Blake Ridge methane seeps: characterization of a soft-sediment, chemosynthetically based ecosystem

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Abstract

Observations from the first submersible reconnaissance of the Blake Ridge Diapir provide the geological and ecological contexts for chemosynthetic communities established in close association with methane seeps. The seeps mark the loci of focused venting of methane from the gas hydrate reservoir, and, in one location (Hole 996D of the Ocean Drilling Program), methane emitted at the seafloor was observed forming gas hydrate on the underside of a carbonate overhang. Megafaunal elements of a chemosynthetically based community mapped onto dive tracks provide a preliminary overview of faunal distributions and habitat heterogeneity. Dense mussel beds were prominent and covered 20×20 m areas. The nearly non-overlapping distributions of mussels and clams indicate that there may be local (meter-scale) variations in fluid flux and chemistry within the seep site. Preliminary evidence suggests that the mussels are host to two symbiont types (sulfide-oxidizing thiotrophs and methanotrophs), while the clams derive their nutrition only from thiotrophic bacteria. Invertebrate biomass is dominated by mussels (*Bathymodiolus heckerae*) that reach lengths of up to 364 mm and, to a lesser extent, by small (22 mm length) vesicomyid clams (*Vesicomya* cf. *venusta*). Taking into account biomass distributions among taxa, symbiont characteristics of the bivalves, and stable-isotope analyses, the relative importance of methanotrophic vs thiotrophic bacteria in the overall nutrition of the invertebrate assemblage is on the order of 60% vs 40% (3:2).
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1. Introduction

A quarter of a century has passed since the first exploration of hydrothermal vents, yet the prospect remains for discovery of biogeographically and ecologically distinctive types of chemosynthetic systems in the world's oceans. Exploration and investigation of these systems will allow us to understand the diversity of habitats, species, and adaptations that can be supported by chemosynthesis. In this report, we provide preliminary characterization of a soft-sediment, chemosynthetically based ecosystem associated with a methane hydrate province on the continental margin of the eastern United States. Gas hydrates close to the sediment–seawater interface are also known from other regions, including the Gulf of Mexico (MacDonald et al., 1994), the Barbados accretionary complex (Olu et al., 1996), the Barents Sea (Egorov et al., 1999), and the Cascadia margin off Oregon (Suess et al., 1999; Sahling et al., 2002).

The focus site for this study lies near the intersection of the Carolina Rise and the Blake Ridge (Fig. 1). This area of the South Atlantic Bight has long been recognized as a major gas hydrate province within the US Exclusive Economic Zone (e.g., Markl et al., 1970; Tucholke et al., 1977; Paull and Dillon, 1981). Over most of the region, the top of the methane hydrate deposit probably lies at depths greater than 100 m below
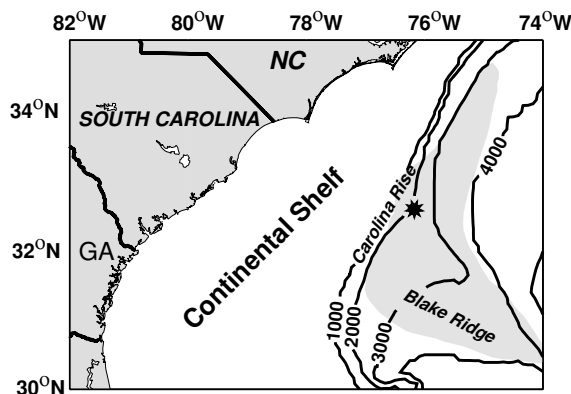

Fig. 1. Blake Ridge Diapir study site location. Star indicates Blake Ridge Diapir; shaded off-shore area delineates region of gas-hydrate deposits; contour lines are in 1000-m intervals.

seafloor (mbsf; Paull et al., 1996). At some locations, however, gas hydrate and underlying free gas occur close to the seafloor, and interaction of the hydrate reservoir with geologic, oceanographic, and other processes leads to the development of focused seeps.

The US Atlantic continental margin south of $34°N$ is among the most extensively mapped gas-hydrate provinces in the world's oceans. Several generations of seismic surveys (e.g., Tucholke et al., 1977; Rowe and Gettrust, 1993; Taylor et al., 1999; Holbrook, 2000) map a regionally extensive bottom-simulating reflector (BSR) in this area. The BSR is a negative-impedance contrast reflector that marks the phase boundary between overlying gas hydrate and underlying free gas. While gas hydrates are known to occur on the Blake Ridge at locations with no BSR (e.g., Paull et al., 1996), the presence of a BSR beneath a large part of the Blake Ridge implies widespread occurrence of gas hydrates.

A line of about 20 salt diapirs begins near the intersection of the Blake Ridge with the Carolina Rise and extends northward on the eastern side of the Carolina Trough (Dillon et al., 1982). The diapirs rise to within 600 m of the seafloor and disrupt the overlying sediments. Interaction between the Blake Ridge Diapir (the southern-most diapir) and the underlying methane-hydrate reservoir was extensively investigated by Taylor et al. (2000). The high thermal conductivity of the diapir alters the local stability field for methane hydrates, causing upward warping of the BSR and shifting of the gas hydrate and free-gas system to shallower levels in the sedimentary section. At the same time, partial dissolution of the salt diapir raises local pore-water salinities, further inhibiting gas-hydrate stability and possibly contributing to the increased mobility of fluids in the sedimentary section (Taylor et al., 2000). Emplacement of the diapir has been accompanied by the development of faults that act as conduits for the transfer of free gas and waters rich in dissolved gas toward the seafloor (Paull et al., 1995).

Seismic reflection profiles across the Blake Ridge Diapir (e.g., USGS CH-06-92 Line 37) show a prominent BSR that shoals over the diapir, and a fault that extends from the BSR to nearly
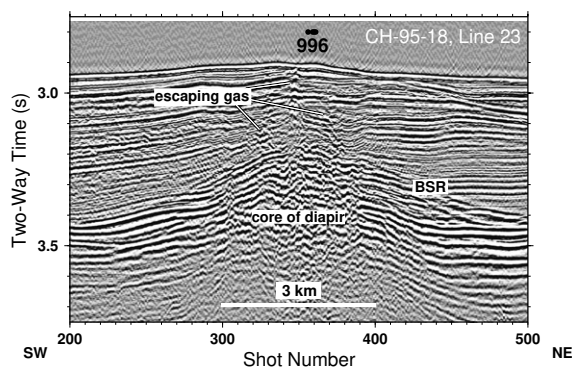
Fig. 2. Seismic profile across the Blake Ridge Diapir showing the prominent Bottom-Simulating Reflector (BSR). Single channel seismic data (line 23 of CH-95-18) collected by the USGS across the Blake Ridge Diapir during a cruise aboard the R/V *Cape Hatteras* in 1995 (Taylor et al., 1999). The raw data have been slightly reprocessed. The seismic line crosses the diapir from southwest to northeast. The BSR is warped upward and disrupted over the core of the diapir, particularly between shots 340 and 370. Vertically oriented features above the core of the diapir mark gas migration paths.

the seafloor (Fig. 2). Chemosynthetic communities and gas-rich plumes rising up to 320 m in the water column have been detected where the fault system intersects the seafloor (Paull et al., 1995, 1996). Sediments consisting of hemipelagic silt and clay with 20–40% pelagic carbonate (Paull et al., 1995; Dillon and Max, 2000b) drape the diapir. These sediments, which were deposited by strong, south-flowing near-bottom currents, were accreted rapidly (up to 48 cm ka^{-1}) during the late Pleistocene interval (Paull et al., 1996).

Leg 164 of the Ocean Drilling Program (ODP; Paull et al., 1996, 2000) drilled 5 holes along an ∼80 m, east-west transect across the Blake Diapir. Three of the holes had total depths of 50–63 m, while two holes near the center of the diapir were drilled to less than 10 m below the seafloor. The recovered cores contained typical hemipelagic sediments along with authigenic carbonates and gas hydrates (Paull et al., 2000). Earlier research on the Blake Ridge Diapir revealed fluid-flow pathways and fluid-related features within the sedimentary section (e.g., Taylor et al., 2000). High concentrations of methane and sulfide in pore waters (1000–3400 µM CH$_4$; 1300 µM H$_2$S) and widespread occurrences of authigenic carbo-

nates and gas hydrates were documented in core material, and collection of mussels at the tops of cores provided evidence for the existence of a chemosynthetic community on the crest of the diapir (Paull et al., 1996).

The geographic location of the Blake Ridge methane seep raises questions about the biogeographical affinities of its fauna. The closest known deep-sea seep sites are those of the Barbados region to the southeast (Jollivet et al., 1990; Olu et al., 1996, 1997) and of the Florida Escarpment, on the opposite side of the Florida peninsula (Paull et al., 1984; Hecker, 1985). There is a perception that seeps support faunas that are more endemic to local regions than hydrothermal vents (Sibuet and Olu, 1998); comparisons of species lists and genetic differentiation in species from these sites can be used to test this hypothesis.

In this paper, we report new data that enhance our understanding of the geological context and ecological setting for the chemosynthetically based community on the Blake Diapir. Further accounts of methane hydrate formation, foraminiferal biology and ecology, and quantitative analyses of the invertebrate assemblage associated with Blake Ridge mussel beds will be presented elsewhere.

