

CHARACTERISTICS OF VESICOMYID CLAMS AND THEIR ENVIRONMENT AT
THE BLAKE RIDGE COLD SEEP

A Thesis

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The Faculty of the Department of Biology
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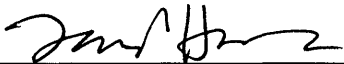
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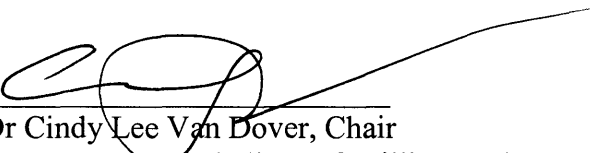
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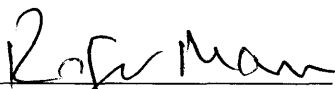
APPROVAL SHEET


This thesis is submitted in partial fulfillment of
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To my father, Captain Phillip J. Heyl, who taught me to accept challenges, and to my mother, Karen B. Hunter, who helped me follow them through.

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Note: Sulfide and sulfate porewater data reported herein make up a portion of the Ph.D. thesis of W. Gillhooly in Prof. S. Macko's lab at the University of Virginia, and are used here with their permission as part of a collaboration between the Van Dover and Macko laboratories. T.P. Heyl assisted in all aspects of the sulfide and sulfate chemical analyses, including collection of samples, extraction of porewater fluids, and wet chemistry analyses in the laboratory of Prof. Macko. Mineral sulfide data reported herein was analyzed by Randy Chambers at the College of William and Mary's KECK laboratory. Gillhooly, Macko and Chambers will be co-authors on any publication resulting from the work reported in this thesis.

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ABSTRACT

Vesicomyid clams are one of the dominant megafaunal taxa found in deep-sea cold-seep communities. They rely on chemoautotrophic sulfide-oxidizing symbionts located in their gill tissues. Since their survival depends on a flux of sulfide, clam populations may be viewed as 'flux indicators' within chemosynthetic ecosystems. At the Blake Ridge methane hydrate seep, extensive beds of dead clams are interspersed with smaller (> 1 m diameter) circular patches of live clams or a combination of live clams and shell beds. The patchy distribution of live clams may reflect underlying heterogeneity in sulfide flux or in other environmental parameters that influence the health and reproductive condition of the clams.

The research effort included sediment geochemical analyses from 4 distinct microhabitats (background sediments, clam beds, a combination of clam/shell beds, and shell beds) within the seep site. Porewater chemistry indicates sulfide concentrations are five times higher (10.3-14mM) in shell beds than in clam beds (0.1-3.1mM) and this could be the cause of massive clam mortality. Gametogenic characteristics of vesicomyid clams (collected from different microhabitats in July 2003) were investigated but there was no correlation between health of clams and geochemistry within or among microhabitats at the seep.

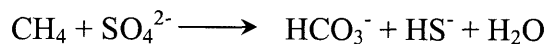
This study suggests that Blake Ridge clams have a broad range of sulfide tolerance that limits local and population-wide extinction of the species but they have a critical sulfide tolerance limit above which mortalities due to toxicity occur.

CHARACTERISTICS OF VESICOMYID CLAMS AND THEIR ENVIRONMENT
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INTRODUCTION

Cold seeps are found in environments where reduced sulfur and methane emerge from seafloor sediments without an appreciable temperature anomaly. These environments are among the most recently discovered marine habitats; the first seep was discovered just 20 yr ago in 1984, on the Florida Escarpment in the Gulf of Mexico (Paull et al. 1984). Seeps support dense communities of invertebrates that rely on the flux of chemicals that flow through the ocean floor for chemosynthesis. Methane-rich fluids of thermogenic and/or biogenic origin and the microbial reduction of sulfate to sulfide are the two principal sources of reduced compounds that can be oxidized by microorganisms to yield ATP/energy (Sibuet & Olu 1998). Anaerobic oxidation of methane (AOM) via methanogens in sediments, presumably coupled to sulfate reduction (Beauchamp et al. 1989, Masuzawa et al. 1992), generates extremely high concentrations of hydrogen sulfide in pore waters, and in many places facilitates formation of carbonates. AOM is carried out by two or more groups of archaea that typically live in syntrophic consortia with sulfate-reducing bacteria in the *Dusulfosarcina/Desulfococcus* and *Desulfobulbu* groups (Orphan et al. 2002, Knittel et al. 2003). The overall reaction

involves the oxidation of methane and the reduction of sulfate, producing the formation of bicarbonate and hydrogen sulfide:



Hydrogen sulfide, which is frequently found in marine sediments, is oxidized by several groups of symbiont-bearing megafaunal species that depend on chemoautotrophic nutrition (Fisher 1990). Where there are reducing environments in the deep sea, sulfide-oxidizing endosymbionts hosted by invertebrates are typically found. Thiotrophic and/or methanotrophic symbionts are found within the gills of mussels and clams, and provide nutrition by chemosynthesis. Of the 211 seep species discovered so far, 64 are symbiont-bearing species (Sibuet & Olu 1998). Sulfide is taken up through the foot of clams and passed to their endosymbiotic chemoautotrophic bacteria, which oxidize the sulfide, fix inorganic carbon, and translocate fixed organic material to their host (Masuzawa et al. 1992).

There is extensive spatial heterogeneity of microbes and higher organisms at seeps (Levin in press). The habitability of seeps to species in the larval pool, which is important in determining the local distribution of species within seeps, depends on the concentration of sulfide or methane. Survival and growth of seep species is linked to variation in their requirements or tolerances of sulfide or other reduced compounds (Barry et al. 1997). Large (megafaunal) seep organisms influence the sediment environment, providing physical structure and modulating geochemistry through oxygenation (pumping) and ion uptake activities (Levin in press).

Seep clams are usually members of the family Vesicomidae and are one of the three dominant taxa present at most seeps (Sibuet & Olu 1998, Kojima 2002). There are

many species in the genera *Calyptogena* and *Vesicomya* (Goffredi et al. 2003). These megafaunal organisms are often aligned linearly along geological structures at the base of steps, in depressions, or in cracks (Suess et al. 1998). Spatial distribution of seep bivalves in relation to geochemistry has been studied along the western United States (Levin et al. 2003, Sahling et al. 2002, Barry et al. 1997, Barry & Kochevar 1998), in Sagami Bay (Hashimoto et al. 1995) and along various other continental margins. This study characterizes an undescribed genus and species of vesicomylid clam from the Blake Ridge cold seep in relation to physical and chemical conditions in which it lives.

GEOLOGICAL SETTING AND GLOBAL DISTRIBUTION OF SEEPS

Cold seeps are among the most geologically diverse of the reducing environments explored to date. Since the first discovery of cold seep environments (Paull et al. 1984), related chemically based systems have been found among a variety of settings including continental margins, transform faults, mass wasting sites (Hovland & Judd 1988, Sibuet & Olu 1998), subduction zones (Suess et al. 1985, Kulm et al. 1986, Laubier et al. 1986, Ohta and Laubier 1987), pockmarks (Dando et al. 1991), and within whale falls (Smith et al. 1998). Many fossil seeps have been discovered (or reinterpreted) as well (Campbell et al. 2002). Seeps have been identified in the Atlantic, the Eastern and Western Pacific oceans, the Gulf of Mexico and the Mediterranean Sea along tectonically active and passive continental margins and are related to geological processes such as tectonically induced high-fluid pressures, petroleum or natural gas escape, artesian flow, or catastrophic erosion and slides (Sibuet & Olu 1998). Seeps are host to

chemosynthetically based benthic invertebrate communities from depths of < 15 m (Montagna et al. 1987) to > 7,400 m in the Japan Trench (Fujikura et al. 1999).

On active margins, cold seeps are found to depths of 6000 m in the Eastern Mediterranean, the Aleutian trench, the Peru Trench, the Japan Trench, the Barbados prism, and the Oregon continental margin (Sibuet & Olu 1998). Pockmarks (as seen at the Blake Ridge diapir) are known to be related to seafloor methane seepage though the formation mechanism is unclear (Dando et al. 1991). New settings may be discovered where spreading ridges (e.g., Chile Triple Junction), or seamounts (e.g., Aleutian Archipelago) encounter subduction zones, or when seepage occurs within oxygen minima (Schmaljohann et al. 2001, Salas & Woodside 2002). Mass wasting from earthquakes, tsunamis, or turbidity currents may also generate or expose reduced sediments and yield seep communities (e.g., Mayer et al. 1988).

On passive continental margins, seep communities are associated with hypersaline, cold sulfide seeps at the base of the Florida escarpment in the Gulf of Mexico (Paull et al. 1984), hydrocarbon seeps on the Louisiana slope in the Gulf of Mexico (Kennicutt et al. 1985) and methane hydrate seeps at the Blake Ridge in the Atlantic Bight off of South Carolina (Paull et al. 1994). Formation and dissociation of gas hydrate outcrops can drive short-term, small-scale variation in chemosynthetic communities in the Gulf of Mexico (MacDonald et al. 2003).

Seep ecosystems may be fuelled by a variety of organic hydrocarbon sources, including methane, petroleum, other hydrocarbon gasses, and gas hydrates. These hydrocarbon sources are ultimately of photosynthetic origin because they are generated from accumulations of marine or terrestrial organic matter.

Melting of the Earth's ice sheets caused eustatic sea level to rise by 125 m over the past 18,000 years, which increased hydrostatic pressure on continental slopes by approximately 1.25 MPa (e.g. Chappell & Shackleton 1986). Methane hydrate formed from free gas below the gas hydrate/free gas interface because of the increase in pressure (Carpenter 1981, Kayen & Lee 1991, Paull et al. 1991, 1996, Ruppel 1997). Sediment burial over time slowly brings the solid gas hydrate to higher temperatures until it is no longer stable and dissociates to gas-saturated water and free gas bubbles (Paull et al. 1994). Hydrate formation influences the porosity and permeability of the near-surface sediments as well as fluids locally (Paull et al 1994).

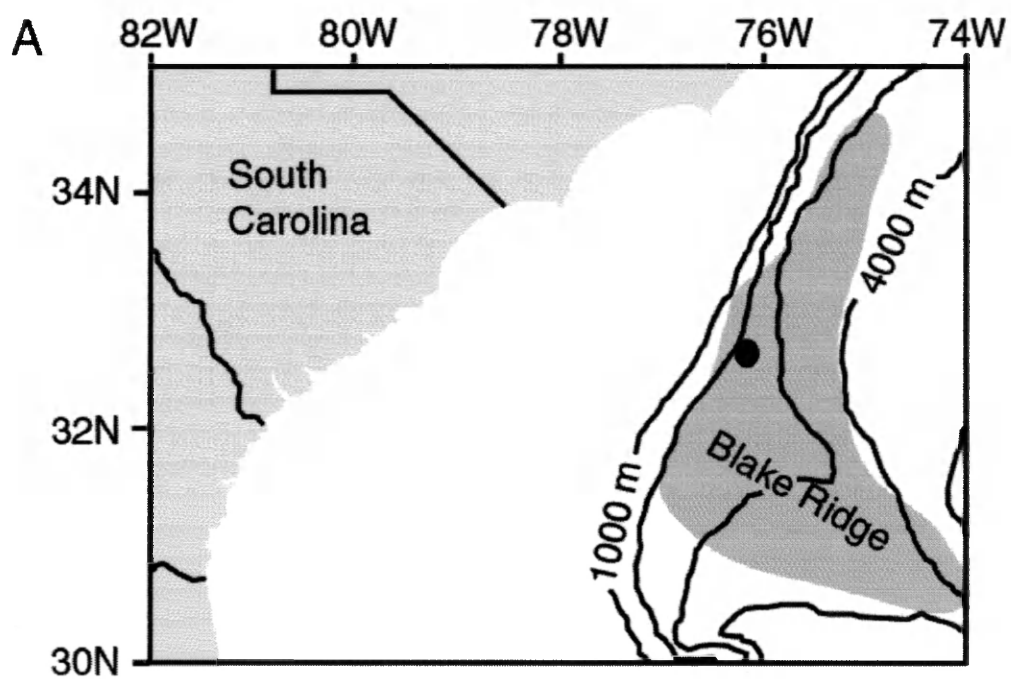
BLAKE RIDGE SEEP

Geological setting. The Blake Ridge cold seep is a soft-sediment, chemosynthetically based ecosystem on the continental margin of the Eastern United States that lies in deep water (2155 m) ~ 200 miles off the coast of South Carolina (Fig. 1). It has a constant ambient seawater temperature of approximately 2-3°C and is hosted by a salt diapir (Dillon et al. 1982). It lies in an area of the South Atlantic Bight that is recognized as a major methane hydrate province within the US Exclusive Economic Zone (e.g., Markl et al. 1970, Tucholke et al. 1977, Paull and Dillon 1981).

The Blake Ridge and adjacent Carolina rise are areas where methane hydrates appear to be extensive, supporting a linear system of approximately 19 diapirs rising from near the base of the Carolina trough (Dillon et al. 1982). The crest of the Blake Ridge diapir is a dynamic system where solid hydrate, gas bubbles, and dissolved gas are created and consumed continuously (Paull et al. 1994). Methane is the predominant gas

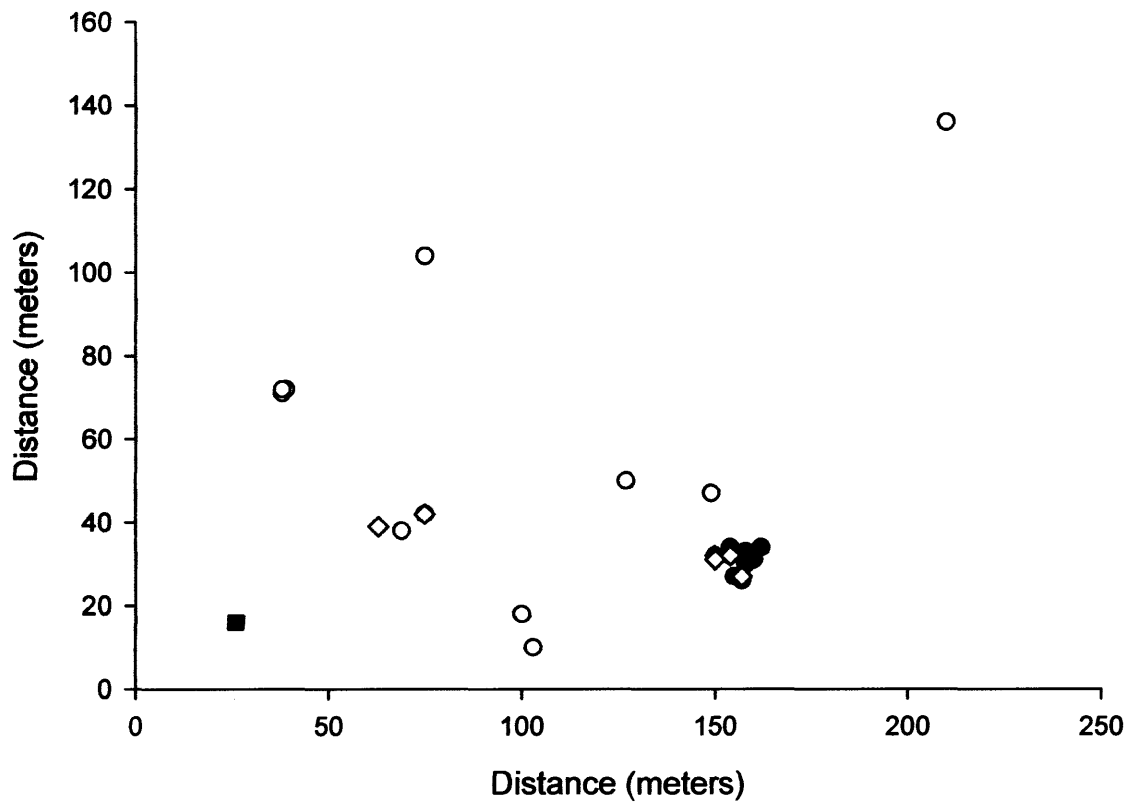
and is generated in sediment through bacterially mediated reactions (Paull et al. 1994, 2000). The methane migrates laterally and vertically and eventually precipitates into a solid hydrate under high pressure and low temperature conditions.

