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Ecology of Bacterial Communities in the Schistosomiasis Vector Snail *Biomphalaria glabrata*

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Abstract. The internal colony-forming bacterial flora of the schistosome intermediate host snail *Biomphalaria glabrata* (Say) has been characterized in ca. 500 individual snails from Puerto Rico, Guadeloupe, and St. Lucia, and from laboratory aquaria. Freshly captured wild snails harbor 2–40 $\times$ 10^6 CFU·g^{-1}, and healthy aquarium snails harbor 4–16 $\times$ 10^7 CFU·g^{-1}, whereas moribund individuals have 4–10 times as many bacteria as healthy individuals from the same habitats. *Pseudomonas* spp. are the most common predominant bacteria in normal snails, whereas *Acinetobacter*, *Aeromonas*, and *Moraxella* spp. predominate in moribund snails. External bacterial populations in water appear to have little effect on the composition and size of the flora in any snail. In addition to normal (healthy) and moribund snails, a third group of snails has been distinguished on the basis of internal bacterial density and predominating genera. These "high-density" snails may have undergone stresses and may harbor opportunistic pathogens. The microfloras of wild and laboratory-reared snails can be altered and stimulated to increase in density by crowding the snails or treating them with antibiotics.

Introduction

The freshwater planorbid pulmonate gastropod *Biophalaria glabrata* (Say) is the principal New World intermediate host of the trematode *Schistosoma mansoni*, a causative agent of schistosomiasis. Snail control is an essential and effective strategy for controlling schistosome transmission [17]. However, biological control of host snails has been successful in only a few instances and remains controversial [11]. Recognizing the great success of bacterial agents in insect control, we have been investigating the possibility of using bacteria as control agents against snail populations. As a first step toward this aim, we have investigated the role of bacteria in the ecology of *B. glabrata*.

B. glabrata populations are widely distributed in streams, lakes, marshes, irrigation drains, and reservoirs of the Caribbean, Brazil, and Venezuela. The ecology of *B. glabrata* has been described by Sturrock [26] and Jobin [16], while most aspects of

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schistosomiasis transmission and ecology are extensively reviewed and abstracted in Warren and Newill [30], Warren and Hoffman [31], and Cohen [5]. We studied the microbiology of snails from a variety of natural and artificial habitats typifying the distribution of *B. glabrata* in the Caribbean.

The primary aims of our study were to: (a) characterize the bacterial flora of individual snails in different wild and laboratory-reared populations, and (b) investigate some of the factors influencing the composition and dynamics of bacterial populations in the snails. Our approach was based on two assumptions: (a) that the complete analysis of almost any natural bacterial community is impossible, and (b) that each snail could harbor a discrete and unique bacterial flora. These considerations led us to perform a few simple analyses on a larger number of snails, rather than more exhaustive analyses of fewer snails. We believe that this strategy has yielded a more ecologically valid picture of the association of *B. glabrata* with bacteria than the latter alternative would have done. During our study we enumerated and identified the principal aerobic heterotrophic, colony-forming bacterial inhabitants of over 400 individual snails from wild and aquarium populations. In addition to examining the bacterial inhabitants of healthy (normal) snails from the field and laboratory, we analyzed the bacterial contents of moribund snails encountered during sampling and experiments. Comparison of bacterial populations in wild and laboratory-reared snails, and of those from healthy and moribund snails, has revealed that the snail microflora is quite plastic and has the potential for rapid qualitative and quantitative changes in its composition. We also studied the dynamics of snail bacterial communities by perturbing the snails (and thus their bacterial populations) and then comparing the size and composition of the bacterial populations to those from unperturbed snails. Artificial disturbance of animal and plant populations has been used as an experimental technique in population ecology [7, 15], and we feel that it can be a valuable tool in microbial ecology as well.

We recognize that the aerobic heterotrophic colony-forming bacterial populations may not represent a majority of the bacteria in *B. glabrata*, but we restricted our study to them since only these organisms are amenable to isolation, identification, and cultural study.

Materials and Methods

Collection of Wild Snails

Wild snails were sampled from populations in Puerto Rico in June 1977, Guadeloupe in July 1978, and St. Lucia in July 1978 and July 1979. The locales surveyed were representative of a typical range of *B. glabrata* habitats and schistosomiasis transmission foci. The snail habitats and ecology of *B. glabrata* in Puerto Rico, Guadeloupe, and St. Lucia are described in Jobin [16], Pointier [21], and Sturrock [26], respectively. Our sites are described in Table 1.

Snails were sampled using standardized techniques and 25 × 25 cm screen dippers [16]. Most habitats were sampled only until enough snails for analyses on that day were collected. Three collections were made in Mahaut, St. Lucia, in 1979. Individual snails were removed from the rinsed screen dippers with forceps and placed in groups of 5–10 in presterilized Whirl-Pak plastic bags (Nasco, Inc., Fort Atkinson, Wis.) containing 5–10 ml of local water for immediate transport to the laboratory. Puerto Rican snails were analyzed at the Center for Energy and Environment in San Juan. St. Lucian and Guadeloupe snails were analyzed at the Research and Control Department in Castries, St. Lucia. Although the Guadeloupe snails were flown to St. Lucia for analysis, the interval between collection and analysis was not much longer than the interval for any other snails. Upon return to the laboratory, snails were kept in small tanks containing stream water (Puerto Rico) or rain water (St. Lucia) for < 1–12 h prior to analysis.

Table 1. *Biomphalaria glabrata* collection sites

Location	Date	Description	Treatment
Aibonito, P.R.	6·22·77	Highland marsh	Shell removed[a]
Humacao, P.R.	6·27·77	Rocky stream	Shell removed[a]
Trujillo Alto, P.R.	6·27·77	Urban stream	Shell removed[a]
Mal Pico, P.R.	6·28·77	Roadside drain	Shell removed[a]
Rio Piedras, P.R.	6·29·77	Lily pond	Shell removed[a]
Morne Fortune, St. Lucia	7·12·78	Outdoor concrete tank at lab	None
Marquis Valley, St. Lucia	7·13·78	Cow pasture stream	None
Soufriere, St. Lucia	7·21·78	Stream near geothermal area	None
Mahaut, St. Lucia	7·7·18·79	Cultivated dasheen marsh	Half disinfected[b]
Grande Etang, Guadeloupe	7·18·78	Lake	None
Beaugendre, Guadeloupe	7·18·78	Urban river	None

[a] Shells too heavily calcified for homogenization.
[b] See Methods.

Culture and Sampling of Laboratory Snails

Snails were reared in the laboratory in 10 or 15 gallon (38 or 57 liter) all-glass aquariums equipped with undergravel filtration systems and continuous aeration. Each aquarium had ca. 1 inch of 6 x 16 mesh crushed dolomite substrate, and the water temperature was maintained at 25.0 ± 0.5°C with thermostatic heaters. All the aquaria contained glass-distilled, deionized water, 75% of which was replaced every 2–4 weeks. The aquaria were disassembled and rinsed, and gravel and water were entirely replaced every 8–12 weeks. The snails were fed washed romaine lettuce 3 times each week.

Snail colonies were derived from the NIH-AID strain of Puerto Rican albino *B. glabrata* [23] or from *B. glabrata* collected at Borricaud, Guadeloupe, in July 1978. Individual snails were sampled from the aquaria with alcohol-flamed forceps into sterile plastic petri dishes.

