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Parasitism in Deep-Sea Chemosynthetic Mussels: Parasitism in Bathymodiolus Mussels from Deep-Sea Seep and Hydrothermal Vents

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PARASITISM IN DEEP-SEA CHEMOSYNTHETIC MUSSELS

Parasitism in Bathymodiolus Mussels from Deep-Sea Seep and Hydrothermal Vents

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A Thesis

Presented to

The Faculty of the Department of Biology

The College of William and Mary in Virginia

In Partial Fulfillment

Of the Requirements for the Degree of

Master of Arts

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by

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APPROVAL SHEET

This thesis is submitted in partial fulfillment of the requirements for the degree of

Master of Arts

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ABSTRACT

Bivalve species, especially mussels, are biomass dominants in many deep-sea chemosynthetic ecosystems. As in shallow-water environments, parasites are likely to be important factors in the population dynamics of bivalve populations in chemosynthetic ecosystems, but there has been little study of parasitism in deep-sea seep or vent molluscs.

In this study, parasite types, diversity, prevalence, infection density, and non-infectious indicators of stress or disease as related to host age, reproductive condition, and endosymbiont density were assessed in mussels (*B. heckerae*) from two seeps sites and mussels (*B. puteoserpentis*) from two vent sites. Ten microbial or parasitic agents were identified in histological sections. Parasite types included three viral-like gut inclusions, two rickettsia-like gill inclusions, a rickettsia-like mantle inclusion, a bacterial gill-rosette, a chlamydia-like gut inclusion, gill-dwelling ciliates, and an unidentified inclusion in gut tissues. Parasite species richness was greater in seep mussels compared to vent mussels, with the seep mussels possessing 9 types of parasites compared to 2 in the vent mussels. One of the viral-like inclusions infecting the seep mussel *B. heckerae* was pathogenic, causing lysis of the digestive tubules. The prevalence and intensity of infection by this pathogen were greater in hosts with shell lengths less than 100 mm. Mussels from all four sites also exhibited intense infiltration of tissues and blood spaces by abnormal hemocytes. The hemocytic infiltration (hemocytosis) showed variable degrees of severity that were not associated with other host factors examined.

PARASITISM IN DEEP-SEA CHEMOSYNTHETIC MUSSELS

INTRODUCTION

Parasitic infections can impair growth, reproduction, competitive ability, stress tolerance, and survival of host species (Kautsky 1982, Price et al. 1986, Esch et al. 1990, Calvo-Ugarteburu & McQuaid 1998). In addition, parasites may regulate host population structure and influence coexisting species, such as the host's prey, predators or competitors (Anderson 1978, Anderson & May 1978, Price et al. 1986, Calvo-Ugarteburu & McQuaid 1998). Parasitism is thus an important factor to consider in studies of the ecology and dynamics of populations within any ecosystem (Esch at al. 1990, Powell at al. 1999, Montaudouin et al. 2000). The paucity of studies of parasitism in organisms living in bathyal environments is a consequence of both the relative inaccessibility of the deep sea and the low biomass and abundance of the organisms living there. Chemosynthetic environments (hydrothermal vents and cold seeps) in the deep sea are celebrated for the high biomass and abundance of organisms adapted for life under extreme conditions, yet surprisingly little is known about parasitism in organisms living in these environments.

Hydrothermal vents are submarine hot springs located along mid-ocean ridges. They are dynamic, ephemeral ecosystems characterized by metal-rich fluids and temperatures elevated above those of the ambient deep sea (see Van Dover 2000). Deep-sea seeps are located primarily on continental margins where reduced compounds diffuse from the sea floor into the overlying water column. Seeps occur in a variety of settings (Sibuet & Olu 1998) and include petroleum seeps, which are common in the Gulf of Mexico (MacDonald et al. 1990), brine seeps found on the Florida Escarpment (Paull et al. 1984), and gas-hydrate seeps, including the methane-hydrate seep at Blake Ridge, off the coast of South Carolina (Van Dover et al. 2003). In contrast to hydrothermal vents, seeps are thought to be more stable, longer-lived ecosystems (Sibuet & Olu 1998). Ecological studies in these environments are still in their infancy and investigations of parasite burdens, pathology, and disease in species living at seeps and vents are scarce.

Bathymodiolin mussels (f. Mytilidae) are one of the dominant megafaunal taxa found at seeps and vents. Bathymodiolin mussels at vents typically live in water between 5-15°C (Van Dover 2000); mussels at seeps are generally at ambient sea-water temperatures (~ 2°C; Sibuet & Olu 1998). Mussels play key roles in seep and vent ecosystems: they serve as physical habitats for other invertebrates, and they house chemoautotrophic, endosymbiotic bacteria within gill epithelial cells called bacteriocytes (Cavanaugh 1983, Le Pennec & Hily 1984). The endosymbionts account for a large proportion of the primary production in seep and vent communities. The energy required for carbon-dioxide fixation is obtained by oxidation of reduced compounds in the endosymbionts; fixed carbon is translocated to the host mussel (Cavanaugh 1983). Bathymodiolin mussels can obtain nutrition from single (e.g., thiotrophic bacteria) or dual (e.g. thiotrophic and methanotrophic bacteria) symbioses. Healthy mussels retain a functional gut and an ability to filter feed on particulate organic matter (Le Pennec et al.1990), but in the absence of reduced compounds, gill condition deteriorates (Raulfs et al. in press).Although diseases of commercially valuable intertidal bivalves have been extensively studied, little is known about parasites in bivalves found at deep-sea seeps, and nothing is known of parasites in bivalves found at hydrothermal vents. Extreme conditions in chemosynthetic ecosystems include high concentrations of metals at vents, brines and hydrocarbons at some seeps, and sulfide-rich effluents or pore-waters. Organisms endemic to chemosynthetic environments have adapted to these environments and require what would normally be considered noxious conditions.

Powell et al. (1999) examined the relationship between elevated concentrations of polycyclic aromatic hydrocarbons (PAH) and parasite burdens in mussels, *Bathymodiolus* sp., from 4 hydrocarbon seep sites in the Gulf of Mexico (depth 550-650 m). In coastal mussels, PAHs disrupt membranes, damage lysozymes, and inhibit hemocyte phagocytic ability (Pipe & Coles 1995, Fernley et al. 2000). Unlike shallow-water mussels, the mussels from hydrocarbon seeps were obligately associated with high PAH concentrations. Five types of parasites were documented in the hydrocarbon-seep mussels, including extracellular gill ciliates, bacterial gill rosettes, *Bucephalus*-like trematodes, and chlamydia/rickettsia-like gill and gut inclusions (Powell et al. 1999). In comparison to mussels from intertidal sites, the mussels from hydrocarbon seeps had similar types of parasites, but were more heavily parasitized. Infections of *Bucephalus*-like trematodes caused severe castration of hydrocarbon-seep mussels, leaving 40 % of the populations in the study reproductively compromised (Powell et al. 1999).

To explore further the relationship between parasite burdens in bathymodiolin mussels and chemosynthetic environments, we conducted a comparative study of parasite burdens in *Bathymodiolus heckerae* from two deep-sea seeps and *B. puteoserpentis* from two deep-sea hydrothermal vents. Both species contain methanotrophic and thiotrophic endosymbiotic bacteria. Differences in types of parasites and abundance of parasites were documented between the two geologically and ecologically distinct habitat types. Prevalence, infection densities, and noninfectious indicators of stress or disease as they relate to age, reproductive condition, and an index of endosymbiont density were also assessed.

MATERIALS AND METHODS

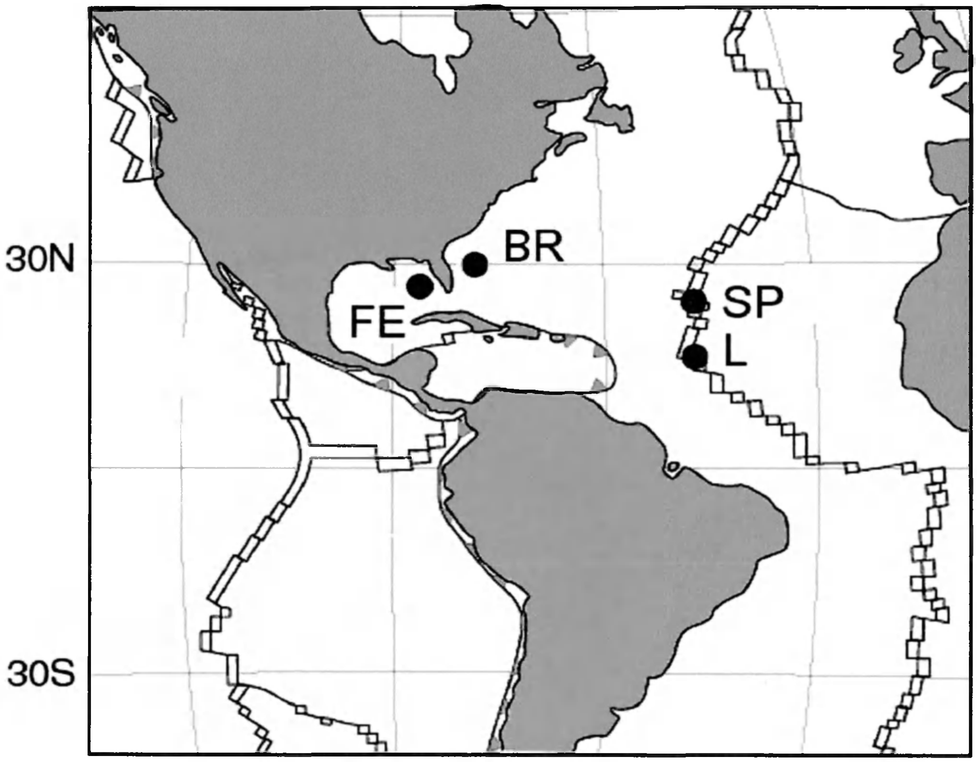
Specimens of *Bathymodiolus heckerae* were collected using the deep-sea submersible *Alvin* from two seep sites: a cold-water, saline site at the Florida Escarpment (26° 2′N 84° 55′W, 3300 m; sampled in October 2000) and a gas-hydrate site off the Carolina coast (Fig. 1; Blake Ridge 32° 31′N 76° 12′W, 2155 m; sampled in September 2001). Species of *B. puteoserpentis* were collected from the Logatchev (15° 45′N 44° 58′W, 3300m, sampled in July 2001; Batuyev et al. 1994) and Snake Pit (23° 22′N 44° 56′W, 3600m, sampled in July 2001; Karson & Brown 1988).

