	0	0	0	0	0	1
Polychaeta sp.3	0	0	0	1	0	0
Polychaeta sp.4	0	0	0	0	0	1
Oligochaete						
Oligochaete sp.1	0	0	0	0	1	0
Oligochaete sp.2	0	0	0	0	0	1
Tubeworm sp.1	0	0	0	1	0	0
Mollusca						
Bivalvia						
Bathymodiolus sp.1	1	0	0	0	0	0
Ladella sublevis	0	0	1	0	0	0

Table 1. Presence (1)/absence (0) of all species found at sites from both 2019 and 2021 data.

Gastropoda						
Fucaria sp.1	0	0	1	0	0	0
Snail sp.1B	0	0	0	0	1	0
Snail sp.2A	0	0	0	1	0	0
Snail sp.2B	0	0	0	1	0	0
Snail sp.3A	0	0	0	0	1	0
Solariella sp.1	0	0	0	0	0	1
Provanna sp.1	0	0	0	0	0	1
Snail sp.5B	0	0	0	0	0	1
Snail sp.6B	0	0	0	0	0	1
Prosipho sp.1	0	0	1	0	0	0
Provanna sp.2	0	0	1	0	0	0
Snail sp.9B	0	0	0	0	0	1
Snail sp.10B	0	0	1	0	0	0
Mohnia sp. l	1	0	0	0	0	0
Whelk sp.1B	0	0	0	0	1	0
Eosipho c.f. canetae	1	0	0	0	0	0
Whelk sp.2B	0	0	0	0	0	1
Whelk sp.3A	0	0	1	0	0	0
Whelk sp.3B	0	0	0	0	0	1
Eulepetopsis c.f. vitrea	0	0	1	0	0	0
Polyplacophora						
Chiton sp.1	1	0	0	0	0	0
Leptochiton sp.1	1	0	0	0	0	0
Arthropoda						
Alvinocaris stactophila	0	1	0	0	0	1
Alvinocaris sp.1	0	0	0	1	0	0
Alvinocaris muricola	0	0	0	1	0	0
Alvinocaris williamsi	1	0	0	0	0	0
Amphipod sp.1	0	0	0	1	0	0
Isopoda sp.1	0	0	0	0	1	0
Munidopsis sp.1	1	1	0	0	0	0
Munidopsis sp.2	0	0	0	1	0	0
Shrimp sp.1	1	0	0	0	0	0
Echinodermata						
Asteroidea						
Juvenile steroid sp.1	0	0	0	0	0	1
Schlerasterias tanneri	0	0	1	0	0	0
Ophiuroidea						

Ophioctenella acies	0	0	1	1	0	0
Juvenile ophiuroid sp.1	0	0	1	0	1	1
Holothuroidea						
Chiridota sp.	0	0	1	1	0	0
Sea cucumber sp.1	0	0	1	0	0	0