Measurement of Snails

In order to compute the density of bacteria in each snail (bacteria/g snail tissue), it was necessary to have an estimate of each snail's weight. We did not weigh the dissected body of each snail to be analyzed because it was not always possible to obtain accurate snail weights in field laboratories and because we wished to avoid contaminating the snails. Thus we estimated snail fresh tissue weights by constructing regressions of fresh tissue weight on snail diameter. For each snail population (Puerto Rico field and lab, St. Lucia field, and Guadeloupe field and lab), from 16 to 80 preserved snails were measured to the nearest 0.5 mm across the largest shell diameter and weighed to the nearest 0.01 g after removal of the shell. The weights of snails we subsequently analyzed were estimated from shell diamters using the appropriate regression. In Ducklow et al. [9] all densities were estimated from the Puerto Rican field snail regression. The data have been recalculated as described for this paper. In all cases, power regressions of the form $w = aD^b$ gave the best fit; correlation coefficients ranged from 0.84 to 0.94.

Analysis of Bacterial Populations in Snails—Contribution of External Shell Bacteria to Total Population

We determined the contribution of the bacterial populations on laboratory snail shells to the total snail bacterial populations by treating snails in several ways. Different snails were subjected to each of the following

treatments: dissection and removal of the shell, swabbing with ethyl ether, brief immersion in 95% ethanol (without allowing ethanol to enter the aperture) followed by flaming, or no treatment. Following these treatments, the bacterial populations in each snail were enumerated as described below.

Snails collected in the field at Mahaut, St. Lucia, were subjected to two cleaning treatments prior to enumeration procedures. Half of the snails in each sample were swabbed thoroughly with cotton-tipped applicator sticks dipped in sterile 0.1% buffered peptone (see below) to remove the visible external contamination. The other half of the sampled snails were swabbed in a solution of 0.1% Hyamine 10-X (Rohm and Haas Co., Philadelphia, PA) and 0.01% Triton N-101 (Sigma Chemical Co., St. Louis, MO). Then each snail was soaked for 5 min in a one-tenth strength concentration of the same solution. Prior tests had shown that snails soaked up to 1 h in this solution were not harmed. Both groups of snails were then analyzed for bacterial content as described in the next section. In each case, no significant difference was found between the bacterial numbers in the disinfected and undisinfected snails. Thus we analyzed untreated snails in all other instances except the Puerto Rican samples. These snails had shells that were too heavily calcified for homogenization.

Enumeration of Bacterial Populations in Snails

We adopted a simple and reasonably rapid enumeration procedure with which groups of snails could be processed in field laboratories. Since we were interested in obtaining any potentially pathogenic organisms that may have been infecting any part of each snail, we chose not to analyze individual portions of the snail microflora, such as the flora in the excised gastrointestinal tract. Each cleaned, surface-sterilized, dissected, or untreated whole snail (specific treatments described in Table 1) was analyzed for its aerobic heterotrophic bacterial content as follows:

After the largest shell diameter was measured, each snail was placed in a preautoclaved 15-ml glass Ten Broeck tissue grinder containing 9 ml of 0.1% peptone in 0.0375 M Na_2HPO_4-NaH_2PO_4 buffer at pH 7.2. Each grinder also contained ca. 1 g of washed precombusted (600°C, 18 h) quartz sand to facilitate grinding. Each snail was then gound in the homogenizer using an alcohol-flamed glass pestle until no tissue fragments were visible. Then the mixture in the foam-stoppered grinder was vortexed at high speed for 1 minute.

Serial decimal dilutions of the homogenate were carried out into 9.0 ml dilution blanks containing the buffered peptone mixture. Finally, 0.1 ml portions of 3 predetermined suitable dilutions were inoculated onto duplicate agar plates containing standard methods agar (TGY) containing in grams per liter: trypticase peptone, 5.0; yeast extract, 2.5; dextrose, 1.0; and agar, 15.0 (BBL, Cockeysville, MD). The colonies on the plates were counted after incubation at room temperature for 5–7 days.

Identification and Analysis of Bacterial Populations in Snails

We did not carry out the extensive, time-consuming, and expensive analysis of all the colony-forming bacteria found in any of the snails. Rather, we tried to determine the identity of the numerically predominant colony-forming bacterial species in each snail. After the colonies were counted on each plate, the plates were examined carefully as described in Ducklow et al. [9] in order to identify, enumerate, and isolate the principal colony former from each snail. One or two typical colonies of the predominant type were picked with a sterile inoculating loop from a representative plate for each snail. These dominant colonies were counted so that the population size of the dominant isolate in each snail could be derived. All the isolates were purified by repeated streaking on TGY plates. The isolates were maintained on TGY agar slants in screw-topped tubes. These predominant colony types were characterized and identified as described in Ducklow et al. [9].

Water Samples

The bacterial content of stream and aquarium water was measured by carrying out plate counts on TGY medium. Predominant colony types from each water sample were isolated and identified. For this study water was collected at Mahaut, St. Lucia, from snail sampling sites and returned to the laboratory in Whirl-Pak bags.

In order to assess the influence of the bacterial populations in the water on bacterial densities in snails, snails and water were sampled weekly over an 11-week period in each of two laboratory aquaria. In one tank the snail population consisted of snails from about 2–7.0 mm in diameter. Snails larger than 7.0 mm diameter were transferred to the second aquarium, which contained snails from less than 2 to over 18 mm in diameter. Thus two large and two small snails, and four water samples were made each week. The snails and water samples were analyzed as described above. Sediments and aquarium gravel were not analyzed.

Experimental Perturbation of Bacterial Populations in Snails

Three different experimental treatments were performed in order to perturb the bacterial populations in different groups of snails. After each treatment bacteria were enumerated, and predominant isolates were isolated from the treated snails.

In one set of experiments, snails captured in the field were returned to Cambridge and placed in aquaria in order to see if their microflora was altered by the change in habitat. A group of snails from Soufriere, St. Lucia was immersed in a Nitex screen cage (Tetko, Inc., Elmsford, NY) for 1 day at Morne Panache, St. Lucia, then returned to Cambridge with 24 h of removal from the temporary habitat. These snails were kept in a fresh aquarium for 3 days and then analyzed.

In a similar experiment, snails collected in Borricaud, Grande Terre, Guadeloupe, were returned to a fresh aquarium in Cambridge and used to initiate a new colony. After 3 months the original 20 snails had produced several hundred offspring. Ten of the first-generation offspring were sampled to test the effect of aquarium cultivation on a genetically similar population of snails.

In a second series of experiments, we tested the effects of crowding, feeding, and starvation on snail bacteria floras in the following manner. Snails collected at Mahaut, St. Lucia, or from the aquarium populations of Puerto Rican albino *B. glabrata* were kept for up to 8 days in 50 ml beakers containing rain water (St. Lucia) or 0.45 μm membrane-filtered, autoclaved aquarium water (FAW) (Cambridge). Five snails were incubated in 40 ml of water, and snails were removed from the beakers daily for analysis. Enough snails were used in each experiment so that crowding conditions could be maintained as snails were removed and sacrificed during the experiments. Water was changed each day in each beaker. Half the snails in each experiment were fed washed romaine lettuce (Cambridge) or boiled dasheen leaves (*Colocasia esculenta*) (St. Lucia) throughout the experiment, while the remaining snails were starved. The aquarium snails were homogenized with their shells untreated, while the St. Lucian snails were surface sterilized with Hyamine-Triton as described above, prior to homogenization.

A third series of experiments was similar to the experiments just described, except that snails were incubated for varying periods of time in solutions of different antibiotics. The purpose of these treatments was to determine the effect of in situ inhibition of specific bacterial populations on the total bacterial community in the treated snails. In the experiments 5 snails were incubated in 40 ml of FAW containing antibiotic solutions for 24–48 h. Control snails from the aquarium and controls incubated simultaneously in plain FAW were also analyzed in each experiment. The antibiotics and concentrations used are shown in Table 7.