2. Materials and methods

Four *Alvin* dives were conducted at the Blake Ridge Diapir site (ODP Site 996; 32°29.623′N, 76°11.467′W; 2155 m depth) on September 25–28, 2001. A map of megafaunal distributions (mussels, clams, cake urchins) was generated from transponder navigation and digital video records from dives 3709, 3711, and 3712. Push cores were used to sample xenophyophores (P. Granuloreticulosa), bacterial mats, and sediments. Clams were collected with a suction sampler (1/4″ mesh); all other animals were collected with the *Alvin* manipulators or 26-cm-diameter quantitative mussel-bed samplers (described in Van Dover, 2002).

Size-frequency distributions of mussels were determined from length measurements of individuals collected in quantitative mussel samplers. They thus represent samples unbiased by the limitations of manipulator collections. Mussels

<1 cm are not included in this analysis but the data will be presented elsewhere upon completion of the study of the invertebrate assemblage associated with mussel beds. Size-frequency distributions of clams were determined from a single suction sample taken within 2 closely spaced, small patches of live and dead individuals. Mean lengths ± 1 s.d. are reported where appropriate.

Transmission electron microscopy (TEM) was used to provide a preliminary characterization of putative symbiont types in mussels and clams. Samples of gill tissue from mussels and clams were dissected and fixed for 2 h in a 3% solution of glutaraldehyde and 0.1 M phosphate buffer with 0.25 M sucrose (pH 7.4). The tissues were rinsed in the same buffer and post-fixed for 2 h in a 1% solution of osmium tetroxide. Specimens were then dehydrated in a graded acetone series, stained with 2% uranyl acetate, and infiltrated with Embed 812 epoxy embedding medium. Thin sections were stained with lead citrate and examined using a Zeiss EM 109 electron microscope.

For molecular analyses, DNA from the Blake Ridge samples was extracted using the DNeasy Tissue Kit (Qiagen, Valencia, CA). MtDNA was amplified using universal COI primers (519 bp; HCO-2198 and LCO-1490) and 16S rDNA primers (511 bp; 16Sar and 16Sbr), based on published or ongoing molecular systematic studies of these taxa (Peek et al., 1997, 2000). PCR products were sequenced directly using an ABI 3100 sequencer according to the manufacturers protocol. In all cases, both forward and reverse strands were sequenced and aligned. Sequence divergence estimates from other vesicomyids (Peek et al., 1997, 2000) were determined via Paup 4.0b. Novel sequences were deposited in Genbank under accession numbers AY163386 and AY163387 (COI) and AY163388 and AY163389 (16S) for Blake Ridge vesicomyids and AY163260 (COI) for Blake Ridge shrimp.

Adenosine triphosphate (ATP) content was determined for selected fragments of xenophyophore tests to assist in distinguishing living and dead specimens. Methods are detailed in Bernhard (1992).

Samples of animal tissues for stable isotope analyses were dried and ground to a fine powder and placed into tin capsules. The samples were converted to CO_2, N_2, and SO_2 for isotope analysis with a Carlo Erba elemental analyzer coupled to an OPTIMA stable isotope ratio mass spectrometer (Micromass, Manchester, UK). Carbon and nitrogen isotopes were determined with a single combustion in a dual-furnace system composed of an oxidation furnace at $1020^{\circ}C$ and a reduction furnace at $650^{\circ}C$. Samples for sulfur-isotope analyses were separately pyrolyzed at $1050^{\circ}C$ in a combination oxidation and reduction single-furnace system. The resulting gases were purified by gas chromatography, chemically dried, and directly injected into the source of the mass spectrometer by continuous flow. Stable-isotope ratios are reported as follows:

$$\delta^X E = [R_{\text{sample}}/R_{\text{standard}} - 1]10^3 (\text{‰}).$$

X is the heavy isotope of the element E; R is the abundance ratio of the heavy to light isotopes ($^{13}C/^{12}C$, $^{15}N/^{14}N$ or $^{34}S/^{32}S$) of that element. The international standard for carbon is the PeeDee Belemnite limestone (PDB), for nitrogen, atmospheric N_2 (air), for sulfur, the Canyon Diablo Troilite (CDT); those standards have defined $\delta^X E$ values of 0.0‰. In the laboratory, the samples were measured against tanks of CO_2, N_2 and SO_2 that had been calibrated against the international standards. For sulfur and carbon, the value was corrected for mass overlap with isotopes of oxygen. Reproducibility of the measurement is typically better than ± 0.2‰. Mean δ values ± 1 s.d. are reported where appropriate.

3. Results and discussion

3.1. Submersible observations of the geological setting

Four *Alvin* dives targeted the crest of the Blake Ridge Diapir at ODP Site 996. The terrain observed from the submersible ranged from flat to rugged, hummocky surfaces draped by fine, readily suspended silt-clay sediment that varied in color from yellow to grey. The prominent morphologic feature on the ridge crest at 2154 m is a circular depression (50 m diameter, 4 m deep)

surrounded by a steeply dipping, smooth rim. The floor of the depression is covered by beds of densely packed, live and dead large mussels and by fields of vesicomyid clams. From ODP studies, it is known that methane and hydrogen sulfide are present in abundance directly below the mussel beds (Paull et al., 1996). A faint linear scarp, which is interpreted to represent the seafloor expression of the fault, divides the depression into two unequal sectors.

The prominent depression at the Blake Ridge Diapir is probably related to seafloor methane seepage, but the process that led to its formation is unknown. Hovland and Judd (1988) and Uchupi et al. (1996) suggest that gas accumulates beneath a relatively impermeable seal (such as carbonate). Developing fractures link the gas pocket to the overlying ocean and are the means for release of the excess pressure and pockmark formation. Alternatively, the depression may be the surface expression of salt withdrawal resulting from subsurface chemical dissolution, as is often observed on top of salt diapirs in the Gulf of Mexico (Aharon et al., 1997).

Isolated, massive carbonate blocks are scattered among the mussel beds and outcrop at the edge of the depression (Fig. 3). The well-indurated carbonate rocks are composed primarily of carbonate-cemented mussel shells (biocalcirudites or chemoherms; Aharon, 1994; Aharon et al., 1997) and are similar to the indurated carbonate beds recovered by ODP from the subsurface (Paull et al., 1996). The subsurface carbonates at this site yield $\delta^{13}C$ values as light as –40.8‰ (Paull et al., 1995), indicating derivation of the carbonate carbon from microbial anaerobic methane oxidation (Aharon, 2000; Naehr et al., 2000). Radiocarbon measurements in marine sediments overlying the methane-derived carbonate beds yield ages of 15,610 yr before present (BP) at 28 cm and 39,160 yr BP at 3.88 m (Paull et al., 1995). Carbon in this overlying sediment is presumed to be derived from ambient seawater rather than from old, migrating methane, and its radiocarbon date indicates that venting of methane and deposition of carbonate have occurred at the Blake Ridge Diapir since the late Pleistocene. Paull et al. (1996) speculate that carbonate blocks scattered on the seafloor
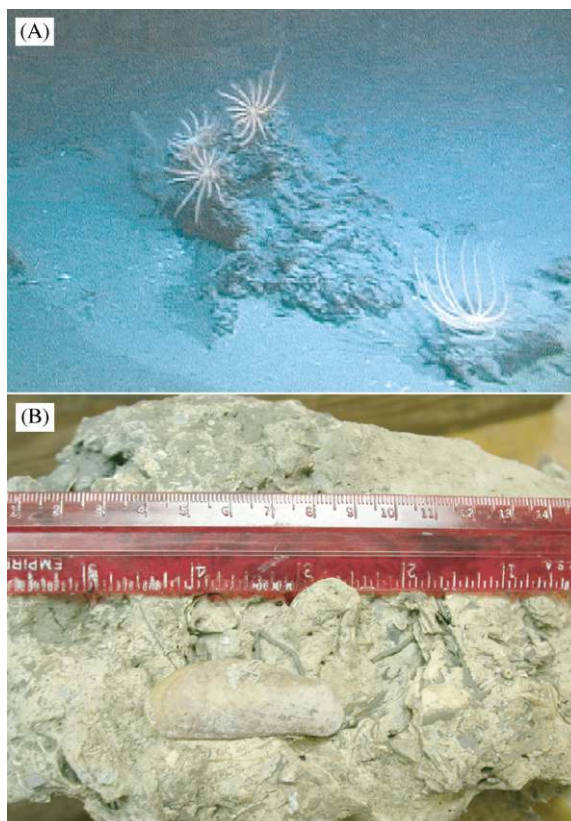

Fig. 3. Massive carbonate blocks. (A) In situ photograph showing carbonate outcrop colonized by suspension-feeding brisingid seastars. (B) Deck-shot of a carbonate block and a small specimen of the seep mussel, *Bathymodiolus heckerae*.

represent gas-hydrate-rafted dropstones. Submersible observations suggest instead that the large carbonate blocks within the depression are likely to have formed at the sediment-water interface. This interpretation is based on the presence of contiguous, massive constructions and crusts of carbonates surrounded by dense mussel beds, and by the presence of narrow, stacked, vertically oriented carbonate orifices that serve as venting conduits for methane and colonization sites for mussels.