Table 2. Total species list and counts from 2021.
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Cridaria Nomerea Nomerea Sponge sp.1 0 0 0 1 0 Sponge sp.2 0 0 0 5 0 Nemertea Nemertea 0 0 1 0 Sipunculid Sipunculid sp.2 0 0 0 1 0 Sipunculid sp.2 0 0 0 1 0 0 Sipunculid sp.2 0 0 1 0 0 0 Aubierus mucronatus 0 1 0 0 0 0 0 Bivalvia 10 0	Species	Brine Pool	Fl. Escarpment	Blake Ridge	Baltimore Canyon	Chincoteague
Šponge sp.1 0 0 0 1 0 Sponge sp.2 0 0 0 5 0 Nemertean sp.1 0 0 0 1 0 Amelid Sipunculid sp.2 0 0 0 1 0 Sipunculid sp.2 0 0 1 0 0 0 Itaubierus mucronatus 0 1 0 0 0 0 Medarobranchiata 10 0 0 1 0 0 Tubeworm sp.1 0 0 1 0 0 0 Mediasublevis 0 25 0 0 0 0 Snail sp.1B 0 0 0 300 30 30 30 Provanna sp.1 0 0 0 0 0 33 0 0 346 Snail sp.5B 0 0 0 0 0 2 0 346						
Spong's p.2 0 0 0 5 0 Nemertean sp.1 0 0 0 0 1 0 Sipunculid Sipunculid sp.2 0 0 0 1 0 Sipunculid sp.2 0 0 0 1 0 0 0 Memetrean sp.1 0 0 0 0 1 0 0 0 Sipunculid sp.2 0 0 1 0 <t< td=""><td>Sponge cf. Sycon</td><td>0</td><td>0</td><td>0</td><td>1</td><td>0</td></t<>	Sponge cf. Sycon	0	0	0	1	0
Nemerican sp.1 0 0 0 1 0 Annelid 3ipunculid sp.2 0 0 1 0 Polychaeta 1 0 0 0 0 0 Laubierus mucronatus 0 1 0 <td>Sponge sp.1</td> <td>0</td> <td>0</td> <td>0</td> <td>1</td> <td>0</td>	Sponge sp.1	0	0	0	1	0
Nemertean sp.1 0 0 0 1 0 Annelid Sipunculid Sipunculid sp.2 0 0 0 1 0 Sipunculid sp.2 0 0 0 0 1 0 0 Caubierus mucronatus 0 1 0	Sponge sp.2	0	0	0	5	0
Annelid Sipunculid sp.2 0 0 0 1 0 Polychaeta <td>Nemertea</td> <td></td> <td></td> <td></td> <td></td> <td></td>	Nemertea					
Sipunculid splanculid splanculid <td>Nemertean sp.1</td> <td>0</td> <td>0</td> <td>0</td> <td>1</td> <td>0</td>	Nemertean sp.1	0	0	0	1	0
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Laubierus mucronatus 0 1 0 0 0 M. dendrobranchiata 10 0 0 0 0 0 0 Tubeworm sp.1 0 0 1 0 0 0 0 Mollusca 0 0 0 0 0 Snail sp.1B 0 25 0<	Sipunculid sp.2	0	0	0	1	0
M. dendrobranchiata 10 0 0 0 0 Tubeworm sp.1 0 0 1 0 0 Mollusca 0 0 0 0 0 Bivalvia Ladella sublevis 0 25 0 0 0 0 0 Snail sp.1B 0 0 0 0 2 0	Polychaeta					
Tubeworm sp.1 0 0 1 0 0 Mollusca Bivalvia -	Laubierus mucronatus	0	1	0	0	0
Mollusca Bivalvia Jadella sublevis 0 25 0 <t< td=""><td>M. dendrobranchiata</td><td>10</td><td>0</td><td>0</td><td>0</td><td>0</td></t<>	M. dendrobranchiata	10	0	0	0	0
Bivalvia Ladella sublevis 0 25 0 0 0 Gastropoda -	Tubeworm sp.1	0	0	1	0	0
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Provana sp.1 0 0 0 0 835 Snail sp.5B 0 0 0 0 346 Snail sp.6B 0 0 0 0 835 Prosipho sp.1 0 20 0 0 835 Prosipho sp.1 0 20 0 0 0 Provanna sp.2 0 2000 0 0 0 Snail sp.9B 0 0 0 0 2 Snail sp.9B 0 0 0 0 2 Snail sp.10B 0 3 0 0 0 Whelk sp.1B 0 0 0 15 0 Whelk sp.3B 0 0 0 4 0 0 0 0 Polyplacophora Imployed Imployed 0 0 0 0 Amphipod sp.1 0 0 80 0 0 0	Snail sp.2B	0	0	0	9	0
Snail sp.5B 0 0 0 346 Snail sp.6B 0 0 0 835 Prosipho sp.1 0 20 0 0 0 Provanna sp.2 0 2000 0 0 0 0 Snail sp.9B 0 <td>Solariella sp.1</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>300</td>	Solariella sp.1	0	0	0	0	300
Snail sp.6B 0 0 0 0 835 Prosipho sp.1 0 20 0 </td <td>Provanna sp.1</td> <td>0</td> <td>0</td> <td>0</td> <td>0</td> <td>835</td>	Provanna sp.1	0	0	0	0	835
Prosibo sp.1 0 20 0 0 0 Provana sp.2 0 2000 0<	Snail sp.5B	0	0	0	0	346
Provana sp.2 0 2000 0 0 0 Snail sp.9B 0 0 0 0 2 Snail sp.10B 0 3 0 0 0 Whelk sp.1B 0 0 0 0 0 Whelk sp.2B 0 0 0 0 4 Whelk sp.3B 0 0 0 4 Eulepetopsis c.f. vitrea 0 2603 0 0 Polyplacophora	Snail sp.6B	0	0	0	0	835
Snail sp.9B 0 0 0 2 Snail sp.10B 0 3 0 0 0 Whelk sp.1B 0 0 0 15 0 Whelk sp.2B 0 0 0 0 4 Whelk sp.3B 0 0 0 4 6 0 0 4 Eulepetopsis c.f. vitrea 0 2603 0 0 0 0 4 Polyplacophora -	Prosipho sp.1	0	20	0	0	0
Snail sp.10B 0 3 0 0 0 Whelk sp.1B 0 0 0 15 0 Whelk sp.2B 0 0 0 0 4 Whelk sp.3B 0 0 0 4 Eulepetopsis c.f. vitrea 0 2603 0 0 0 Polyplacophora 4 0 0 0 0 Arthropoda 0 0 80 0 0	Provanna sp.2	0	2000	0	0	0
Whelk sp.1B 0 0 0 15 0 Whelk sp.2B 0 0 0 0 4 Whelk sp.3B 0 0 0 0 4 Eulepetopsis c.f. vitrea 0 2603 0 0 0 Polyplacophora	Snail sp.9B	0	0	0	0	2
Whelk sp.2B 0 0 0 4 Whelk sp.3B 0 0 0 0 4 Eulepetopsis c.f. vitrea 0 2603 0 0 0 0 Polyplacophora - - - - 0 0 0 0 Arthropoda - - - - - - 0 0 0 0	Snail sp.10B	0	3	0	0	0
Whelk sp.3B0004Eulepetopsis c.f. vitrea02603000Polyplacophora I 40000Leptochiton sp.1400000Arthropoda I 008000	Whelk sp.1B	0	0	0	15	0
Eulepetopsis c.f. vitrea02603000Polyplacophora	Whelk sp.2B	0	0	0	0	4
Polyplacophora Leptochiton sp. 140000Arthropoda Amphipod sp.1008000	Whelk sp.3B	0	0	0	0	4
Leptochiton sp.140000Arthropoda008000	Eulepetopsis c.f. vitrea	0	2603	0	0	0
Arthropoda Amphipod sp.1008000	Polyplacophora					
Arthropoda Amphipod sp.1008000	Leptochiton sp.1	4	0	0	0	0
	Arthropoda					
<i>Munidopsis sp.1</i> 150 0 0 0 0	Amphipod sp.1	0	0	80	0	0
	Munidopsis sp. l	150	0	0	0	0