In addition to the normal plate counts on TGY plates, homogenized snail dilutions were also inoculated onto TGY plates containing 50 μg/ml of the appropriate antibiotic in order to enumerate the antibiotic-resistant bacteria inside each treated or untreated snail. These plates were prepared by adding filter-sterilized antibiotic solutions (0.2 μm membrane filtered through Millex filter units, Millipore Corp., Bedford, MA) to the cooled molten agar shortly before it was poured. These antibiotic-containing plates were used within 24 h of preparation.

Two additional groups of snails were analyzed. It was possible to sample moribund snails from the aquariums. These were analyzed in order to determine if moribund, dying snails had a different microflora from normal snails. During the experiments in which St. Lucian snails were incubated in beakers, it was also possible to sample and analyze the moribund snails that occassionally occurred, to compare their flora with that of their healthy counterparts.

As part of a study on the rearing of axenic snails, we cultured axenic albino *B. glabrata* by procedures described in detail in Chernin [3]. Several of our axenically reared snails became contaminated during feeding or transfer to fresh medium. After 2 months of cultivation in the contaminated state, these individuals were analyzed as described above in order to characterize the bacterial populations established in the formerly unoccupied snails.

Results

1. Description and Analysis of the Snail Bacterial Flora

1.1. Laboratory-reared snails have denser bacterial populations than wild snails, and moribund snails have denser bacterial populations than normal (healthy) snails. Aquatic snails, like most other animals, undoubtedly posess several distinct bacterial communities. In normal, healthy snails, it seems reasonable to postulate the existence of at least 3 such communities: a gut microflora, a fouling community attached to the exterior of the shell, and perhaps a community colonizing the mantle cavity and epidermis. Our analyses do not differentiate among these different groups of bacterial populations, but rather pool them together. Thus our analyses are only a first-order approximation of the character of bacterial populations associated with *B. glabrata*.

The external surfaces of the shell of aquarium-reared *B. glabrata* are remarkably clean, as evidenced by scanning electron microscopy of shell surfaces. The same surfaces on snails from natural habitats are heavily fouled. Gross contamination with mud, egg masses, and fecal material can be observed on freshly collected snails.

Much of this external or fouling community of microorganisms is not intimately associated with normal snails. Many of these organisms are incidental contaminants living on the shell. The shell merely serves as an attachment site for such organisms, which attach just as readily to rocks, dead leaves, detritus, and other nonliving surfaces [13]. We found that the shell populations do not contribute significantly to the total bacterial flora of each snail (see Methods).

The results of enumerations of the bacterial contents of 338 snails from 14 field and laboratory populations are shown in Table 2. The bacterial density in wild snails collected from 11 different Caribbean locations ranges from 1.5×10^6 to 5.9×10^7 colony-forming units (CFU) per gram of snail tissue. Some snail populations have lower bacterial densities than others. For instance, the Puerto Rican snails generally have 2×10^6 to 2×10^7 CFU·g^{-1}, whereas the snails from Guadeloupe harbor populations ranging from 1 to 4×10^7 CFU·g^{-1}.

B. glabrata reared in laboratory aquaria have bacterial densities an order of magnitude higher than those from wild snails. The 3 samples of laboratory PR-1 snails shown in the table are from our colonies of the albino strain of *B. glabrata*, which has been in laboratory culture at the National Institutes of Health for over 30 years [23]. It is conceivable that at least part of the reason for increased bacterial densities in lab-reared snails is genetic. However, we observed similar densities of bacteria in snails from a colony derived from snails collected at Borricaud, Guadeloupe, 4 months previously. All the snails analyzed were first-generation offspring of the founder snails. Thus it appears that some factor related to laboratory cultivation may be responsible for the difference in snail bacterial density between wild and aquarium populations.

A final set of snail populations shown in Table 2 suggests that environmental-physiological factors may be important in influencing bacterial densities in snails. Several snails collected during the St. Lucia dry season (Feb. 1980) were also analyzed. These snails were lying on the surface of dry banana drains and presumably were considerably stressed from desiccation. The bacterial density in these snails was the highest we observed in any freshly caught wild snails.

During experiments with wild snails in St. Lucia, we encountered a few moribund individuals. These snails were partially or fully contracted into their shells and did not

Table 2. Bacterial densities in individual *B. glabrata* snails from field, laboratory, and experimental populations

Sample	*N*	Bacterial density in snail CFU/g snail tissue[a] 95% conf. interval	Mean
Field Populations			
Aibonito, P.R.	13	4.8– 12.9 × 10^6	7.9 × 10^6
Humacao, P.R.	9	1.9– 4.8 × 10^6	3.0 × 10^6
Trujillo Alto, P. R.	13	13.2– 36.0 × 10^6	22.5 × 10^6
Mal Pico, P.R.	33	9.7– 17.9 × 10^6	13.0 × 10^6
Rio Piedras P.R.	29	1.5– 3.6 × 10^6	2.3 × 10^6
Morne Fortune, St. Lucia	9	12.0– 24.2 × 10^6	17.4 × 10^6
Marquis Valley, St. Lucia	19	32.6– 59.4 × 10^6	44.5 × 10^6
Soufriere, St. Lucia	29	9.7– 24.2 × 10^6	15.6 × 10^6
Mahaut, St. Lucia[b]	12	9.8– 36.0 × 10^6	18.6 × 10^6
Mahaut, St. Lucia[b]	18	6.7– 14.7 × 10^6	9.9 × 10^6
Mahaut, St. Lucia[b]	19	7.1– 32.6 × 10^6	15.2 × 10^6
Grande Etang, Guadeloupe	12	13.1– 32.6 × 10^6	20.2 × 10^6
Beaugendre, Guadeloupe	13	19.3– 44.0 × 10^6	28.9 × 10^6
Laboratory populations			
Laboratory PR-1 1[c]	10	44.0–146.1 × 10^6	80.1 × 10^6
Laboratory PR-1 2[c]	20	31.5– 93.1 × 10^6	53.8 × 10^6
Laboratory PR-1 3[c]	30	20.2– 78.4 × 10^6	39.8 × 10^6
Laboratory Guadeloupe	10	82.5–294.2 × 10^6	158.2 × 10^6
Naturally and experimentally stressed snails			
Mahaut, St. Lucia moribund	8	24.2–197.3 × 10^6	67.7 × 10^6
Laboratory PR-1 moribund	13	259.3–977.0 × 10^6	500 × 10^6
Soufriere, transferred to laboratory	4	76.1–325.2 × 10^6	157 × 10^6
Contaminated, formerly axenic PR-1	9	4693–16066 × 10^6	8817 × 10^6
St. Lucia dry season	6		76 × 10^6

[a] Data transformed to natural logs for analysis.

[b] Mahaut samples 1, 2, and 3 were collected on July 7, 10, and 18, 1979.

[c] PR-1 sample 1 was collection of snails 10–16 mm dia. sampled 10·24·78. Samples 2 and 3 were collections of snails of 12–18 mm and 5–7 mm dia., respectively, sampled over a period of 11 weeks from 2 separate aquaria.

move about over a period of 6–10 h of observation in a small glass tank, but they responded to gentle prodding. Similar snails were routinely observed on the bottom of our laboratory aquaria. Prolonged observation of moribund individuals showed that these snails almost always died instead of resuming their normal behavior. All the moribund snails sampled were observed to respond to tactile stimuli or to possess a heartbeat. Table 2 shows that these snails had bacterial densities higher than those in the healthy snails from the same populations. These results show that before snails die, and

well before they begin to decompose, their bacterial populations grow to abnormally high levels not usually observed in most normal, healthy snails.