FIGURE 1A
SITE AND SAMPLE LOCATIONS



The Blake Ridge seep site.

FIGURE 1B
SITE AND SAMPLE LOCATIONS



Location of pushcores:

Background (O), Clam beds (□), Clam/shell beds (◇) Shell beds Δ).

Venting of microbially generated gases occurs at the sediment surface above the Blake Ridge Diapir (Paull et al. 1995). Plumes and venting fluids emanate from a seafloor pockmark that lies along a fault that extends downward toward a dome in the bottom-simulating reflector (BSR). The BSR is negative impedance contrast sediment layer evident in seismic reflector profiles and marks the top of the stable gas hydrate and the underlying free gas (Holbrook 2000). The high thermal conductivity of the diapir alters the local stability field for methane hydrates, causing upward warping of the bottom simulating reflector and shifts the gas hydrate and free-gas system to a shallower depth (Taylor et al. 2000). Gas-rich plumes rising up to 320 m in the water column have been detected acoustically where the fault system intersects the seafloor (Paull et al. 1995).

Cold seeps (e.g. Blake Ridge Diapir) have greater longevity and stability compared to hydrothermal vents (Sibuet & Olu 1998), but they are still subject to transient shifts in the loci and intensity of seepage (Sibuet & Olu 1998, Carson et al. 2003, Levin et al. 2003). The effects of shifting seepage could have an effect on the overall health and condition of chemosynthetic organisms present within these environments.

Biological setting. The dominant megafauna present at the Blake Ridge methane hydrate seep are two species of bivalve, *Bathymodiolus heckerae*, the giant (up to 360 mm) seep mussel and an undescribed genus and species of small (1-2 cm) clams from the family Vesicomidae (Van Dover et al. 2003, referred to as *Vesicomya* cf. *venusta* but more closely related to cf. *Calyplogena birmani*, Kryolova, pers. com.). Both species of bivalve house chemoautotrophic, endosymbiotic bacteria in their gill tissues (Van Dover

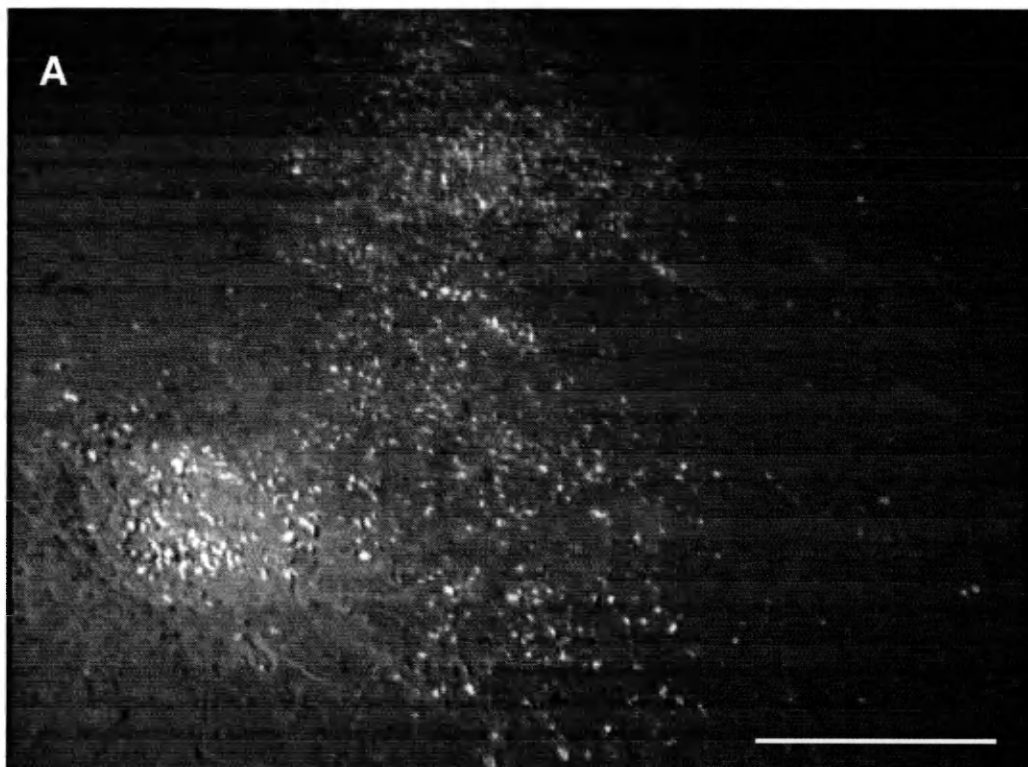
et al. 2003). *Bathymodiolus heckeriae* receives nutrition from two symbiotic bacterial morphotypes: sulfide-oxidizing thiotrophs and methanotrophs, while the clams rely solely on thiotrophs (Van Dover et al. 2003). Chemoautotrophic bacteria in vesicomid clams derive energy for carbon-dioxide fixation through the oxidation of sulfide (Felbeck et al. 1981). The digestive tract and filter-feeding apparatus of vesicomid clams are reduced (Boss & Turner 1980), suggesting that the primary source of nutrition in these bivalves is organic carbon produced by the endosymbionts.

Mussel and clam beds cover 20 x 20 m areas at the Blake Ridge seep (Van Dover et al. 2003). Extensive beds of dead clams interspersed with smaller (< 1 m diameter) clam beds or a combination of live clams and shell beds (Fig. 2) and the nearly non-overlapping distribution of clams and mussels suggests local (meter-scale or less) variations in fluid flux and chemistry within the seep site (Van Dover et al. 2003). Because the presence of clams in a chemosynthetic environment is presumed to be indicative of porewater fluids with elevated concentrations of sulfide (Olu et al. 1997), these organisms are considered 'flux indicators' (Torres et al. 2001, Sahling et al. 2002, Levin et al. 2003). Vesicomid clams at Blake Ridge live half-burrowed at the sediment surface, allowing access to available sulfide in the sediment and oxygen from seawater.

Vesicomid clams exhibit high mortalities at the Blake Ridge seep (Van Dover et al. 2003). Massive bivalve mortality has frequently been observed at cold seeps (e.g. Mayer et al. 1988, Jollivet et al. 1990, Olu et al. 1996), but the cause of such mortality is still mainly speculative. Predation is a common cause of clam mortality in shallow-water ecosystems, but recently deceased clams show no evidence of damage by crabs, octopus, or fish predation at this site (Van Dover et al. 2003). Parasitism has been suggested by

FIGURE 2A

IN SITU PHOTOGRAPHS OF THE DISTRIBUTION OF VESICOMYID
CLAMS AT THE BLAKE RIDGE SEEP SITE.



Clam bed showing distribution in small circular patches. Scale bar = 30 cm.

FIGURE 2B
IN SITU PHOTOGRAPHS OF THE DISTRIBUTION OF VESICOMYID
CLAMS AT THE BLAKE RIDGE SEEP SITE.



Pushcore sampling in a shell bed. Scale bar = 10 cm.

Ward et al. (2004) as a cause of mortality in mussel populations at the Blake Ridge site. These authors found a pathogenic viral-like parasite that invades the epithelium of the digestive tubules and results in deterioration of tissues but an investigation of parasite burdens on vesicomid clams from Blake Ridge by Mills et al. (submitted) found no evidence of pathogenic parasites in high enough abundance to influence mortalities in Blake Ridge clams.

Patchy distribution of live and dead clams may reflect underlying temporal and/or spatial heterogeneity in sulfide flux or other environmental parameters that influence the health and reproductive condition of the clams. Cessation or reduction of fluid flux from conduits in seep sediments was considered a likely cause for bivalve death at the Blake Ridge Diapir (Van Dover et al. 2003), based on the nutritional reliance of vesicomid clams on the delivery of sulfides to the seafloor. Narrow sulfide requirements of at least some vesicomid clam species (Goffredi & Barry 2002) suggest that mortality could also be due to sulfide toxicity caused by an increase in fluid flux, which interferes with the cytochrome c oxidase system of the clams. Dead clams at the Blake Ridge seep are relatively uniform in size (~ 1-2 cm) and extent of erosion of the periostracum and shell, suggesting that the die-off was synchronous and pandemic (Van Dover et al. 2003). Sulfide and methane have been detected in porewaters of sediments from the Blake Ridge diapir (Paull et al 1995), but little information is available concerning spatial gradients or patchiness of chemicals within the seep or the effect of environmental factors on the health and reproductive condition of the megafauna.

REPRODUCTIVE STRATEGIES IN SEEP BIVALVES

The deep sea has historically been considered temporally homogenous with little or no seasonal variation in physical parameters and hence it was hypothesized that inhabitants would reproduce continuously (Orton 1920, Rokop 1974). Based on gametogenic condition indices and quantitative measurements of gonadal tissue, the majority of deep-sea invertebrates studied to date do have continuous reproduction (Scheltema 1972, Rokop 1974, 1979), though this is not always the rule. Protobranch bivalves *Yoldiella jeffreysi* and *Ledella pustulosa* from the Rockall Trough (Lightfoot et al. 1979) are known to reproduce seasonally.

Within individuals, *Bathymodiolus thermophilus*, from hydrothermal vent systems on the East Pacific Rise, has a wide range of oocyte sizes indicative of asynchronous development and reproduces continuously (Tyler & Young 1999). Vesicomid and mytilid bivalves have phylogenetic constraints on reproductive processes and early life history stages (Tyler & Young 1999).

To date, seven species of vesicomid bivalves have been studied for reproductive characteristics: *Calyptogena magnifica* from the East Pacific Rise, *C. kilmeri* and *C. pacifica* from Monterey Canyon, *C. soyoae* from Japan, *C. lauberi* from the Tenryu Canyon, *C. phaseoliformis* from the Japan Trench, and *C. diagonalis* from the Cascadia subduction zone (Table 1, modified from Tyler & Young 1999). Gametogenesis within all vesicomids is intragonadal (Lisin et al. 1997). Oocytes of all species are large (~ 220 μm) in maximum diameter, indicating lecithotrophic larval development.

Among deep-sea bivalves, lecithotrophic development generally predominates and direct development and brood care are rare (60 % of bathyal species have

TABLE 1

Table 1. Environmental and reproductive variables in vesicomimid and solemyid bivalves in chemosynthetic communities

Species	Location (sulfide data)	Sulfide Range (mM)	Source (Sulfide data)	Maximum oocyte diameter (μm)	Periodicity of reproduction	Larval development	Source (Reproductive)
<i>Vesicomimid sp.</i>	Blake Ridge	0.1-3 mM	Present study	197	Asynchronous	Lecithotrophic	Present study
<i>C. magnifica</i>	EPR ¹	n/a	n/a	309	Asynchronous		Berg 1985
<i>C. kilmeri</i>	Monterey, USA	0-6 mM	Barry et al. 1997	220	Seasonal		Lisin et al. 1997
<i>C. pacifica</i>	Monterey, USA	1-2 mM	Sahling et al. 2002	220	Asynchronous		Lisin et al. 1996
<i>C. soyae</i>	Japan	0.05-0.60	Hashimoto et al. 1995	n/a	Asynchronous		Endow & Ohta 1980
<i>C. laubieri</i>	Japan	n/a	n/a	~200	Asynchronous		Fiala-Medioni & Le Pennec 1989
<i>C. phaseoliformis</i>	Japan	n/a	n/a	~200	Asynchronous		Fiala-Medioni & Le Pennec 1989
<i>Acharax sp.</i>	CCM ² , Oregon	0-0.3 mM	Sahling et al. 2002	660	Asynchronous		Beninger & Le Pennec 1997

¹ EPR = East Pacific Rise² CCM = Cascadia Convergent Margin

lecithotrophic development and 30 % direct development, 70-75 % lecithotrophy and 15 % direct development for abyssal species; Knudsen 1979). Deep-sea bivalves tend to have smaller gonads for a given shell length than those of shallow-water forms (Gage & Tyler 1991). Smaller gonads physically limit the number of larvae produced. To ensure successful recruitment in these deep-sea environments, a non planktotrophic mode of larval development is favored (Jablonski & Lutz 1983) though it is not possible to make a distinction between pelagic lecithotrophic development, direct development or brood care by the parent organism.

Seasonal variability in reproduction has been documented in some deep-sea (4000 m) organisms (see Tyler 1988 for review) and can be compared to shallow-water organisms that respond to cyclic and fluctuating external conditions based on photosynthetic input from surface waters (e.g. Navarro & Iglesias 1995). When these exogenous cues are reduced or absent, different cues (currents, tides, seismic activity, temperature, chemical flux) are supposed to influence synchronous spawning events (Fujioka et al. 1997).