Munidopsis sp.2	0	0	2	0	0
Shrimp sp.1	8	0	0	0	0
Echinodermata					
Asteroidea					
Juvenile steroid sp.1	0	0	0	0	1
Ophiuroidea					
Ophioctenella acies	0	1268	11	0	0
Juvenile ophiuroid sp.1	0	20	0	1	3
Holothuroidea					
Chiridota sp.1	0	29	0	0	0

Table 3. Presence/absence of	of species	from 2019.
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Species	Brine Pool	Bush Hill	Fl. Escarpment	Blake Ridge	Baltimore Canyon	Chincoteague
Cnidaria			•	2	•	~~~~~~
Anemone sp.1	0	0	0	1	0	0
Anemone sp.2	0	0	1	0	0	0
Zoanthinaria	1	0	0	0	0	0
Annelida						
Sipunculid						
Sipunculid sp.1	0	0	0	1	0	0
Sipunculid sp.2	0	0	0	0	1	0
Sipunculid sp.3	0	1	0	0	0	0
Polychaeta						
M. dendrobranchiata	1	0	0	0	0	0
Cossura sp.1	0	0	0	1	0	0
Macrochaeta clavicornis	0	0	0	0	1	0
Nereis sp.1	0	0	0	0	0	1
Nicomache sp.1	0	0	0	0	1	0
Nicomache sp.2	0	0	0	1	0	0
Nicomache sp.3	0	0	0	0	0	1
Polychaetae sp.1	0	0	0	0	1	0
Polychaeta sp.2	0	0	0	0	0	1
Polychaeta sp.3	0	0	0	1	0	0
Polychaeta sp.4	0	0	0	0	0	1
Oligochaete						
Oligochaete sp.1	0	0	0	0	1	0
Oligochaete sp.2	0	0	0	0	0	1
Mollusca						
Bivalvia						
Bathymodiolus sp.1	1	0	0	0	0	0
Gastropoda						
Fucaria sp.1	0	0	1	0	0	0
Snail sp.2A	0	0	0	1	0	0
Snail sp.3A	0	0	0	0	1	0
Mohnia sp.1	1	0	0	0	0	0
Eosipho c.f. canetae	1	0	0	0	0	0
Whelk sp.3A	0	0	1	0	0	0
Sea Slug sp.1	0	0	1	0	0	0
Polyplacophora						