3.2. Gas hydrates

During ODP Leg 164, gas hydrate was recovered from all five boreholes at Site 996, at depths

ranging from just below the seafloor (Hole 996D) to greater than 60 mbsf (Hole 996A) (Paull et al., 1996). No hydrates were documented on the seafloor, despite extensive acoustical and photographic surveys prior to drilling (Paull et al., 1995, 1996; Dillon and Max, 2000b). During the September 2001 *Alvin* dives, outcrops of massive, layered hydrate (Figs. 4A and B) were observed beneath contorted and chaotically oriented carbonate overhangs near the seafloor, proximal to the location of Hole 996D of ODP Leg 164. The hydrates occur as layers (up to 15-cm thick) of white, crystalline "bubbles", and are in the shape of upside-down ice-cream cones. Large ($\sim$1 cm) bubbles (Fig. 4C) were observed drifting upward through the water column from a seafloor emission site. Hydrate first formed around the bubble, which then continued rising before becoming plastered onto the surface of the hydrate layer. The in situ temperature of the layered hydrate was 3.14°C, slightly lower than the bottom-water temperature of 3.20°C. Clumps of gas hydrate broken loose during insertion of the temperature probe were buoyant.

3.3. Origin and nature of the outcropping gas hydrates

The methane gas occluded in the gas hydrates recovered from the Blake Ridge Diapir sediments at ODP Site 996 had high methane/(ethane + propane) ratios (> 1000, Lorenson and Collet, 2000) and δ^{13}C and δD values characteristic of biogenic methane (δ^{13}C = −67.5 ± 1.9‰ and δD = −181.7 ± 22.7‰ for $n = 7$, Lorenson and Collet, 2000; Paull et al., 2000), leading Lorenson and Collett (2000) to conclude that the methane is of microbial origin. Not all of the gas vented on the Blake Ridge Diapir must be generated by contemporary, local microbial activity within the sediments, however. The occurrence of faults and flow-related disturbances in the shallow sediment imply that much of the gas seeping from the crest of the diapir probably migrates there from deeper levels or the surrounding area (Egeberg, 2000; Musgrave and Hiroki, 2000).

Ambient conditions (3.2°C, 21.6 MPa) are conducive for formation of stable gas hydrates on the
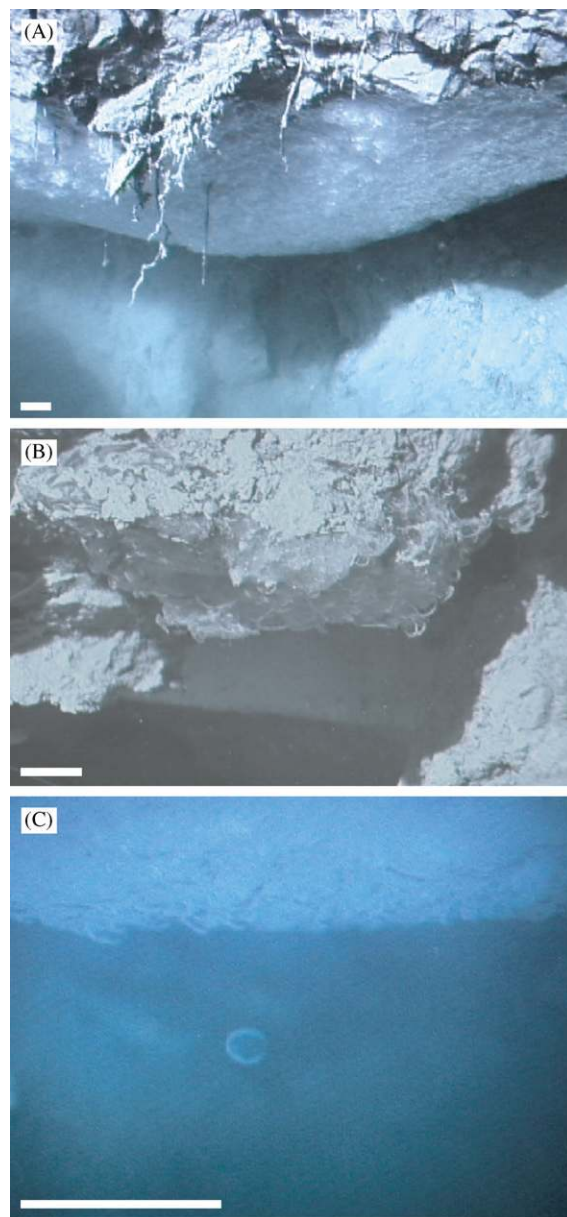
Fig. 4. Massive layered gas hydrates. (A) Hydrate trapped under sediment-covered carbonate cap. (B) Close-up of hydrate-enveloped bubbles. (C) Hydrate formation around a gas bubble during its ascent; the bubble will rise to accumulate against other bubbles trapped by the over-lying carbonate layer. Scale bars = $\sim$5 cm.

seafloor (Dillon and Max, 2000a), and plumes of methane were detected venting into the water column (Paull et al., 1995). Yet no gas hydrate

out-crops were observed on the seafloor prior to drilling. The absence of gas-hydrate mounds and the occurrence of hydrate outcrops only as massive under-coatings of collapsed beds require an explanation.

Hydrates are rarely observed at the seafloor, even where pressure and temperature conditions are within the stability field for methane-hydrate formation, since the overlying seawater is under-saturated in methane. Notable exceptions are isolated locations in the Gulf of Mexico (Macdonald et al., 1994), where gas-hydrate mounds form at the seafloor at some focused seeps. The concentration of methane in the plumes observed by Paull et al. (1995) was 4 μM, much lower than the 79 mM (calculated from the formulation of Egeberg and Dickens, 1999) required to saturate the bottom water with methane and to form gas hydrate. We postulate that the gas hydrate observed on the September 2001 *Alvin* dives formed beneath carbonate outcrops because the rock ledges isolated pockets of seawater from the larger circulation system, generating localized pools saturated in methane. Because of this local saturation effect, gas bubbles emitted in the methane plumes remain undissolved; the plumes themselves need not have high concentrations of methane. Initiation of gas-hydrate formation around bubbles, which represent minimum energy surfaces, has been observed in a variety of laboratory experiments (e.g., Tohidi et al., 2001) as well as in field settings where seeping gases were captured in inverted vessels (e.g., Brewer et al., 1997). The hydrate-encased gas bubbles remain buoyant relative to the surrounding seawater, explaining their continued ascent until they encounter the overhanging carbonate.

3.4. Types and distributions of organisms

Dominant megafauna were present as largely non-overlapping populations of bathymodiolid mussels and vesicomyid clams (Figs. 5–7). DNA sequence analysis revealed that the mussels
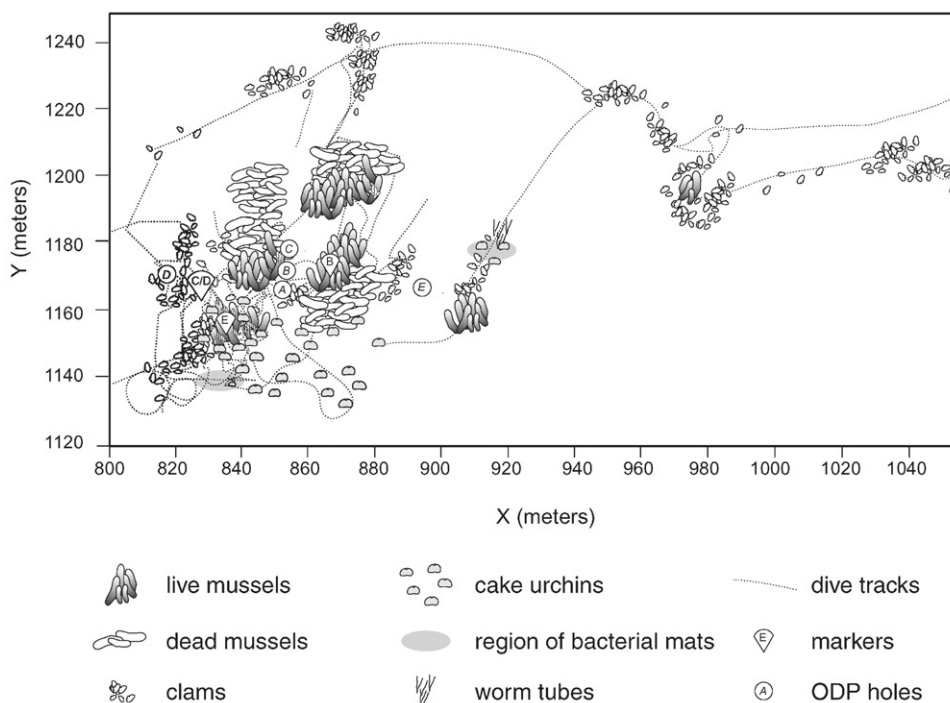

