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Population Size Structure and Feeding Biology of *Bathynerita naticoidea* Clarke 1989 (Gastropoda: Neritacea) from Gulf of Mexico Hydrocarbon Seeps

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***Bathynerita naticoidea* is a numerically dominant gastropod in upper continental slope chemosynthetic communities of the northern Gulf of Mexico. A comparison of population size structure at four sites off Louisiana revealed site-specific differences in mean shell size consistent with different recruitment histories and growth rates. Where individuals grow to the largest size, population numbers are low and recruitment seems to be limited. Where individuals grow to the smallest size, populations are high and recruitment seems to be high. These patterns appear to parallel the population size pattern of the beds of *Bathymodiolus childressi* Gustafson et. al. 1998 inhabited by the snail, which suggests a link between the control of the two. Analysis of gut contents and fecal matter of *B. naticoidea* and the organic film on the shell surface of *B. childressi* confirmed initial assumptions that the snail feeds by radular browsing. Free-living bacteria are abundant on mussel surfaces and are ingested by the snail. The presence of bacteria in the gut and feces was, however, lower, possibly because of dilution by mucus and digestion. It is proposed that *B. childressi* provides more than a passive surface for organic film development. The mussel may control the organic film development, thus controlling availability of food to the snail.**

Bathynerita naticoidea Clarke 1989 (Fig. 1) is a numerically dominant gastropod in upper continental slope chemosynthetic communities in the Gulf of Mexico (Clarke, 1989; Warén and Bouchet, 1993; Carney, 1994) and similar depths on the Barbados Excretionary Prism (Olu et al., 1996). Related, if not identical, species have been associated with chemosynthetic systems since the Miocene; *Nerita* (*Thalassonerita*) *megastoma* Moroni in Italian deposits (Taviani, 1994) and *Thalassonerita? eocenia* Squires and Goedert in the Humptulips Formation of the Olympic Peninsula, Washington State (Squires and Goedert, 1996). The habitats of contemporary *B. naticoidea* in the northwest Gulf of Mexico are sites of hydrocarbon seepage in a region where geological processes of sediment movement on the continental slope interact with migrating salt layers within the slope. The result is an unusually complex seafloor topography with numerous geochemically unique local environments characterized by combinations of methane, hydrogen sulfide, liquid petroleum, and brine seepage (Roberts and Carney, 1997). These environments are colonized by symbiont-hosting metazoans only below a depth of ~400 m. Below that bathymetric threshold, dense aggregations of mussels and vestimentiferan tubeworms are present. Between 400 and 1,000 m, the depth limit of the Johnson Sea-Link submersible, selected examples of this habitat have been well studied. It is here that *B. naticoidea* is abundant on the surface of mussel shells, predominantly *Bathymodilous childressi* (Gustafson et al., 1998). The logistical complexity of research below 1000 m has limited the extent of seep exploration, but studies of other Gulf of Mexico seep sites, such as Alaminos Canyon at 2200 m and the west Florida escarpment at 3300 m, indicate geographic or bathymetric restriction. *B. naticoidea* appears to be absent from these systems, and there is a shift in the dominant mussel from *B. childressi* to *B. brooksi* and *B. heckeri* (Gustafson et al., 1998).

The work reported herein was intended to answer two questions that grew out of initial field observations of *B. naticoidea*. The first concerned population differences over a geographic region. The species composition of the seep community above 1000 m has proved to be remarkably uniform for both the large substrate-forming organisms and the smaller associated fauna (Carney, 1994). It is well established, however, that the population structure and growth of the dominant chemosynthetic mussel, *B. childressi*, show site-to-site variation linked to local geochemical processes (Nix et al., 1995). It was, therefore, questioned whether the population of a dominant heterotrophic associate would show similar variation. This question was addressed by intersite sampling and determination of size-frequency relationships. The second question concerned feeding.

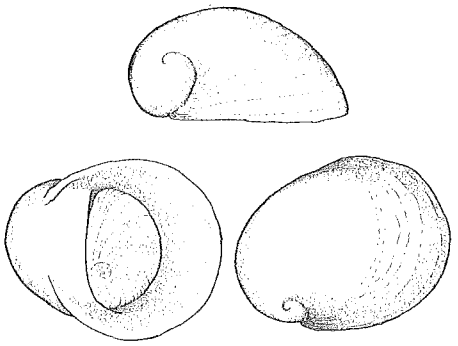

Fig. 1. Lateral, dorsal, and ventral views of a typical *Bathynerita naticoidea* specimen collected at GC-184. The color is white, and the shell lacks markings and sculpture except of growth lines. Usually, the apical region has undergone some dissolution. The specimen is 12 mm in length.

What is it within the seep community that supports such an abundant population of consumers? This work is a portion of a larger study of the organism-level biology and ecology of *B. naticoidea* (Zande, 1994).