Chiton sp.1	1	0	0	0	0	0
Leptochiton sp. 1	1	0	0	0	0	0
Arthropoda						
Alvinocaris stactophila	0	1	0	0	0	1
Alvinocaris sp.1	0	0	0	1	0	0
Alvinocaris muricola	0	0	0	1	0	0
Alvinocaris williamsi	1	0	0	0	0	0
Isopoda sp.1	0	0	0	0	1	0
Munidopsis sp.1	1	1	0	0	0	0
Echinodermata						
Asteroidea						
Schlerasterias tanneri	0	0	1	0	0	0
Holothuroidea						
Chiridota sp.1	0	0	0	1	0	0
Sea cucumber sp.1	0	0	1	0	0	0

Table 4. Collection and diversity information for each site sampled in this study for both 2019 and 2021 data sets. Species richness, abundance, and diversity indices do not include the mussel foundation species. The following abbreviations are used: S(species richness), N(number of individuals), d(Margalef's Richness), J'(Pilou's Evenness), and H'(Shannon Weiner Diversity).

Site	Longitude	Latitude	Depth(m)	Foundation species	S	Ν	d	J'	H'(loge)
Baltimore Canyon	-73.822	38.048	388	G. childressi	9	39	2.184	0.7878	1.731
Blake Ridge	-76.191	32.494	2167	B. heckerae	4	94	0.6603	0.3741	0.5186
Brine Pool	-91.279	27.723	651	G. childressi	4	172	5828	0.3714	0.5149
Bush Hill	-91.504	27.776	562	G. childressi	-	-	-	-	-
Chincoteague Florida	-74.102	37.541	1028	G. childressi	9	2330	1.032	0.6019	1.322
Escarpment	-84.911	26.028	3287	B. heckerae	9	5969	0.9201	0.5232	1.15

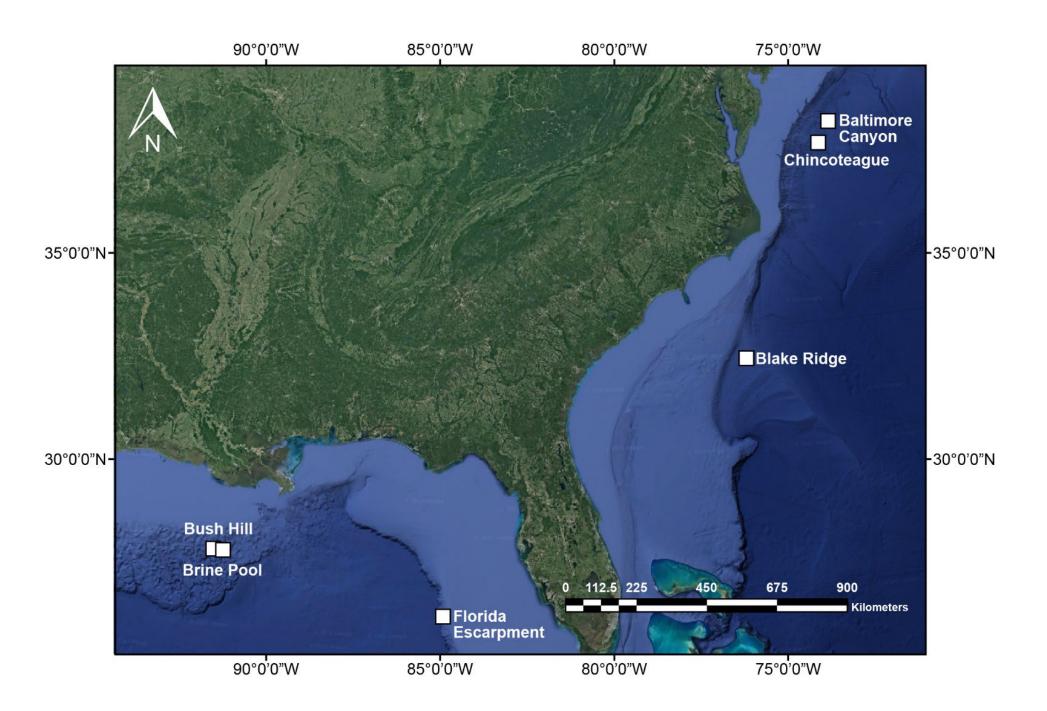


Fig. 1. Map of the sites used in this study from both the 2019 and 2021 cruises. A green box indicates the site with the corresponding dive number.