Mussels were sorted by length and 30 to 32 specimens were systematically selected to represent an even distribution across the range of sizes available. Shell lengths were recorded for each specimen. Within 3 hr of collection, mussels were removed from the shell, tagged, fixed in Davidson's solution (Humason 1972) for 24 hr, and stored in 70 % ethanol. Number and lengths of commensal polychaetes (*Branchipolynoe seepensis*) in the mantle cavities of the mussels were determined.

Wet weight was determined for whole, fixed mussels without the shell. An oblique, ~ 5 mm-thick, transverse tissue slice, containing mantle, gonad, digestive tract, and gill was dissected from each specimen. This tissue slice was dehydrated in a graded ethanol series, embedded in paraffin, and transverse sections were taken at 5-6 μm.

FIGURE 1

LOCATIONS OF THE FLORIDA ESCARPMENT (FE) AND BLAKE RIDGE (BR) SEEP SITES AND THE LOGATCHEV (L) AND SNAKE PIT (SP) VENT SITES



Sections were stained with Gill's hematoxylin and eosin (H&E; Stevens 1990) or Ordway-Machiavello for rickettsia (Humason 1972) to identify parasite types and prevalence (# of host individuals infected with any parasite; Margolis et al. 1982) using light microscopy. A Spot camera (Diagnostic Instruments) was used for capturing images for light microscopy. Contrasts were adjusted and photomosaics were generated using Adobe Photoshop (Adobe Systems).

Using an ocular grid, parasite densities (number of parasites per area standardized to 500 μm^2 of tissue; Margolis et al. 1982) were determined for infected individuals. Five grids were examined per section for three sections that were separated by ~1 mm or more. Body burden (mean infection densities for all parasitic infections) of infected individuals was also determined. A quantitative measure of bacterial endosymbiont density was determined for *Bathymodiolus puteoserpentis* by measuring the area of the gill bacteriocytes occupied by the endosymbiotic bacteria to derive a ratio of the area occupied by the endosymbiotic bacteria to the area of the gill bacteriocytes. Cells used to measure bacterial endosymbiont density were located on sections of the transverse gill filaments in approximately the same region of each mussel. Host sex and gonad stage were measured. Semi-quantitative scales were assigned for intensity of hemocyte infiltration per cross-section (Table 1; Fig. 2a & b) and density and stage of development of gonads (Table 2) and the presence of neoplastic tumors in the gill epithelia were recorded. Statistical tests were performed using MINITAB software, version 13.20, 2000. Comparisons of means among sites were determined using Kruskal-Wallis.

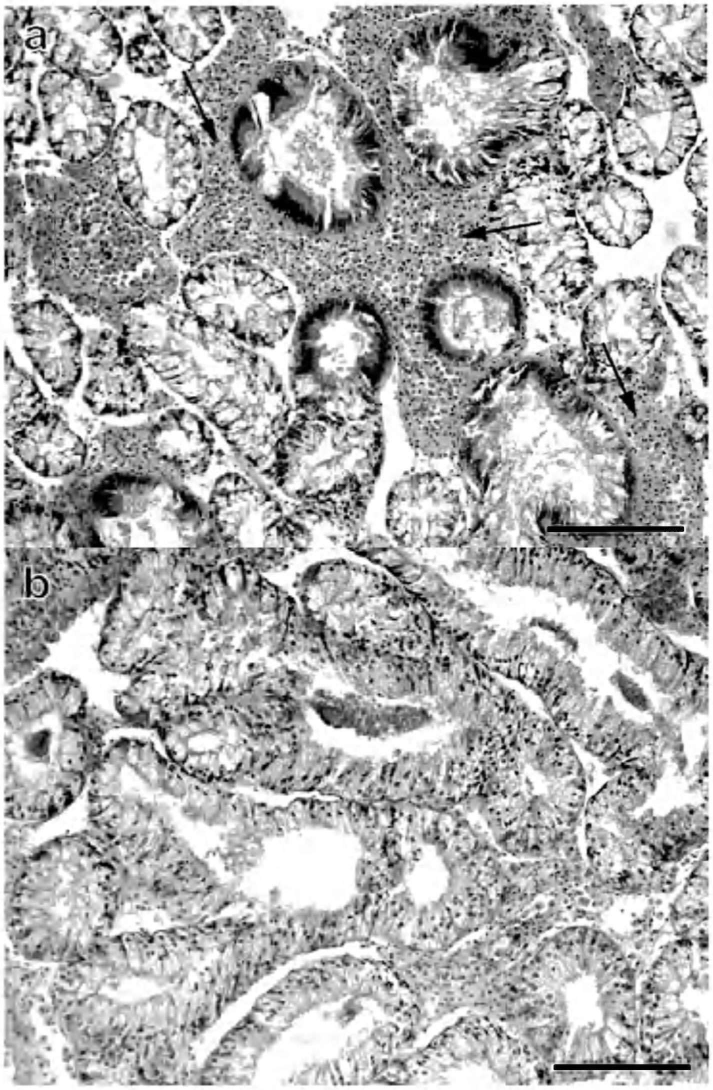
TABLE 1

INDEX OF INTENSITY OF HEMOCYTE INFILTRATION

Intensity	Description
0	No areas of infiltration
1	Slight infiltration, small, focal, usually only around intestine
2	Several small foci of infiltration or a few larger areas; sometimes occurring in more than one type of tissue
3	Disseminating foci ranging from small to large areas; infiltration extensive and usually in several or all types of tissue
4	A few blood vessels enlarged due to heavy infiltration of hemocytes
5	Most blood vessels enlarged due to massive infiltration

FIGURE 2

HEMOCYTE INFILTRATION



a) Heavy infiltration of hemocytes (arrows) around the digestive diverticula and intestine.Scale bar = 200 µm. b) Healthy visceral mass tissue without hemocyte infiltration. Scale bar = 200 µm

TABLE 2

INDEX OF GONADAL DEVELOPMENT

Gonad Stage	Description
1	Undifferentiated; few areas in visceral mass with gonad
2	Early stages of developing gametes; several areas of visceral mass with small regions of gonadal development
3	Approximately equal proportions of developing and mature gametes; large areas of visceral mass taken up by gonads
4	Early stages of gametogenesis rare; follicles contain mature gametes; visceral mass densely packed with gonad

If significant results ($p < 0.05$) were obtained, ANOVA and Tukey's pairwise comparisons were used to evaluate significant differences among sample pairs. Multi-dimensional scaling (MDS; PRIMER v5; Clarke & Gorley 2001) was used to examine similarities among individuals based on the parasite type- density matrix, using Bray-Curtis coefficients calculated from non-transformed data. The 16 individuals not infected by any parasite were excluded from the MDS analysis. MDS plots position individuals on a unit-less 2-dimensional map; closely spaced points represent the most similar individuals in a multivariate space based on parasite types and density. Analysis of similarity (ANOSIM subroutine of PRIMER v5) was used to determine significant differences between groups of individuals evident in MDS plots. ANOSIM provides R statistics, where $R > 0.75$, groups are well-separated; when $0.75 > R > 0.5$, groups are over-lapping but clearly different; when $R < 0.25$, groups are not separable (Clarke and Gorley, 2001). Factors contributing to these differences of parasite infection densities were determined from similarity percentages (SIMPER subroutine in PRIMER v5). The Shannon-Weiner diversity index ($H'_{\log(e)}$) was calculated using the DIVERSE subroutine in PRIMER v5. This diversity index takes into account both species richness (the number of parasite types) and evenness (the distribution of individuals among species; Hayek and Buzas 1997).