An initial supposition that *B. naticoidea* is a grazer of surface films is supported by morphology. Shallow-water Neritacea are known to graze algal films (Hughes, 1986). Although *B. naticoidea* has a radula with distinct modifications (Warén and Bouchet, 1993), it shares with all species in the Neritaceam superfamily a rhipidoglossan radula associated with grazing (Fretter and Graham, 1962). A trophic link to chemosynthesis has been demonstrated from ^{13}C and ^{15}N stable isotope analysis; the tissues of *B. naticoidea* resemble that of the chemosynthetic mussels of the community (MacAvoy et al., in press; Kennicutt, pers. comm.). The possibility that these isotopic values reflected symbiosis within *B. naticoidea* can be rejected. Although there are three confirmed and two suspected symbiont-hosting gastropods found in hydrothermal systems (de Burgh and Singla, 1984; Stein et al., 1988; Warén and Bouchet, 1993), *B. naticoidea* shows no evidence of such a relationship. Its gills are typical for the superfamily, not hypertrophied, lack internal symbionts, and have shown no enzyme activity for chemosynthesis. Assays for ribulose-1,5-bisphosphate carboxylase-oxygenase, adenosine triphosphate sulfurylase, and methanol dehydrogenase were negative (C. Fisher, pers. comm.). A fungal associate on the gill has been reported (Zande, 1999), but there are no specific indications of a chemosynthetic symbiosis.

Methods and Materials

The four sites sampled (Fig. 2, Table 1) were chosen to meet multiple objectives of the Chemosynthetic Ecosystems Study (MacDonald et al., 1995), which is primarily concerned with making initial comparisons and contrasts of the ecology, geochemistry, and geology of hydrocarbon seep communities at multiple locations. Preliminary surveys had established that the sites had seep communities, offered a range of bottom topography, and were logistically convenient. It was anticipated that all four would have sufficient community development to allow for replicated long-term sampling. The sites, designated by the seafloor lease blocks in which they lay, were within the Green Canyon (GC) region, specifically in blocks 185, 234, 233, and 272. The specific bottom feature sampled in block GC-185 was a large carbonate-topped diapir called "Bush Hill" because of the abundance of chemosynthetic tubeworm clumps on its summit (MacDonald et al., 1989). GC-234 and GC-272 both contained carbonate outcrops with hydrocarbon seepage and chemosynthetic communities. Once extensive sampling began, however, only one small mussel bed was located and sampled at GC-272, whereas extensive scattered beds were found at GC-234. The community at GC-233 was unique in being a brine pool (MacDonald et al., 1990a), a 22 × 11 m elliptical crater filled with a heavier-than-seawater brine and surrounded by a ring of mussels.

The platform for sampling was the Johnson Sea-Link submersible operated by the Harbor Branch Oceanographic Institution of Fort Pierce, Florida. Operating at depths down to 1000 m, beds of the chemosynthetic mussel *B. childressi* ranging in size from ~2 to >100 m^2 were located and sampled with a hydraulic grab integral to the manipulator of the submersible. The cylindrical grab was designed to take a uniform sample area of 341 cm^2, which allowed the samples to be considered quantitative. Caution in treating the samples as quantitative is necessitated by the difficulty in equating grab volume to the mussel shell surface area actually sampled. The grab sampled the entire thickness of the mat, scraping the mussels from the underlying substrate.

Each of the four samples consisted of the within-site pooled specimens from multiple grabs (Table 1). Within site pooling was considered a priori the appropriate design for two reasons. First, *B. naticoidea* has been observed to be highly mobile and forming temporary aggregates. Analysis at the individual grab level

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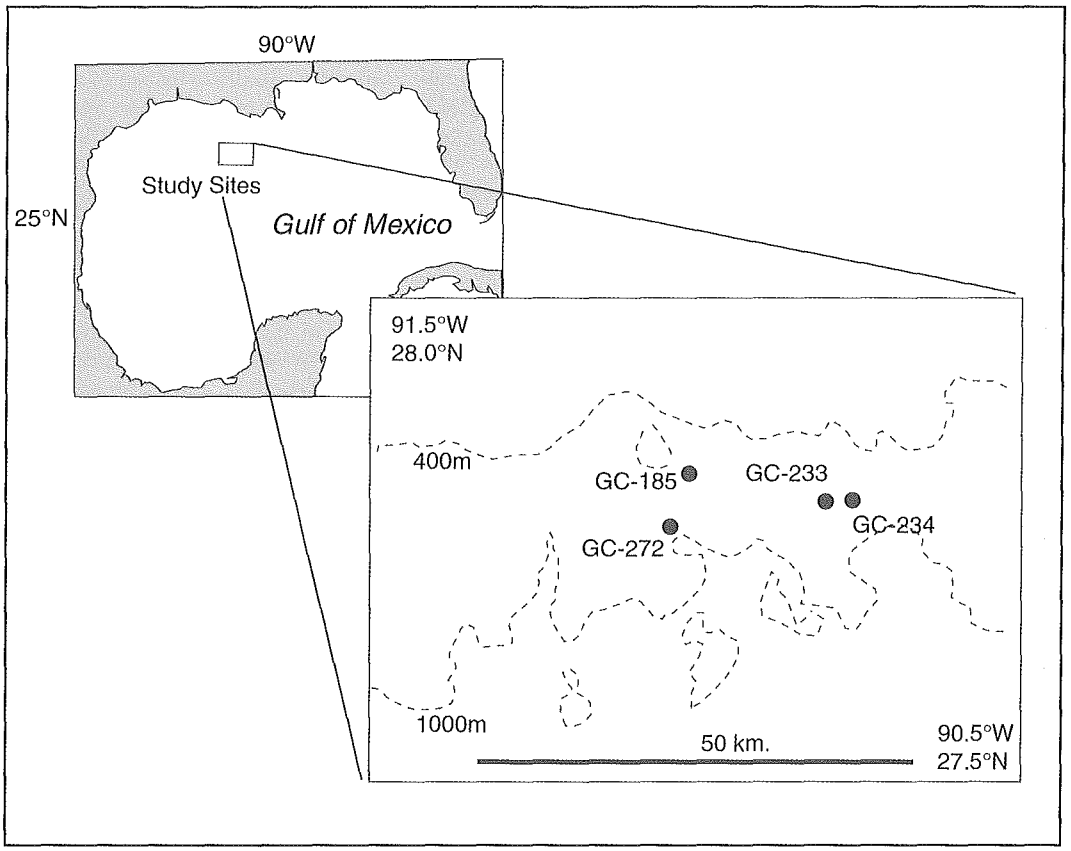