Fig. 5. Map of major megafaunal distributions at the Blake Ridge Diapir, with locations of *Alvin* dive tracks, ODP holes (A–E), and markers deployed (B–E).
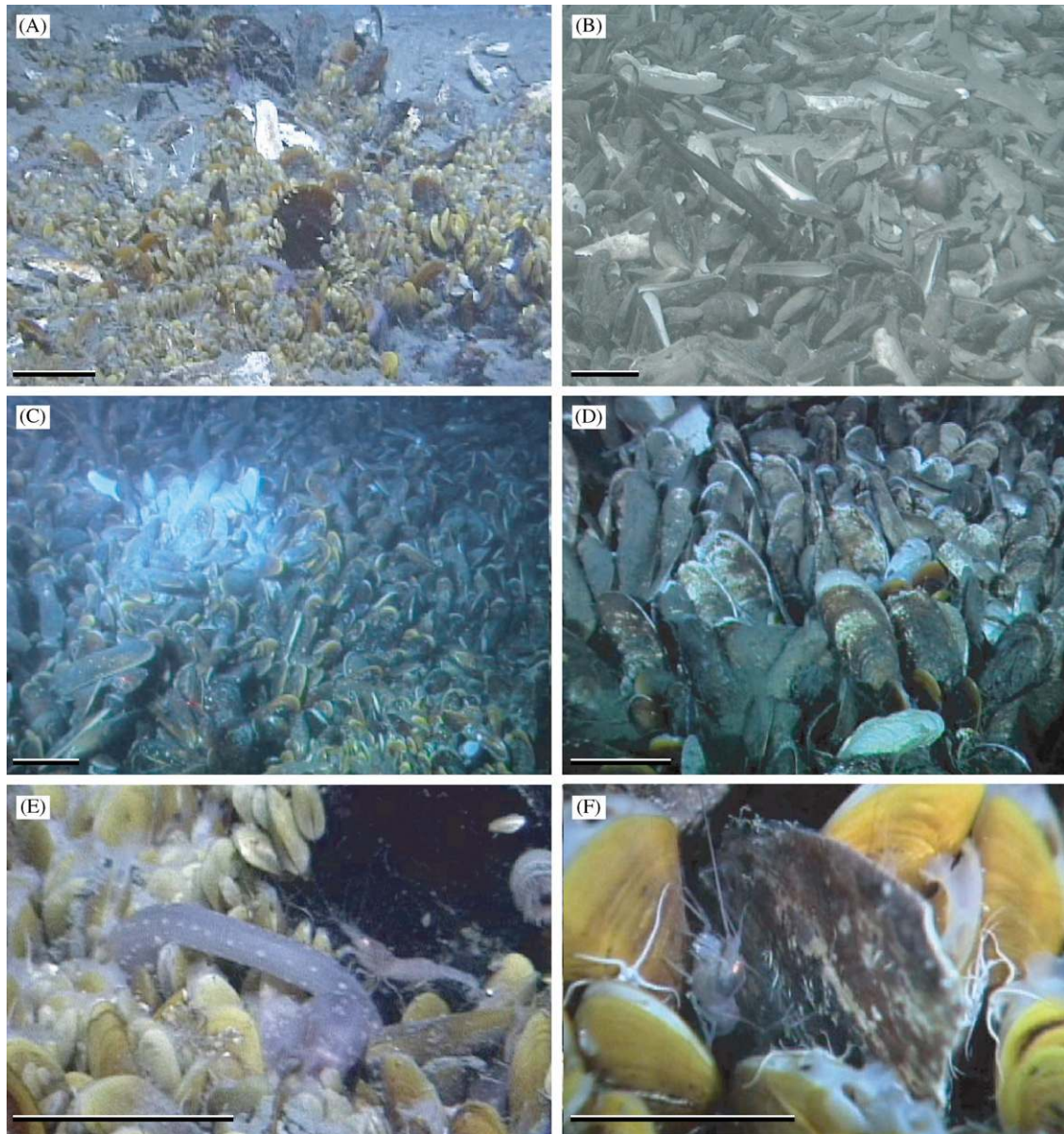
Fig. 6. *Bathymodiolus heckerae* mussel beds. (A) Juvenile and adult mussels at Marker 'E'. (B) Dead mussels and octopus. (C) Extensive bed of live mussels of relatively uniform size, partially covered by bacterial mats, at Marker 'B'. (D) Dead mussels at the eastward periphery of Marker 'B'. (E) Mussels with a chiridotid holothurian and *Alvinocaris* sp. (F) Mussels with *Alvinocaris* sp. and ophiuroids. Scale bars: $A–D = 10$ cm, $E, F = 5$ cm.

are probably *Bathymodiolus heckerae*, which also occurs at West Florida Escarpment seeps (Gustafson et al., 1998). Won et al. (in press) report that sequence divergence of the mitochon-drial ND4 gene (nicotinomid adenine dinucleotide dehydrogenase subunit 4) between Blake mussels and Florida Escarpment specimens is low, 1.4% (± 0.5 SE). Although the sample sizes were small
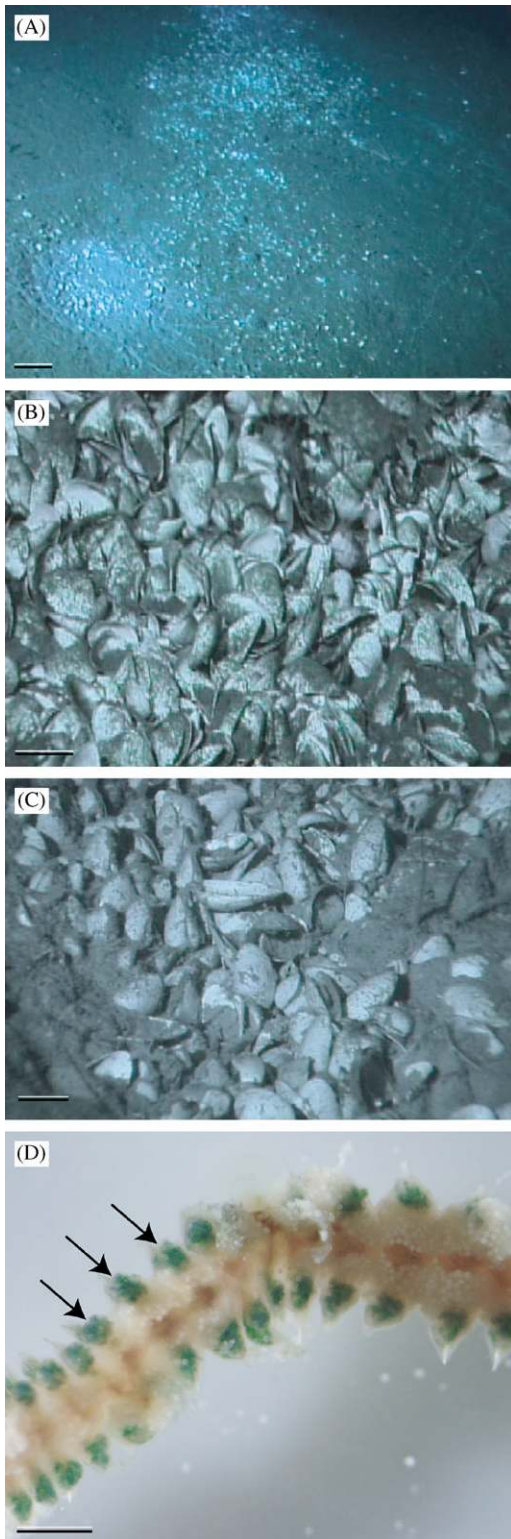
($N = 2$ for each population), this level of divergence is consistent with levels found within other named species of *Bathymodiolus* (generally $< 2.0\%$), and well below the range of distances observed between named species of this genus (11.3 to 30.6%). Additional samples across the entire size range of the Blake Ridge mussels should be analyzed before concluding that only a single mussel species exists at this site. The Blake mussels were much larger on average (one live individual was 364 mm in length) than *B. heckerae* from the Florida Escarpment (230 mm on average, Van Dover and Turnipseed, pers. obs.), and large individuals have a "boomerang" shape that resembles another large species, *Bathymodiolus boomerang*, found at diapiric seeps on the Barbados Accretionary prism (von Cosel and Olu, 1998). Unfortunately, the evolutionary relationships of *B. heckerae* and *B. boomerang* are not known, as specimens of the latter were unavailable for molecular analyses. Comparisons of shell growth rates between these sites are needed to determine whether the large mussels found at Blake are older or grow faster than conspecific mussels from the West Florida Escarpment. No commensal polynoids were recovered from hundreds of mussels sampled at Blake, whereas mussels from both the West Florida Escarpment and Barbados seeps support a high incidence of commensal polynoid polychaetes (Van Dover, pers. obs.; Olu et al., 1996).