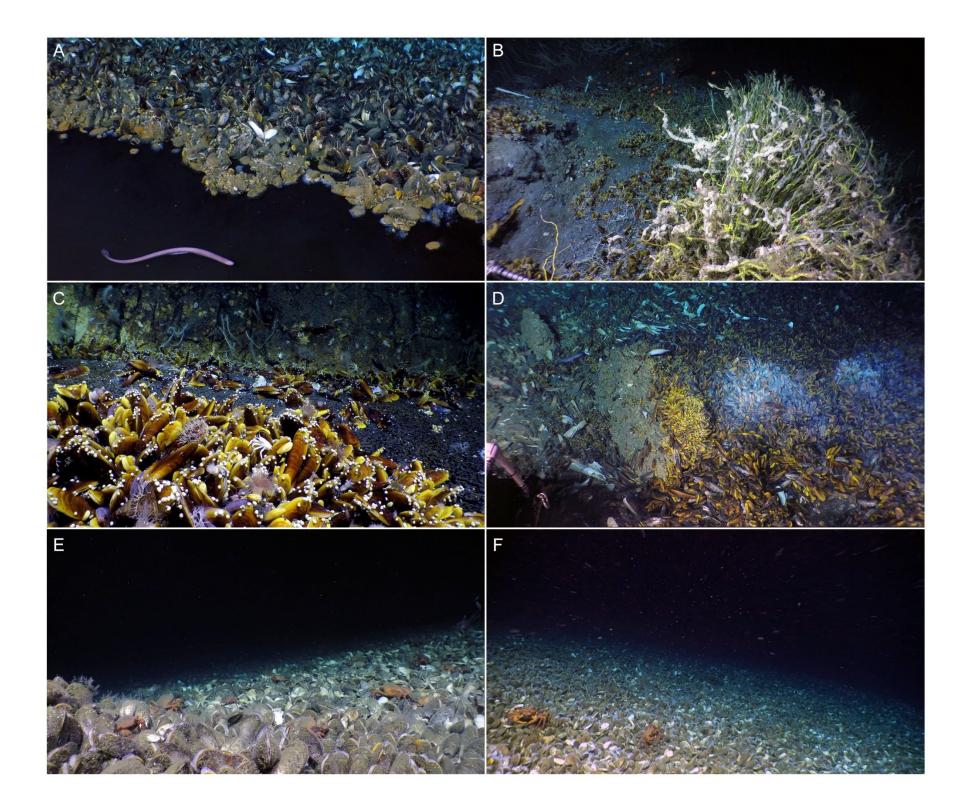


Fig. 2. Photographs of each individual site. (A: Brine Pool; B: Bush Hill; C: Florida Escarpment; D: Blake Ridge; E: Chincoteague; F: Baltimore Canyon).