Fig. 2. The continental slope of the northern Gulf of Mexico has numerous hydrocarbon seep sites inhabited by chemosynthetic communities. Four such sites were sampled in the present study.

would, therefore, reflect only transient patterns. Second, logistical constraints of submersible work and intrasite differences in habitat extent prevented equal sampling effort and a consistent sampling strategy at all sites. Results of analysis at the individual grab level would, therefore, be confounded with sampling artifacts. Pooling suitability was verified post hoc through examination of descriptive statistics, means, standard deviations and ranges, tests for normality, and tests for homogeneity of samples within sites by use of SAS procedures. Intersite comparisons on the pooled samples were tested by two statistical procedures: analysis of variance tested similarity of mean shell length, and similarity of length-frequency relationships were tested by the Kolmogorov-Smirnov (K-S) method (Sokal and

TABLE 1. *Bathynerites naticoidea* samples, grab effort, and catch per grab statistics and variance/mean examination of aggregation.

Site, depth (m)	Latitude, longitude	Grab effort	Specimen condition	Mean catch	Variance	Variance/mean
GC-185, 550	27°47.00′N, 91°30.40′W	17	Live	62.0	6977.3	112.5
			Dead	8.0	110.8	13.8
GC-234, 550	27°44.70′N, 91°13.20′W	9	Live	67.6	6326.5	93.7
			Dead	6.6	140.0	21.4
GC-233, 650	27°43.40′N, 91°16.70′W	8	Live	22.2	203.4	9.1
			Dead	4.5	31.1	6.9
GC-272	27°41.20′N, 91°13.28′W	7	Live	3.3	16.6	5.0
			Dead	15.1	129.8	8.6

Rohlf, 1981). In order to maintain an overall acceptance criteria of $\alpha = 0.05$ during multiple inter-site comparisons, Bonferroni's criteria of 0.0083 was adopted.

Sample processing for size analysis began on deck. Collections were sieved through a 500-μm screen to remove any sediment and preserved in bulk in 10% seawater formalin. Within 1–2 wk of collecting, the formalin solution was drained, the samples rinsed, then soaked in tap water for 24–48 hr, and stored in 80% ethanol. Samples were subsequently sorted to species and *B. naticoidea* was separated for additional study. Because the shell of *B. naticoidea* is simple, unadorned, and very consistent in proportions, length provided an adequate measure of overall size. Length was measured to the nearest tenth of a millimeter along the axis of the shell outline, as defined by Murty and Rao (1978), by use of a dissecting microscope with attached camera lucida.

For the feeding study, it was assumed that feeding biology for the species was similar at all sites, and intersite differences were not sought. Samples for feeding studies consisted of guts dissected from preserved specimens, fecal material collected from live specimens at sea, and the perisotracum-covered surface of mussel shell. The samples were examined by a combination of three techniques. Scanning electron microscopy (SEM) was used to provide visual, descriptive information and used similar specimen processing for all samples: ethanol dehydration, critical point drying, and gold-palladium sputter coating. Electron microprobe analysis was used to determine the identity of the mineral components and to verify the organic nature of others. Epifluorescent microscopy that used acridine orange and 4′,6′-diamidino-2-phenylindole (DAPI) was used to label live bacteria or other microorganisms.