A similar phenomenon was observed in two groups of experimentally manipulated snails. Wild *B. glabrata* were collected at Soufriere, St. Lucia, and kept for 1 day in an open cage in a stream at Morne Panache, St. Lucia, then returned to Cambridge and analyzed after 3 days of incubation in a laboratory aquarium. These snails also had much higher densities than those observed for the normal snails from Soufriere. Wild snails are capable of attaining bacterial density values equal to those for the laboratory strains of *B. glabrata*.

The final sample contained snails reared from individual disinfected eggs under axenic conditions in test tubes. During feeding or transfer, some of these bacteria-free snails were contaminated and thus acquired potentially atypical bacterial populations. These snails were found to possess exceptionally large bacterial populations 3–4 orders of magnitude higher than normal aquarium snails. The reasons for these high bacterial levels are not known, but stresses from transport or confinement may influence the normal regulatory mechanisms controlling the size of bacterial populations in the snails.

1.2. External bacterial densities do not significantly influence the bacterial population levels in the snails. It seemed likely that differences in snail bacterial densities might be caused by differences in bacterial density in water. In order to examine these possibilities, we sampled both water and snail bacteria on different days at Mahaut, St. Lucia. In another experiment, the water and snail bacteria were sampled over an 11 week period from two separate aquaria containing different sizes of PR-1 albino snails. The data from these experiments are shown in Table 3. In each case, there was no significant difference between the bacterial densities in the matched groups of snails. However, in both sets of water samples, the matched samples had highly significant differences in bacterial density. Although in each experiment one sample had a significantly larger population of bacteria in the water, the snails from both samples had similar bacterial densities. These experiments do not show that snails inhabiting water with exceptionally high or low bacterial concentrations would harbor different densities of bacteria. But they do show that snail bacterial densities vary independently of densities of bacteria in the surrounding water.

Table 3 also shows that equal concentrations of bacteria were found in aquarium water (sample 2) and in the stream water at Mahaut, St. Lucia (sample 3). However, the wild St. Lucian snails have much lower bacterial densities than the snails reared in the aquaria. Clearly factors other than the level of bacteria in water inhabited by snails control the density of bacterial populations in or on the snails.

1.3. Relatively few species of bacteria predominate in snail bacterial populations. More species predominate in wild snails than in laboratory snails, and more predominate in normal than in moribund snails. In order to further characterize the bacterial populations inhabiting *B. glabrata*, we isolated and identified the numerically dominant colony types from most of the snails analyzed. The distribution of the predominant bacterial genera sampled from snails in each collection is shown in Table 4. Various *Pseudomonas* species were the most commonly occurring predominant organisms in both wild and laboratory-reared snails. Members of *Acinetobacter*, *Enterobacter*, and *Aeromonas* were also common in wild snails, whereas *Acinetobacter*, *Aeromonas*, *Citrobacter*, and *Moraxella* are the other genera most frequently en-

Table 3. Relationship between bacterial densities in water and snails from aquarium and wild populations[a]

Sample	Bacterial density[b]	Single-Classification ANOVA[c]		
		MS	F	*P*
Mahaut snails 2	$6.7–14.7 \times 10^6$	14.802	0.985	$0.25 < P < 0.50$
Mahaut snails 3	$7.1–32.6 \times 10^6$			
Mahaut water 2	$7.1–15.6 \times 10^3$	3.182	70.39	$P < 0.001$
Mahaut water 3	$2.8–5.9 \times 10^4$			
PR-1 aquarium snails 2	$31.5–93.1 \times 10^6$	11.56	1.34	$0.25 < P < 0.50$
PR-1 aquarium snails 3	$20.2–78.4 \times 10^6$			
PR-1 aquarium water 2	$2.1–5.6 \times 10^4$	28.66	42.42	$P < 0.001$
PR-1 aquarium water 3	$4.1–8.6 \times 10^3$			

[a] Wild and laboratory populations as described in methods and Table 2. Water data were pooled from samples taken before and after snail collecting.
[b] Snail bacterial densities are CFU·g^{-1} tissue; water densities are CFU·ml^{-1}, expressed as 95% confidence limts.
[c] ANOVA tests performed on ln-transformed data.

Table 4. Distribution of numerically predominant colony-forming bacteria in wild and laboratory-reared *Biomphalaria glabrata* populations and accompanying water samples

Population or sample:	Wild snails		Laboratory snails		Water samples	
	Healthy	Moribund	Healthy	Moribund	St. Lucia	Aquarium
No. sampled:	103	8	49	19	13	25
Predominant genus	(Percentage of snails or water samples with each genus predominant)					
Pseudomonas spp.	23	0	34	0	15	28
Acinetobacter lwoffi	11	38	12	0	15	36
Aeromonas hydrophilia	09	50	18	0	0	0
Moraxella spp.	03	0	18	89	07	20
Shigella sonnei	10	0	0	0	0	0
Enterobacter spp.	11	0	0	0	23	08
Citrobacter freundii	06	12	12	11	0	0
Providencia rettgeri	05	0	0	0	0	0
Klebsiella spp.	0	0	02	0	0	0
Vibrio extorquens	08	0	0	0	0	0
Flavobacterium sp.	0	0	02	0	0	04
Miscellaneous gram +	09	0	0	0	07	04
No organism predominant	07	0	0	0	31	0
	102	100	98	100	98	100

countered in lab-reared snails. In general, more kinds of organisms predominated in the wild snail populations than in aquarium populations. Only in a few wild snails could no predominant organism be distinguished among the different colonies on the agar plates. Both of these observations suggest that more bacterial species predominate in wild snails than in aquarium snails.

Table 4 also reveals that only four genera of bacteria were found in moribund snails. *Moraxella* spp. are especially favored in dying laboratory snails, whereas *Aeromonas* and *Acinetobacter* exploit dying wild snails. *Pseudomonas* spp., the most common dominant organisms in healthy snails, were never dominant, and were not numerous in any dying snails, where they are apparently outcompeted by other, more opportunistic organisms.

A comparison of the distributions of predominant organisms isolated from water samples with those from snails suggests a reason for the predominance of some genera but not others in the snails. *Pseudomonas* and *Acinetobacter* spp. were common in both St. Lucian stream water and aquarium water and in both groups of snails. However, several bacterial genera that predominated in wild or lab snails were not found to predominate in any of the water samples (e. g., *Aeromonas*, *Shigella*, *Citrobacter*). The populations of bacteria in the water may influence those populations in snails, but as the data on the sizes of bacterial populations also indicted, that influence is not exceptionally powerful.

The data on the bacterial populations inhabiting wild snails show that a variety of bacteral species are potentially capable of gaining numerical dominance over all the other species in snail. However, in view of the hundreds to thousands of species of bacteria present in snail habitats, it is important to recognize that comparatively few species actually predominate in the snails.

1.4. The bacterial flora of high-density snails is qualitatively and quantitatively distinct from that of most normal snails. In order to examine the relationship between individual snails and their dominant bacterial species, we noted the relative bacterial density of each snail and the contribution of its predominant bacterial species to the total microflora. Within each sample of snails, the bacterial density for each snail was compared to the mean density for that sample. The resulting "colonization ratios" are a measure of the degree of bacterial colonization within each snail. In a collection of three snails with bacterial densities of 1.0, 2.0, and 3.0×10^7 CFU·g^{-1}, the colonization ratios for each snail would be 0.5, 1.0, and 1.5, respectively. A snail with a *Pseudomonas* sp. predominant and making up 1.5×10^7 CFU·g^{-1} out of a total of 3.0×10^7 CFU would have the dominant organism contributing 50% of the total CFU. For all the snails in each sample, and for all snails in which each different genus of bacteria dominated, the mean contribution was calculated. Thus in three snails with *Pseudomonas* sp. making up 30%, 40%, and 50% of the total CFU in the snails, the mean contribution for *Pseudomonas* would be 40%. If each snail in a sample of three snails had a different dominant contributing 50%, the mean percentage of dominants in the sample would be 50%. From these data several patterns of bacterial colonization and dominance of individual snails can be observed.