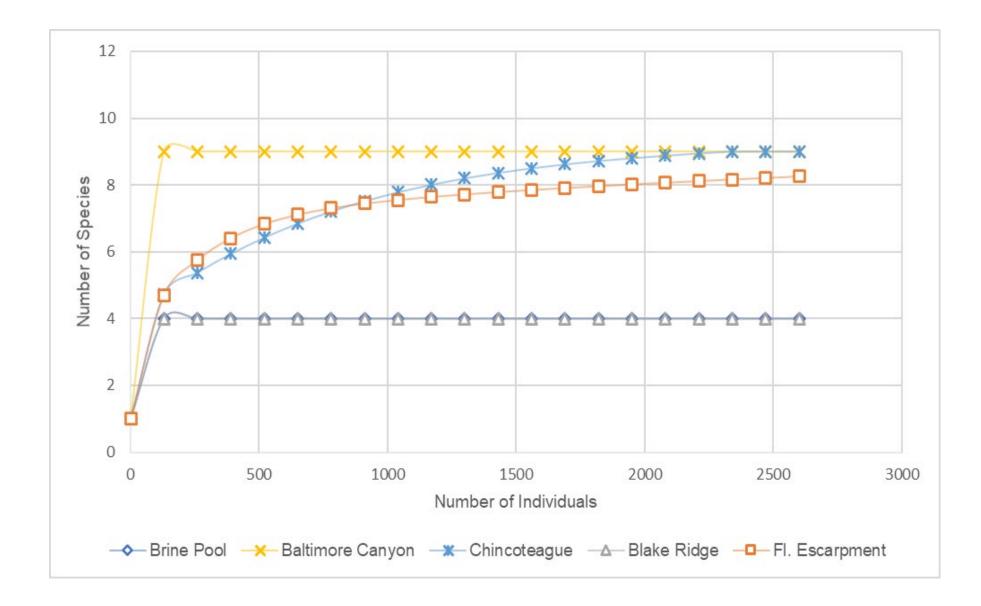


Fig. 3. Species accumulation curves for mussel-associated fauna using 2021 data. Note: Brine Pool line is directly underneath Blake Ridge and thus not visible.

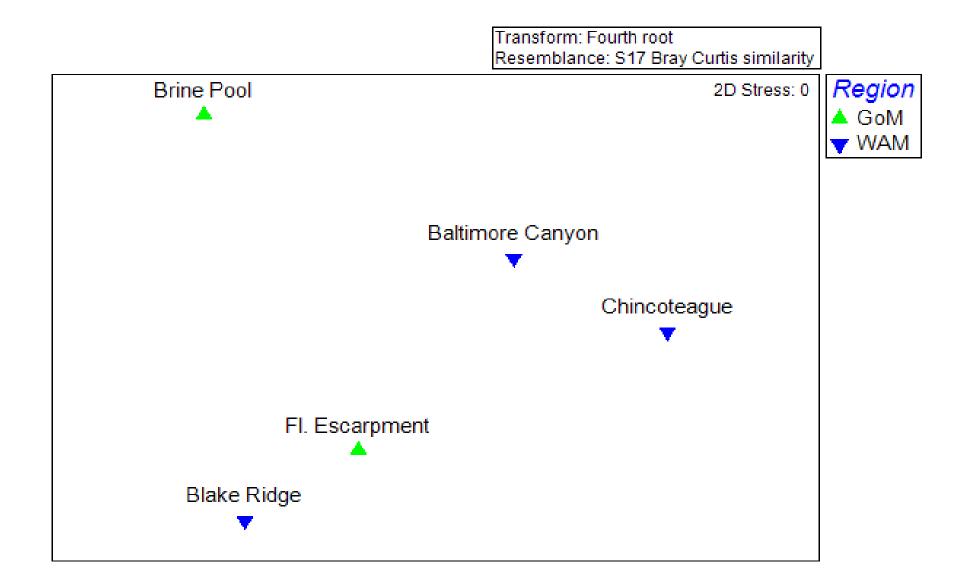


Fig. 4. Multidimensional scaling plot of community similarity among mussel associated communities. Similarity is estimated by the Bray-Curtis similarity index based on fourth root transformed species densities using 2021 data. (GoM=Gulf of Mexico; WAM=Western Atlantic margin)

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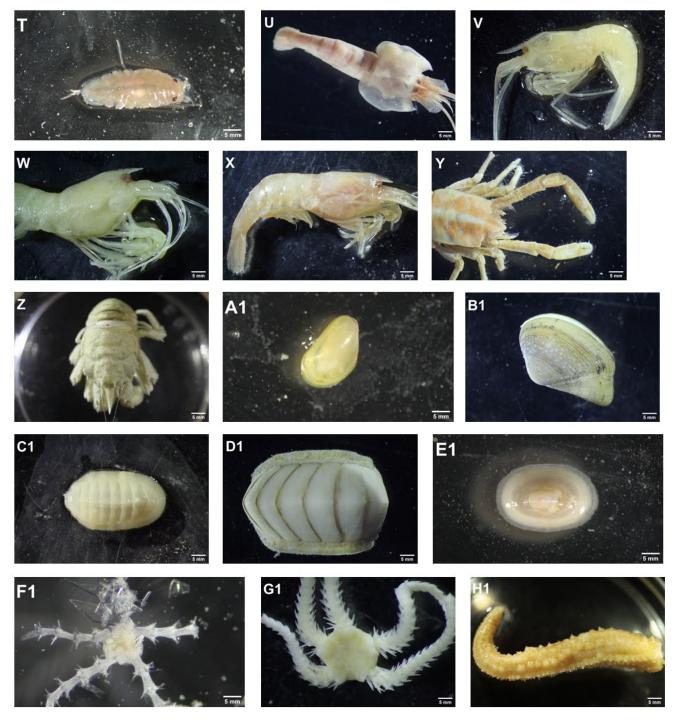
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Appendix: Photographs of associated fauna collected from methane seeps.