Gut contents identification and quantification was carried out on guts dissected from eight formalin-preserved specimens, two from each of four sites. The contents were disaggregated and the resulting paste smeared over 1.27-cm diameter Cambridge pin mounts by use of double-sided tape and prepared for SEM by dehydration in 100% ethanol, critical point dried, and sputter coated with gold palladium. A field of ~1 mm^2 was located under the SEM at a magnification of ×30. Then a large-field photo mosaic of the entire area was created with an effective magnification of ~×575, sufficient to identify bacteria and larger particles. A semiquantitative description of the gut contents was developed by point counting and identification of objects lying at 200 preselected random points. Elemental analyses of the gut contents were done by use of a JOEL 733XMA electron microprobe. The gut contents of a single preserved specimen from each site were removed, press-tab mounted onto carbon planchets, and air dried. Each planchet was then attached to a separate pin mount, carbon coated by use of a Balzers MED010 carbon coater, and examined with the microprobe.

Qualitative microbial analyses were performed on the gut contents of 12 preserved specimens from GC-234 and five preserved specimens from GC-233. The gut contents were placed in separate sterilized vials and rinsed with filtered (0.45 μm) deionized water. Two hundred microliters of 0.1% acridine orange solution prepared with deionized water was added to each vial. The vials were then mixed and allowed to stain for 2 min. Each resulting suspension was filtered onto 0.2-μm nucleopore black filters and rinsed with filtered (0.45 μm) deionized water. Filters were mounted onto a slide coated with immersion oil and covered with immersion oil and a cover slip. The slides were then examined at a magnification of ×100 by use of a Zeiss Axioskop epifluorescence microscope.

Fresh fecal material was collected during the September 1991 cruise by maintaining 25 live specimens from GC 185 in petri dishes without food in a shipboard cold room (4–6 C) for 3 d. The fecal material produced was pipetted off, fixed in 10% seawater formalin, distilled water rinsed, and then prepared from SEM examination in the standard manner. A single shell of a living *B. childressi* was used to provide a qualitative sense of the materials found there. The shell was removed from a fresh Johnson Sea-Link sample and preserved immediately in 10% seawater formalin. Within 1–2 wk of collecting, the formalin solution was drained. The shell was then rinsed in distilled water, pieces removed from three regions (mantle edge, middle, and upper umbo), and the pieces prepared for SEM examination. Qualitative analyses of microbial populations on the shell surface was performed on single pieces of shell removed from each of the regions. They were placed in separate sterilized vials, water rinsed, transferred to 2 ml filtered (0.45 μm) sodium pyrophosphate, and sonicated for ~10 sec to dislodge microorganisms. Acridine orange staining, filtration, and slide preparation were the same as for gut contents. A second method of qualitative microbial analysis that used DAPI (Porter and Feng, 1980) were performed on periostracum separated

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TABLE 2. Analysis of variance and Kolmogorov comparison results grabs pooled within sites.

Site		Average length, mm	SD	*n*
GC-233		12.8	2.73	178
GC-185		10.1	2.29	1054
GC-272		9.8	1.08	23
GC-234		7.9	2.23	608
ANOVA		Model	Error	Total
Degrees of freedom		3	1859	1862
Sums of squares		3741.67	9887.7	
Mean square		1247.22	5.319	
F ratio				234.5
Probability (*P*)				<0.0001
Kolmogorov 2-sample statistic	GC-233	GC-185	GC-272	GC-234
GC-233	—	6.62 ($P < 0.001$)	3.50 ($P < 0.001$)	8.7 ($P < 0.001$)
GC-185		—	1.5 ($P = 0.017$)	8.9 ($P < 0.001$)
GC-272			—	2.29 ($P < 0.001$)
GC-234				—

from the calcified portion of the shell. Pieces of periostracum were removed from each of the three regions, placed in separate sterilized vials, and water rinsed. Staining consisted of 5 hr immersion in added 0.1 μg/mL sufficient to cover each piece of periostracum. Final filtration, slide preparation, and examination were as for gut contents.

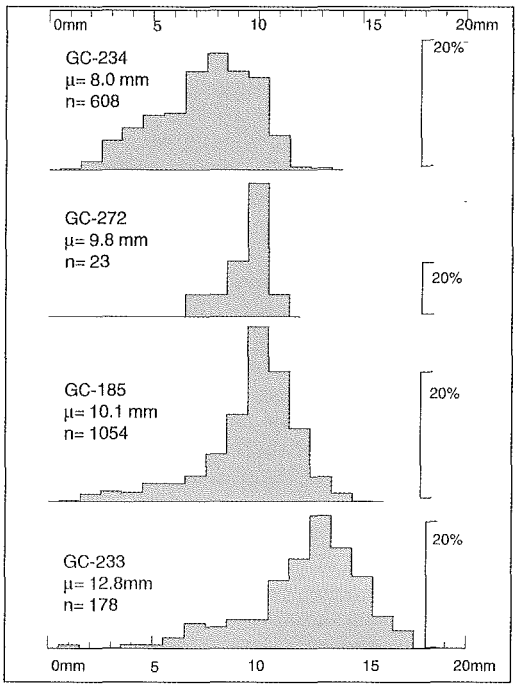

Fig. 3. Size frequency histograms for living specimens reveals a distinct difference in modal size and abundance of smaller individuals between GC-234 and GC-233. The populations at GC-185 and GC-272 appear to be similar and intermediate.