The mean contribution of all the different predominant bacterial genera toward the total microflora in each snail sample is shown in Table 5, along with the number of different genera that predominate in the sample. Some samples (e.g., Mahaut #2) have 5–7 genera of weakly dominant bacteria which make up less than 20% of the total microflora in the snails. Other snail populations have less diverse bacterial populations, with relatively few genera contributing 50–65% of the total microflora. In moribund snails, the predominant bacterial species account for over 50% of the total bacteria present.

Table 5. Distribution of high-density snails and occurrence of predominant bacteria in different snail populations

Sample	Mean percent of dominant bacterial genera in total microflora	No. of different predominant genera in sample	Percent of high-density snails[a]
Field Populations			
Aibonito, P.R.	–	–	23
Humacao, P.R.	–	–	11
Trujillo Alto, P.R.	–	–	15
Mal Pico, P.R.	–	–	18
Rio Piedras, P.R.	–	–	28
Morne Fortune, St. Lucia	43 ± 6	4	11
Marquis Valley, St. Lucia	49 ± 6	4	11
Soufriere, St. Lucia	57 ± 4	6	31
Mahaut, St. Lucia 1	34 ± 6	5	17
Mahaut, St. Lucia 2	14 ± 3	7	17
Mahaut, St. Lucia 3	55 ± 9	6	37
Grande Etang, Guadeloupe	55 ± 8	3	33
Beaugendre, Guadeloupe	40 ± 7	3	23
Laboratory populations			
Laboratory PR-1 1	36 ± 4	3	20
Laboratory PR-1 2	44 ± 5	5	35
Laboratory PR-1 3	33 ± 4	3	14
Laboratory Guadeloupe	51 ± 4	2	10
Naturally and experimentally stressed snails			
Mahaut, St. Lucia moribund	56 ± 12	3	–
Laboratory PR-1 moribund	65 ± 7	2	–
Soufriere, transferred to laboratory	–		–
Contaminated, formerly axenic PR-1	37 ± 5	3	–

[a] Colonization ratios > 2.0. See Results section 1.4 for explanation.

Colonization ratios indicate the relative density of the microbial population in each snail. Colonization ratios of 2.0 or greater were arbitrarily taken to denote high bacterial density snails. In most cases a bacterial density of twice the sample mean for a given snail sample was beyond the upper 99% confidence limit for bacterial density. Such high-density snails were considered to be colonized with abnormally high populations of bacteria. The percentage of high bacterial density snails in each sample is also shown in Table 5. At least 10% of the snails in each population fell into the high-density category. There was a tendency for the samples with over 30% high-density snails to have the dominant bacterium making up over 50% of the total microflora. The dominant bacteria made up 59 ± 4% of the microflora in the 46 high-density snails, but comprised only 39 ± 2% of the microflora in all the normal-bacterial-density snails. Snails sampled from field and laboratory populations that had abnormally high densities of bacteria are also characterized by a greater degree of dominance of the individual microfloras by the dominant organisms.

Table 6. Patterns of bacterial colonization and dominance of the aquatic snail *Biomphalaria glabrata* by different bacterial genera

Genus	Frequency of occurrence[a]		Mean colonization ratio[b]	Mean percent of genus in microflora[b]
	Normal	High density		
Acinetobacter spp.	11.3	13.5	1.3± 1.3	46± 5
Aeromonas hydrophilia	13.2	10.8	1.7± 0.3	51± 6
Citrobacter freundii	4.7	18.9	1.7± 0.4	48± 5
Enterobacter spp.	8.5	5.4	1.1± 0.2	40± 10
Moraxella spp.	8.5	8.1	2.6± 1.4	55± 7
Pseudomonas spp.	33.0	16.2	1.1± 1.2	41± 4
Shigella sonnei	7.5	5.4	0.9± 0.4	27± 7
Vibrio extorquens	2.8	8.1	3.3± 0.4	75± 7

[a] Percent of snails in which indicated organism occurs as the dominant bacterial species.
[b] See Results for details of calculation. Data are presented as mean ± S E.

Grouping the data by predominant genus instead of snail population revealed that high-density snails are characterized by the presence of *Citrobacter*, *Moraxella*, and *Vibrio* spp. The first three columns of Table 6 reveal that the major bacterial species occur as dominants in different frequencies in normal- and high-bacterial-density snails. *Citrobacter freundii* and *Vibrio extorquens* are isolated as predominant colony formers more than 3 times as often from the high-density snails, apparently at the expense of pseudomonads, which occur about twice as often in the normal snails. The data on colonization ratios shows that *Moraxella* spp. and *V. extorquens* tend to be found in snails with high bacterial densities, since their mean colonization ratios are greater than 2.0. All the other genera have mean ratios of 0.9–1.7, indicating that they tend to predominate in normally colonized snails. Although *C. freudii* occurs more frequently in high-density snails, it does so in those snails with ratios of 2.0–3.0 as well as in normally colonized individuals, accounting for its lower mean ratio of 1.7. Conversely, *V. exorquens* and *Moraxella* spp. were found to predominate in snails with very dense microfloras (ratios up to 50), which accounts for the high mean ratios ascribed to those genera. In addition to occurring in high-density snails, *V. extorquens* also tended to dominate the microbial populations to a greater extent than other dominant species. When *V. extorquens* was predominant it tended to make up over 70% of the bacterial population of a snail, whereas most of the other organisms usually comprised about 50% of the microflora in their snails.

Taken together, these data reveal that three different groups of snails can be distinguished with respect to bacterial density: normally colonized snails, with colonization ratios of less than 2.0; high-density snails (ratios >2.0); and moribund snails, with very high colonization ratios (usually >4.0). Furthermore, different patterns of dominance by the numerically superior bacterial species in the snail microfloras were observed. Normally colonized snails were usually inhabited by *Pseudomonas*, *Acinetobacter*, or *Aeromonas* spp., which tended to make up 40–50% of the microflora. High bacterial density snails were colonzied with *Citrobacter*, *Vibrio*, or *Moraxella* spp. *V. extorquens* was a principal dominant in snails from wild populations;

Moraxella predominated in high-density and moribund aquarium snails; and *Citrobacter* was a less important predominant organism in both groups.

2. *Experimental Pertubation of Snail Bacterial Populations*

2.1. Crowding results in increased bacterial populations. In order to understand the processes by which certain bacteria come to dominate the microflora of certain snails, we performed simple perturbation experiments and then analyzed the bacterial inhabitants of disturbed and undisturbed snails. In this way some of the dynamic behavior of snail microfloras was inferred. Incubation at densities of 5 snails/40 ml water resulted in the "crowding phenomenon" [4, 32]. Snails held under crowded conditions have been reported to exhibit decreased growth, fecundity, and respiration and increased mortality, possibly as a result of the accumulation of mildly toxic excretory products.

The results of two crowding experiments with captured wild St. Lucian snails are shown in Figs. 1 and 2. Crowding of the snails resulted in elevation of the snail bacterial densities above the levels found in the initial, undisturbed snail population. Snails fed daily had higher bacterial densities than snails that were starved during the experiment. Populations in both groups of snails showed declines after the initial increases, but stayed above the precrowding levels throughout the experiment.