The small ovate clams found at the Blake site (Figs. 7A–C) morphologically resemble *Vesicomya venusta* Dall 1886, a species first described from specimens dredged off of Havana (1480 m) and Cape Fear (1350 m) (G. Oliver, pers. comm.). At the molecular level, the Blake Ridge clams were highly divergent from other Atlantic basin vesicomyids. Mitochondrial *COI* (cytochrome *c* oxidase subunit-I) and 16S ribosomal sequences revealed

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Fig. 7. *Vesicomya* cf. *venusta* beds and a commensal polychaete. (A) Overview of linear patch of live and dead clams. (B) Close-up of live and dead clams. (C) Dead clams. (D) Mid-body segments of a nautiliniellid polychaete found in clam mantle cavities; the green pigment in parapodial lobes (arrows) is a fluorescent protein of unknown function. Scale bars: $A = 10$ cm, B, $C = 2$ cm, $D = 1$ mm.

the Blake Ridge clams to be distinct from an undescribed species that occurs in deeper water off Barbados (5000 m), at the West Florida Escarpment seep site (3313 m), and at the Logatchev vent site (14°47′, 3038 m) on the Mid-Atlantic Ridge (Peek et al., 2000). Sequence divergence between this undescribed Atlantic species and the Blake Ridge specimens is substantially greater ($>9.5\%$ and $>5.8\%$ for *COI* and 16S, respectively) than intraspecific levels of divergence typically found for vesicomyids (<2.0–3.0%, Peek et al., 1997, 2000). Shallower species from the Gulf of Mexico, *Calyptogena ponderosa* and *V. chordata*, can also be ruled out as relatives, as they differ by 10.7% and 14.2%, respectively, for *COI*. We cannot rule out a relationship with ovate clams from 1000–2000 m on the South Barbados Accretionary Prism, which Jollivet et al. (1990) recognized as being similar to *V. chuni* Thiele and Jaeckle (1931), although the Barbados clams appear to be significantly larger. Divergence of the Blake specimens from named Pacific Ocean species of vesicomyids (i.e., *Calyptogena pacifica*, *C. magnifica*, *C. elongata*, *C. kilmeri*, and *Vesicomya gigas*) was also great ($>10.7\%$ for *COI* and $>5.6\%$ for 16S). Based on morphological considerations, geographic and bathymetric location, and molecular uniqueness from available samples, we consider the Blake Ridge clams to be *Vesicomya* cf. *venusta*. A large proportion of the Blake Ridge clams ($\sim60\%$) hosted 1–4 commensal nautiliniellid polychaetes in their mantle cavities (Fig. 7D). A subset of these nautiliniellids contained a green fluorescent pigment of unknown consequence. Nautiliniellids were also found in *Vesicomya* sp. of Barbados seeps (Olu et al., 1996).

Cake urchins (*Sarsiaster griegi*; Figs. 8A and B) and xenophyophores (Fig. 8C) were also common megafaunal elements, occurring just at the margin of the mussel beds and not in distant peripheral areas. To our knowledge, this is the first record of these two taxa in close association with seeps. Until recently, *Sarsiaster griegi* was known from just a single specimen in the NE Atlantic, but it has now been collected from the Gulf of Mexico and the Blake Ridge site (D. Pawson, pers. comm.).

Xenophyophores are reportedly common in areas with high organic input (e.g., Levin and Thomas, 1988; Richardson, 2001), so their presence at the seep was not unexpected. The Blake xenophyophores appeared to comprise a single morphotype, probably of the genus *Syringammina*, which is known in both the Atlantic and Pacific, from bathyal to abyssal depths (800–4850 m; Tendal and Gooday, 1981; Gooday, 1996). They were patchily distributed, often occurring as groups of 4–5 specimens within a few centimeters of the mussel beds. Patchiness on scales of centimeters to meters is not unusual for this group (e.g., Tendal and Gooday, 1981; Levin and Thomas, 1988). The largest xenophyophores were ~6–7 cm in diameter. Both live and dead xenophyophores were observed on the seafloor. Live specimens were distinguishable by the greater amount of fine sediment covering their tests (Fig. 8C), a likely consequence of the "sticky" nature of their cytoplasm. This distinction was confirmed by laboratory observation of pseudopodial strands extending from the tests of sediment-covered specimens and by the presence of high ATP concentrations in fragments of sediment-covered specimens but not in fragments of sediment-depauperate tests (J. Bernhard, unpubl. data). On-going ultrastructural studies are investigating whether Blake Ridge xenophyophores have adaptations to the seep environment (e.g., symbionts), although the xenophyophores may be sufficiently removed from the localized seep activity to warrant no specific physiological adaptations.

Within the mussel beds, several macro- and megafaunal invertebrates were common. These included a deposit-feeding chiridotid holothurian (Fig. 6E), which resembles the chiridotid of Florida Escarpment seeps, and deposit-feeding sipunculids. Sipunculids have also been reported at Barbados (Olu et al., 1996) and Gulf of Mexico seeps (MacAvoy et al., 2002); based on external examination, the Blake sipunculid appears to be different from any of the Gulf seep species (M. Rice, pers. comm.). We observed numerous alvinocarid shrimp (Fig. 6F) among the mussels. These shrimp are morphologically similar to *Alvinocaris muricola* from Florida Escarpment seeps, but they differ in some diagnostic features, including the number, position, and length of the
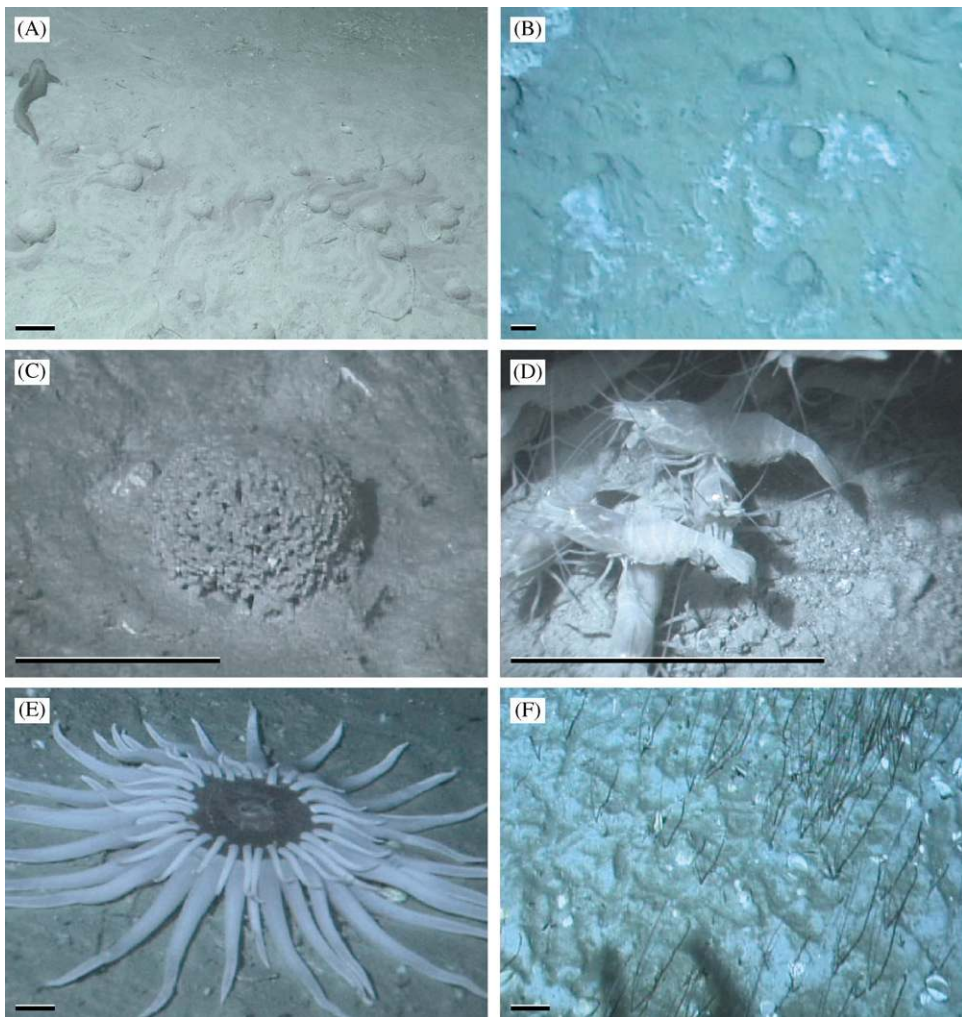
Fig. 8. Miscellaneous organisms. (A) Cake urchins *Sarsiaster greigi*, and a macrourid fish. (B) Cake urchins associated with bacterial mats at the edge of mussel beds. (C) Xenophyophore. (D) *Alvinocaris* sp. at methane hydrate. (E) Large solitary anemone. (F) Upright worm tubes. Scale bars = 5 cm.

telson setae and the presence of a prominent anterior spine on the 3rd maxilliped. This degree of difference is sufficient to warrant description of a new morphospecies. The Blake Ridge shrimp is almost as different from *A. stactophila* from Louisiana seeps as it is from vent shrimp in the genus *Rimicaris* spp. (Kimura two-parameter distance = ~15%), based on preliminary analysis of 600 base pairs of mitochondrial Cytochrome Oxidase I gene. Samples of *A. muricola* were not available for molecular comparisons. Shrimp were also observed in the vicinity of exposed methane hydrates, in the absence of other megafaunal invertebrates (Fig. 8D). Close-up video images suggest that they are the same species as the shrimp that are abundant in the mussel beds. Also among the mussels were large numbers of ophiuroids (Fig. 6F) that are morphologically similar to *Ophioctenella acies* of Mid-Atlantic Ridge vent sites (Logatchev, Snake Pit) and Florida Escarpment seeps (P. Tyler, pers. comm.). Nematodes and chaetopterid, maldanid, and capitellid

polychaetes were abundant within Blake Ridge mussel-bed samples. Large predatory or omnivorous species included galatheid crabs, octopods, and fish. A large (~ 0.5 m diameter) solitary anemone (Fig. 8E), localized patches of upright worm tubes (Fig. 8F), and suspension-feeding brisingid seastars on carbonate outcrops (Fig. 3A) were observed but not collected.