RESULTS

Size structure.—The average catch of live specimens varied appreciably among sites, with GC-185 and GC-234 averaging in excess of 60 individuals per grab (Table 1). GC-233 had an average of about one-third that amount, whereas at GC-272 dead specimens predominated. Typical of benthic fauna, the catch per grab was highly aggregated, as evidenced by large variance : mean ratios. Live specimens were extremely aggregated, with dead shells being noticeably but less dramatically aggregated. This pattern of highly clumped live specimens and more evenly distributed dead specimens was repeated at all sites except GC-272, where there was an atypical abundance of dead specimens and a paucity of live.

With respect to size, there was great similarity between live and dead specimens. A test for similar mean size could not be rejected (μ-live = 9.66 mm, μ-dead = 9.80 mm, $t = -0.821$, probability of greater t value by chance, 0.41). In spite of this similarity, subsequent intersite analysis was limited to live specimens. Significant site-to-site differences in live *B. naticoidea* size were found in the analysis of variance (Table 2) and were obvious in the size frequency diagrams (Fig. 3). By inspection, it is apparent that GC-233 site had the highest frequency of large specimens ($\mu = 12.8 \pm 2.97$ mm) and GC-234 the lowest ($\mu = 8.1 \pm 2.31$ mm). In-

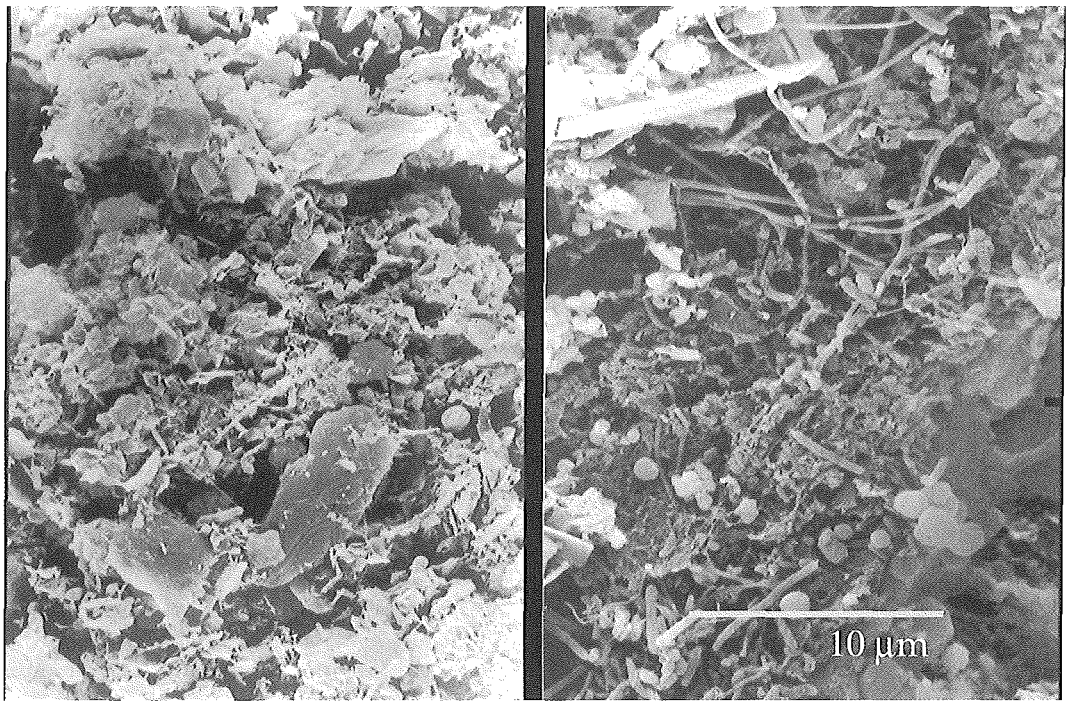

Fig. 4. The surface of *Bathymodiolus childressi,* left, contains a rich and diverse microbial fauna and decomposing perisotracum. Gut material from field-collected *Bathynerita naticoidea,* right, have markedly fewer microbes than the mussel surfaces but contain decomposing perisotracum and very small mineral detritus from the seep system.

termediate mean sizes were found at GC-185 (μ = 10.1 ± 2.32 mm) and GC-272 (μ = 9.8 ± 3.22 mm). K-S pairwise tests produced complimentary results for the overall size-frequency relationships. Significant differences (Bonferroni's criteria for significance $P < 0.00833$) were again detected between all pairings of sites except GC-185 and GC-272. These two sites may be considered as having similar size distributions; however, the unequal sample sizes limited our ability to detect real differences that might exist.