All species of *Calymene* previously examined appear to spawn freely into the water column. *C. soyoae*, a cold seep clam, will spawn in response to a 0.1 to 0.2°C temperature rise in Sagami Bay, Japan. Spawning can be induced by experimentally increasing the temperature (Fujiwara et al. 1998), but the increment of temperature used was at least an order or magnitude greater than the natural thermal increment. Eleven independent natural spawning events have been observed in *C. soyoae* over a 1.5 yr period (Momma et al. 1995). Reproductive seasonality was not observed in *C. pacifica* (Berg 1985), which is consistent with other studies of vesicomid clams, indicating continuous reproduction (Gage & Tyler 1991).

It is difficult to make assumptions about the reproductive success of megafaunal communities due to a lack of time-series studies on reproduction in deep-sea bivalves on scales

appropriate to their gametogenic cycles. The only reproductive study to date that has examined gametogenesis in a time-series design during different times of the year is that of two species of cold seep clam from the Monterey Canyon, *Calyptogena kilmeri* and *C. pacifica* (600 m and 900 m).

Synchronous gametogenesis was reported for these clams, with increased reproductive effort in the late fall and spring (Lisin et al. 1997). Evidence for synchronous reproduction in chemosynthetic organisms allows for speculation on how synchrony is established and maintained in these environments. Environmental cues such as tidal or chemically generated signals have been hypothesized to be controlling factors (Zal et al. 1995).

In this study, the reproductive biology of an undescribed genus and species of vesicomyid clam was investigated. Reproductive condition was used as a proxy for health and condition of clams in relation to sediment geochemistry at the Blake Ridge seep.

METHODS

SAMPLING AND INITIAL CORE PROCESSING

Fifty-three pushcores (6.35-cm inner diameter, 30-cm length) were collected at the Blake Ridge Diapir (32° 29.623'N, 76°11.467'W; 2155 m; July-August 2003; Fig. 1B) using the deep-sea submersible *Alvin*. Multiple pushcores were collected from discrete areas of live clams or clam shells, as defined by submersible observations at the time of sampling (Fig. 2B).

Pushcores from clam beds were further categorized on deck based on the relative proportion of live clams to clam shells (see Appendix 1). Pushcores were also collected from non-seep, background sediments devoid of vesicomid shells or other flux indicators. Five of these background samples were taken at the erosional face of the Blake Ridge depression, ~ 2 km from the active seep site. Three background cores were taken within 1-3 m of active seeps at the Blake Ridge Diapir. Sampling effort was thus: clam bed, 9 pushcores; clam/shell bed, 2 pushcores; shell bed, 10 pushcores; background sediments, 5 pushcores. Penetration depths of pushcores varied from 11 cm to 28 cm.

Cores were stored in a cold room (4°C) on recovery and vertical redox potential (Eh in mV) profiles (1-cm intervals) were obtained from undisturbed cores by inserting a UNISENSE platinum Eh electrode (0.5 mm diameter; 3 mm length; Moore et al., 1993) through the sediment surface. Redox potential was read on a portable pH-millivolt meter (Denver Instruments)

connected to a saturated calomel electrode suspended in the overlying water. Values were corrected to the hydrogen reference electrode scale by adding +244 mV to each measurement (Bagander & Niemisto 1978). Calibration of the electrodes was verified by measuring the redox potential of quinhydrone dissolved in buffers (pH 4, 7; Bohn 1971).

After the Eh profiles were taken, pushcores were sub-sampled for grain-size analyses, organic carbon and nitrogen content, sulfide and sulfate concentrations. Each core was sectioned into 2-cm intervals and extruded under nitrogen. For pushcores with layers of live clams and/or clam shells at the sediment-water interface, the "0" index was the surface of the sediment layer underlying the shells.

Only a subset of the pushcores collected was analyzed for a suite of geochemical and physical parameters (i.e., grain size, organic carbon and total nitrogen (i.e. ammonia and organic N), sulfide, sulfate and mineral sulfides (AVS + CRS); Appendix 1).

GEOCHEMICAL AND PHYSICAL ANALYSES

Porewaters were squeezed from 2-cm sections of cores using Reeburgh-style squeezers (Reeburgh 1967) and collected in 7 mL vacutainers purged with nitrogen and pretreated with 0.3 mL of saturated zinc acetate solution. Porewater sulfide (Cline 1969) and sulfate concentrations (Gieskes et al. 1991) were analyzed at the University of Virginia. Analytical precision was within $\pm 5\%$ for both methods.

Sediment samples (1 cc) were analyzed for acid volatile sulfides (AVS: particulate FeS and residual dissolved H₂S) and chromium reduced sulfides (CRS: particulate FeS₂ and elemental sulfur) from pushcores after porewater extraction. Mineral sulfides (AVS + CRS) were analyzed at the College of William & Mary's Keck Laboratory. A 1 normal HCl extraction

was used for acid volatile sulfides. A boiling hot chromium concentrated acid extraction was used for chromium reduced sulfides. Both extractions were followed by an analyses of the sulfide trap (Cline 1969).

Sediment sub-samples for grain-size analyses were taken from the 0-1 cm interval after extraction of porewaters. Sediments were dried and homogenized. Percent sand, silt, and clay were determined by sieving and gravimetric techniques.

Dried, homogenized sediment from the surface 0-1 cm interval (~ 15 mg was acidified in silver capsules with 10 % HCl to remove inorganic carbonates and dried) was analyzed for total organic C (TOC) and total N (TN) content using an EA 1108 Elemental Analyzer (GV Instruments, Manchester, UK) at the Virginia Institute of Marine Science.

BIOLOGICAL ANALYSES

The top section of cores containing clams and/or shells was washed through a 65 μm sieve. All large clams (> 5 mm length; including shells) and shells were removed, measured (shell length, ± 0.1 mm) and live clams were counted. Clam tissues were fixed in Davidson's solution (Humason 1972) for 24 h, and then stored in 70% ethanol. Material retained on the sieve was preserved in 10% buffered formalin, stored in 70% EtOH, and subsequently sorted under a dissecting microscope to enumerate juvenile clams (< 5 mm).

Subsets of 38 female and 24 male adult individuals were selected from among the largest clams (11 – 25 mm shell length) within pushcores for histology (see Appendix 1). Tissue slices taken from the mid-body region (where gonadal tissue is concentrated) were dehydrated through a graded ethanol series and embedded in paraffin. Transverse sections (6 μm) were stained with Gill's hematoxylin and eosin (H&E; Stevens 1990).

Gonadal tissues were analyzed under light microscopy with a Zeiss Axioskop 2 compound microscope (females) or an Olympus SZX12 dissecting microscope (males). Oocyte feret diameter (the theoretical diameter of an object if it were spherical in shape) and volume in females, and areas of somatic and reproductive tissues in males were determined from digital photographs taken with a Diagnostic Instruments Spot camera, using Sigma Scan Pro 2.0 software. Oocyte characteristics were analyzed in 3 histological sections from each female. The sections were systematically selected from the mid-dorsal region and were separated by 200 μm . All pre-vitellogenic and vitellogenic oocytes with the nucleus and nucleolus evident (Pearse 1965) were characterized, counted, and measured on each section (i.e., 50 to 150 oocytes per individual). From these data, mean oocyte diameters and oocyte size-frequency distributions were determined. A gonadal index (modified from Kennedy 1977, Eversole 1997) was used to rank different developmental stages of gametogenesis within an individual: inactive, early developing, developing, ripe, and spent). Somatic and reproductive areas in males were measured from single histological sections from the mid-dorsal body region and were used to calculate the relative proportion of reproductive to somatic tissue (referred to herein as % gonad).

The periostracum of the adult clam shell was observed with an Olympus SZX12 dissecting microscope.

STATISTICAL ANALYSES

Data collected from individual pushcores for percentage composition of sand, silt and clay, and organic carbon and total nitrogen were square-root transformed and analyzed using one-way ANOVAs and Tukey's post-hoc analysis (MINITAB) to investigate differences among

the four microhabitats sampled. Statistical analyses, using a stratified ANOVA (two error terms were tested –“within” clam and “among” clam variance. The residual error term from the “among” clam variance was used) as part of the R-software system (Ihaka & Gentleman 1996), were used to test for correlations between biological parameters and sulfide concentration in all pushcores.

RESULTS

SEDIMENT CHARACTERIZATION BY HABITAT

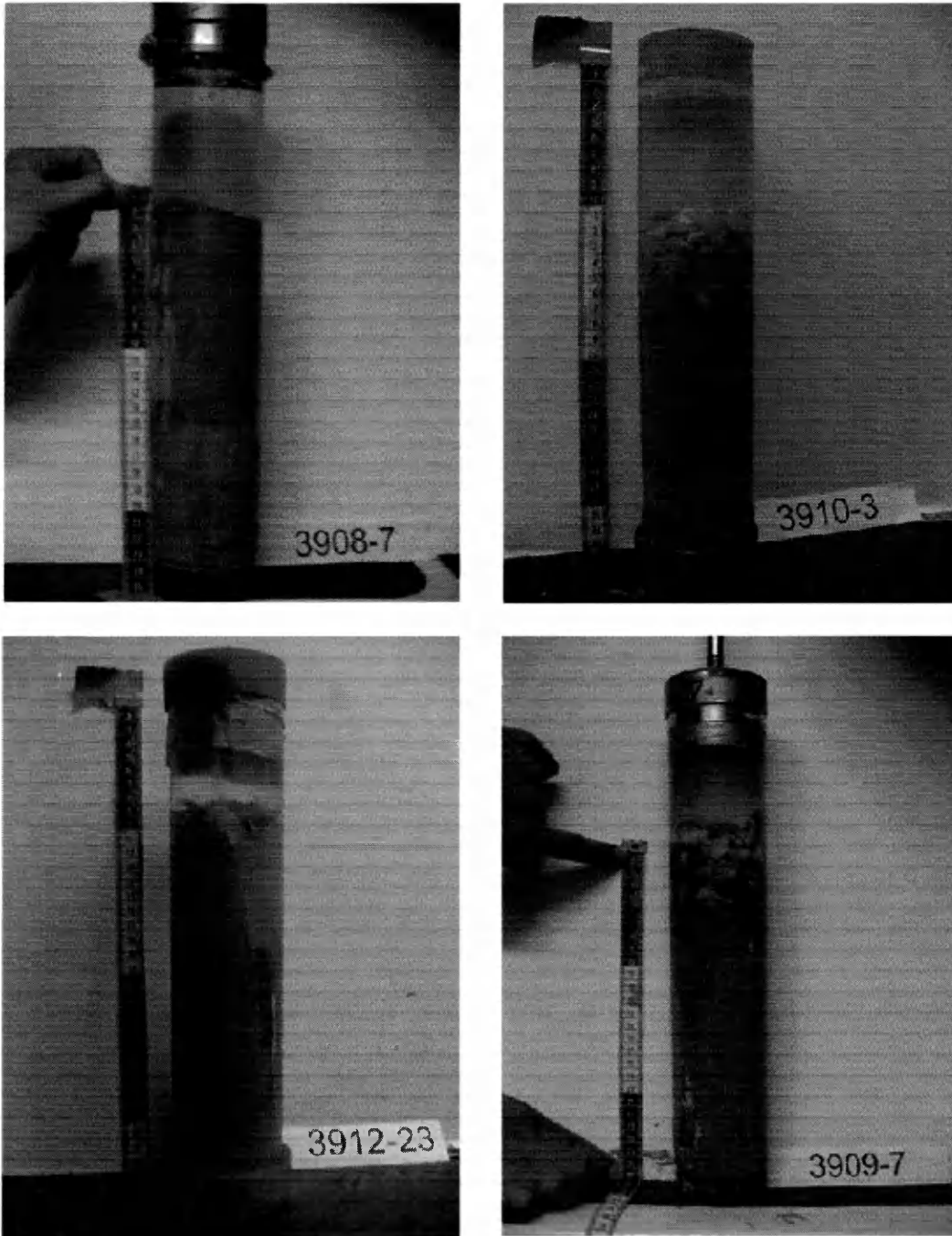
Sediments in pushcores from the 4 different microhabitats (background, clam beds, clam/shell beds and shell beds) were distinct (Fig. 3). Layers of light brown or tan sediments in pushcores from background sites suggested oxidizing conditions, which was confirmed by Eh values between 0 and +200 mV throughout the core (Fig. 4A). Sulfide concentrations in porewaters of background sediments were low, ranging from 0.7 to 19.3 μM (mean \pm s.d.: $3.5 \pm 4.28 \mu\text{M}$); sulfide concentrations $>10 \mu\text{M}$ were restricted to the 7+ cm layers. Porewater sulfate concentrations ranged from 14.2 to 33.1 μM ($4.1 \pm 4.67 \mu\text{M}$).