Patches of white were visible on the seafloor. These patches ranged from about 10–80 cm in maximum dimension. The patches were generally located in soft sediments outside the mussel beds, although they were also noted on mussels (Fig. 6C). Visual inspection indicated that most of the sampled patches were very thin (~ 1–2 mm) bacterial mats. Microscopic examination revealed that the mats were not composed of filamentous *Beggiatoa* sp. bacteria, which comprise bacterial mats in the Gulf of Mexico (Larkin and Henk, 1996). Blake Ridge Diapir mats may be composed of a sulfide-oxidizing *Thiovulum* species, which constructs mat-like, inorganic 'veils' (e.g., Fenchel and Glud, 1998), or the sulfur-oxidizing *Arcobacter* sp., which precipitates fibrous sulfur (Wirsen et al., 2002), but further characterization is required to confirm this. Thicker white mats resembling foam were also observed; microscopic examination indicates a composition similar to that of the thinner mat samples (i.e., *Thiovulum* sp. or *Arcobacter* sp.). Sediments immediately below the mats were black and smelled strongly of hydrogen sulfide and hydrocarbons. Preliminary examination of meiofaunal abundance in the Blake Ridge bacterial mat samples indicates a depauperate fauna compared to that of Gulf of Mexico seeps (Robinson and Bernhard, unpublished). Different geochemical regimes at Blake Ridge and Gulf of Mexico seeps likely account for these distinctions.

In overview, the Blake Ridge community shares some species with Florida Escarpment (and other Gulf of Mexico) seeps and with Barbados seeps (Table 1), but the degree of overlap in species composition among sites is incompletely assessed at present. Detailed studies of community composition of the invertebrate species associated with Florida Escarpment and Blake Ridge mussel beds

Table 1
Preliminary list of numerically or biomass-dominant taxa from the Blake Ridge site and the record of occurrence of these taxa at Florida Escarpment (FE) and Barbados (BA) seeps

Blake Ridge	FE	BA
Granuloreticulosa		
cf. *Syringammina* sp.	−	−
Cnidaria		
Anthozoa (unidentified)	+	+
Platyhelminthes		
Turbellaria (unidentified)	−	−
Annelida		
Cl. Polychaeta		
Nautiliniella n. sp.	−	?
F. Maldanidae	+	−
F. Chaetopteridae	−	+
F. Capitellidae	+	−
Sipunculida (unidentified)	−	?
Mollusca		
Cl. Bivalvia		
Bathymodiolus heckerae	+	?
Vesicomya cf. *venusta*	−	?
Cl. Cephalopoda		
Octopoda (unidentified)	+	+
Arthropoda		
Cl. Crustacea		
Munidopsis sp.	+	+
Alvinocaris cf. *muricola*	?	?
Nematoda (unidentified)	+	+
Echinodermata		
Cl. Echinoidea		
Sarsiaster greigi	−	−
Cl. Holothuroidea		
Chiridota sp.	+	−
Cl. Ophiuroidea		
Ophioctenella ?acies	+	−
Cl. Asteroidea		
Brisingia sp.	−	−

Florida Escarpment records from Turnipseed and Van Dover (unpublished); Barbados records from Sibuet and Olu (1998); ($+$) = present; ($-$) = not reported; (?) = species that may be shared, but for which taxonomic resolution is pending.

are underway and will provide a robust comparison of species and community similarities between these two sites.

3.5. Bivalve symbioses

TEM of Blake mussel (*Bathymodiolus heckerae*) gills (Fig. 9A) documented the presence of two distinct morphotypes of bacteria-like cells that we interpret to be methanotrophic and sulfide-oxidizing bacterial symbionts. Both morphotypes had cell envelopes typical of gram-negative bacteria and were contained within vacuoles surrounded by a peribacterial membrane; vacuoles contained one or both morphotypes. The larger, cocci-shaped morphotypes ($\sim 1.8\,\mu m$ in diameter) contained stacks of complex intracytoplasmic membranes, characteristic of Type I or Type X methanotrophic bacteria (Cavanaugh et al., 1992; Cavanaugh,

Fig. 9. TEM of bivalve gill tissues. (A) *Bathymodiolus heckerae*. (B) *Vesicomya* cf. *venusta*. Arrows point to some of the putative thiotrophic symbionts; M = putative methanotrophs; n = nucleus. Scale bars = 1 μm.

1994). The smaller, cocci-shaped morphotypes ($<0.5\,\mu m$ in diameter) lacked intracellular membranes and morphologically resembled sulfide-oxidizing bacteria seen in other mussel/bacterial dual symbioses (Cavanaugh et al., 1987; Cavanaugh et al., 1992).

The presence of methanotrophic symbionts in the Blake Ridge mussels is supported by the carbon isotopic composition ($\delta^{13}C = -55.7 \pm$ 1.9‰; $n = 10$ individuals) of the gills (Brooks et al., 1987; Kennicutt et al., 1992; see also Fisher, 1990; Van Dover, 2000 for reviews of interpretations of stable isotope data in vent and seep organisms). Dual symbionts are also known in *B. heckerae* from the Florida Escarpment (Cavanaugh et al., 1987) and in the Barbados *B. boomerang* (von Cosel and Olu, 1998). In contrast, *Vesicomya* cf. *venusta* from the Blake Ridge Diapir likely supports only sulfide-oxidizing bacteria, based on TEM observations (Fig. 9B) and isotopic data ($\delta^{13}C_{gill} = -36.4 \pm 1.8$‰; $n = 10$ individuals). *Vesicomya* sp. from Barbados seeps also hosts only sulfide-oxidizing bacteria (Olu et al., 1996). The Blake Ridge mussel and clam carbon isotope values mirror the isotopic differences observed in $\delta^{13}C$ values of shallow Gulf of Mexico seep mussels (off Louisiana) that rely on methanotrophy (-56.9 to -49.0‰) and seep vestimentiferans that rely on sulfide oxidation (-38.6 to -31.6‰; Brooks et al., 1987). The Louisiana seep mussels, however, do not support dual symbionts, and the similarity in the $\delta^{13}C$ values of their bulk tissues to those of the dual-symbiont-hosting Blake Ridge mussels may be coincidental, perhaps due to different original methane signatures.

Because of their requirements for reduced compounds (methane or sulfide), the two Blake Ridge bivalve species may serve as flux indicators for these compounds, as has been observed for bivalve species at Hydrate Ridge on the Cascadia convergent margin (Sahling et al., 2002). Moreover, their typically discrete, non-overlapping distributions suggest underlying differences in flux and chemical conditions, but hydrological, microbial, and geochemical distinctions have yet to be measured between clam and mussel habitats. It is also plausible that discrete distributions of clams and mussels could be explained by competition for
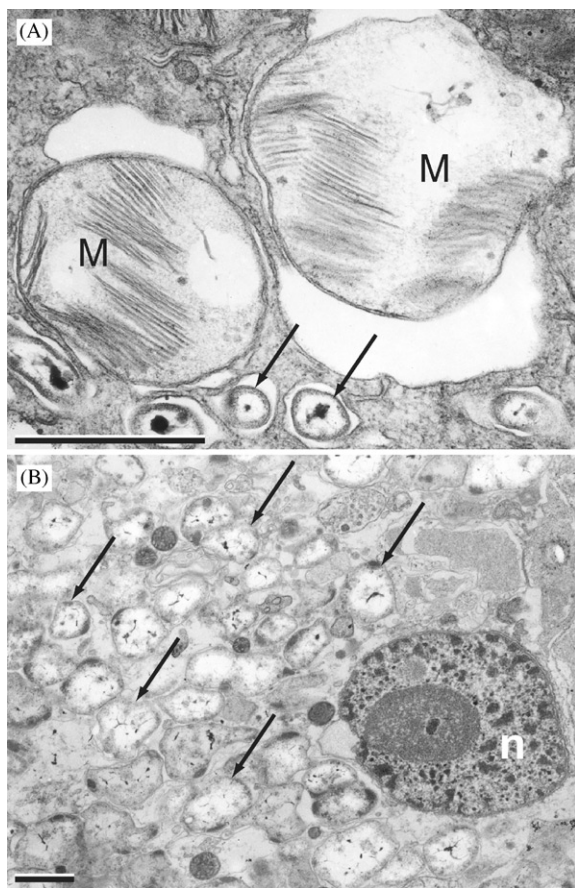
sulfide, as has been observed between interacting species of clams in Monterey Canyon seep settings (Barry et al., 1997).