Feeding study.—The apparent texture, color, and consistency of the gut content smears and the field-collected fecal material of all specimens from all sites was quite similar. Light microscopy and low-magnification (×30) SEM revealed amorphous aggregations of light- and dark-colored material without readily identifiable components (Fig. 4). At the higher power (×575) SEM and point counting of gut content smears, seven categories of material could be found in varying percentages in all specimens (Table 3). Approximately 48% of the gut contents could be classified only as unidentified amorphous material. Mucus that may have been produced by the gastropod during feeding represented ~30% of the gut contents. Small organic spheres, possibly microbial in origin, represented 10%. Decomposing mussel periostracum and byssal fibers in the form of small flakes and fibers comprised 5%. Readily identifiable organism parts such as crustacean appendages, sponge spicules, and echinoderm ossicles comprised only ~1% of the contents. Mineral grains comprised ~5%. The same seven categories of gut contents could be found in field-collected feces.

Electron microprobe analyses confirmed the suspected nature of three gut content categories seen in the gut content smears. The X-ray spectra of mossy strands and flakes and fibers were consistent with organic material. The mineral nature of the crystalline platelets was confirmed by X-ray spectra characteristic of silica, magnesium, aluminum, potassium, and sodium associated with clays, feldspar, quartz, calcite, aragonite, and dolomite. Certain spherical crystalline objects contained magnesium sulfate, potassium sulfate, and sodium chloride, all soluble salts that may be artifacts of processing. Other spherical and fragmented crystalline objects, however, contained sulfates

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TABLE 3. Gut content categories determined by point counting.

Category	Percentage of contents	Description
Amorphous	47.9 ± 4.5%	No consistent and characteristic shape, unidentifiable. Only limited live bacteria content confirmed by epifluorescence.
Mossy strands	29 ± 6.7%	These objects came in a variety of sizes (2 to >50 μm in diameter) throughout the gut material within and around amorphous aggregations (Fig. 4a). They were suspected to be mucus aggregations. X-ray spectra confirmed organic nature.
Small spheres	9.9 ± 3.8%	Comparatively uniform 1–2-μm diameter spheres associated with all larger gut content particles, possibly microbial.
Mineral grains	5.6 ± 4.2%	Mixed crystalline grains 10–15 μm across tabular, platelet-shaped, spherical, and fragmented. Mineral nature confirmed by X-ray spectra.
Flakes and fibers	4.8 ± 1.9%	Thin flakes 10–40 × 10–20 μm and flat fibers 50–150 μm long suspected to be derived from mussel periostracum and byssal fibers. X-ray spectra confirmed organic nature.
Gut lining	1.8 ± 2.1%	Gut epithelium was occasionally observed and was considered an artifact of preparation.
Invertebrate parts	1.1 ± 1.5%	Partial crustacean appendages, optically amorphous silica sponge spicules, and optically crystalline carbonate echinoderm ossicles.

of barium, calcium, and strontium, indicating local formation within the seep environment (Dr. Harry Roberts, Dr. Paul Aharon, pers. comm.). These sulfates did not account for the total amount of sulfur detected by the microprobe. The remaining sulfur must be present its elemental form in much smaller sizes.

Epifluorescent light microscopy of gut contents and fecal material indicated that intact bacteria were present but not abundant. Most of the material that formed the amorphous category was stained by acridine orange, which indicates a bacterial origin. Scattered bacterial/microbial aggregates of spheres, rods, and isolated filamentous chains of various sizes (1 to >50 μm) were present and usually centered around smaller aggregations of amorphous gut material. Individual spheres ~5 μm in diameter resembled in size and shape the fungal bodies at the gills of the gastropod (Zande, 1999).

Examination of mussel shell surface revealed a bacteria-rich film. Scanning electron micrographs of shell surfaces revealed a variety of bacteria, other microorganisms, detritus, and pieces of byssal fibers (Fig. 4) on all three shell sections; aggregations of spherical (0.5–2.5 μm), rod-shaped (1.5–2.5 μm), and filamentous (3 to >20 μm) bacteria were especially abundant within pits and lesions of the periostracum. The larger microbes are similar in size and shape to the fungal bodies. Detrital material contained a few coccolithophore tests, but other indications of plankton input were absent. Epifluorescent light micrographs of stained periostracum and shell revealed the presence of spherical, rod-shaped, and filamentous chains of bacteria and microorganisms similar in size to the microbes found in gut content smears. Individual spheres, rods, and filaments were scattered throughout the field of view as well as aggregated around pieces of periostracum or detrital particles. DAPI staining, however, failed to detect the presence of bacteria or other microorganisms within the periostracum. Because acridine orange and SEMs identified microbes on the shell surface, the lack of staining by DAPI suggests that the protocol used for this stain is not appropriate to identify microbes on and within mussel periostracum.

DISCUSSION

Intersite population differences.—When intersite average catch data are compared with intersite size-frequency data, a complex picture of differences in *B. naticoidea* samples emerges with no simple correlation of population density and specimen size. The sites with the most dra-

matic difference in population densities, CG-185 versus GC-272, have a statistically indistinguishable mean shell lengths. The sites with similar average densities, GC-185 versus GC-234, have significantly different size structures. In contrast to these comparisons, GC-233 is distinct in having very large specimens and only moderate population densities. The intersite relationships are simplified, to some extent, if GC-272 with its low population density, very small area of chemosynthetic habitat, and high number of dead specimens is treated as a special case ecologically and statistically. This can be justified by a consideration of the general nature of the sites.