RESULTS

The parasite fauna

Ten types of parasites (9 in *Bathymodiolus heckerae,* 2 in *B. puteoserpentis*) were identified in histological sections: three viral-like inclusions in the gut, two rickettsia-like inclusions in the gill, rickettsia-like inclusions in the mantle, bacterial rosettes in the gill, chlamydia-like inclusions in the gut, gill ciliates, and an unidentified inclusion in the gut.

One of the viral-like inclusions in the gut, referred to hereafter as viral inclusion I, was observed only in the vent mussel *Bathymodiolus puteoserpentis,* where it occurred in the digestive diverticula (Fig. 3a). These eosinophilic inclusions were generally spherical in shape with an average diameter of 20 μm (n = 20; Fig. 3b). Occasionally, the cell contents were peripherally located in the host cell or the cell was hypertrophied (Fig. 3b).

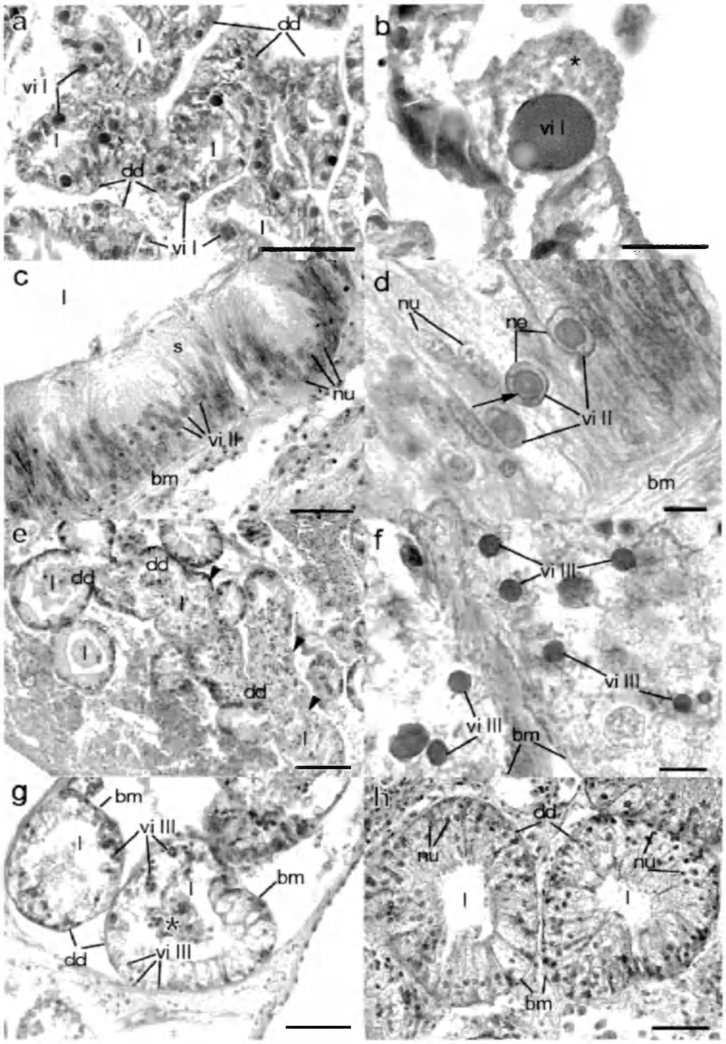
Two viral-like inclusions in the gut were associated with the seep mussel *Bathymodiolus heckerae.* One, hereafter referred to as viral inclusion II, occurred in the nuclei of stomach and intestinal epithelia of mussels from the Florida Escarpment (Fig. 3c). The inclusion had a waxy, eosinophilic appearance and was oval or spherical in shape with an average diameter of 11 μm (n = 20). The inclusion was surrounded by what we infer to be the hypertrophied nuclear envelope of the host cell. Lighter staining rings were occasionally visible within the inclusion (Fig. 3d). No obvious tissue pathology was associated with this infection.

A third viral-like inclusion in the gut, referred to as viral inclusion III, infected the epithelial nuclei of the digestive diverticula and intestines of *B. heckerae* from both seep sites (Fig. 3e). Infections were characterized by small (4.8 µm diameter; n = 25) eosinophilic spherical bodies within host cells, free in the lumen of the digestive tract (Fig. 3e & f), or occasionally in the connective tissue of the visceral mass. The spherical bodies showed no internal structure at the level of light microscopy. Severe tissue pathology was often associated with infection by viral inclusion III (Fig. 3e, f, & g). In moderately or heavily infected mussels, infected cells had either lysed or had sloughed into the lumen of the digestive tubules (Fig. 3g). Widespread necrosis of the digestive tissue was evident with large regions of the digestive tissue destroyed, leaving a mass of viral inclusions among the remnants of the cells (Fig. 3e & f). In adjacent healthy mussel tissues, nuclei were basally located and individual cells were easily distinguishable (Fig. 3h).

One of the rickettsia-like inclusions in the gill, referred to as gill rickettsia I, was found in both mussel species from all four sites. These basophilic, intracytoplasmic inclusions were spherical, with an average diameter of 20 µm (n = 20) and could be found in all cell types along the entire length of the gill filament (Fig. 4a & b). Many inclusions had a filamentous internal structure (Fig. 4b) while other inclusions appeared smooth.

FIGURE 3

VIRAL-LIKE INCLUSIONS FROM *BATHYMODIOLUS* SPP.



a) An individual heavily infected with viral inclusion I in the digestive diverticula. Scale bar = 200 µm. b) Viral inclusion I in the digestive diverticula causing hypertrophy and necrosis of the host cell (asterisk). Scale bar = 20 µm. c) Viral inclusion II infecting stomach epithelial cells. Scale bar = 50µm. d) Detail of viral inclusion II showing the hypertrophied nuclear envelope (arrow). Scale bar = 10 µm. e) Viral inclusion III infecting digestive diverticula and intestinal tissue. Viral-like inclusion bodies visible in the lumen and tissues with necrotic areas of digestive diverticula. Scale bar = 200 µm. f) Viral inclusion III within two tubules of the digestive diverticula. Scale bar = 10 µm. g) Digestive diverticula infected with viral inclusion III with necrotic tissue caused by the virus. A mass of viral-like inclusions and necrotic cells are visible in one tubule (asterisk). Scale bar = 60 µm. h) Uninfected digestive diverticula for comparison. Scale bar = 50 µm. bm, basement membrane; dd, digestive diverticula; i, intestine; l, lumen; ne, nuclear envelope; nu, nucleus; s, stomach; vi I, viral inclusion I; vi II, viral inclusion II; vi III, viral inclusion III

A second type of rickettsia-like inclusion in the gill, referred to as gill rickettsia II was found only in *Bathymodiolus heckerae* from the Florida Escarpment. The intracytoplasmic inclusions were basophilic and were found along the entire length of thegill filament within the host bacteriocytes (Fig. 4c). These inclusions were morphologically different from gill rickettsia I, being larger (average diameter 37 μm; n = 20) and more irregular in shape (Fig. 4c & d). These inclusions also had a filamentous internal structure (Fig. 4d). Both types of gill rickettsia were capable of displacing the endosymbiotic bacteria to the periphery of the host cell.

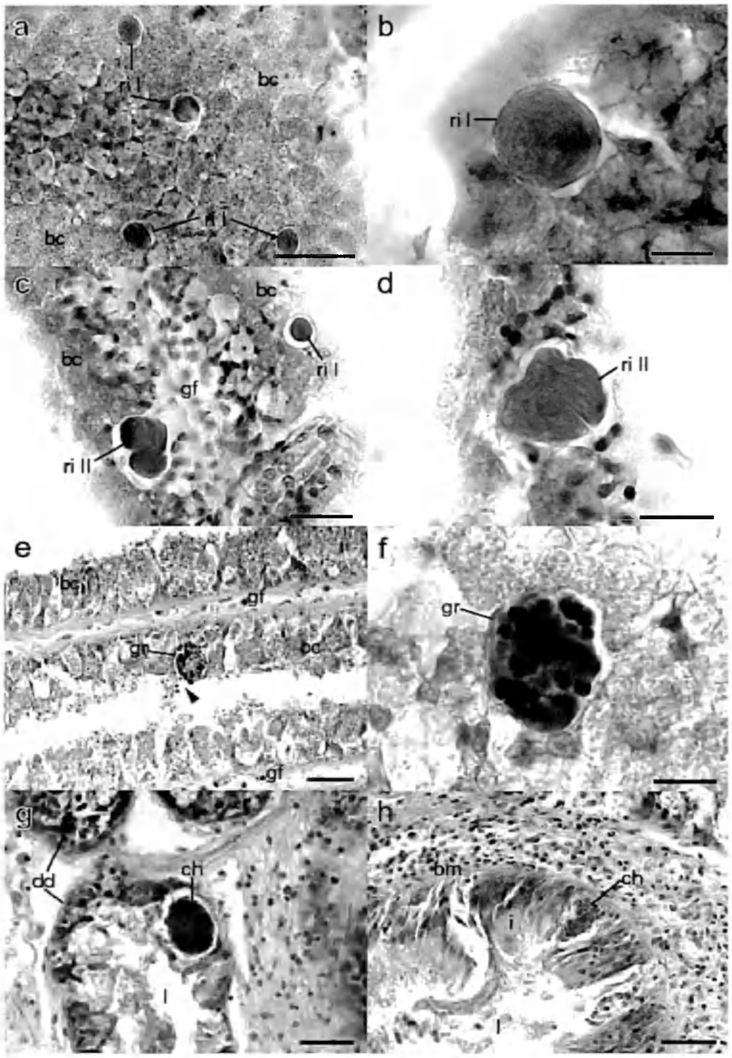
The third type of rickettsia-like inclusion, referred to as mantle rickettsia, was found in the cytoplasm of mantle epithelial cells in *Bathymodiolus heckerae*. These inclusions were morphologically similar to gill rickettsia I, but due to their different location within the host, they were considered a different parasite. No obvious tissue pathology was observed with this parasite.