3.6. Bivalve size-frequency distributions and mortality

Mussel beds within the study area were roughly circular to ovate in shape (~ 20 m diameter) and were distinctive in the size-frequency distributions of mussels within them (e.g., Fig. 10). The mussel bed at Marker E was distinctive in being characterized by many juvenile mussels attached to much larger mussels (Fig. 6A). This particular bed was also colonized by a large number of pediveliger and early post-larval mussel stages.

Mussel patches were often edged by a 1–2-m-wide band of empty valves (Fig. 6B). Mussel mortality could not be attributed to a specific cause, although the uniform size of the empty, paired valves within most patches suggests a
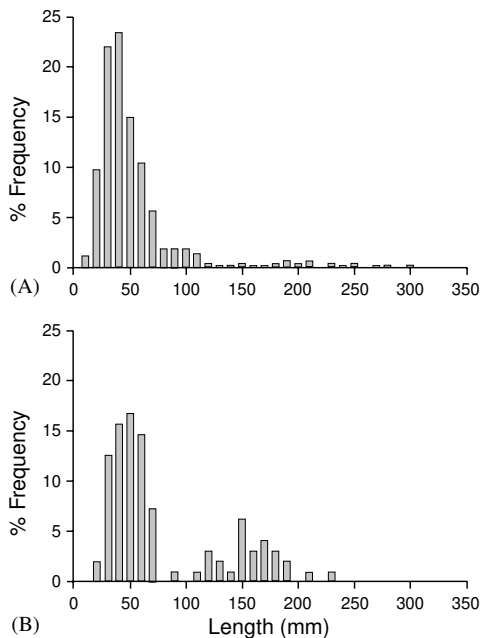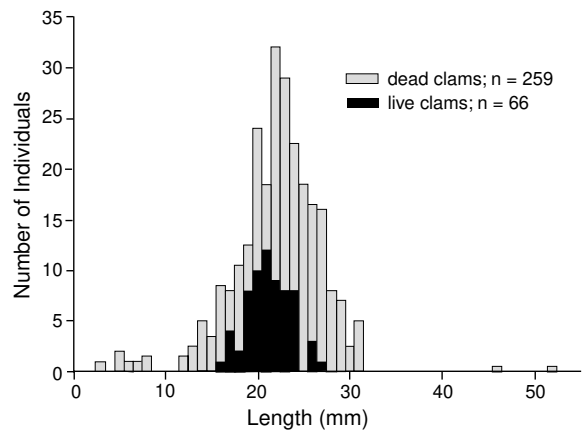

Fig. 11. Clam (*Vesicomya* cf. *venusta*) size-frequency distributions in a suction sample from two circular patches of clams. Black bars = live clams (2 valves); grey bars = valves of dead clams divided by 2; *n* = number of individuals (2 valves).

common cause and timing of mortality. The extent of the seep may fluctuate and mussels at the perimeter may die when the seep ebbs. Large predators, including fish (chimaerids, macrourids, zoarcids) and octopods (Fig. 6B), seem unlikely to explain the specific pattern of mortality observed, given the presence of paired shells without apparent damage. Clam patches occurred as roughly linear bands ~ 1-m in width (Fig. 7A) or as small circular patches (< 1 m diameter) dominated by small (1–2 cm length) live and dead individuals. The live clams are relatively uniform in length (20.7 ± 2.3 mm; Fig. 11) and give the appearance of a cohort of recruits that settled over a short period of time (days to weeks), but other explanations are plausible. The relative uniformity in size of the dead valves (21.6 ± 5.0 mm; Fig. 11) and the similar degree of erosion of these valves (not illustrated) suggest that mortality may have been massive. As with the mussel populations, massive mortality among the clams suggests a common cause and discrete timing, such as might result from fluctuations in fluid flux.

3.7. Food–web relationships

The chemosynthetic community at the Blake Ridge Diapir includes primary producers, grazers, suspension feeders, deposit feeders, predators, and

Fig. 10. Mussel (*Bathymodiolus heckerae*) size-frequency distributions. (A) Mussels from bed with large number of juveniles at Marker E; *n* = 441. (B) Mussels from bed at Marker B and near ODP Hole B; *n* = 95. (See Fig. 6 for marker and ODP Hole locations.)

Table 2
Inferred feeding guilds of invertebrates in the Blake Ridge Diapir chemosynthetic communities

| Guild						
Primary producers	Primary consumers				Secondary + consumers	
Autotrophic microorganisms	Symbiont hosts	Deposit feeders	Suspension feeders	Grazers	Predators, scavengers, & omnivores	Decomposers
Mussel symbionts	Mussels	Xenophyophores	Mussels	Shrimp	Anemones	Nematodes
Clam symbionts	Clams	Polychaetes	Xenophyophores	Galatheids	Octopus	
Free-living		Sipunculids	Polychaetes		Galatheids	
Bacteria		Ophiuroids	Brisingid seastars		Fish	
		Cake urchins			Flat worms	
		Holothurians				
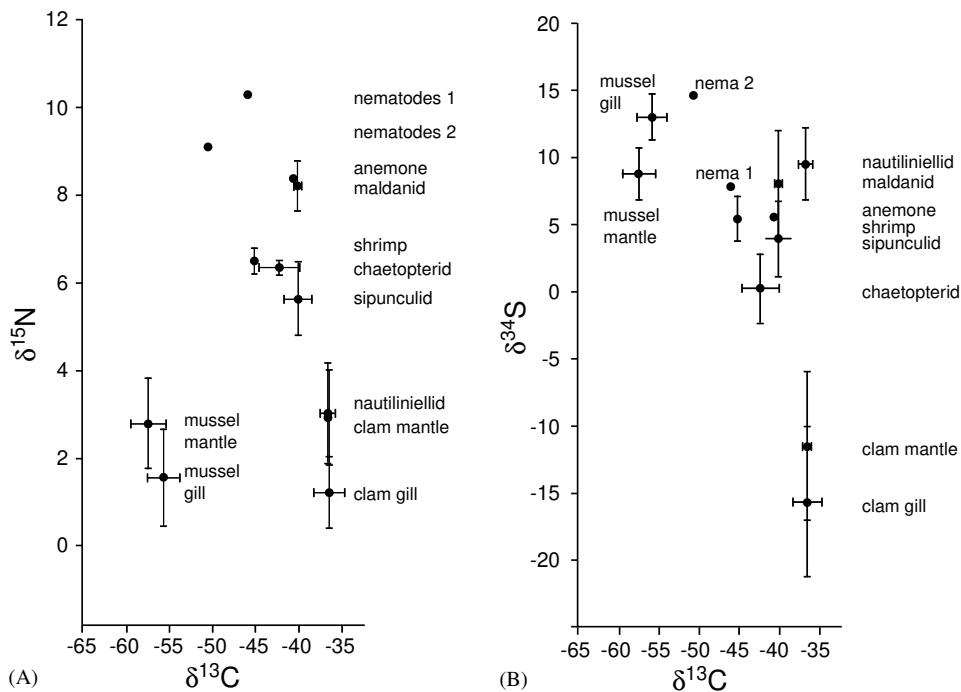
Fig. 12. Stable-isotope composition of selected Blake Ridge invertebrates. (A) $\delta^{15}N$ vs. $\delta^{13}C$ (‰). (B) $\delta^{34}S$ vs. $\delta^{13}C$ (‰). Number of individuals analyzed: mussel (*Bathymodiolus heckerae*) paired gill and mantle, $n = 10$; clam (*Vesicomya* cf. *venusta*) paired gill and mantle, $n = 10$; clam commensal polychaete (*Nautiliniella* n. sp.), $n = 18$ except for $\delta^{34}S$, where $n = 16$; sipunculid, $n = 3$; chaetopterid polychaete, $n = 4$; maldanid polychaete, $n = 3$; anemone, $n = 1$; nematodes, 2 samples of pooled specimens (12 and 62 individuals).