CG-272 is a steeply sloping region with scattered carbonate outcrops; samples came from a small shelf a few hundred square meters in extent. The location sampled was a single mussel bed ~10 m^2 in extent on that shelf. The number of dead mussels and gastropods in those samples were quite high, which suggests a relatively recent mortality event. In contrast, GC-185 and GC-234 are both large sites with numerous mussel beds. All samples from GC-185 and GC-234 were taken from multiple mussel beds on a sloping region with carbonate outcrops and extensive development of tubeworm fields. The mussel bed at GC-233 was large and continuous.

An especially intriguing result of this study is that the site-specific size distributions of *B. naticoidea* generally parallel the size distributions of the habitat-forming mussel populations. The mussels at GC-234 have consistently been found to be the smallest and the mussels at GC-233 the largest, with GC-272 and GC-185 occupying close intermediate positions. The same site ordering has been found by measurement of sampled mussel populations (MacDonald et al., 1990b) and by actual mark and recapture growth studies on the mussels (Nix et al., 1995; Smith et al., 2000). The growth studies showed a population dominated by smaller mussels at GC-234 consisted of young, rapidly growing individuals. Sites where large specimens predominated had an accumulation of older, slower growing individuals.

Lacking estimates of *B. naticoidea* growth rates, it can be proposed but not proved that the population structure is generated by mechanisms similar to those controlling the mussels; age and recruitment of young rather than growth rate per se being more important. Because *B. naticoidea* undergoes development to a benthic crawling stage while in an attached egg capsule (Zande, 1994), recruitment must largely depend on within-site reproduction. GC-234 and GC-185 support roughly equal densities of *B. naticoidea,* but the former site has experienced greater reproductive success and recruitment of young. In spite of a very large mussel bed, GC-233 supports a smaller population *B. naticoidea,* and experiences the least reproductive success and recruitment of young, yet the individuals reach the largest size. If size can be equated with age, it can be suggested that the apparently lower carrying capacity at the brine pool is associated with a greater stability of resources.

The close parallels between mussels and *B. naticoidea* suggest that in some way both share a common site-specific history of recruitment, mortality, and growth. In the case of the mussels, this history must be directly linked to the seepage of chemosynthesis-supporting methane emissions. Chemical and geological variations between and within seep sites are known to exist (MacDonald et al., 1989, 1990b, 1990c; Roberts et al., 1990; Fisher, pers. comm.). It is more difficult to determine which factors influence the heterotrophic populations in such a similar manner. Populations of a true mussel predator might fluctuate with the prey population, but what regulates a browser like *B. naticoidea*? There are two primary possibilities. First, *B. naticoidea* is so dependent on free-living chemosynthetic bacteria that its populations respond to gas emissions in a manner independent of but similar to the mussels. Second, that the food supply of *B. naticoidea* is directly controlled by the mussel, causing the populations to vary in parallel.

Because the four study sites proved to be different in the extend of chemosynthetic fauna development, the question arises as to whether the conclusion that population size structure differences between sites might be an artifact of sampling. Perhaps within the numerous scattered mussel beds at GC-185 and GC-234 there might be aggregates of certain size classes that resemble the size structure found in the large single bed at GC-233 and the small single bed at GC-272. This possibility can be examined by partitioning the sums of squares for error in the main ANOVA (Table 2) into the variation due to different grabs within each site (Table 4). The hypothesis tested at each site is that all grabs produced the same average specimen size. The results differed among the four sites. At the extensively developed sites GC-185 and GC-234, the hypothesis must be rejected; different grabs produced significantly dissimilar average specimen sizes. However, the extent of size aggregation is not dramatic and explains only 29.5% and 28.4% of the variation in size at each site. At GC-233's large continu-

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TABLE 4. Analysis of variance result with grabs nested in sites.

Site	ANOVA table	Model	Error	Total
GC-185	Degrees of freedom	15	1038	1053
	Sums of squares	72,915	174,593	247,507
	Mean square	4,861	168	
	F ratio			28.9
	P			<0.0001
	Percentage of variance (%)			29.5
GC-234	Degrees of freedom	7	600	607
	Sums of squares	38,624	97,347	135,971
	Mean square	5,518	162	
	F ratio			34
	P			<0.0001
	Percentage of variance (%)			28.4
GC-272	Degrees of freedom	3	19	22
	Sums of squares	337	824	1161
	Mean square	112.4	43.4	
	F ratio			2.6
	P			0.08
	Percentage of variance (%)			29.1
GC-233	Degrees of freedom	7	170	177
	Sums of squares	2,444	56,785	59,230
	Mean square	349.2	334.0	
	F ratio			1.1
	P			0.40
	Percentage of variance (%)			4.0
Total (error from Table 2)	Degrees of freedom			1859
	Sums of squares			443,869

ous mussel bed, the hypothesis cannot be rejected; sample size structure within the site appears uniform. Once again, GC-272's small single mussel bed is problematic. Virtually the same percentage of within-site size variation is explained as at GC-185 and GC-234, which indicates essentially the same results. Actual hypothesis rejection or acceptance is criteria sensitive (single testing or multiple testing), largely because of small sample size.