Bacterial gill rosettes similar to those described by Powell et al. (1999) were observed in bacteriocytes. They were associated with the host bacteriocytes of *Bathymodiolus heckerae*, averaged 9 μm (n = 15) in diameter, and were located within or penetrating the membrane of the host gill cell (Fig. 4e). The rosette structures were composed of groups of 30 or more basophilic, spherical bodies, each with an average diameter of 2.8 μm (n = 25; Fig. 4f). No tissue damage was observed except where rosettes penetrated the gill cell membranes.

Chlamydia-like intracytoplasmic inclusions occurred in epithelial cells of the

FIGURE 4

BACTERIA-LIKE INFECTIONS IN *BATHYMODIOLUS* SPP.



a) Moderate infection with gill rickettsia I. The rickettsial inclusions are located in gill bacteriocytes. Scale bar = 50 µm. b) Gill rickettsia I with a filamentous internal structure. Scale bar = 10 µm. c) Gill filament infected with both gill rickettsia I and gill rickettsia II. Note the difference in size and shape of the two inclusions. Scale bar = 30 µm. d) Gill rickettsia II with a filamentous internal structure and irregular shape. Scale bar = 20 µm. e) Gill rosettes located among bacteriocytes of the host gill filament. The gill rosette appears to be breaking through the host gill cell (arrowhead). Scale bar = 30 µm. f) A gill rosette composed of several spherical bodies with an average diameter of 2.8 µm. Scale bar = 10 µm. g) A tightly compacted chlamydia-like inclusion in the digestive diverticula. Scale bar = 40 µm. h) A more diffuse chlamydia-like inclusion in the intestine. Scale bar = 40 µm. bc, bacteriocytes; bm, basement membrane; ch, chlamydia-like inclusion; dd, digestive diverticula; gf, gill filament; gr, gill rosette; i, intestine; l, lumen; ri I, gill rickettsia I; ri II, gill rickettsia II stomach, intestine, and digestive diverticula of *Bathymodiolus heckerae.* These parasites were basophilic, finely granular, and irregular in shape and size (average diameter 5 µm; n = 20). The finely granular inclusions appeared either tightly compacted (Fig. 4g) or diffuse (Fig. 4h). Host cells were hypertrophied or lysed by the inclusion (Fig. 4g & h).

Ciliates were found among the gills of *Bathymodiolus heckerae* (Fig. 5a). They had an average length of 30 µm (n = 15) and were associated with, but did not appear to be attached to, the gill filaments of the host mussel (fig. 5a). No obvious tissue pathology was observed.

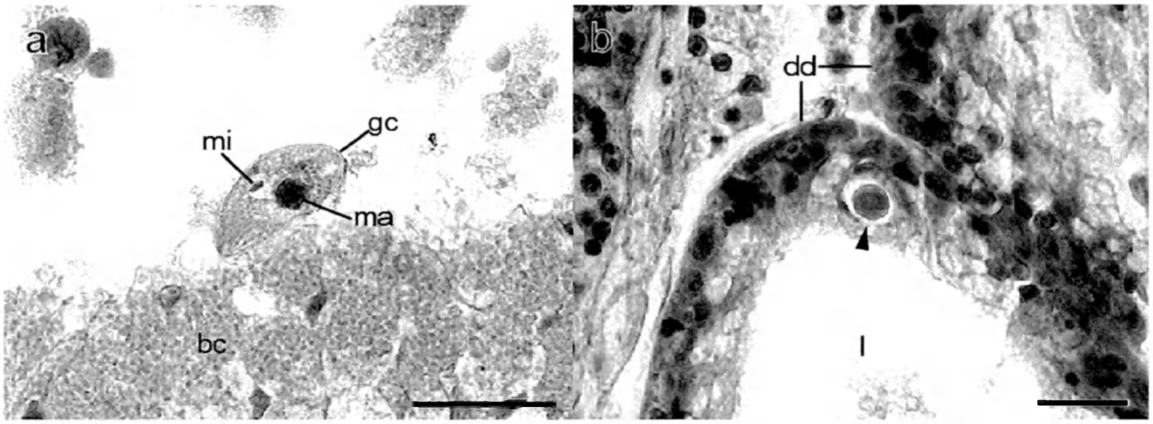
An unidentified parasite occurred only in *Bathymodiolus heckerae.* It was characterized by a slightly refringent, intracytoplasmic, basophilic, spherical inclusion with an average diameter of 8 µm (n = 10; Fig 5b). These inclusions were located in the epithelial cells of the intestine and digestive diverticula of the host. With the exception of a slight hypertrophy of the host cell, no obvious tissue pathology was observed.

Polychaetes

Commensal polynoid polychaetes (*Branchipolynoe seepensis*) were retained between the gill filaments of 2 vent mussels and subsequently sectioned (Fig. 6a). Displacement of gill filaments and some physical trauma of the filaments resulting in necrosis or poor condition were attributed to the polychaetes. In *Bathymodiolus puteoserpentis,* the commensal polychaete had a prevalence of 30-40 %. An even greater prevalence of the commensal polychaete was observed in *B. heckerae* from the Florida

FIGURE 5

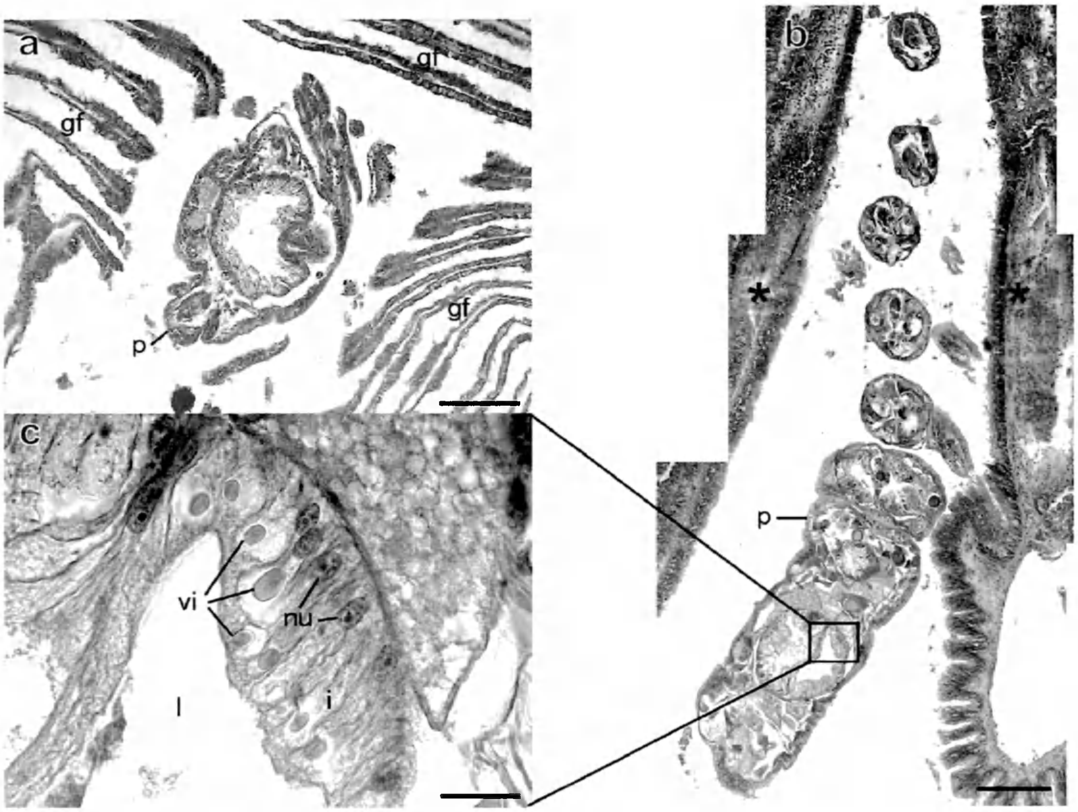
GILL CILIATES AND AN UNIDENTIFIED PARASITE FROM *BATHYMODIOLUS* SPP.



a) Gill ciliate showing a micro- and macro-nucleus. Scale bar = 30 μm. b) Unidentified intracytoplasmic inclusion (arrowhead) in a digestive diverticulum. Scale bar = 20 μm. bc, bacteriocytes; dd, digestive diverticula; gc, gill ciliate; l, lumen; ma, macro-nucleus; mi, micro-nucleus

FIGURE 6

COMMENSAL POLYCHAETES IN *BATHYMODIOLUS* SPP.



a) Polynoid polychaete, *Branchipolynoe seepensis,* between gill filaments of the vent mussel *B. puteoserpentis*. Scale bar = 200 µm. b) Nautiliniellid polychaete between the demibranchs (asterisks) of *B. heckerae* from Florida Escarpment. Scale bar = 200 µm. c) Viral-like inclusions in the intestine of the nautiliniellid. Scale bar = 20 µm. gf, gill filaments; i, intestine; l, lumen; nu, nucleus; p, polychaete; vi, viral-like inclusion

Escarpment seep site (>60 %). *Branchipolynoe seepensis* was not observed in *Bathymodiolus heckerae* from the Blake Ridge seep.