decomposers (Table 2). Stable-isotope data (Fig. 12) and dietary inferences based on knowledge of shallow-water and hydrothermal-vent

analogues are used to provide a preliminary assessment of trophic relationships (Fig. 13). As reported above, populations of the two
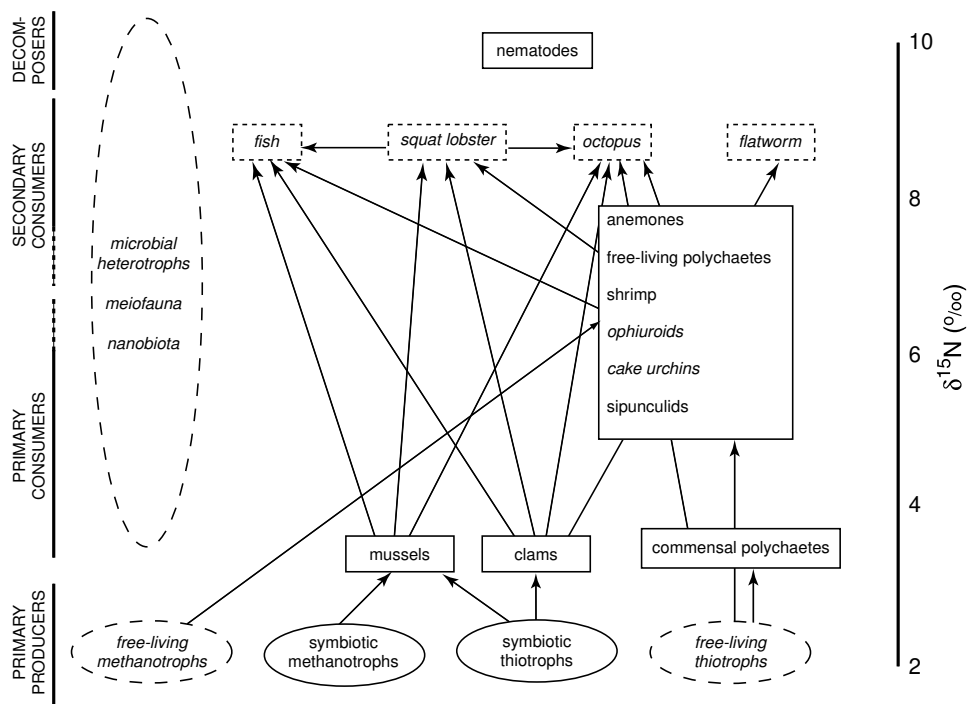
Fig. 13. Food web model for the Blake Ridge Diapir mussel and clam beds. Italicized entries and dashed envelopes indicate taxa for which isotopic data are not available. Positions in the food web for these taxa are inferred from behavior, distribution, and feeding characteristics of the taxa in other habitats. All other taxa are positioned vertically according to their nitrogen isotopic ratios. Dashed lines beneath Primary Consumer and Secondary Consumer legends represent a mixotrophic region. Note that nitrogen values for the symbiotic and free-living autotrophs are approximated by analyses of bivalve gills that include a mix of symbiont and host organic material.

symbiont-bearing bivalves have distinctive carbon isotopic compositions, consistent with differences in their autotrophic symbiont populations and with likely carbon sources (a mix of methane and seawater CO_2 for mussels, seawater and porewater CO_2 for clams). For both the mussels and clams, we can reasonably assume that photosynthetically derived organic carbon is likely not important in their nutrition (Fisher, 1990). The relative importance of methanotrophy vs. sulfide oxidation in the nutrition of the mussels may be estimated by assuming a two-end-member mixing equation (Fry and Sherr, 1984) in which photosynthetically derived organic material plays a negligible role. In this model, $\delta^{13}C_{consumer} = f(\delta^{13}C_{source\ 1}) + (1-f)(\delta^{13}C_{source\ 2})$, and f is the proportion of source 1 used. If the $\delta^{13}C$ value of methanotrophically generated biomass is assumed to be that

of the source methane (−67.8‰; Paull et al., 1995, 2000) and the $\delta^{13}C$ value of thiotrophically generated biomass is that of the clams (−36‰; this study), then these mussels (−56‰) derive approximately 60% of their organic carbon from methanotrophic symbionts and approximately 40% of their carbon from sulfide-oxidizing symbionts. This contrasts with the situation in mussels of Gulf of Mexico seeps, where only methanotrophs are symbiotic in the gills and the carbon isotopic composition of mussel tissues is similar to that of the source methane ($\delta^{13}C_{mussel} = -40.6‰$; $\delta^{13}C_{methane} = -41.2‰$; Brooks et al., 1987). This simple approach to assessing the relative importance of methanotrophy and thiotrophy neglects the potential for differential fractionation effects related to microbial-host interactions and translocation of nutrients.

Sulfur isotopic compositions were also distinctive between the two bivalve species (*Vesicomya* cf. *venusta* $\delta^{34}S_{gill} = -15.6 \pm 5.6‰$; *Bathymodiolus heckerae* $\delta^{34}S_{gill} = 13.0 \pm 1.7‰$; Fig. 12B) and may be interpreted to indicate reliance on hydrogen sulfide as a sulfur source in clams (Brooks et al., 1987; Vetter and Fry, 1998; MacAvoy et al., 2002) and a combination of seawater sulfate (20‰; Michener and Schell, 1994) and hydrogen sulfide ($\sim -10‰$; Michener and Schell, 1994) as sulfur sources in mussels. The $\delta^{34}S$ value of mussel gill tissue was significantly more positive ($\sim 7‰$) than that of the mussel mantle tissue (paired *t*-test; $P < 0.001$). Using a 2-source mixing equation, we conclude that mussels could derive as much as $\sim 75\%$ of their organic sulfur from seawater sulfate rather than from sulfide, suggesting that sulfide uptake and oxidation is not as critical to the organic sulfur budget in mussels as it is in clams. This model, however, does not account for differential fraction of sulfur by microbes and host–microbe interactions, nor does it take into account the effect of inorganic sulfur storage in the host tissues.

Florida seep clams (*Calyptogena* cf. *kaikoi*; Van Dover et al., 2002) and mussels (*B. heckerae*) have carbon and sulfur isotopic compositions (Cary et al., 1989; Vetter and Fry, 1998) that mirror those of the Blake Ridge clams and mussels, respectively. The isotopic data suggest nutritional similarities within these bivalve taxa despite their disparate geological and geochemical settings (brine seepage from the carbonate platform at the Florida Escarpment and methane-hydrate deposits at Blake Ridge). This observation suggests congruence in microbial processes that generate and use methane and hydrogen sulfide even under different physical regimes.

Nitrogen sources for both bivalve species are inferred to have the same isotopic composition; enrichment in ^{15}N from gill to mantle tissues is consistent with the trophic characterization of mussels and clams as primary consumers of organic material derived from gill symbionts (Fisher, 1990).

The $\delta^{13}C$ and $\delta^{15}N$ values of the commensal nautiliniellid polychaetes (Fig. 12A) suggest that these worms might derive nutrition from tissues of their host, but the mean sulfur isotopic composition of the polychaete is so distinctive ($\Delta\delta^{34}S \geqslant 20‰$) from that of the host clams (Fig. 12B) that an alternative diet must be inferred. One alternative feeding strategy is that ciliary activity of the clam gills moves sufficient volumes of seawater to allow the polychaetes to collect suspended organic particles either from gill mucus or a worm-generated mucus net. Mucus-net feeding in polychaetes is not uncommon and is known in, for example, the maldanid *Praxillura maculata* (McDaniel and Banse, 1979) and the nereid *Nereis diversicolor* (Riisgaard et al., 1992).

Higher trophic-level organisms (fish, octopus) were not captured. Small nematodes had the most positive $\delta^{15}N$ values of any organism examined. These nematodes could be members of a decomposer guild, involved in recycling of locally produced organic material. Two turbellarian flatworms (Platyhelminthes) were collected from gaping clams, suggesting that the flatworms are predators or scavengers.

Based on examination of gut contents, sipunculids from the mussel beds and cake urchins from the peripheral muds ingest large volumes of sediment and might be among the most sensitive indicators of the role of surface-derived organic material (SDOM) in the seep food web. Body-wall tissues of three sipunculids had $\delta^{13}C$ values averaging $\sim -40‰$ rather than the $-22‰$ expected if they were dependent on SDOM (Van Dover et al., 1992). Assuming a 2-member mixing model and the same $\delta^{13}C$ values for organic carbon derived from methanotrophic and sulfide-oxidizing bacteria as discussed for mussels and clams above, methanotrophically derived organic material contributes less than 15% of the organic carbon in sipunculid tissues. If, however, a 2-member mixing model is constructed that dismisses the role of thiotrophs in favor of methanotrophically and photosynthetically derived organic carbon, then the methanotrophic contribution would be $> 40\%$. Given that the sulfur isotopic composition of the sipunculids is more like that of the mussels, this latter scenario seems plausible. A 3-end-member mixing model that incorporates photosynthetically derived carbon cannot be discounted. Fatty-acid biomarkers and their

stable-isotope compositions would prove helpful in determining the relative importance of different carbon sources, but these were outside the scope of the present study.

Given the importance of methanotrophic microorganisms in the nutrition of mussels determined from carbon isotopes (60% of mussel organic carbon assuming a 2-source mixing model), and the dominance of mussels in the community, the bulk of the organic carbon at the Blake Ridge Diapir is derived from methane. Based on carbon-isotope compositions of seven other members of the Blake Ridge seep community (average $\delta^{13}C = -41.8‰$) and the number of organisms potentially linked to thiotrophs (Fig. 13), sulfide-oxidation by free-living thiotrophic bacteria may contribute to the nutritional base of the food web, but the sulfur-isotope data for these species (average $\delta^{34}S = 6.3‰$) is more consistent with dependence on sulfur with a seawater sulfate signal ($\delta^{34}S = 7–15‰$) than sulfidic sulfur ($< 0‰$). The possibility of a major role of photosynthetically derived organic material in the food web cannot be ruled out using bulk isotope data alone. Further isotopic characterization of potential nutritional resources is needed before the partitioning of thiotrophically, methanotrophically, and photosynthetically derived organic material within the Blake Ridge Diapir food web can be determined with greater resolution.

Acknowledgements

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