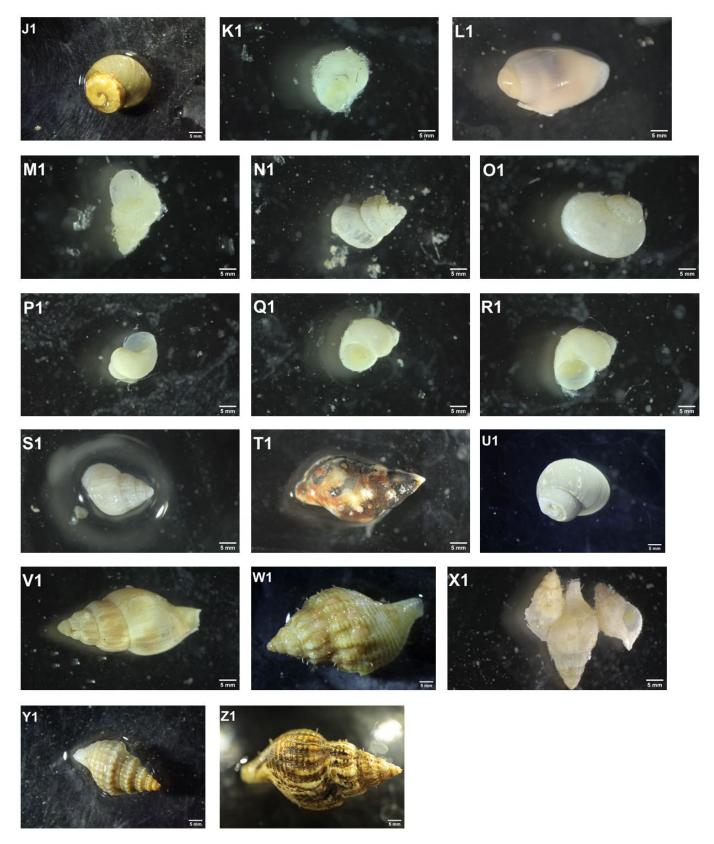


A. Zooanthinaria B. Sponge sp.1 C. Anemone sp.1 D. Oligochaeta sp.1 E. Polychaeta sp.2 F. *Macrochaeta clavicornis* G. Sipunculid sp.1 H. Sipunculid sp.2 I. *M. dendrobranchiata* J. *Cossura* sp.1 K. Polychaeta sp.2 L. *Nicomache* sp.3 M. Oligochaeta sp.2 N. *Nereis* sp.1 O. *Nicomache* sp.2 P. Polychaeta sp.4 Q. Sipunculid sp.3 R. Tubeworm sp.1 S. *Labierus mucronatus*





T. Isopoda sp.1 U. Shrimp sp.1 V. Alvinocaris muricola W. Alvinocaris stactophila X. Alvinocaris williamsi
Y. Munidopsis sp.1 Z. Munidopsis sp.2 A1. Bathymodiolus sp.1 B1. Ladella sublevis C1. Chiton sp.1
D1. Leptochiton sp.1 E1. Eulepetopsis vitrea F1. Juvenile ophiuroid G1. Ophioctanella acies H1. Schlerasteria stanneri H1. Chiridota sp.1



J1. *Fucaria* sp.1 K1. Snail sp.1B L1. Snail sp.2A M1. Snail sp.2B N1. Snail sp.3A O1. *Solariella* sp.1
P1. *Provanna* sp.1 Q1. Snail sp.5B R1. Snail sp.6B S1. Snail sp.9B T1. Snail sp.10B U1. *Provanna* sp.2
V1. Whelk sp.1B W1. *Eosipho canetae* X1. Whelk sp.2B Y1. Whelk sp.3A Z1. *Mohnia* sp.1