In 3 *Bathymodiolus heckerae* from the Florida Escarpment, 4 nautiliniellid polychaetes (possibly *Laubierus mucronatus* based on characteristics of the setae) were observed in section (Fig. 6b). The polychaetes were located between the ascending and descending filaments of the demibranchs. Little evidence of damage on the surrounding filaments was observed, although cells in the adjacent gill tissue were detached from the filament or the tissue was in poor condition. Three of the polychaetes were infected with viral-like inclusions in the digestive tract (Fig. 6c).

Parasite diversity and abundance

Parasite diversity (H') was highest (H' = 0.92) in *Bathymodiolus heckerae* from the Florida Escarpment (Table 3). Parasite diversities at the other 3 sites were considerably lower, with H' values of < 0.20. Species richness (number of parasite types) was greater *B. heckerae* than in *B. puteoserpentis* (Table 3 & Fig. 7a). Mussels from the Florida Escarpment were infected with 9 different types of parasites and *B. heckerae* from the Blake Ridge were infected with a subset of at least 6 of these. Viral inclusion I was unique to *B. puteoserpentis* at the vents. Gill rickettsia I was observed in both mussel species at all four sites.

The number of parasite types in infected individuals (Fig. 7b) was species and site dependent. *Bathymodiolus heckerae* from Florida Escarpment seeps had ~ 4 times more

TABLE 3

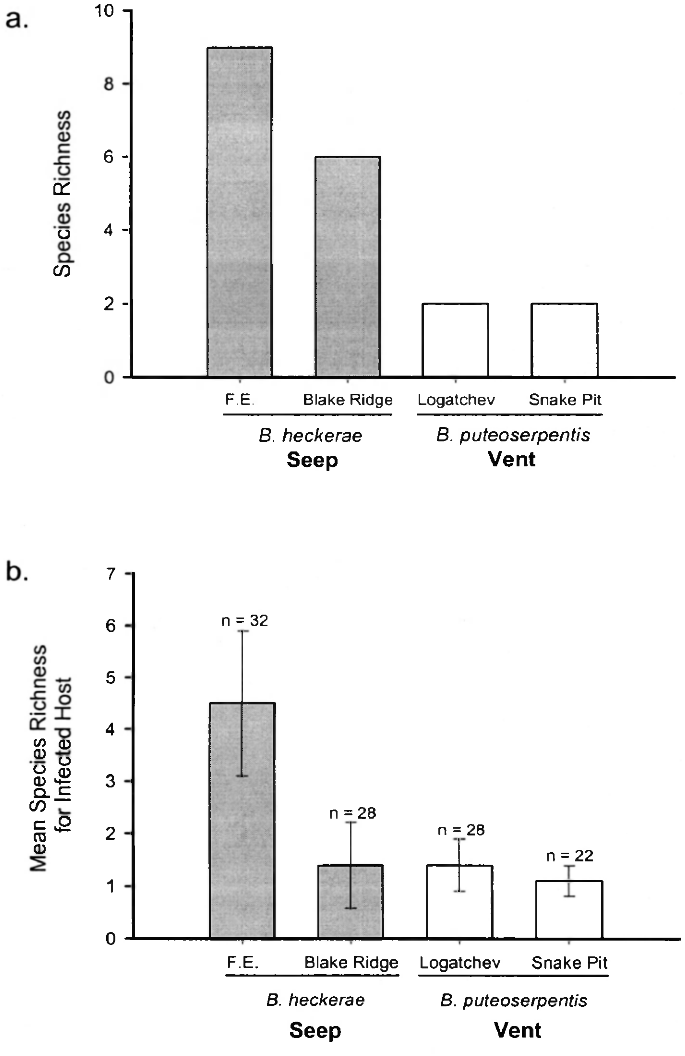
PREVALENCE AND DENSITIES OF INFECTION

Prevalence (% of individuals infected) and densities of infection per 500 μm^2 of tissue for parasitic agents and data on polychaete infestations. n = number of individuals sampled.

	Bathymodiolus heckerae		***Bathymodiolus puteoserpentis***	
	Seep Sites		Hydrothermal Vent Sites	
	Fl. Escarpment	Blake Ridge	Logatchev	Snake Pit
n	32	32	32	30
Gill Rickettsia I				
Prevalence	94 %	13 %	87 %	20 %
Density (± s.d.)	0.3 (± 0.34)	0.1 (± 0.09)	1.1 (± 1.20)	0.06 (± 0.04)
Gill Rickettsia II				
Prevalence	69 %	0	0	0
Density (± s.d.)	0.3 (± 0.34)	0	0	0
Mantle Rickettsia				
Prevalence	3 %	3 %	0	0
Density (± s.d.)	0.03	0.7	0	0
Bacterial Gill Rosette				
Prevalence	34 %	0	0	0
Density (± s.d.)	0.2 (±0.35)	0	0	0
Chlamydia-like Inclusion				
Prevalence	21 %	3 %	0	0
Density (± s.d.)	0.3 (± 0.56)	0.25	0	0
Gill Ciliate				
Prevalence	40 %	3 %	0	0
Density (± s.d.)	0.04 (± 0.05)	0.02	0	0
Unidentified Gut Inclusion				
Prevalence	50 %	12 %	0	0
Density (± s.d.)	0.08 (± 0.05)	0.03 (± 0.02)	0	0
***B. seepensis* polychaetes**				
Prevalence	62 %	0	31 %	40 %
# polychaetes per individual	1.5 (± 1.26)	0	1.1 (± 0.55)	1.7 (± 1.24)

FIGURE 7

PARASITE SPECIES RICHNESS AND MEAN SPECIES RICHNESS PER INFECTED MUSSEL

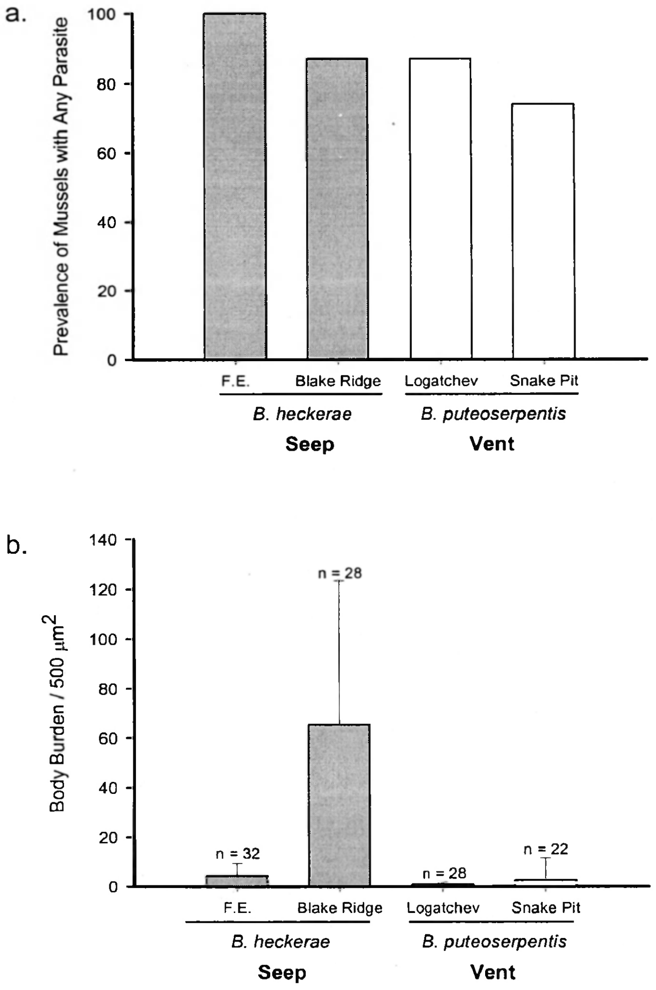


a) Parasite species richness (# of parasite species) in *Bathymodiolus heckerae* and *B. puteoserpentis* and b) Mean species richness for infected *Bathymodiolus heckerae* and *B. puteoserpentis* mussels by site. F.E. = Florida Escarpment. n = number of individuals parasites per individual on average than did *B. heckerae* from Blake Ridge (Kruskal-Wallis: $p < 0.001$). Infected individuals of *B. puteoserpentis* from the Logatchev vent had a slightly but significantly greater number of parasites per individuals on average (Fig. 7b) than did infected individuals from Snake Pit (1.1; Kruskal-Wallis: $p = 0.017$).