Pushcores from live vesicomid clam beds had 2-to-4 cm-thick layers of clams. Clams were stacked on one another, with no consistent orientation (i.e., siphons were not necessarily oriented vertically into the water column), and were free of any covering of sediment. Clam density (clams > 5 mm shell length) per pushcore ranged from 9 to 48 individuals per pushcore. Clam shells were present but rare in the clam-bed pushcores. Sediment immediately beneath the clams (~ 0 -2 cm interval) was light-brown, followed by a steep gradient to dark-brown-black coloration. A typical Eh profile for pushcores from clam beds included a 0-2 cm oxidizing layer, with reducing conditions below 2 cm (Fig. 4B). Three of 23 pushcores from clam beds were

completely reducing throughout the sediment profile. Sulfide concentrations in porewaters below 3 cm ranged from 0.01

FIGURE 3

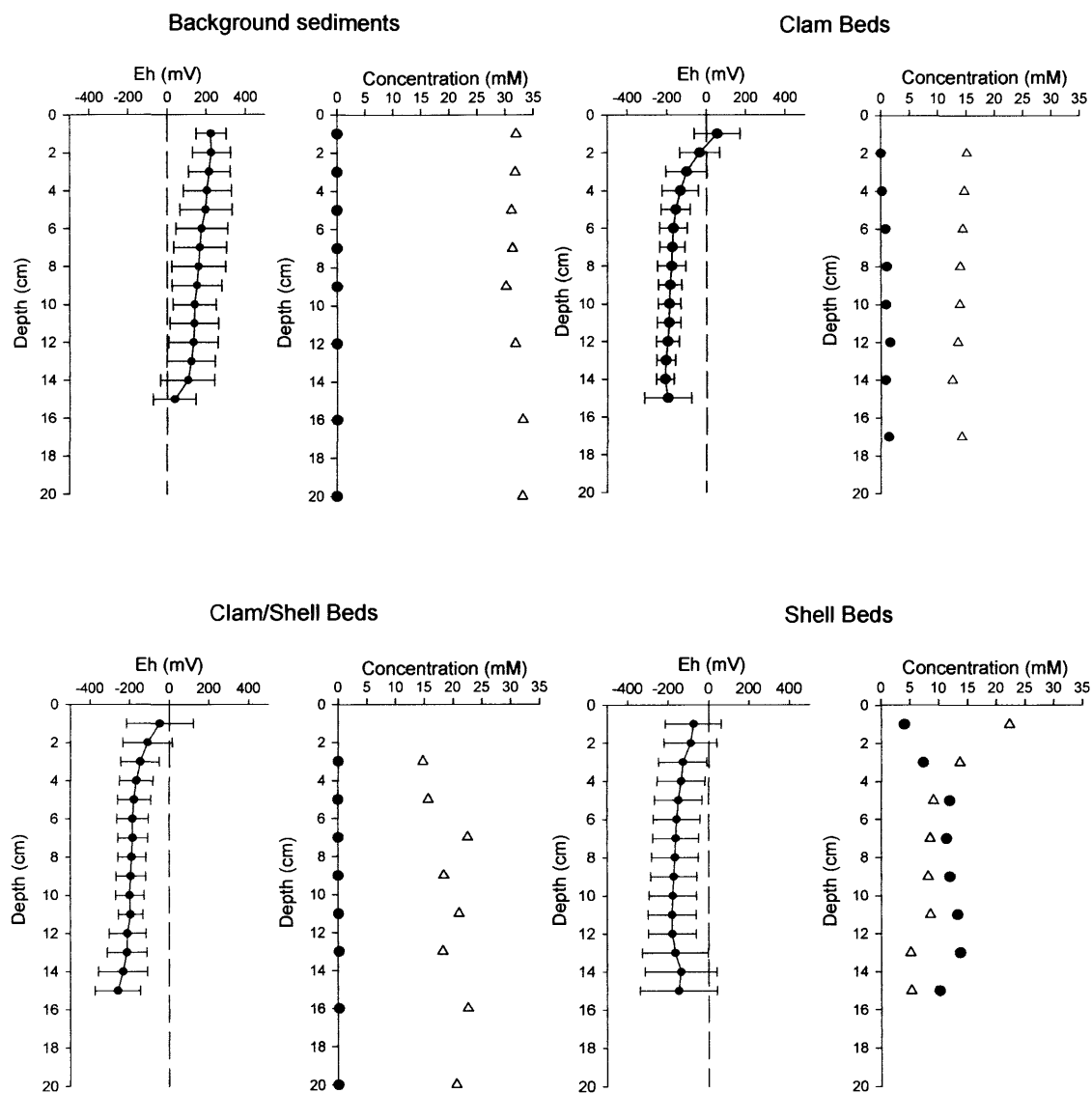
PHOTOS OF REPRESENTATIVE PUSHCORES FROM 4 MICROHABITATS AT BLAKE
RIDGE



(A) Background. (B) Clam beds. (C) Clam/shell beds. (D) Shell beds.

FIGURE 4

REDOX POTENTIAL, SULFIDE, AND SULFATE CONCENTRATION (mM) PROFILES IN REPRESENTATIVE PUSHCORES FROM 4 MICROHABITATS AT BLAKE RIDGE



Redox potential [Eh (mV) \pm s.d.], sulfide (●), and sulfate (Δ) concentration (mM) profiles in representative pushcores from 4 microhabitats at the Blake Ridge seep. (A) Background cores. (B) Clam beds. (C) Clam/shell beds. (D) Shell beds.

to 3.6 mM (1.4 ± 1.95 mM), except in core #3910-1, where porewater sulfide concentrations were as high as 8.8 mM at depth and were also high (1.3 mM) at the sediment-seawater interface. The sulfide maximum in clam bed pushcores was always at or below 10 cm. Sulfate concentrations ranged from 10 to 33 mM (18.0 ± 4.24 mM) and typically varied by only a few mM with depth within a given core. Sulfide and sulfate concentrations in core #3910-1 did not match this pattern; instead, sulfate concentrations decreased as sulfide concentrations increased down the core.

The 2 pushcores from clam/shell beds contained mostly shells. The shells formed layers up to 4 cm thick on the sediment surface, and always included 1 to 10 live individuals > 5 mm. One of these pushcores (# 3912-23) contained the highest density of juveniles (47 ind.) of any pushcore from Blake Ridge. The sediment was light-brown beneath the shells and extended to depths of ~8 cm before turning dark brown-black. Eh profiles indicated that the reducing zone began at 1 cm below the shell layer (Fig. 4C). Porewater sulfide concentrations ranged from 0 to 1.1 mM (0.3 ± 0.39 mM). Sulfate concentrations ranged from 14 to 23 mM (18.7 ± 2.71 mM).

Of the 17 pushcores from shell beds, 15 contained only shells, and 2 contained 1 live clam (> 5 mm) each. Shell layers in these pushcores were dusted with sediment and were thick, up to 8 cm, with broken shells mixed with sediment at depth. The periostracal layer of the shells were abraded in patches extending from the umbo region to the edge of the shell, but the shells on the surface of the sediment were not markedly dissolved, nor did they show signs of predation. No light-colored sediments were observed in pushcores from the shell-bed habitat, with the exception of 1 anomalous pushcore (#3909-10, discussed separately below). Eh profiles indicate the sediments were reducing throughout the cores from the shell beds (Fig. 4D). Sulfide

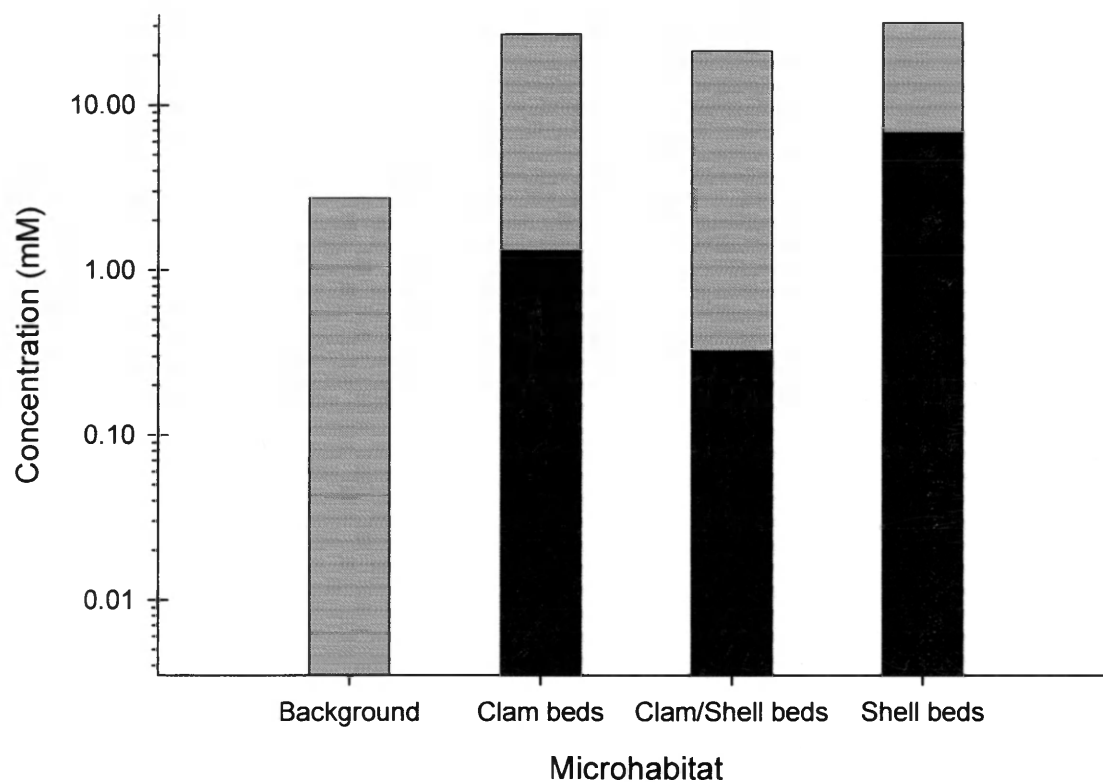
concentrations in the surface sediment layers of these cores were four times higher than levels observed in clam-bed surface sediments (3.3 to 12.1 mM in 6 of the 8 pushcores from shell beds). Sulfate concentrations in shell beds decreased from the surface to ~5 cm and sulfide concentrations increased from the surface to ~5 cm. Porewater sulfide concentrations below the sulfate reduction zone in shell-bed sediments were an order of magnitude higher than in any other pushcore sediments, up to 17.5 mM. Sulfate concentrations below the sulfate reduction zone in pushcore sediments from shell beds were typically < 1mM, i.e., an order of magnitude lower than those observed from other sample sites. The anomalous pushcore #3909-10 had sediment coloration and porewater chemistry characteristics that matched those of pushcores from live clam beds, but 3909-10 contained no live clams.

Average porewater across all depth intervals and mineral sulfides (FeS and FeS₂) from top (1 cm) and bottom (8 cm) intervals within the four microhabitats sampled were significantly different ($p < 0.05$; Fig. 5A) but there was no significant difference in mineral sulfides among the four microhabitats.

Sulfide retention is the amount of sulfide held within sediment layers (not diffused into the water column or lost through uptake of megafaunal organisms). Retention correlations from Blake Ridge show a greater retention of sulfide within shell beds with the exception of one anomalous pushcore (3909-10) that was sampled in a shell bed but falls along the regression line for sulfide retention in clam beds (Fig. 5B). Pushcores from clam beds have lower sediment sulfide retention with the exception of another anomalous pushcore (#3910-1) that falls along the regression line for shell beds but had 20 adult clams.

FIGURE 5A

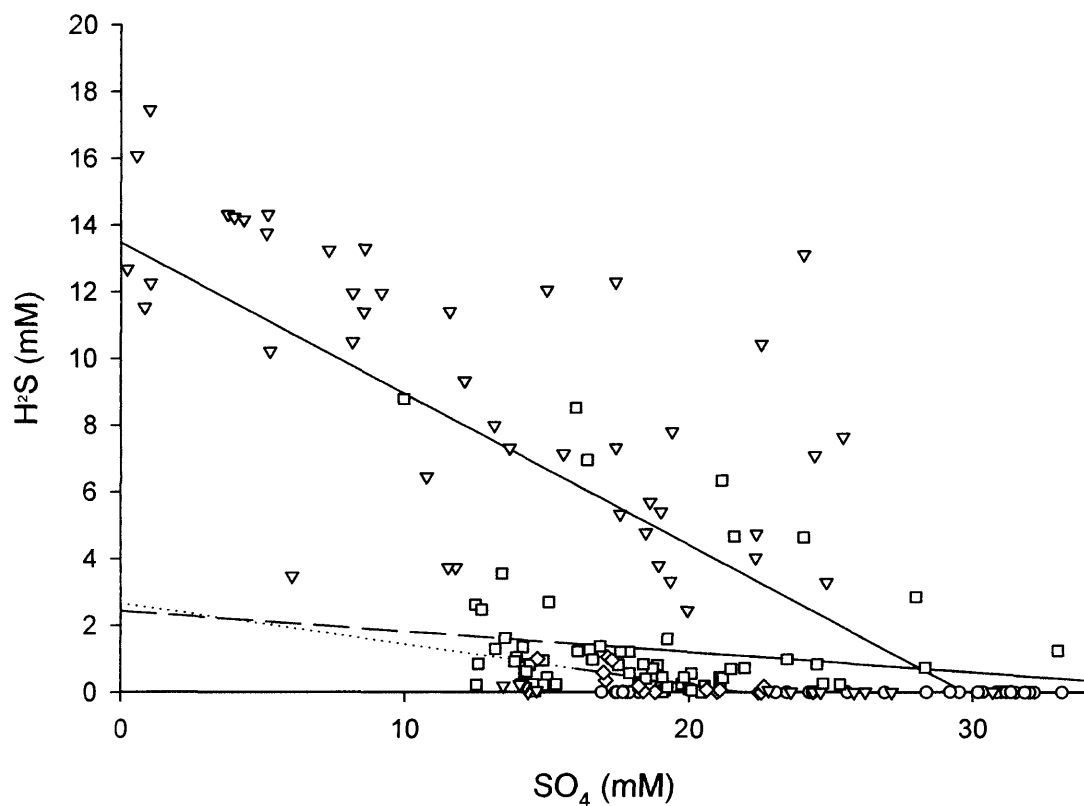
CONCENTRATIONS OF POREWATER AND MINERAL SULFIDES ACROSS ALL INTERVALS WITHIN PUSHCORES FROM 4 MICROHABITATS AT BLAKE RIDGE



Average concentrations of porewater sulfide and mineral sulfides (AVS + CRS) across all intervals within pushcores from 4 microhabitats at the Blake Ridge seep: ■ = porewater sulfide from Background cores (n = 37 intervals in 5 pushcores), Clam beds (n = 68 intervals in 9 pushcores), Shell beds (n = 59 intervals in 2 pushcores), Clam/Shell beds (n = 14 intervals in 10 pushcores). ▨ = Mineral sulfides from Background cores (n = 2), Clam beds (n = 2), Shell beds (n = 2), Clam/Shell beds (n = 2).

FIGURE 5B

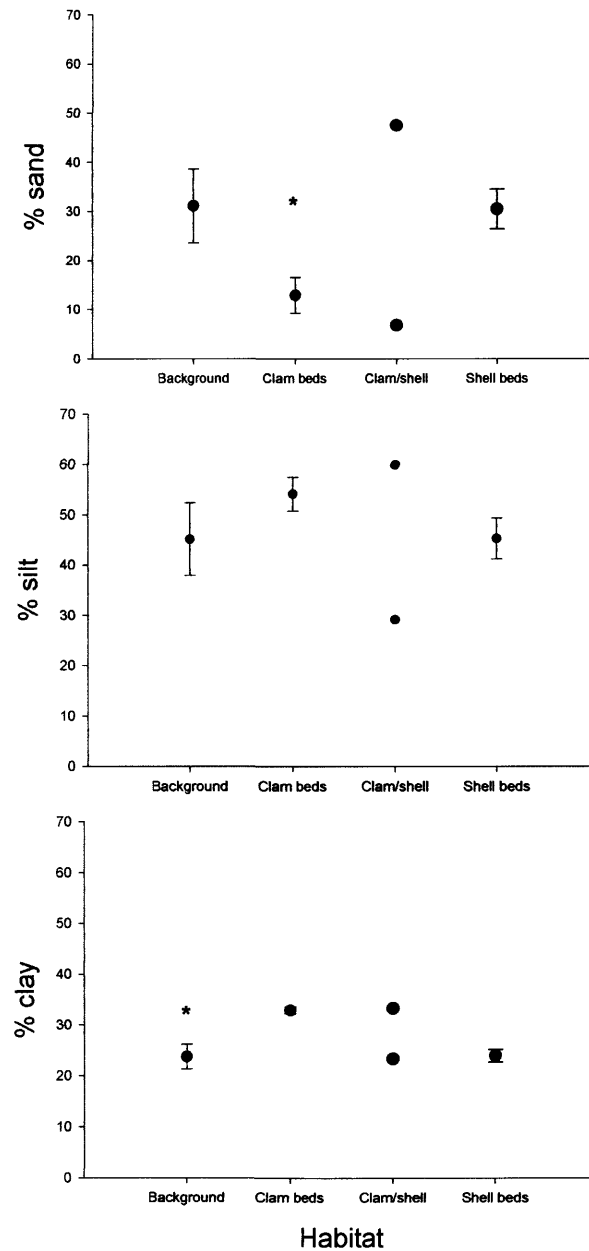
SULFIDE RETENTION AT THE 4 MICROHABITATS AT BLAKE RIDGE



Sulfide retention in Background sites (\circ), Clam beds (\square), Clam/Shell beds (\diamond) and Shell beds (Δ) from the Blake Ridge seep. Dashed regression line through Clam beds, dotted regression line through Clam/shell beds, Solid regression line through Shell beds.