In addition to population size increases, crowding resulted in shifts in the patterns of bacterial dominance. In the first experiment, the sample from which the experimental snails were taken had *Acinetobacter* spp. dominant in about half the snails sampled, with *Shigella*, *Aeromonas*, *Pseudomonas*, and *Moraxella* also occurring as dominants. However, *Acinetobacter* was isolated as the dominant organism in 22 of the 32 incubated snails sampled. The trends in the second experiment were not as clear. *Flavobacterium* spp. and *Escherichia coli* were never isolated as dominants in the original snail sample, but became dominant in some of the crowded snails. *Aeromonas hydrophilia* was dominant in 9% of the original snails analyzed and in 28% of the crowded snails analyzed. There were no discernible trends in the proportions of the microbial populations contributed by the predominant organisms.

A similar pattern was observed when this experiment was performed using laboratory snails (Fig. 3). Snails fed throughout the experiment showed an immediate increase in bacterial density and maintained densities above initial levels throughout the experiment. In the starved snails the bacterial density remained essentially unchanged until the fourth day, when it increased to about 8 times the original density. *Moraxella* spp. were most commonly isolated as dominants from both fed and starved snails. These experiments show that changes in snail bacterial populations can be induced by stresses in short periods of time. The elevation of bacterial densities in crowded wild snails to levels equal to those in laboratory snails suggests that the densities of bacteria observed in laboratory snails may be a result of accumulation of waste materials in the laboratory aquaria. The additional increments in bacterial density observed in crowded lab snails, in addition to those seen in moribund lab snails and the contaminated, formerly axenic lab snails, suggest that healthy snails do not maintain their bacterial densities at maximal levels under normal conditions. There appears to be a considerable potential for further growth in most snail bacterial populations.

2.2. Antibiotic treatment results in selective stimulation of specific bacterial populations. The results of incubations of snails in 11 different antibiotics, with ac-

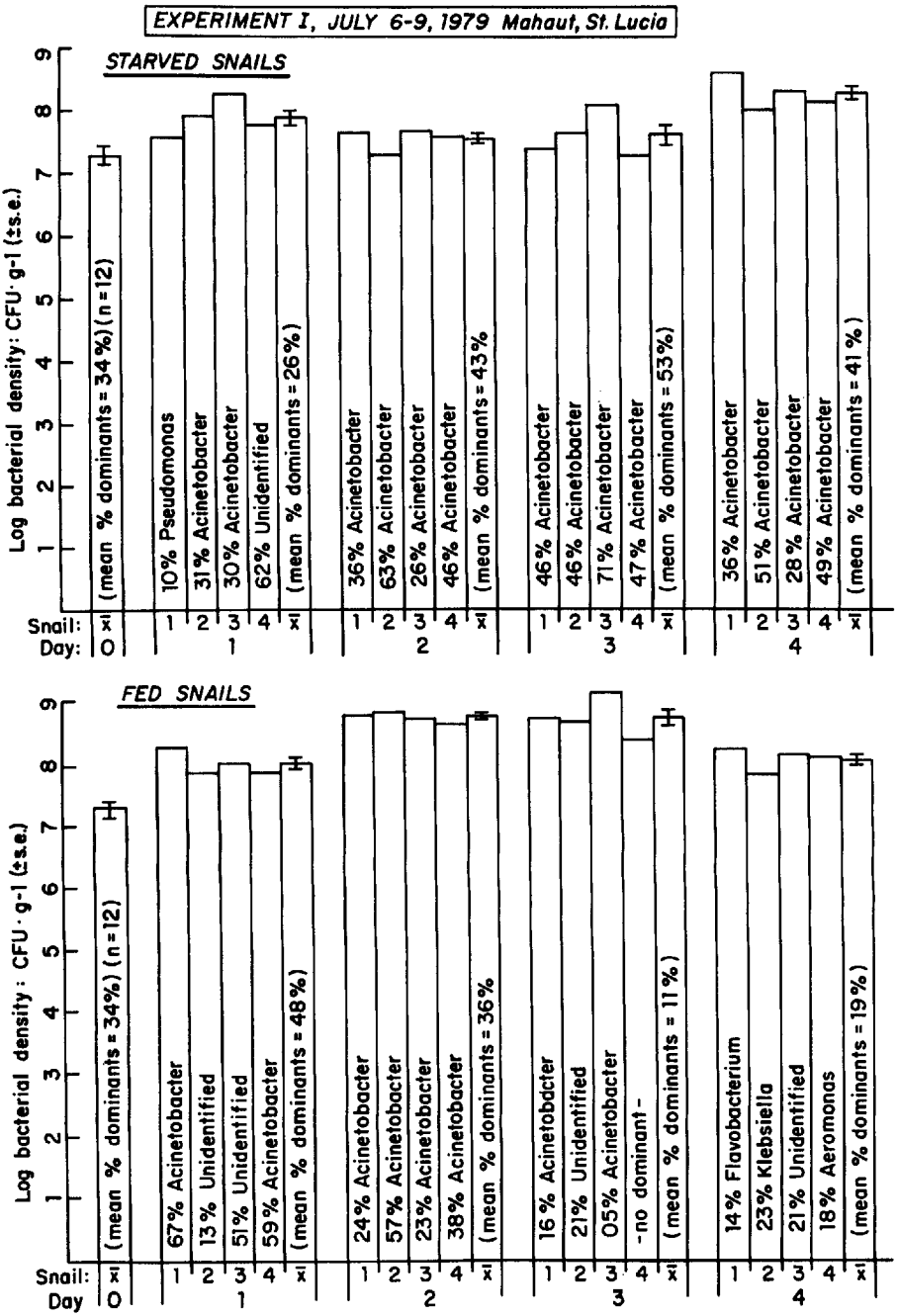

Fig. 1. Bacterial densities in wild St. Lucian *B. glabrata* subjected to crowding. Day 0 bars represent the mean bacterial density in the population from which the experimental snails were taken. For each day of incubation, bars for each sacrificed snail and the mean for that day are included. Within each bar the identity of the predominant bacterial genus from that snail and its contribution to the total microflora are given.