More than 70 % of the mussels sampled from all four sites were parasitized by one or more types of parasites. At the Florida Escarpment site, 100 % of the mussels were infected with at least one type of parasite (Table 3 & Fig. 8a). Despite large numbers of infected individuals, total body burdens were relatively low except in mussels from the Blake Ridge seep (Tukey's, $p < 0.001$; Fig. 8b). Individuals from the four sites mapped as significantly different groups on MDS plots based on the parasite type-density matrix (Fig. 9; ANOSIM, $R > 0.40$; excluding uninfected hosts). Differences in parasite burdens between *Bathymodiolus heckerae* and *B. puteoserpentis* ($R \geq 0.8$) and between *B. heckerae* from Florida Escarpment and Blake Ridge ($R = 0.43$) were attributable to infection by viral inclusion III, which accounted for an average of 68 % of the differences (SIMPER). *B. heckerae* from the Florida Escarpment mapped into two significantly different groups ($R = 0.58$; ANOSIM; Fig. 9). Infection with viral inclusion III accounted for 67 % of the difference between these groups (SIMPER). *B. heckerae* individuals infected with viral inclusion III mapped closest to the *B. heckerae* individuals from Blake Ridge that were also infected with the virus (Fig. 9). Viral-like gut inclusions occurred in mussels from all four sites, but *Bathymodiolus heckerae* had a greater prevalence of viral inclusions than *B. puteoserpentis* (Fig. 10a). Sixty percent of *B. puteoserpentis* at Snake

FIGURE 8

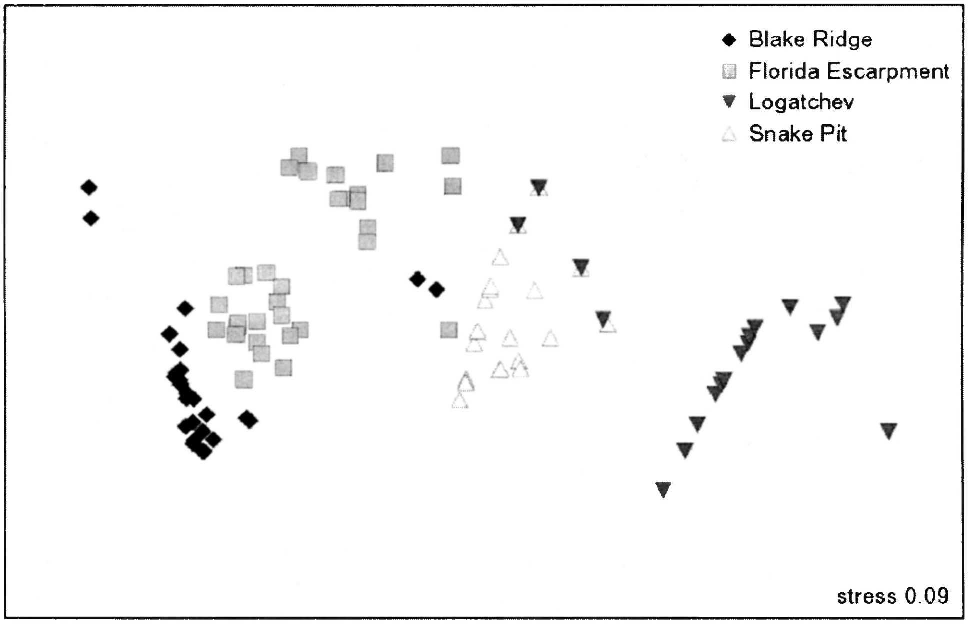
PREVALENCE AND BODY BURDEN



a) Prevalence of *Bathymodiolus heckerae* and *B. puteoserpentis* mussels infected with any parasite. b) Body burden (Mean infection densities for all parasitic infections) of infected *Bathymodiolus heckerae* and *B. puteoserpentis* mussels per 500 μm^2 of tissue. F.E. = Florida Escarpment. n = number of individuals

FIGURE 9

BODY BURDENS OF ALL MUSSELS INFECTED WITH AT LEAST ONE PARASITE ANALYZED BY MDS



Pit were infected with viral inclusion I, but less than 30 % of the mussels from the Logatchev site were infected with the same or similar viral inclusion I. More than 90 % of the *B. heckerae* from Florida Escarpment were infected with either viral inclusion II or viral inclusion III; of these, 53 % were infected by both viruses. Almost 90 % of *B. heckerae* from Blake Ridge were infected with the pathogenic viral inclusion III. Infection density of viral inclusion III was significantly greater in *B. heckerae* from Blake Ridge than from the Florida Escarpment (Tukey's: $p < 0.001$; Fig. 10b). Host size was an important factor in infection in *B. heckerae.* Mean infection density for viral inclusion III was greater in individuals with shell lengths less than 100 mm (Kruskal-Wallis with outlier, 0.005; Kruskal-Wallis without outlier: $p < 0.007$; Florida Escarpment, Fig 11a; Blake Ridge, Fig. 11b). Mussels with shell lengths greater than 100 mm were not infected or only slightly infected with viral inclusion III (Fig. 11a & b).

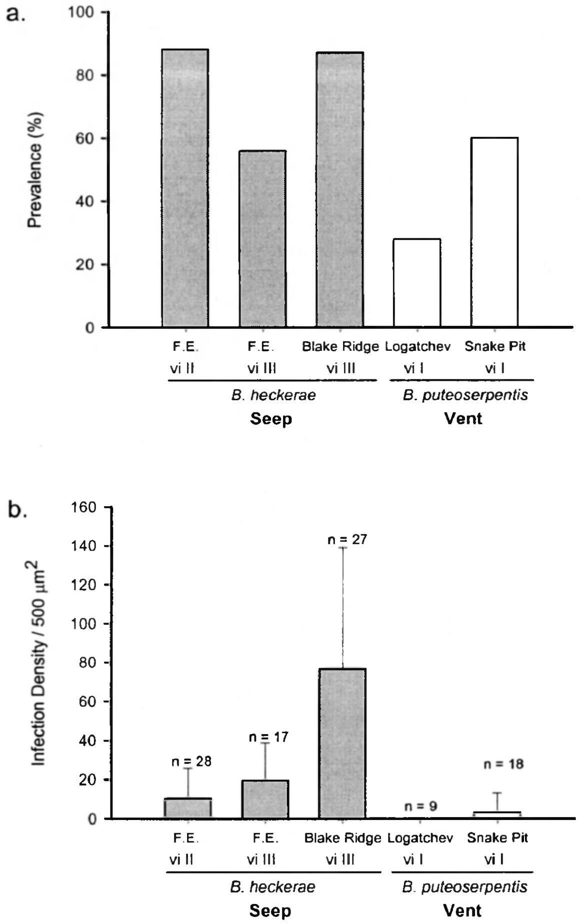
Gill rickettsia I was present in both species of mussels, but its prevalence varied between habitats. *Bathymodiolus puteoserpentis* from Logatchev vents had a significantly higher density of gill rickettsia I than *B. puteoserpentis* or *B. heckerae* from the other sites (Tukey's: $p < 0.02$).

Noninfectious diseases

Heavy hemocyte infiltration (Fig. 2a) occurred in mussels from all four sites (Table 4). Neoplastic gill epithelial tumors were observed in *B. puteoserpentis* from the Logatchev vent site and *Bathymodiolus heckerae* from the Blake Ridge seep site (Fig.

FIGURE 10

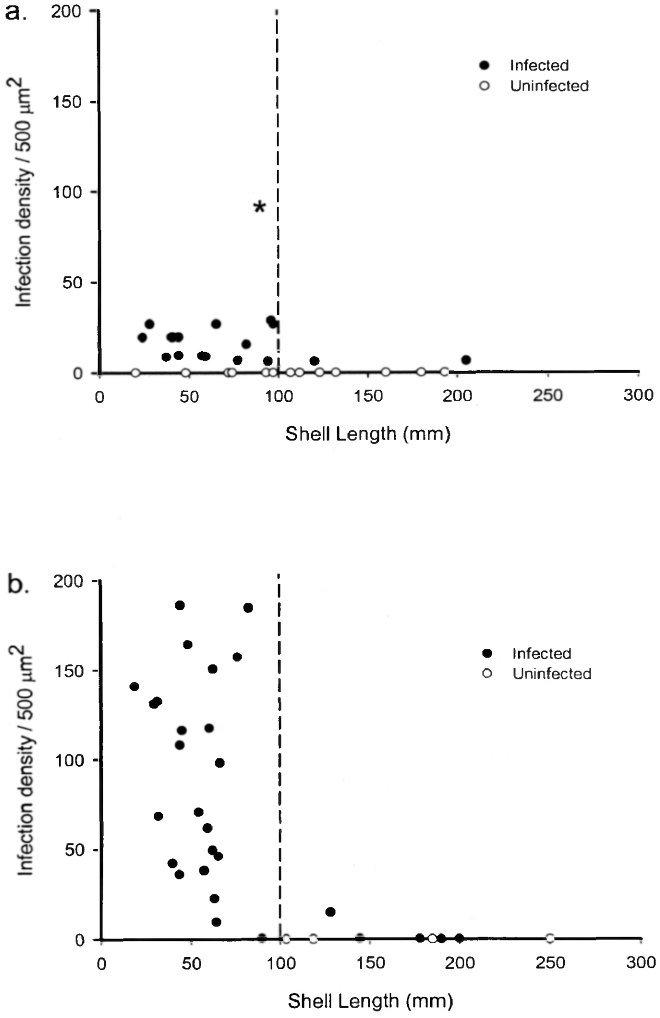
PARASITE PREVALENCE AND INFECTION DENSITIES OF MUSSELS INFECTED WITH VIRAL-LIKE GUT INCLUSIONS



a) Prevalence of viral-like gut inclusions in *Bathymodiolus heckerae* and *B. puteoserpentis* b) Density of infection of viral-like gut inclusions per 500 µm^2 of *Bathymodiolus heckerae* and *B. puteoserpentis* digestive tissue. F.E. = Florida Escarpment. n = number of individuals. Vi = viral inclusion