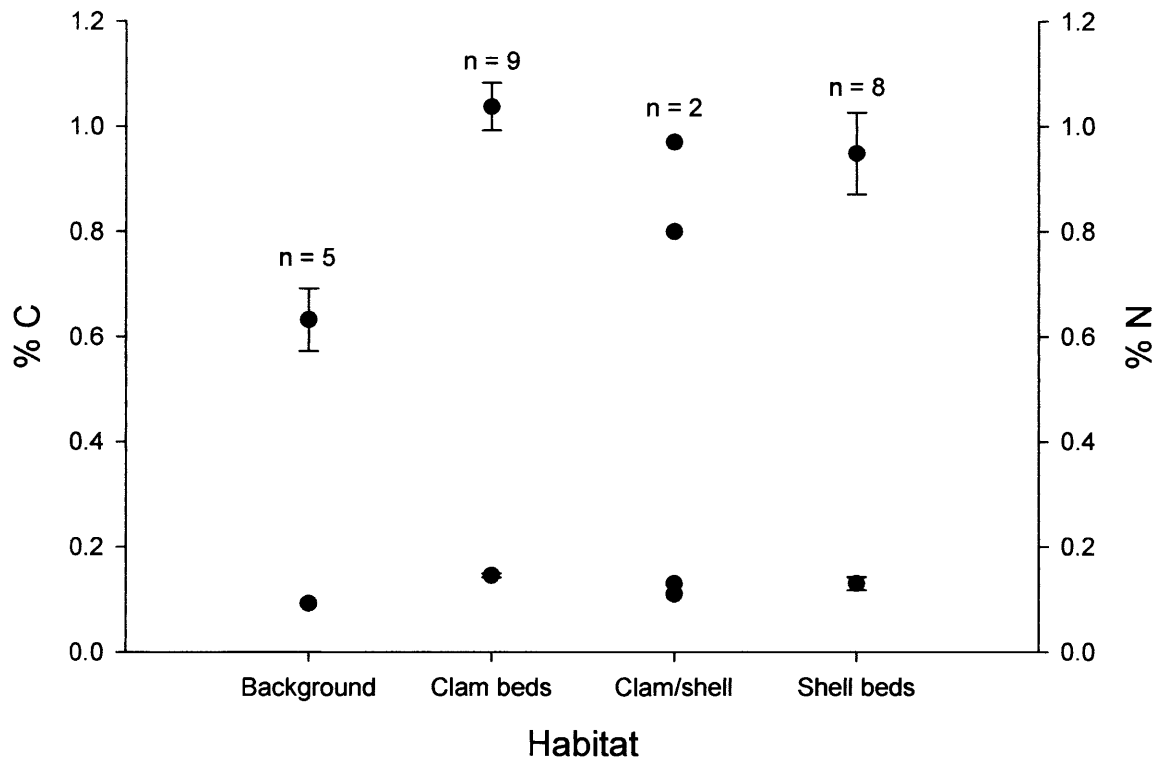
FIGURE 6

GRAIN SIZE FOR SEDIMENTS SAMPLED FROM BLAKE RIDGE



Grain size for sediments sampled from the Blake Ridge seep [% sand, silt and clay \pm s.e.].
 Background sites (n = 5), Clam beds (n = 9), Clam/shell beds (n = 2),
 Shell beds (n = 7).

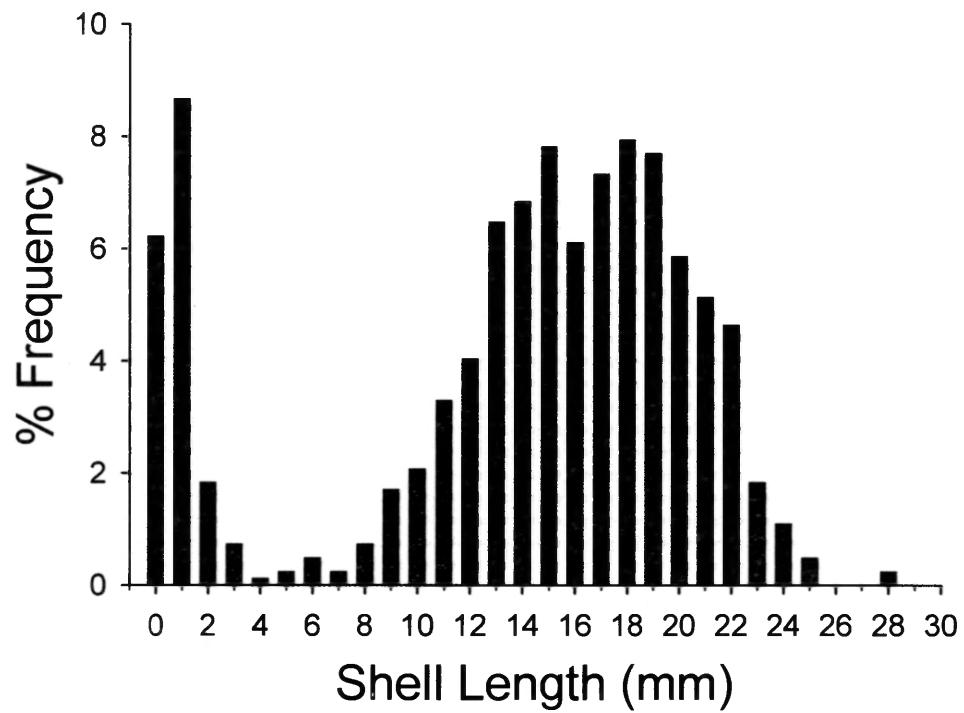
FIGURE 7
PERCENT ORGANIC CARBON AND TOTAL NITROGEN IN BLAKE RIDGE
SEDIMENTS



Percent organic carbon and total nitrogen in sediments analyzed from the Blake Ridge seep (\pm s.e). Background sites (n = 5), Clam beds (n = 9), Clam/shell beds (n = 2), Shell beds (n = 8).

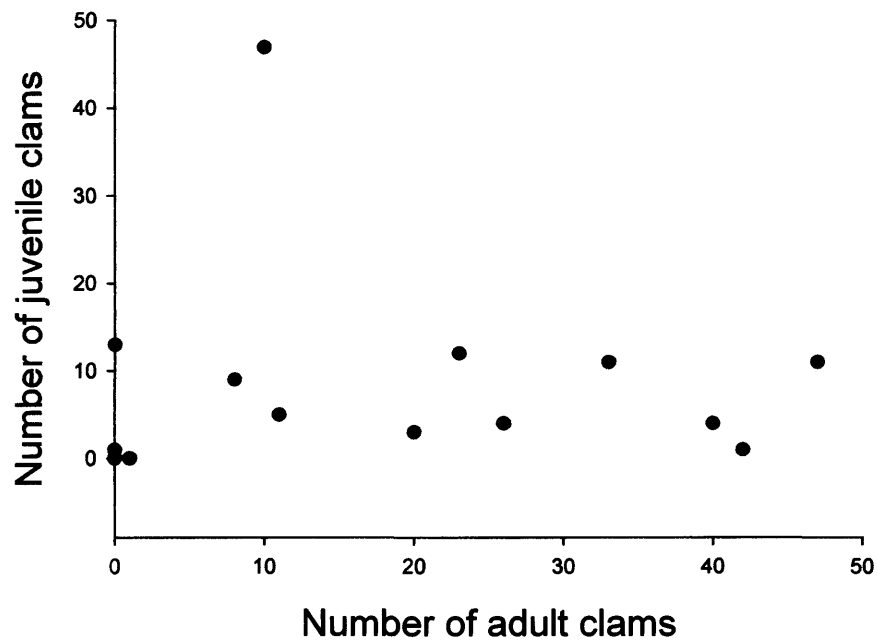
FIGURE 8

SIZE-FREQUENCY DISTRIBUTION OF VESICOMYID CLAM SHELL LENGTHS



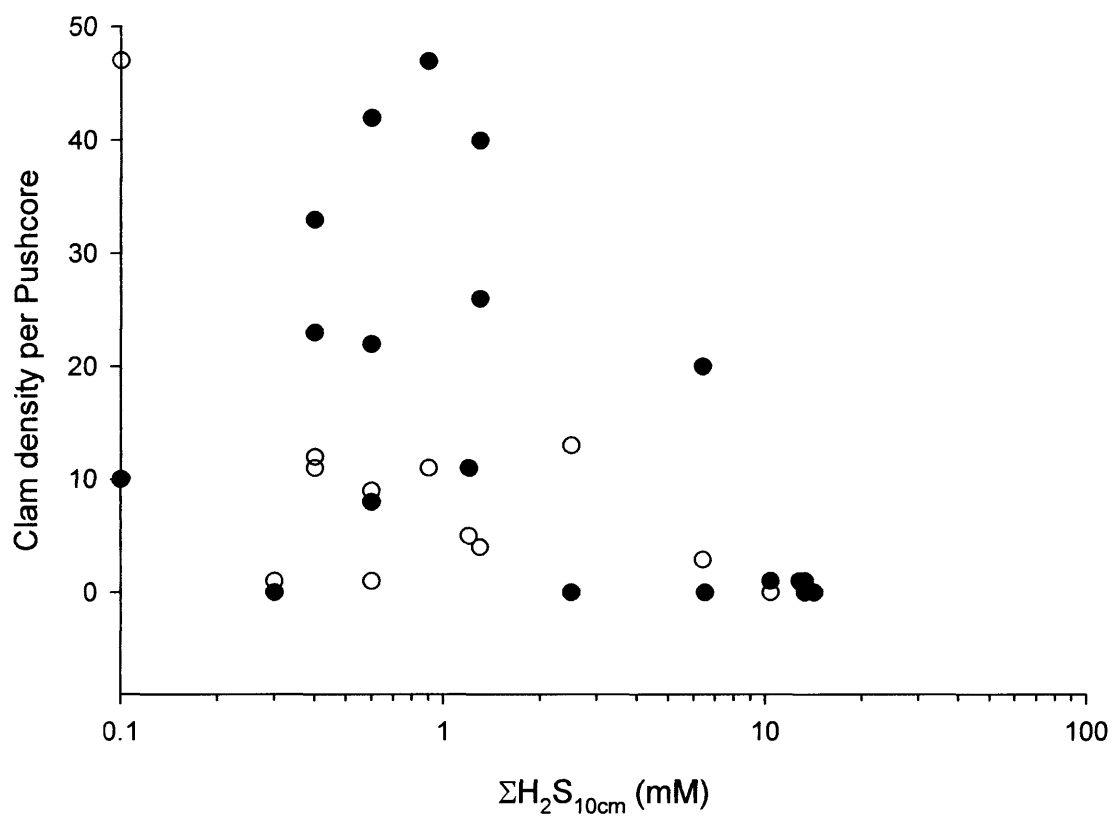
Vesicomyid clams from Blake Ridge. Size-frequency distribution of clam shell lengths (total $n = 818$) from Clam beds ($n = 9$), Clam/shell beds ($n = 2$) and Shell beds ($n = 2$).

FIGURE 9A
VESICOMYID CLAMS FROM BLAKE RIDGE



Relationship between number of juvenile clams (< 5 mm) and adult clams (> 5 mm) per pushcore.

FIGURE 9B
VESICOMYID CLAMS FROM BLAKE RIDGE



Relationship between clam density per pushcore and ΣH_2S_{10cm} . Adult clams (●), Juvenile clams (○).

As discussed above, sulfide concentrations in the clam habitat (defined as the upper 3 cm of sediment through which the clam foot extends) were low, ranging from 0.0 to 1.3 mM (0.3 ± 0.51 mM). In two pushcores, sulfide concentrations in the upper 3 cm were much higher: 5.3 mM (#3909-7; 1 clam) and 7.8 mM (#3910-8; 1 clam). We chose to use the sulfide concentration at 10 cm depth within each core (herein referred to as $\Sigma\text{H}_2\text{S}_{10\text{cm}}$) as the reference sulfide concentration for all subsequent analyses because there were low variances associated with sulfide concentrations in the porewaters of surface sediments.

Adult clam densities were low (10 ind/core) in the one pushcore where the $\Sigma\text{H}_2\text{S}_{10\text{cm}}$ was lowest (0.1 mM), and clams were absent where $\Sigma\text{H}_2\text{S}_{10\text{cm}}$ was > 13 mM (Fig. 9B). Adult clam density was maximal (48 ind/core) at ~ 1 mM; densities were variably low (8 ind/core) to high (to 47 ind/core) between 0.4 and 1.1 mM $\Sigma\text{H}_2\text{S}_{10\text{cm}}$ (Fig. 9B). Juvenile densities were maximal (47 ind/core) in the core where $\Sigma\text{H}_2\text{S}_{10\text{cm}}$ was lowest and low at other $\Sigma\text{H}_2\text{S}_{10\text{cm}}$ concentrations (Fig. 9B). The greatest number of juveniles collected was from one of the pushcores designated a clam/shell bed. One pushcore from shell beds had a higher number (13 ind.) of juveniles but the $\Sigma\text{H}_2\text{S}_{10\text{cm}}$ concentrations from this core were low (2.5 mM) relative to the other shell beds.

There was no significant correlation between shell length and $\Sigma\text{H}_2\text{S}_{10\text{cm}}$ in the sub-sample of clams from Blake Ridge (Fig. 10).