As a final conclusion, the statement that pooled *B. naticoidea* samples show significant size differences between sites must be made cautiously. There is significant within-site variation where numerous patches of habitat exist. Therefore, sites such as GC-185 and GC-234 might contain areas where the population structure resembles that at GC-233. Arguing that the population at GC-233 is truly distinct and not an artifact is the marked greater abundance of the largest individuals found there. Better resolution of within- versus between-site differences remains to be better explored.

Feeding study.—The results of the feeding studies confirm that *B. naticoidea* browses on the surface of mussel shells in an apparently nonselective manner, ingesting any surface film, biotic and abiotic materials in that film, and fragments of the mussel perisotracum and attached byssal fibers. The materials assimilated to provide the primary nutrition source were not identified. It seems most probable that the nutrition source is a mixture of mucus-bound bacteria and labile organic detritus from within the seep system. Unlikely sources of nutrition are the rare invertebrate body parts, refractory periostracum and byssal fibers, and exogenous detritus from the euphotic zone.

The ubiquity of an elemental sulfur component in X-ray analyses of gut content smears strongly indicates that the ingested film contains sulfide-oxidizing bacteria as living cells detected by staining and as bacteria-derived, nonliving detritus. Microorganism populations of this nature have been reported elsewhere within chemosynthetic communities and have been hypothesized to be food sources for vent and seep grazers (Brooks et al., 1987; Hook and Golubic, 1988, 1990, 1992, 1993; Trager and De Niro, 1990; de Angelis et al., 1991; Warén and Bouchet, 1993). Although not found in

any of our analyses, the giant bacterium *Beggiatoa,* common at seeps (Roberts et al., 1990), contains relatively large elemental sulfur grains (Nelson and Jannasch, 1983), and smaller bacteria might produce even smaller grains. The larger, staining, spherical to elliptical, and rod-shaped bacteria, common on shell surfaces but relatively rare in the gut contents, may be free-living sulfide oxidizers. Their apparent paucity in the gut may simply reflect dilution by mucus produced during feeding and rapid digestion. An additional possible sulfide-oxidizing microbe is the ubiquitous, small organic spherical objects found in gut contents, field-collected feces, and on mussel shell. Alternately, these microbes may be the fungal spores found associated with *B. naticoidea* gill tissue.

These results are consistent with the qualitative observations of Warén and Bouchet (1993) on a variety of cold seep gastropods *B. naticoidea, Cantrainea macleani,* and *Cataegis meroglypta* and hydrothermal vent gastropods *Fucaria striata, Protolira valvatoides,* and *Pachydermia sculpta.* The material in the guts of those species was consistent with detritus collected in an organic surface film on the primary seep or vent organisms. That detritus generally consisted of unidentifiable organics, a mineral component, refractory parts of the benthic fauna, and biological detritus from the water column.

The feeding scenario that emerges from the results reported here is that the mussel shell serves as a substrate for the development of an organic film. That film may be primarily derived from mucus-like exudates from the closely packed mussels. The film serves as a microhabitat for bacterial growth and a trap for detrital material. The association of staining bacteria with damaged perisotracum suggests a direct role for the mussel covering in maintaining bacterial populations in the film. Even though all mussel periostracum is a sclerotized protein resistant to biological digestion and mechanical damage (Saleuddin and Petit, 1983), it harbors a microbial community. Hook and Golubic (1988, 1990, 1992, 1993) identified a variety of eukaryotic and prokaryotic microorganisms attacking and digesting the periostracum of *Bathymodiolus* sp. Secondary inhabitants, including chemoautotrophic and heterotrophic bacteria, colonized the resulting labyrinth of tunnels within the periostracum. It was speculated that large populations of grazing gastropods were dependent on these secondary inhabitants.

Combining the apparent nerite-mussel parallels in population dynamics with the information gained about feeding, it can be proposed that *B. naticoidea* populations are directly controlled by the state of the *B. childressi* populations. Because *B. naticoidea* is rarely seen on bare rock surfaces within seep sites and is much less common on vestimentiferan worm tubes adjacent to mussel beds (Ward, 2000), the mechanism of control appears to be the availability of a microbial-detritus film unique to the perisotracum-covered mussel shell. The nutritional content of that film must be linked to the metabolism of the mussel population, even though the film may contain free-living bacteria obtaining chemical substrates of methane and hydrogen sulfide from the environment. A strong possibility is that mussel products such as the proteinaceous periostracum and muco-polysaccharides are a required component of the microbial microhabitat.

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