FIGURE 11

INFECTION DENSITY OF VIRAL INCLUSION III IN RELATION TO MUSSEL SHELL LENGTH



a) Infection density of viral inclusion III infections (per 500 μm^2 of tissue) in mussels from the Florida Escarpment in relation to mussel shell length (mm). * represents outlier. Dashed line indicates 100 mm shell length. b) Infection density of viral inclusion III infections (per 500 μm^2 of tissue) in mussels from the Blake Ridge in relation to shell length (mm). Dashed line indicates 100 mm shell length

12a & b). Hemocyte infiltration was observed in the majority of mussels (107 of 126) and in 42 of these mussels, massive hemocyte infiltration resulted in abnormal expansion of blood vessels (Fig. 12c & d). Most hemocytes appeared to be enlarged, with the nuclei displaced to the side of the cells (Fig 12d). A positive relationship (Pearson's: $p < 0.002$) was observed between hemocyte infiltration and host length in mussels from all four sites.

Index of gonad development

Gonads of *Bathymodiolus heckerae* from Florida Escarpment were more developed than those of *B. heckerae* from the Blake Ridge (Kruskal-Wallis: $p < 0.001$). In *B. heckerae* from the Florida Escarpment, individuals were more evenly distributed across the four categories of gonad development with only 18 % individuals possessing undifferentiated gonads. In *B. heckerae* from Blake Ridge, 81 % of individuals sampled had undifferentiated gonads. There was a negative correlation between total parasite body burden and degree of gonad development in *B. heckerae* (Pearson's: $p < 0.030$); the density of viral inclusion III was negatively correlated with gonad development (Pearson's: $p < 0.040$). There was no difference in gonadal development in *B. puteoserpentis* from Snake Pit and Logatchev.

Index of endosymbiont density

In *Bathymodiolus puteoserpentis*, endosymbiont density was positively correlated

TABLE 4

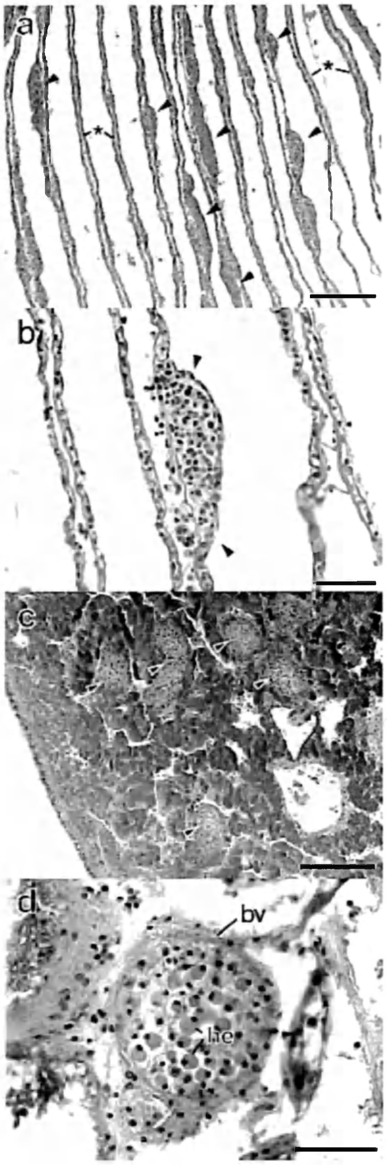
NONINFECTIOUS INDICES OF DISEASE

Prevalence of individuals with gill epithelial tumors and hemocyte infiltration and intensity of infiltration per 500 μm^2 of tissue. n = number of individuals sampled

	Bathymodiolus heckerae		***Bathymodiolus puteoserpentis***	
	Seep Sites		Hydrothermal Vent Sites	
	FE	BR	L	SP
n	32	32	32	30
Prevalence of gill epithelial tumors	9 %	31 %	3 %	6 %
Prevalence of hemocyte infiltration	91 %	97 %	63 %	90 %
Mean hemocyte infiltration (± s.d.)	2.2 (± 1.4)	2.9 (± 2.0)	2.3 (± 1.5)	2.8 (± 1.6)
Mean gonad development (± s.d.)	2.3 (± 0.97)	1.3 (± 0.70)	3 (± 1.13)	2.5 (± 1.10)

FIGURE 12

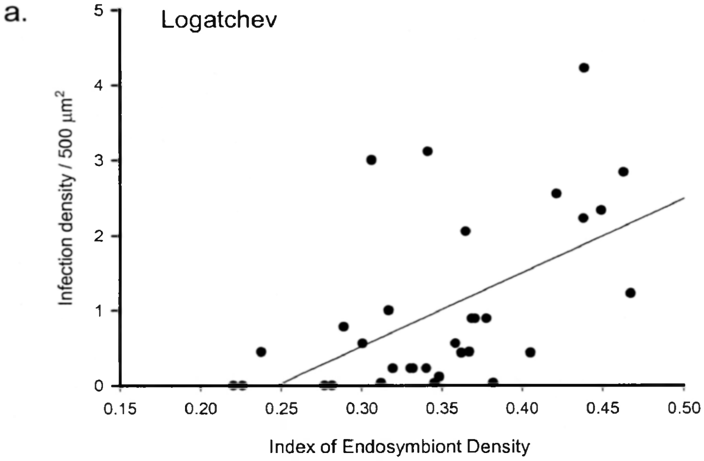
NONINFECTIOUS INDICATORS OF DISEASE

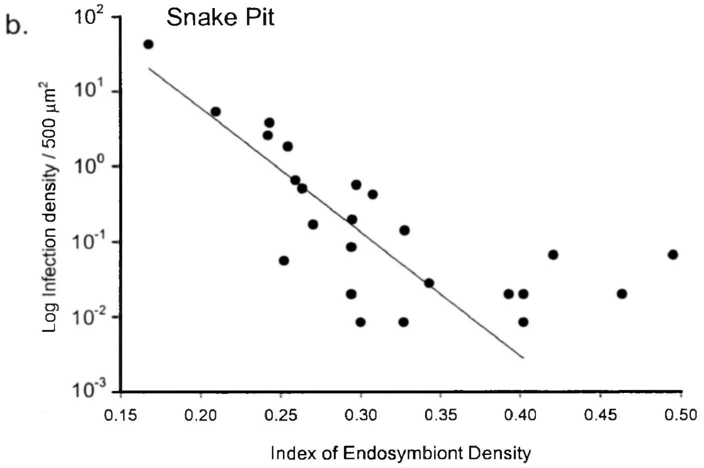


a) Gill filaments with areas of neoplasia (arrowheads) among normal gill filaments (asterisks). Scale bar = 200 µm. b) Neoplasia of gill filament (between arrowheads) demonstrated in figure 11a. Scale bar = 50 µm. c) Hemocytic infiltration causing enlargement of blood vessels (intensity of 5; arrow heads) in visceral mass. Scale bar = 200 µm. d) Enlarged blood vessel filled with abnormal hemocytes. The hemocytes were enlarged with the nuclei displaced to the side of the cells. Scale bar = 50 µm. bv, blood vessel; d, dorsal; he, hemocytes; v, ventral with infection density of gill rickettsia I (Pearson's: $p < 0.020$; Fig 13a). In *B. puteoserpentis* from Snake Pit, endosymbiont density also decreased with greater total body burdens of parasites in individual mussels and with an increase in infection density of viral inclusion I (Pearson's: $p < 0.020$; Fig. 13b).