CLAM REPRODUCTIVE CHARACTERISTICS

General reproductive anatomy and condition: Vesicomid clams from Blake Ridge seeps are gonochoric, with no histological evidence of hermaphroditism. The gonad is the largest organ in the visceral mass of the clam. It surrounds the remnants of the digestive system, which have been reduced to a single tubule. The gonad extends from the dorsal epithelium to the foot

muscle and consists of acini surrounded by interacinal tissue (Fig.11). Within acini, developing gametes are arranged peripherally around a central lumen. There was no evidence for follicle cells associated with oocytes in females at the level of light microscopy. Female gametes were categorized into 3 stages: pre-vitellogenic (~ 15 to 30 μm ; dark-pink-staining with H&E), and 2 vitellogenic stages (early vitellogenic: ~ 30 to 60 μm , pink-staining, fine granules inferred to be yolk; late vitellogenic: ~60 to 200 μm with large, light-pink-staining granules). The earliest gametogenic stages, the oogonia (< 15 μm) were too small to be distinguished reliably from other cell types. Pre-vitellogenic and vitellogenic gametogenic stages were found within each female acinus (Fig. 12A), although the relative proportions of these stages varied within individuals. Only mature sperm were observed in male acini of the specimens examined (Fig. 12B).

Five different stages of reproductive condition were observed in female clams from Blake Ridge (Fig. 13A-E): "inactive": dominated by small (<25 μm), oogonia; "early-developing": dominated by pre-vitellogenic oocytes; "developing": dominated by pre-vitellogenic and early vitellogenic oocytes; "ripe": dominated by late vitellogenic oocytes; "spent": central lumens of acini are expansive; interacinal tissue (epithelium surrounding gonad) structure is disrupted; vitellogenic oocytes are rare. In some ripe individuals (3 of 37), there was evidence for partial release of gametes (i.e., a portion of the gonad appeared to be spent). Most females examined were in the "developing" to "ripe" reproductive stages (Fig. 14). There was no detectable variation in reproductive condition in male clams from Blake Ridge; all males were in a "ripe" condition, i.e., with only mature sperm (Fig. 12B). Percent gonad ranged from 9 to 38 % in males. There was no correlation between % gonad and shell length for males (stratified ANOVA; $p > 0.05$).

FIGURE 10

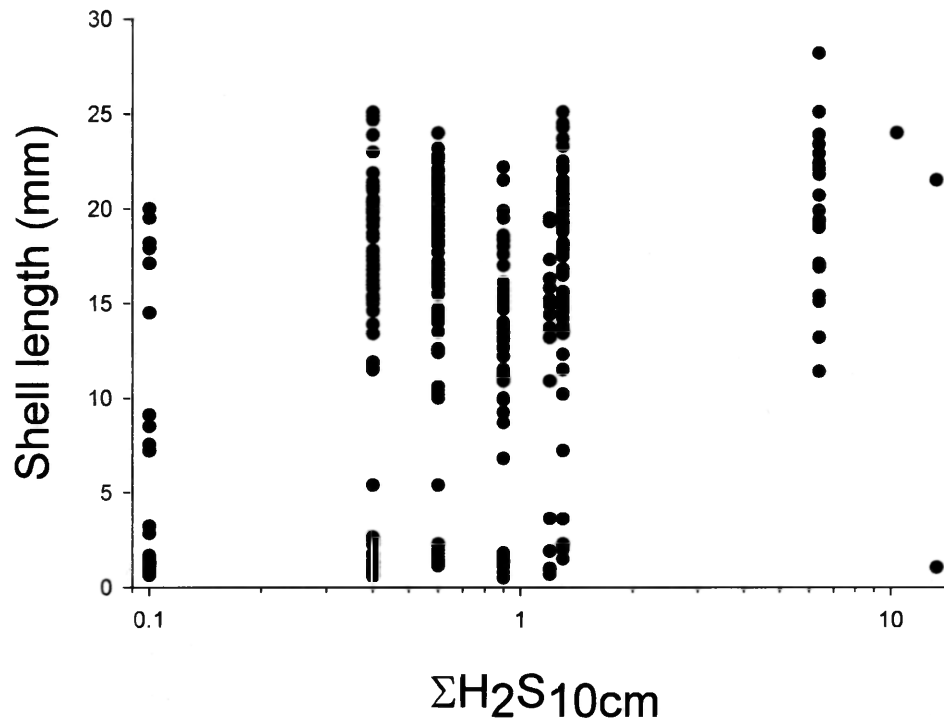
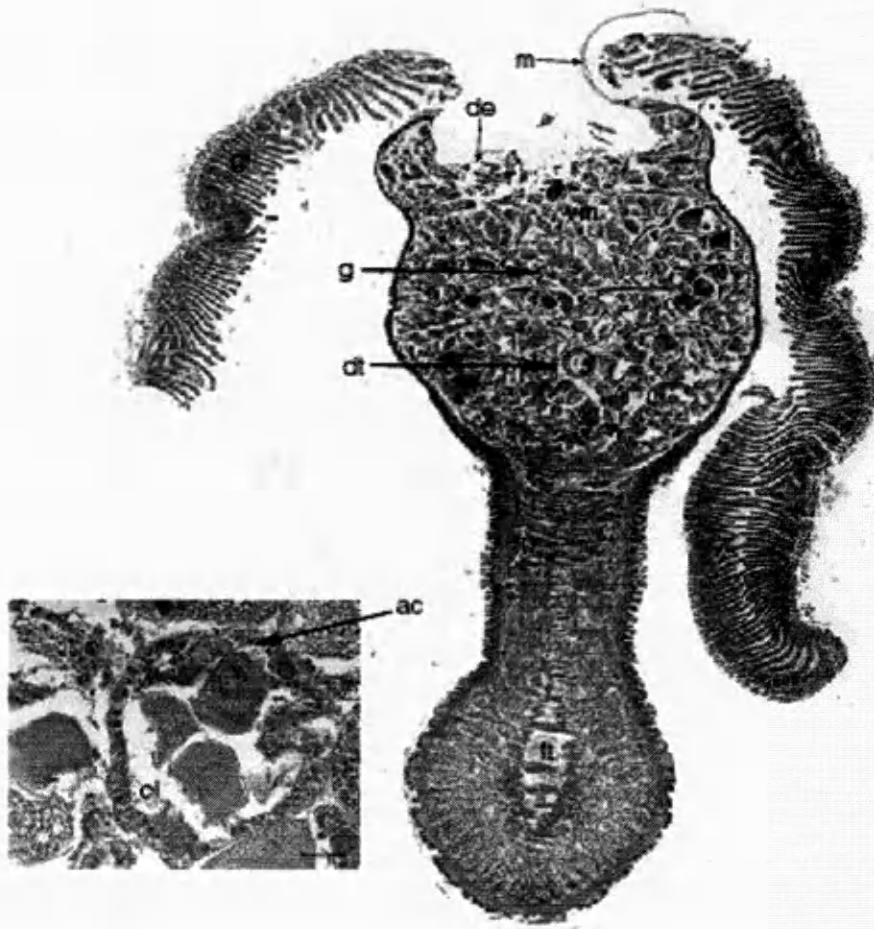
RELATIONSHIP BETWEEN CLAM SHELL LENGTH (mm) AND $\Sigma\text{H}_2\text{S}_{10\text{cm}}$ Vesicomyid clams from Blake Ridge. Relationship between shell length (mm) and $\Sigma\text{H}_2\text{S}_{10\text{cm}}$.

FIGURE 11

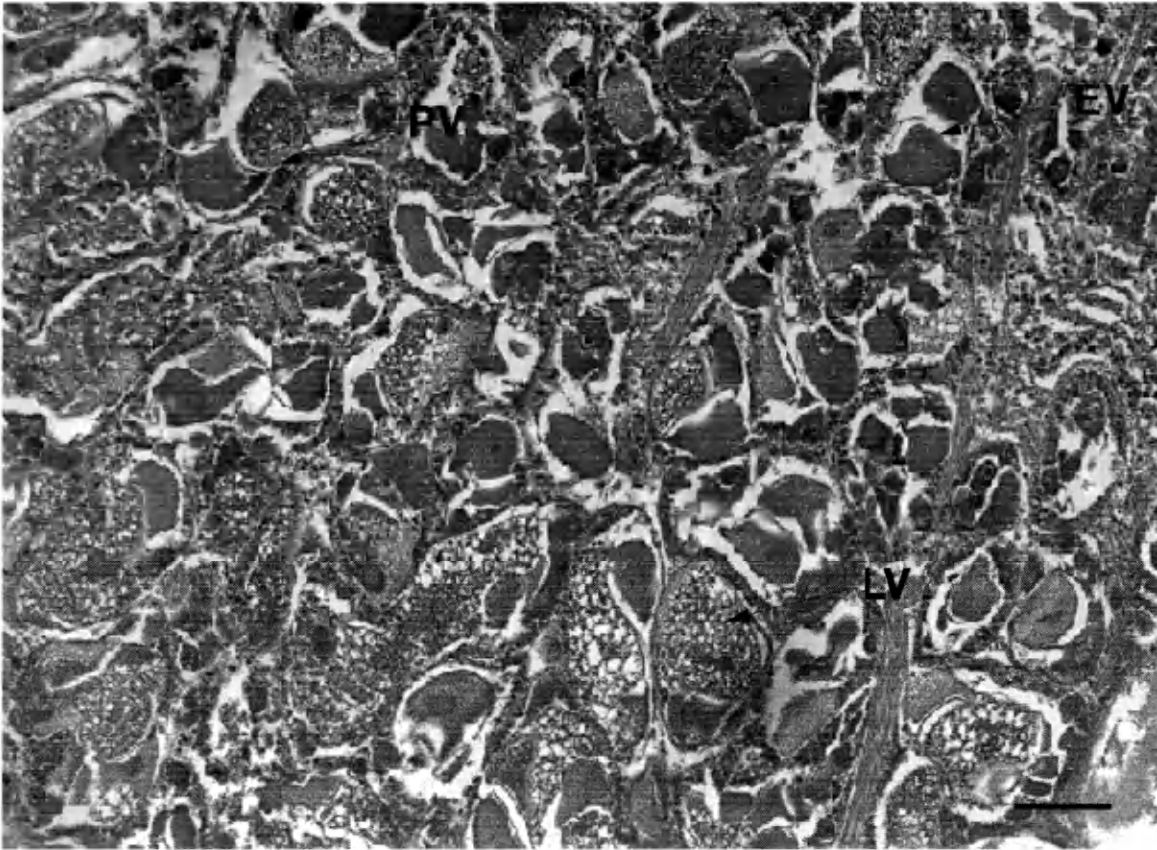
General anatomy of vesicomyid clams from Blake Ridge



Cross-section through the mid-dorsal region of a clam. Digestive tubule (dt); dorsal epithelium (de); foot (ft); gills (gl); gonad (g); interacinal tissue (it); mantle (m); visceral mass (vm). Scale bar = 150 μm Insert: Gonad of female vesicomyid clam. Acinus (ac); central lumen (cl). Scale bar = 20 μm .

FIGURE 12A

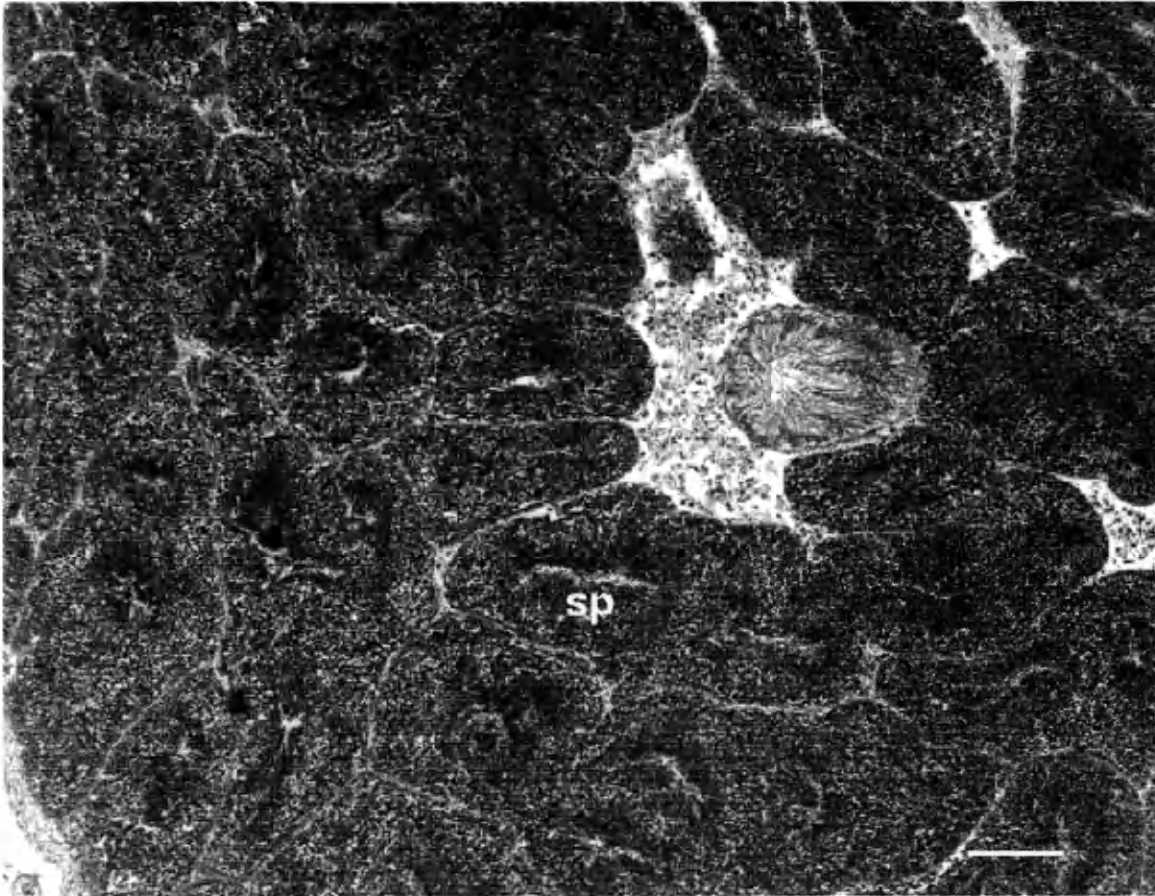
REPRODUCTIVE ANATOMY OF VESICOMYID CLAMS FROM BLAKE RIDGE.