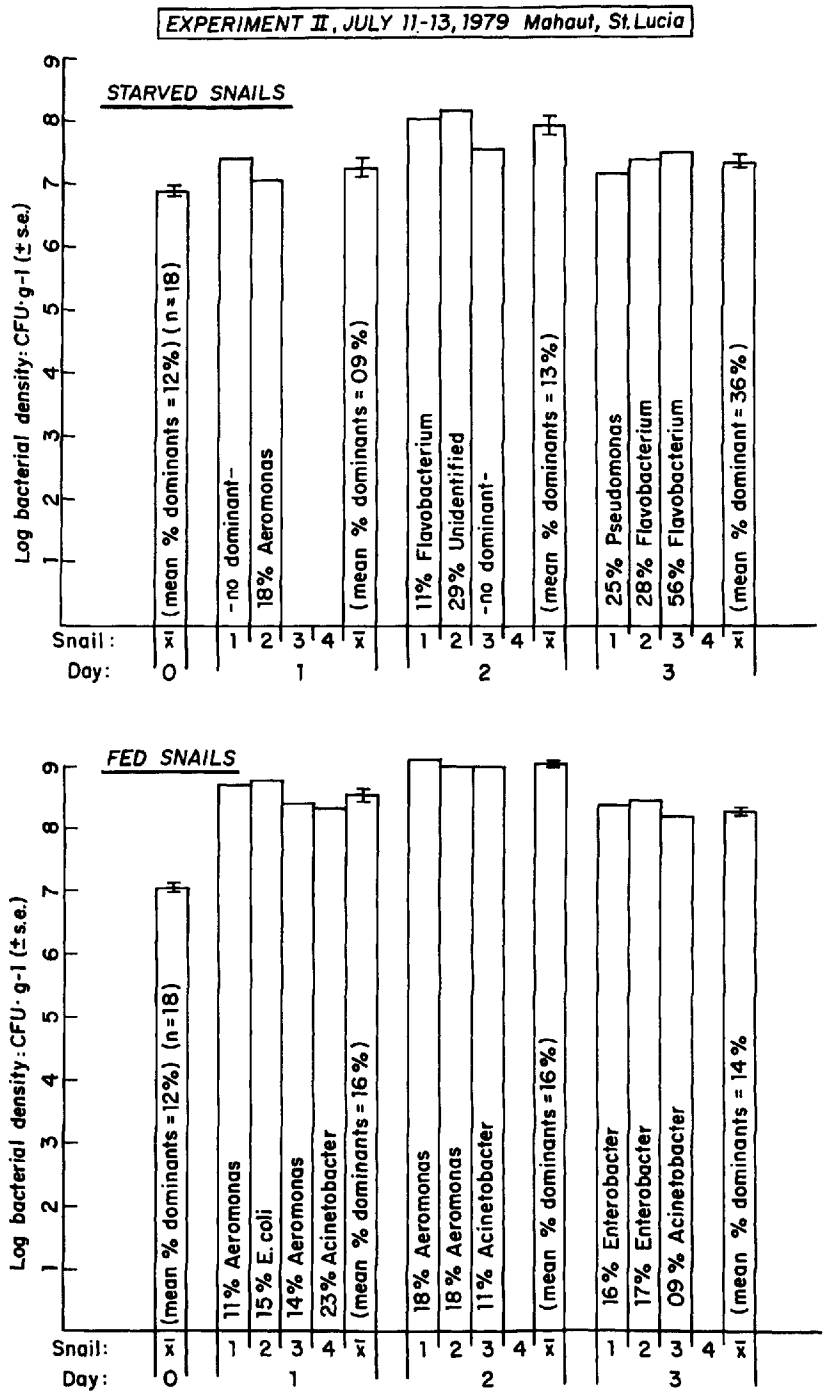

Fig. 2. Bacterial densities in wild St. Lucian *B. glabrata* subjected to crowding. Second experiment. For explanation, see Fig. 1.

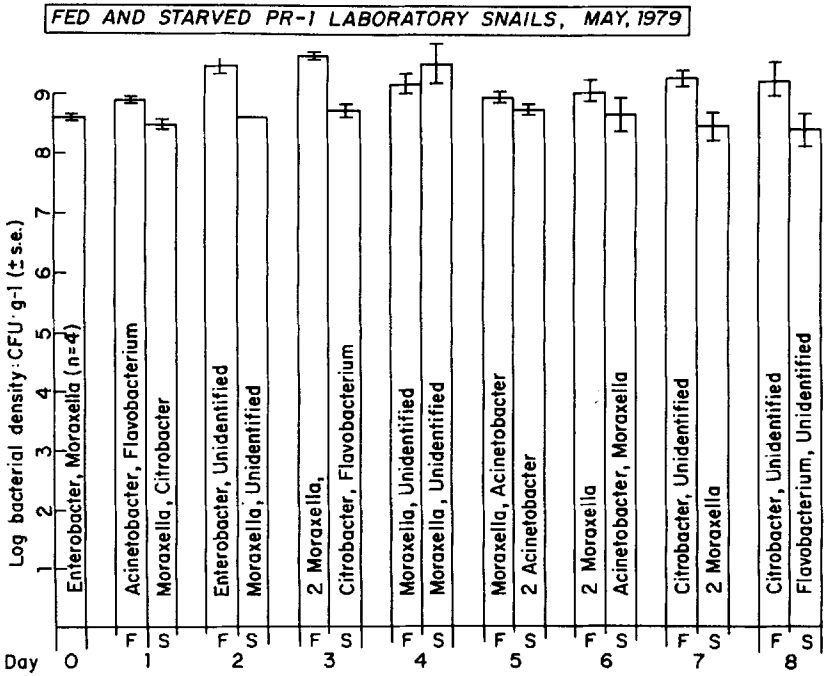

Fig. 3. Bacterial densities in laboratory-reared PR-1 *B. glabrata* subjected to crowding. *F*, fed snails; *S*, starved snails. Day 0 bar represents the mean density in untreated snails from which the incubated snails were taken. Bars represent mean of 2 snails for each group and day.

companying controls, are shown in Table 7. For each antibiotic, the total population of colony-forming bacteria in the antibiotic-treated snails can be compared to the total bacterial population in snails incubated simultaneously in water lacking antibiotics. The antibiotic-resistant fraction of the total bacterial population is also shown, as are the most frequently occurring predominant organisms for each group of AB-treated snails.

In 5 of the 11 experiments, the antibiotic-treated snails had higher densities than control snails after incubation. This was somewhat surprising since we assumed that the main effect of the antibiotics would be to lower the microbial populations inside the snail. Examination of the data showing the fraction of antibiotic-resistant bacteria in each set of snails helps to explain the changes in bacterial populations as a result of antiobiotic treatment. In most cases, the control snails had only a small fraction of their bacterial populations resistant to the antibiotics. However, in most cases incubation in antibiotics resulted in a larger fraction of the population resistant to each antibiotic. Apparently, when the snails are incubated in the antibiotics, selection of antibiotic-resistant bacteria occurs as a result of the differential killing of the antibiotic-sensitive fraction of the population. Then the resistant population may replace the sensitive population, perhaps by occupying vacant habitats or utilizing resources previously monopolized by the antibiotic-resistant population. For instance, in the gentamycin experiment, the gentamycin-resistant bacteria comprised only 0.04% of the total flora of the control snails, but made up 96% of the population in the treated snails. Yet the total

Table 7. Bacterial densities and predominant organisms in antibiotic-treated snails

Antibiotic	Conc. (μg-ml^{-1})	Total pop. (CFU·g^{-1} × 10^6)	Resistant	Predominant organism
			Total	
Penicillin	200	3,243	0.24	*Citrobacter* *Aeromonas*
Control	—	293	0.04	
Streptomycin	200	1,470	0.63	*Moraxella*
Control	—	293	0.001	
Gentamycin	100	211	0.96	*Moraxella* *Flavobacterium*
Control	—	293	0.0004	
Kanamycin	200	438	0.66	*Moraxella*
Control	—	293	0.002	
Tetracycline	10	1,490	0.14	*Pseudomonas* *Moraxella*
Control	—	1,704	0.18	
Polymixin-B	12.5	724	0.50	*Acinetobacter*
Control	—	1,704	0.00006	
Nitrofurantoin	50	210	0.73	*Acinetobacter* *Yeast*
Control	—	511	0.10	
Sulfamerazine	50	1,262	0.51	*Aeromonas*
Control	—	511	0.67	
Colistin	50	98	1.02	*Acinetobacter*
Control	—	511	0.38	
Trimethoprim	200	852	1.35	*Pseudomonas*
Control	—	129	0.11	
Penicillin and streptomycin	200 + 50	436	0.97	*Moraxella*
Control	—	129	0.003	

population remained almost unchanged, suggesting that a simple replacement had taken place. In other cases, the resistant population grew to higher levels than the original total population in the control snails, resulting in increases in the total population in the treated snails. This particular phenomenon is indicated in the kanamycin-treated snails. Even when the total population declined, a shift to a more resistant population occurred, as in polymixin-B- or nitrofurantoin-treated snails. This is probably because the resistant bacteria increase, but not sufficiently to offset the loss of all the sensitive cells. These effects are complicated by several factors. Chief among them is probably the inefficiency of the antibiotics in reaching all the antibiotic-sensitive cells in a treated snail. For instance, in the penicillin- and streptomycin-treated groups, a large increase in population took place without an overwhelming shift to resistant populations. In most of the treated snails, the microflora was dominated by *Moraxella* or *Acinetobacter* spp., which have been implicated in dominance of the bacterial populations in other sets of disturbed snail microfloras.