FIGURE 13

RELATIONSHIPS BETWEEN INFECTION DENSITIES AND INDEX OF ENDOSYMBIONT DENSITY IN THE VENT MUSSEL *BATHYMODIOLUS PUTEOSERPENTIS*





a) Infection density of gill rickettsia I (per 500 μm^2 of tissue) in mussels from Logatchev in relation to the index of endosymbiont density. A similar relationship was observed in mussels from Snake Pit. b) Infection density of viral-inclusion I (per 500 μm^2 of tissue) in mussels from Snake Pit in relation to the index of endosymbiont density (Pearson's: $p = 0.013$). This relationship was not observed in mussels from Logatchev

DISCUSSION

Parasitism in seep and vent mussels

Nine different types of parasites were observed in *Bathymodiolus heckerae* from the seep sites; only two types of parasites were found in *B. puteoserpentis* from the vent sites. Only one type of parasite, gill rickettsia I, was common to mussels from both seep and vent habitats. We speculate that the ephemeral and extreme nature of vents may limit colonization and transmission of parasites, resulting in low parasite diversity in vent organisms (viz., Esch et al. 1990, Price 1990). Low diversity of invertebrates at vents compared to seeps has been attributed to the more ephemeral nature of vents (Craddock et al. 1995, Sibuet & Olu 1998) and to the potential for greater barriers to invasion of vent habitats due to their more extreme conditions (e.g., elevated metal concentrations, greater sulfide flux; Turnipseed et al. 2003). Other factors might also be implicated in determining the lower parasite diversity at hydrothermal vents. For example, invasion of deep-sea habitats by ancestral, shallow-water host species and their parasites may be a function of proximity to shallow-water sites. The Florida Escarpment and Blake Ridge seep sites, located along the continental margins of eastern North America, are closer to shallow-water habitats than are the Logatchev and Snake Pit vent sites. Seep habitats, with a higher diversity of invertebrate taxa, are more likely to have higher parasite diversity due to the greater number of potential hosts than vent habitats (Price 1990).

In shallow-water systems, species richness generally decreases as environmental stressors increase (Warwick & Clarke 1995, Menge & Branch 2001). Environmental stress, such as pollutants may affect the physiology of the host organism, thus negatively affecting a host response to disease-causing agents or restricting available habitat within the host for parasitic agents (Laird 1961, Lauckner 1983, Cheng 1988, Winstead & Couch 1988). Parasites themselves can also be affected by environmental extremes. Deep-sea vent and seep sites have noxious compounds such as sulfides, brines, and metals. These compounds may act as barriers to invasion by parasites and limit infections to microbial agents, as few protozoan and no metazoan parasites other than commensal polychaetes were found in these deep sea mussels.

Pathogenicity: Viral inclusion III

Viral-like infections were the most significant potential disease-causing agents found in the seep and vent mussels. Several lines of evidence, including relationships between mussel size and infection intensities of viral inclusion III, tissue destruction associated with viral inclusion III, undeveloped gonads in mussels infected by viral inclusion III, and *in situ* observations of extensive mussel mortality, suggest that viral inclusion III may be a significant pathogen with a potentially adverse affect on mussel populations at the Blake Ridge seep. Infection by viral inclusion III was pathogenic, causing significant damage to the digestive diverticula and connective tissues in the visceral mass. Although bacterial endosymbionts provide most of the nutrition to seep and vent mussels (Felbeck et al. 1981, Cavanaugh 1983, Childress et al. 1986), bathymodiolin mussels retain the ability to filter feed, which presumably provides some nutrition to the mussels (Le Pennec & Hily 1984, Page et al. 1991). Viruses that form intranuclear inclusion bodies, such as those observed for viral inclusion III, can result in reduced food uptake in bivalves due to necrosis within the digestive tract (see Lauckner 1983). The high prevalence of mussels with undifferentiated gonads from Blake Ridge may be a result of castration by viral infection III or resorption of gonads by an infected host, which is a common response in molluscs to disease (Lauckner 1983).

Several patches of empty valves have been observed in mussel populations from the Blake Ridge, suggesting that mass mortalities have occurred at this site (Van Dover et al. 2003). While the mortality could not be attributed to a specific cause, the valves were relatively uniform in size, suggesting a common cause of death. The mortality was attributed to shifting foci of seepage (Van Dover et al. 2003), but the pathogenicity of viral inclusion III suggests an alternative hypothesis, namely that viral infection may have contributed to the mortality. Mussels with shell lengths greater than 100 mm were either lightly infected or uninfected with viral inclusion III and may represent a subset of the population that was immune to infection or strong enough to overcome the infection. Alternatively, viral inclusion III may be an opportunistic parasite infecting stressed hosts.

Pathogenicity: Hemocytic infiltration

Heavy infiltration by abnormal hemocytes was observed in the majority of mussels sampled. The predominant mechanism of molluscan internal defense involves phagocytosis by circulating hemocytes (Pipe & Coles 1995); and infiltration by hemocytes is part of the immune response to parasitic infection in molluscs (Quayle 1969, Lauckner 1983, Pipe & Coles 1995,). Abnormal hemocytes, as seen in *Bathymodiolus heckerae* and *B. puteoserpentis,* may impair host immune function. The extreme infiltration by hemocytes into tissues and blood vessels was comparable to that observed in diseased, shallow-water bivalves (reviewed in Lauckner 1983). Although hemocyte infiltration can increase with parasitic infection, no correlation was observed between parasite infection densities and hemocyte infiltration in *B. heckerae* or *B. puteoserpentis*. While hemocytes are important in molluscan internal defense, heavy hemocyte infiltration can hinder the normal flow of hemolymph, give rise to a reduction of host tissues needed for vital functions, and has been suggested as a possible cause of mass mortalities (Lauckner 1983, Villalba et al. 1997, Lee et al. 2001).

Prevalences of parasites

Fifty percent of the parasite types observed in *Bathymodiolus heckerae* (gill rickettsia I & II, gill ciliates, gill "rosettes," and gut chlamydia) were similar to parasites present in mussels (*Bathymodiolus* sp.) of relatively shallow (550-650 m) hydrocarbon seeps (Powell et al. 1999), and all but the gill rosettes are similar to common parasites found in coastal-zone mussels (Kim et al. 1998). Parasites resembling gill rosettes occur in the shallow-water lucinid clam *Loripes lucinalis,* which also hosts chemoautotrophic, endosymbiotic bacteria in its gills (Johnson & Pennec 1995, Powell et al. 1999). Trematodes (*Bucephalus*-like), which were the most pathogenic parasite observed by Powell et al. (1999) and which can be common in intertidal mussels (Lauckner 1983, Kim et al. 1998, Montaudaouin et al. 2000), were entirely absent in *B. heckerae* and *B. puteoserpentis* from the deeper vents and seeps. Trematodes were also absent in limpets from deep-sea hydrothermal vents and a deep-sea seep site (Terlizzi pers. comm.). Larval trematodes have a high host specificity and use molluscs as their first intermediate hosts (see Lauckner 1983, Mouritsen et al. 1997). Transmission and infection by trematodes are affected by locality, season and host (Lauckner 1983, Montaudaouin et al. 2000). The absence of trematodes in this study could be the result of factors such as depth, susceptibility to heavy- metal toxicity in trematode larval stages, or the lack of suitable intermediate and definitive hosts.

Polychaetes

Polychaetes are commonly found within the mantle cavities and between the gills of bathymodiolin mussels (Van Dover et al. 1999), but their true relationship with the host mussel is unknown. Gill flatworms (e.g., *Urastoma cyprinae*) are also common inhabitants of mantle cavities in coastal mussels (Villalba et al. 1997). Flatworms can disrupt the structure of gill filaments, cause necrosis of gill tissue, and thereby reduce the feeding capacity of the host resulting in loss of condition. From these observations, Villalba et al. (1997) concluded that the flatworms were parasitic. Both the polynoid (*Branchipolynoe seepensis*) and the nautliniellid (*Laubierus mucronatus*) polychaetes found in vent and seep mussels cause minor tissue damage to the gills and displacement of gill filaments, but there is no strong evidence for a detrimental effect on host condition. This is consistent with observations of Fisher et al. (1988), who found that a species of commensal polynoid polychaete in mussels at the Rose Garden hydrothermal vent (Galapagos Spreading Center; eastern Pacific Ocean) did not cause gross lesions in soft tissues of the infected mussels. The polychaetes likely consume bacterial-laden mucus or pseudofeces, or free-living bacteria rather than tissues of the host mussel.

SUMMARY

In summary, parasite burdens were greater in mussels at seeps than at vents. In some instances, parasites may affect reproductive output and overall health of the mussel populations, as is observed in shallow-water bivalve populations. We suggest that infection by viral inclusion III influences population dynamics of *Bathymodiolus heckerae* at the Blake Ridge seep site, possibly causing widespread mortalities in juvenile mussels. Mussels from both seep and vent sites suffered from severe hemocytic infiltration by abnormal hemocytes. Hemocytic infiltration may be a symptom of disease or it may be an independent agent of mortality. How parasitic infections and hemocytic conditions affect individual mussels and the invertebrate communities associated with deep-sea mussel beds remains to be determined. Therefore, parasitological studies are a necessary addition to the more traditional ecological approaches used in order to obtain an accurate representation of the factors affecting community structure.

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VITA

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Megan Elizabeth Ward was born in Lima, Peru on September 20, 1978. She graduated from McLean High School in McLean, Virginia in 1996. In the Fall of 1996, Megan entered the College of William and Mary where she received a B.S. in Biology and in Interdisciplinary Sciences in Environmental Science in May 2000. In 2001, she began her Master's degree in the laboratory of Cindy Lee Van Dover at the College of William and Mary. During her time in Dr. Van Dover's lab she had the opportunity to dive in the DSV *Alvin* twice. The work presented in this thesis was defended in March 2003.