Female gonad: pre-vitellogenic oocytes (pv); early vitellogenic oocytes (ev); late vitellogenic oocytes (lv).

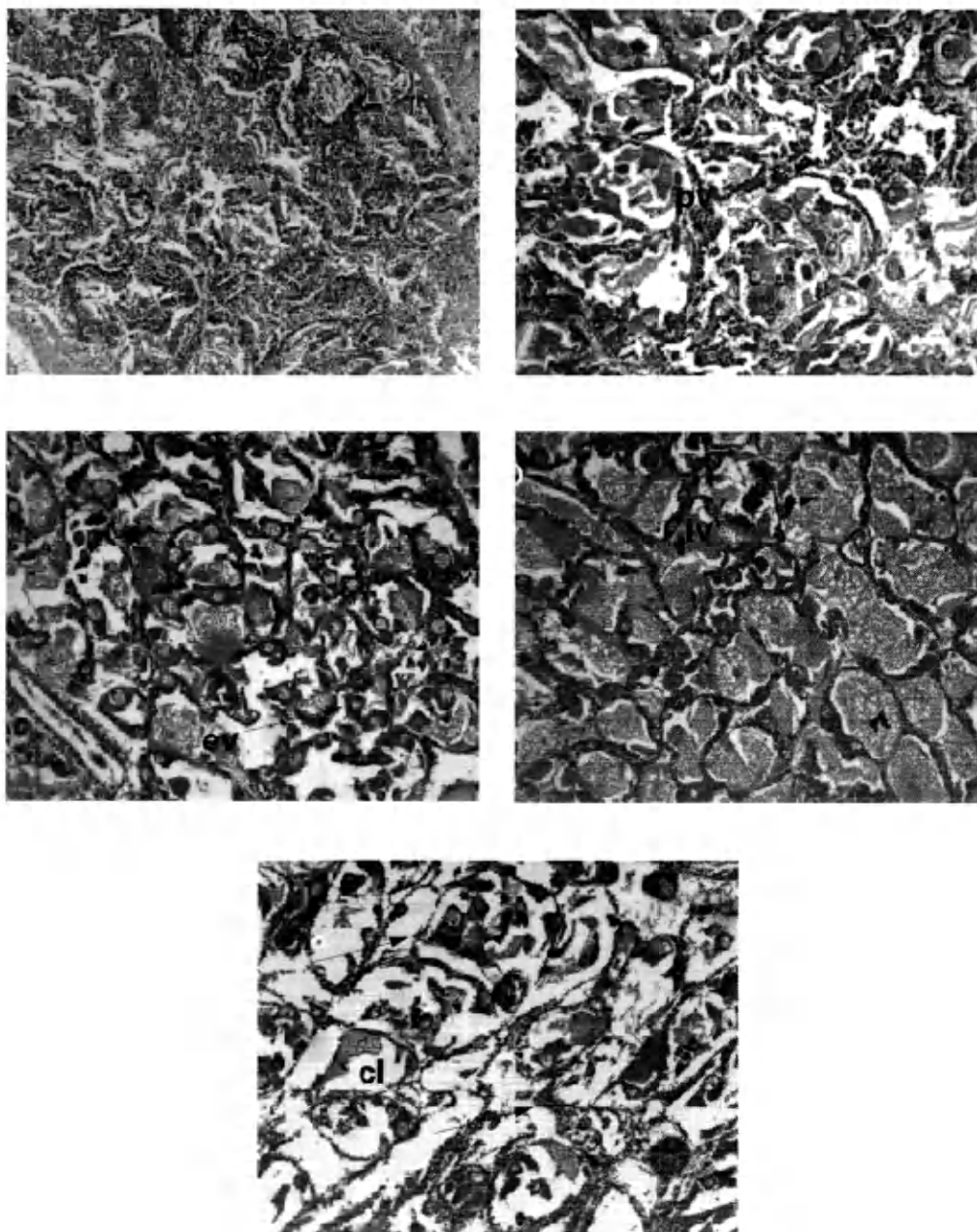
FIGURE 12B

REPRODUCTIVE ANATOMY OF VESICOMYID CLAMS FROM BLAKE RIDGE.



Male gonad: mature sperm (sp). Scale bars = 150 μ m.

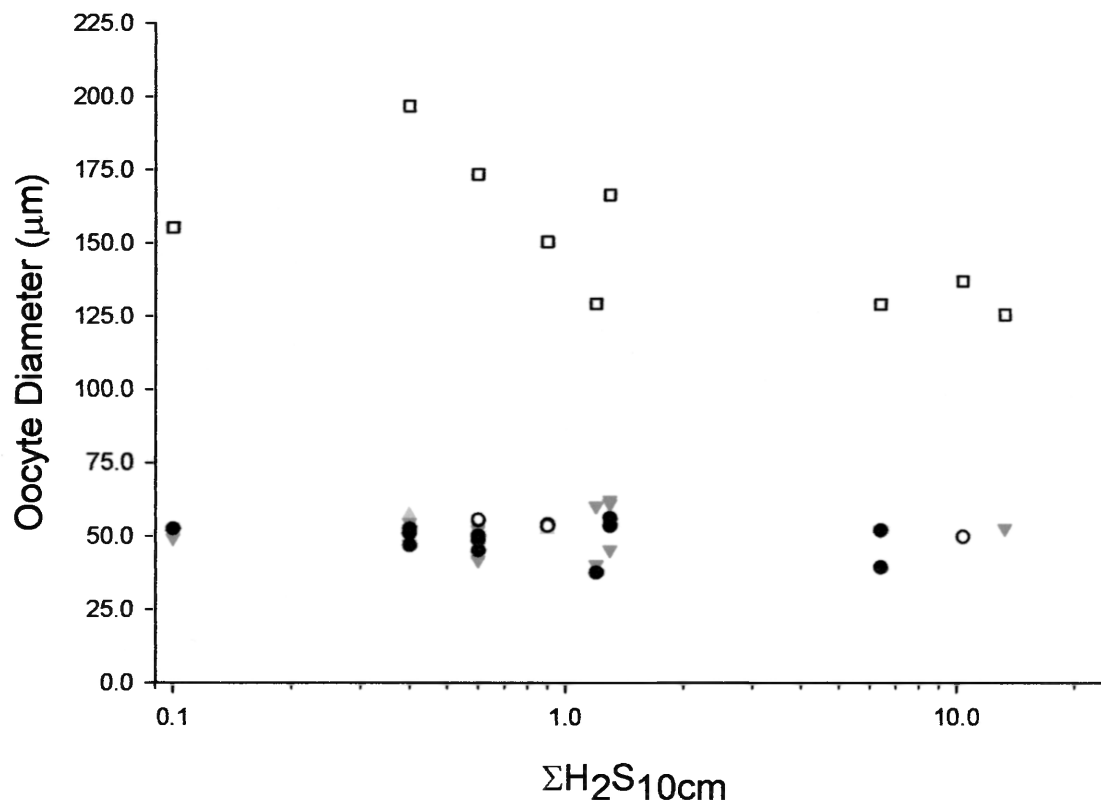
FIGURE 13

STAGES OF REPRODUCTIVE CONDITION IN VESICOMYID CLAMS FROM BLAKE
RIDGE

Stages of reproductive condition in vesicomyid clams from Blake Ridge. (A) Inactive. (B) Early-developing. (C) Developing. (D) Ripe. (E) Spent. Pre-vitellogenic oocytes (pv); early-vitellogenic oocytes (ev); late vitellogenic oocytes (lv); central lumen (cl); arrows: disrupted tissues. Scale bars = 150 μ m.

FIGURE 14

REPRODUCTIVE STAGES, MEAN AND MAXIMUM OOCYTE DIAMETERS IN
VESICOMYID CLAMS FROM BLAKE RIDGE VS. $\Sigma\text{H}_2\text{S}_{10\text{cm}}$



Reproductive stages, mean (○) and maximum (□) oocyte diameters in vesicomyid clams from Blake Ridge vs. $\Sigma\text{H}_2\text{S}_{10\text{cm}}$. ○ = inactive/spent; Δ = early-developing; ▽ = developing; ● = ripe.

Relationship between reproductive characteristics and the environment: There was no significant correlation between mean oocyte diameter and $\Sigma\text{H}_2\text{S}_{10\text{cm}}$ (stratified ANOVA, $p > 0.05$; Fig. 12) or mean oocyte volume and $\Sigma\text{H}_2\text{S}_{10\text{cm}}$ (stratified ANOVA; $p > 0.05$). There was no correlation between reproductive stage of females and $\Sigma\text{H}_2\text{S}_{10\text{cm}}$ within sediments or between % gonad in males and $\Sigma\text{H}_2\text{S}_{10\text{cm}}$ (stratified ANOVA; $p > 0.05$).

DISCUSSION

BLAKE RIDGE VESICOMYID CLAM HABITATS

Sediments sampled from Blake Ridge showed distinct differences in environmental conditions among the four microhabitats. Sediment cores taken from background environments were completely oxidizing in contrast to shell beds, which were completely reducing to a maximum depth of 27 cm. The zone of oxidation within the surface intervals was reducing at 3 cm for clam beds and 5 cm for clam/shell beds. Clams from clam bed habitats have a larger oxidized zone in the surface sediments. An increase in porosity because of a greater abundance of shell and debris within clam/shell beds may influence sediment conditions as well as influences of clams on their environment through bioturbation (Forster 1996). Differences in redox potential were further supported by analyses of sulfide concentrations in each push core.

There were significant differences between percent sand, silt and clay among the four microhabitats with a lower percentage of sand in live clam beds than any of the other three environments. These results suggest that clams are influencing their immediate environment through filtration and bioturbation. The higher amount of sand in clam beds than in clam/shell beds also suggests that the number of clams in the habitat affects the composition of sediment. Shell beds may accumulate sand because the clams are no longer actively filtering it out of the

local habitat. Clam beds also showed a higher percentage of clay than in background sites or shell beds but there was no significant difference between clam beds and clam/shell beds.

Sediments underneath clam beds and shell beds had higher percent organic carbon and nitrogen than those below background sites or mixed clam/shell beds. The sample size for mixed beds was too small ($n = 2$) to speculate on these values but in general, where organic material was present, % C and N was greater. Decomposition of clam tissue and shell in clam beds with high mortalities is a possible cause of higher organic carbon in sediments as well as residual organic carbon methanogenesis in sediment layers.

Sediment porewater geochemistry: Fluid flow is thought to control the distribution and abundance of seep benthos by regulating the availability of reduced compounds (Barry et al. 1997, Olu et al. 1997, Sahling et al. 2002, Levin et al. 2003). Previous studies have shown that gradients in sulfide concentration from peripheral to central zones within seeps (Barry et al. 1997) define gradients in habitability and control the distribution of megafaunal species (Levin et al. 2003, Sahling et al. 2002). Concordance between patterns of porewater sulfide chemistry and the distribution of vesicomid clams at the Blake Ridge seep suggests that porewater sulfide concentration exerts a fundamental control on the composition and structure of vesicomid clam communities (Barry et al. 1997).

Differences in the retention of sulfide within microhabitats may be caused by a combination of physical and biological factors (Roden & Tuttle 1993). The availability of sulfide to chemosynthetic megafauna at seeps is spatially discrete (Sahling et al. 2002) and transient within small physical and temporal scales (Levin et al. in press). Fluid flow records have shown transience on time scales of hours to months with variation coinciding with tidal, lunar, or much longer cycles (Carson & Sreaton 1998). Sulfide can be moved through a system by diffusive

loss of sulfide through sediment layers, oxidation of sulfide at sediment water interface or the precipitation of sulfide when reactions occur with available iron in sediments. Organisms also influence the availability of sulfide through microbially-mediated sulfide oxidation reactions, bioturbation and mixing by clams, and/or the uptake of sulfide by the foot of clams.

Clams at the Blake Ridge diapir generally live in low sulfide concentrations (0.1 mM to 1.3 mM). Due to the low variance in sulfide concentrations at the surface sediment intervals, biological analyses from this study were tested against porewater sulfide concentrations at 10 cm in the pushcores. Increases in sulfide were detected with increasing depth in pushcores with the highest sulfide concentrations from clam beds > 7 cm. One anomalous pushcore (3910-1), showed the highest concentration of sulfide from a clam bed environment (surface _(3 cm) concentrations of 3.1 mM and bottom _(10 cm) concentrations of 6.4 mM). Sediment geochemistry from several other seep sites have similar vertical profiles for sulfide concentration and document distribution of chemosynthetic bivalves in areas of low sulfide with an increase in sulfide with depth (Table 1). *Vesicomya pacifica*, a species of clam from northern California methane seeps, is found in sediments with sub-surface sulfide concentrations up to 2 mM but the clams avoid concentrations > 1 mM (Levin et al. 2003).

The 2 pushcores from clam/shell beds that were analyzed for sulfide concentrations had 7 and 8 clams. Sulfide concentrations at surface intervals (< 5 cm) within clam/shell beds (0.1 to 1.1 mM) were similar to sulfide concentrations from clam beds though there were fewer live clams seen in the clam/shell bed cores. The average concentration of sulfide in clam/shell cores was an order of magnitude lower than those of live clam beds. The layer of shells in the clam/shell pushcores was also greater and could account for the limited number of live clams.

Sediment cores from shell beds show average sulfide concentrations five times higher than in live clam beds as well as a greater retention of sulfide (Fig. 5A, 5B). The greater retention may be the result of no megafauna to take up the available sulfide, or possibly a product of higher sulfide flux in these microenvironments (Roden & Tuttle 1993). Shell beds had high concentrations of sulfide in the surface intervals and an increase in sulfide with depth. This increase of sulfide with depth has been found in several studies of seep environments (Levin et al. 2003, Sahling et al. 2002). High sulfide concentration values found at Blake Ridge were similar to microbial mat environments seen on the Cascadia Convergent Margin (Sahling et al. 2002) and also to barren zones in the center of clam beds in Monterey Bay (Barry et al. 1997).

With the exception of two pushcores, surface sediment layers with high (> 10 mM) sulfide concentrations at Blake Ridge had no clams. Two clams collected from 2 pushcores within shell beds (sulfide concentrations of 3.5 and 7.3 at 3 cm) have survived at higher concentrations. These clams may have been buffered against harsh chemical conditions. Clam shells could serve as a protective barrier from high sulfide flux upward through the sediment layers, providing a shelter from toxicity and allowing clams to situate in optimal sulfide concentrations. It is possible that clams from sediments with high sulfide levels are in a microenvironment in which the flux of sulfide had recently shifted and they are at their upper limit for sulfide tolerance.

As sulfate is reduced to sulfide, sediment profiles show a decrease in sulfate and an increase in sulfide with depth with the sulfate-reduction zone indicating the highest concentration of sulfide and lowest concentration of sulfate (Boetius et al. 2000). Concentrations of sulfate remain relatively constant throughout the sediment cores sampled from live clam beds. Profiles in live clam beds illustrate a zone of sulfate reduction at a greater depth (> 18 cm) than profiles

from dead clam beds (5 – 9 cm), indicating that sulfide is produced much closer to surface sediments in shell beds than in live clam beds. Sulfide and sulfate profiles in some pushcores from shell beds (3 of 8) matched profiles observed in sediment zones with active microbial sulfate reduction (Boetius et al. in press), i.e., sulfate concentrations decreased from the surface to ~5 cm and sulfide concentrations increased from the surface to ~5 cm.

Sulfide rising through conduits underneath live clam beds has a greater vertical distance to the sediment surface. Clams living at the sediment surface are situated in microhabitats where the supply of sulfide originates from greater depths at lower concentrations. Some vesicomid clams are able to maintain a high internal sulfide concentration at lower external sulfide levels based on their sulfide-binding protein capacities (Barry et al. 1997a). *C. pacifica* from the Monterey seeps can concentrate sulfide levels in blood serum by a factor of 10 - 60 times above ambient levels, much greater than *C. kilmeri*, which lives in much higher sulfide concentrations and can only concentrate sulfide 5- 10 times above ambient levels (Barry et al. 1997). Vesicomid clams at Blake Ridge may also be able to withstand low sulfide concentrations in surface sediments without starvation because of higher sulfide-binding capacities.

There are distinct non-overlapping assemblages of mussels and clams at Blake Ridge. The zonation observed between these species at Blake Ridge may be caused by an insufficient or toxic source of sulfide for the clams, or by an excess of methane (Van Dover et al. 2003) necessary for the seep mussel, which harbors endosymbiotic methanotrophs (Cavanaugh 1983).

Several conditions could be responsible for large mortalities in vesicomid clam populations at the Blake Ridge seep. Parasitism has been suggested as a possible cause of mortality in mussel populations (Ward et al. 2004). Pushcores sampled within healthy and unhealthy mussel bed habitats showed no difference in sulfide concentrations (all pushcores <

0.0 mM), which supports this hypothesis. Parasitism in vesicomid clams, however, has been excluded after an investigation of individuals from the same sites and during the same sampling period as those from this study found only non-pathogenic parasites in low abundance (Mills et al. submitted).

Sulfide toxicity rather than starvation may be responsible for large mortalities. Large (~12-15 cm) vesicomid clams (*Calyptogena phaseoliformis*) that live in dense aggregations at the Kodiak seep in the Gulf of Alaska, are known to be very mobile and can select for a particular environment (Levin et al. in press). It may be that the changes in sulfide flux occur too rapidly and clams do not have adequate time to escape harsh conditions. Narcotization has been identified in a species of polychaete worm (Dubilier et al. 1993) and this could also prevent clams from moving out of toxic sulfide conditions within microhabitats.

Live clams at Blake Ridge have a broad range of sulfide tolerances, though the highest densities of adult clams are positioned within a preferred sulfide range (0.1 mM – 3.1 mM). Juvenile density was highest in the sediment pushcores with low (0.1 mM) sulfide levels. The highest number of juveniles (47 ind.) was found from a clam/shell habitat, although the second pushcore sampled from the same environment had a lower (9 ind.) density. A large number of juveniles were seen in one pushcore from a shell bed but sulfide concentrations in this core were similar to a typical clam bed habitat. This suggests that there is some selection for sulfide levels during juvenile settlement.

VESICOMYID CLAM REPRODUCTIVE CHARACTERIZATION

Maximum oocyte diameters from female vesicomid clams suggests that these vesicomid clam species exhibit lecithotrophic development, a life history strategy typical of

many deep-sea bivalves (Ockelmann 1965). Species with lecithotrophic development favor a small number of large oocytes that develop into non-feeding larvae, and spend a relatively short period (days to weeks) transported in the water column. This reproductive and dispersal strategy would be advantageous in a transient and spatially discrete deep-sea seep environment like the Blake Ridge seep.

If egg size is phylogenetically constrained, seep organisms may have selectively favored species with yolky eggs that provide enough nutritive reserves for long-distance dispersal, as is seen in non-vent deep-sea organisms (Young et al. 1997). Bivalve larvae could then withstand long-term dispersal in oligotrophic waters away from the productive seep environment and this would facilitate genetic exchange from the source population to different seep or other chemosynthetic environments. Lecithotrophs from the Antarctic have been found to have a pelagic existence for up to 60 months whereas the maximum larval life of a planktotroph was 10 months (Shilling & Manahan 1994).

Males sampled from the Blake Ridge cold seep were all found to be in a state of maturity. A continuous state of maturity in males has been identified in several deep-sea echinoderm species (Gage & Tyler 1991). This life history strategy ensures that the males are ripe during any given spawning event by a female.

LIFE HISTORY STRATEGIES

Populations of vesicomyid clams and other megafaunal organisms at cold seeps are regulated by environmental parameters (e.g. sulfide concentration, depth, sediment characteristics) and biological (e.g. predation, competition) factors that control the supply of larvae (birth rates, survivorship and transport of larvae) to each seep site (Barry et al. 1997).

Species with phylogenetic constraints on their reproductive biology rely on highly adapted life history strategies to survive in high disturbance environments.

Invertebrates employ several mechanisms to aid in successful fertilization within a population. Seasonally breeding bivalves in the deep sea, as in shallow water systems, produce egg and sperm at approximately the same time in all individuals, release gametes and fertilize externally (Gage & Tyler 1991). The majority of deep-sea bivalve reproductive studies suggest that gametogenesis and spawning is continuous or asynchronous. In order to maximize fertilization success in a spatially discrete and ephemeral environment, many deep sea species form large aggregations of individuals within a population (e.g. *Calyptogena soyoae* up to 1000s of individuals; Momma et al. 1995). Bivalves at the Blake Ridge seep exhibit aggregations that may be a reproductive response as well as a response to patchy food source (i.e. transient chemical flux).

Analyses of oocyte size class for clams from the Blake Ridge diapir indicate a broad range of sizes within the population as well as within individuals, which is consistent with asynchronous (continuous) gametogenesis found in *Calyptogena magnifica* from hydrothermal vents (Berg 1985). A large number of small previtellogenic oocytes were seen in the ovary at the individual and population level in clams from Blake Ridge. Several species of deep-sea invertebrates are known to have large numbers of small oocytes (Tyler & Young 1999). In populations of deep-sea echinoderms, species known to have continuous reproduction, oocyte size frequency counts suggest that there is a large reserve of small previtellogenic oocytes in the ovary, some of which will develop to the maximum size. In many cases, there is evidence of recycling of unspent oocytes and the breakdown of mature oocytes to be used as nurse cells for the remaining developing oocytes (Gage & Tyler 1991).

There was no correlation found between reproductive condition and sulfide concentrations within sediments below clam beds, but this species of vesicomid clams may have a sulfide concentration limit above which there are population-wide levels of extinction. The presence of juvenile clams and the relatively uniform size of adult clam shells in shell beds could suggest that transient and rapid shift in sulfide was responsible for mass mortalities. The presence of both adults and juveniles within a population indicates that predation or other biological responses (e.g. terminal spawning events, life span of species) are an unlikely cause of massive clam mortality.

Blake Ridge clams may be comparable to shallow-water opportunistic species. Opportunistic species live in relatively unstable habitats, mature at an early age, display rapid growth, high fecundities, and high-density-independent mortalities (Mackie 1984). Opportunistic species also occupy broad niches. Bivalves employing this type of life-history strategy typically allocate a relatively large proportion of energy to reproductive development during their adult lifetime. Lack of time-series data limits speculation on population-wide reproductive processes in this species of vesicomid clam but if there are phylogenetic constraints on reproductive biology within vesicomid clams, the survival of the species is dependent on reproductive strategies (high fecundities, dispersal, and survival of larvae). It is suggested that clams from Blake Ridge occupy a broad ecological niche in terms of sediment geochemistry, which allows for greater post-settlement survival once a new habitat has been colonized. Changes in sulfide concentrations within sediments may be of greater importance during larval life stages as an exogenous settlement cue and source of nutrition.

Little information is available regarding responses of chemosynthetic megafauna to temporal and spatial variation in availability of porewater constituents (sulfide and methane) that

result from variability in fluid flow at seeps. Blake Ridge clams appear to have adapted over evolutionary time to tolerate a broad range of sulfide concentrations (0.1-3.1 mM in surface sediments and 0.1-13.3 mM at greater depth) so as the transient environment changes, there is limited local and population-wide extinction of the species. Functional responses such as small-scale migration are likely because species of *Calyptogena* are extremely mobile (Levin et al. in press) and can move horizontally and vertically within the sediment column in search of available sulfide. Movement of Blake Ridge clams is evident from trails across soft sediment surfaces (Fig. 2A). Some *Calyptogena* species are known to survive periods of reduced or halted fluid flow and variable sulfide concentrations by the cessation of pumping or feeding in response to hostile environmental conditions (Sibuet & Olu 1998). Reproduction, recruitment and colonization, and succession are expected and probably rapid in selected, opportunistic species. To further investigate the effect of temporal variation in resource availability (i.e., reduced compounds) on life histories of seep species (reproductive cycles, dispersal abilities) and the ability of seep organisms to cope with temporary cessation of flow or expulsion events that raise sulfide concentrations to toxic levels, in situ biological, geochemical, microbiological and hydrogeological measurements are necessary over extended time periods.

APPENDIX

Appendix 1. Pushcore designations (dive #, core #), clam densities (adults, juveniles), number of individuals used in histological studies from selected cores; identification of cores with corresponding geochemical analyses (sulfide, sulfate), % organic C and total N, grain size characteristics

Dive #	Core #	# Adults	# Juveniles	Histology	Chemistry	% C	% N	% sand	% silt	% clay
Background										
3908	1	0	0	0	Yes	0.64	0.10	56.8	23.0	20.1
3908	3	0	0	0	Yes	0.68	0.09	36.8	32.8	30.5
3908	7	0	0	0	Yes	0.55	0.08	23.9	56.8	19.2
3908	19	0	0	0	Yes	0.47	0.08	25.7	54.3	20.1
3909	24	0	0	0	Yes	0.82	0.11	12.2	58.7	29.0
3908	13	0	0	0	No	*	*	*	*	*
3909	22	0	0	0	No	*	*	*	*	*
3909	23	0	0	0	No	*	*	*	*	*
Clam beds										
3910	1	20	3	4	Yes	0.92	0.16	6.5	60.6	32.9
3910	3	26	4	5	Yes	0.96	0.14	6.6	60.1	33.3
3910	4	34	11	3	Yes	1.00	0.13	23.5	42.0	34.6
3910	5	22	*	6	Yes	0.92	0.13	38.6	32.3	29.0
3910	7	11	5	6	Yes	1.10	0.15	9.7	54.0	36.3
3912	1	40	4	6	Yes	1.10	0.15	6.4	60.6	33.0
3912	4	47	11	5	Yes	1.30	0.16	9.2	57.8	33.0
3912	6	42	1	9	Yes	0.99	0.15	7.4	59.8	32.8
3912	8	23	12	4	Yes	1.05	0.14	8.2	59.9	31.8
3910	2	20	*	0	No	*	*	*	*	*
3912	2	45	*	0	No	*	*	*	*	*
3912	3	35	*	0	No	*	*	*	*	*
3912	7	44	*	0	No	*	*	*	*	*
3912	9	22	*	0	No	*	*	*	*	*
3912	13	35	*	1	No	*	*	*	*	*
3912	14	48	*	0	No	*	*	*	*	*
3912	15	17	*	0	No	*	*	*	*	*
3912	20	32	*	0	No	*	*	*	*	*
3912	22	44	*	0	No	*	*	*	*	*
3912	24	26	*	0	No	*	*	*	*	*
Clam/Shell										
3912	19	8	9	5	Yes	0.97	0.13	6.8	59.9	33.3
3912	23	10	47	5	Yes	0.80	0.11	47.4	29.1	23.4
3910	21	0	*	0	No	*	*	*	*	*
3910	24	5	*	0	No	*	*	*	*	*
3912	5	9	*	0	No	*	*	*	*	*
3912	21	8	*	1	No	*	*	*	*	*
Shell beds										
3909	4	0	1	0	Yes	0.97	0.15	52.5	25.9	21.6
3909	5	0	0	0	Yes	1.00	0.14	30.3	42.0	27.7
3909	7	1	0	1	Yes	0.70	0.13	22.8	56.1	21.1
3909	8	1	0	1	Yes	0.93	0.12	27.2	48.3	24.5
3909	10	0	*	0	Yes	*	*	*	*	*
3909	19	0	0	0	Yes	1.20	0.08	34.6	40.4	25.1
3909	20	0	13	0	Yes	0.70	0.10	22.5	58.0	19.6
3910	10	1	*	0	Yes	*	*	*	*	*
3910	12	0	*	0	Yes	1.30	0.20	*	*	*
3910	20	0	1	0	Yes	0.79	0.12	23.2	46.2	27.9
3909	6	0	*	0	No	*	*	*	*	*
3909	9	0	*	0	No	*	*	*	*	*
3909	21	0	*	0	No	*	*	*	*	*
3910	6	0	*	0	No	*	*	*	*	*
3910	8	0	*	0	No	*	*	*	*	*
3910	15	0	*	0	No	*	*	*	*	*
3910	16	0	*	0	No	*	*	*	*	*
3910	22	0	*	0	No	*	*	*	*	*
3910	23	0	*	0	No	*	*	*	*	*

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VITA

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Taylor Perrine Heyl was born in Bethesda, Maryland on December 14, 1979. She graduated from The Williams School, New London, Connecticut in 1997. Taylor Heyl received her B.S. at the University of New Hampshire, Durham, NH in 2001 with a degree in Marine and Freshwater Biology, spending a year at Eckerd College, St Petersburg, FL in 1998, before returning to UNH to complete her studies.

In addition to her academic studies and teaching assistantships, Taylor Heyl has gained invaluable experience aboard research vessels, which most notably includes two dives on DSV *Alvin* in 2002 to explore seamounts in the Gulf of Alaska and in 2003 to the Blake Ridge methane hydrate seep off South Carolina.

Taylor Heyl defended her Master's thesis on her 25th birthday before embarking on a research placement in January 2005 to Palmer Station, Antarctica as part of the Long Term Ecological Research (LTER) Network.