The patterns revealed by antibiotic treatment of snails and the enumeration of antibiotic-sensitive and antibiotic resistant-bacteria further indicate the dynamic character of the bacterial populations in *B. glabrata*. In these experiments, shifts in dominance from one bacterial population to another were revealed to occur within the treated snails, in addition to the simple increases in population observed previously.

Discussion

By performing a few simple measurements or experimental techniques on a large number of snails from a variety of habitats, we have revealed some qualitative and quantitative patterns in the commensal bacterial populations of the aquatic pulmonate snail *Biomphalaria glabrata*. Prior to our studies, there had been few investigations of the bacteriology of *B. glabrata*, or of snails in general. These studies generally involved the isolation of pathogens or unusual microorganisms from snails. Michelson [18] isolated a *Mycobacterium* sp. from *Helosoma anceps*, and described the effects of its infection on several other snail species. Cole et al. [6] found a morphologically unique bacterium inhabiting *Bulinus jousseauei*. Richards [24] observed different species of spirochetes in three species of snails. None of these organisms were implicated as damaging pathogens or parasites of their hosts. However, these results illustrate the diversity of bacteria inhabiting aquatic snails.

In our study, we have characterized the normal bacterial flora of wild *Biomphalaria glabrata*, and showed how the quantitative and qualitative properties of the flora change when the snails are brought into laboratory culture. The bacterial populations vary greatly in wild snails and retain the potential for significant changes in population size and composition. These findings amplify a concept familiar to field malacologists and parasitologists, but not to many of their laboratory-based colleagues. Snails kept in culture even for short periods of time can differ radically from the wild state. Some factors responsible for these changes can be inferred from our results.

The origin of the normal flora in wild snails is their environment. Snails do not transmit bacterial inocula to their young. Externally disinfected snail eggs give rise to axenic offspring [3, 25]. Thus the bacterial flora derives ultimately from the diet of the snails, and from colonization of the snails by external organisms. However, the environmental conditions within the snails select and determine the ultimate size and composition of the microfloral community. Our results show that the size and composition of bacterial populations in the water in snail habitats have little direct effect on the character of the snail microflora. The normal microflora of *B. glabrata* generally contains 10^6–10^7 CFU/g snail tissue, of which 50% are pseudomonads. Several other bacterial genera also commonly predominate in individual snails.

Tripp [27] showed that *B. glabrata* were quite efficient in eliminating injected foreign materials from their tissue cavities. This at first seems to suggest that environmental bacteria would be repulsed by the cellular defense reactions of the potential host snail. But Tripp tested only gram-positive bacteria or yeasts, which seldom occur as a major part of the normal bacterial flora of *B. glabrata*. Injection of organisms actually derived from healthy snails may have yielded different results. On the other hand, it is likely a great majority of the bacteria we analyzed were sampled from the gastrointestinal tract and epidermis, and not from the interior sinuses of the snails, where Tripp observed the cellular reactions to occur. Of greater potential significance to the formation and maintenance of the microbial flora of these snails are the hemolymph and tissue agglutinins and lysins that have been found in over 80 species of gastropod molluscs [19]. The observation that vitamin E (synthesized by bacteria, but not by snails) is necessary for reproduction in axenic *B. glabrata* [28] shows that the maintenace of a normal bacterial flora may be necessary to the normal functioning of the snail.

Dean et al. [8] studied epizootics in populations of the giant African snail *Achatina fulica*, which has been introduced into Hawaii. They implicated the bacterium *Aeromonas liquefaciens* (syn. *Aeromonas hydrophilia*) in natural epizootics in *Achatina*

populations which occurred following heavy rains. They further suggested that stress was a major factor in predisposing the snails toward *Aeromonas* infections.

Our study provides further evidence that stress can affect the microfloral changes in snails. Several groups of snails from among the snail populations we analyzed possessed higher bacterial densities than normal wild snails, and also had different distributions of predominant bacterial species. We found that three bacterial genera, *Moraxella*, *Citrobacter*, and *Vibrio*, occurred more frequently in high-density or moribund snails. We have also showed that snails subjected to crowding stresses have increased numbers of bacteria. We infer from our surveys of wild and laboratory snails, and conclude directly from crowding experiments, that stress results in increased bacterial populations and changes in the dominant bacterial species in the affected snails.

In addition to characterizing the normal flora of *B. glabrata* we have shown that atypically colonized snails can be found in natural populations. By considering separately these snails with significantly greater bacterial densities than normal ($P \leq 0.01$), we were able to show that these snails had a distinct distribution of predominant bacteria. We hypothesize that these snails experienced stresses that resulted in a different bacterial pattern than that found in normal snails in the same samples. Some stresses that wild snails experience in natural snail habitats include starvation, desiccation, and exposure to high temperatures resulting from being temporarily stranded out of the water. These stresses may also occur during the dry season, when considerable snail mortalities are observed [26].

Snails moved from the field to the laboratory, and snails cultured for extended periods in aquaria have higher bacterial densities and fewer types of predominant organisms than wild snails. Snails cultivated in laboratory aquaria feed and reproduce readily, but are exposed to accumulated metabolic products that should be removed periodically. The minor stresses of aquarium incubation cause the snails to support bacterial densities an order of magnitude greater than normal wild snails.

Moribund snails from the field and laboratory, which have obviously undergone considerable stress, have very high bacterial populations. In these snails the predominant organism usually makes up over 50–70% the total CFU.

Atypically colonized, high bacterial density snails also exist in the aquarium populations. These snails usually harbor a specific bacterial population, *Moraxella* spp., as the numerically dominant bacteria. Since moribund aquarium snails also have very high bacterial densities and have *Moraxella* spp. predominant, it seems reasonable to suggest that some high bacterial density snails may become moribund. It is conceivable that high-density snails in wild populations bear a similar relationship to moribund snails. However, in natural populations this relationship is obscured by the greater diversity of the snail and bacterial populations and greater heterogeneity of the habitat.

There is abundant evidence that high bacterial populations can denote stressed or sick hosts, and that stresses result in diseases in the stressed organisms. Campbell and Podgewaite [2] used high densities of bacteria seen in tissue smears to diagnose diseased gypsy moths (*Porthetria dispar*) from natural populations. Bucher [1] concluded similarly that excessive numbers of bacteria were a sympton of ill health in western Canadian grasshoppers. Epibiotic fouling of Dungeness crab eggs by filamentous bacteria resulted in mortalities [12]. Experimentally applied stresses result in bacterial growth in a variety of host organisms, including corals [10], duckweed [22], and fruit flies [14]. Additional examples are reviewed in Mitchell and Chet [20]. In our experiments crowding stresses to *B. glabrata* resulted in the growth and changes of the bacterial populations in both wild and cultivated snails. The high densities of specific

bacteria found in wild snails (*Vibrio extorquens*) and laboratory-reared snails (*Moraxella* spp.) probably also result from unspecified stresses.

Stress-induced diseases are the result of infections with populations of opportunistic bacteria, which may be members of the normal microflora but exploit permissive conditions such as injuries to host defenses to grow and initiate pathogenesis [29]. Many of the predominant genera isolated from *B. glabrata* have been characterized as opportunists. These include *Pseudomonas*, *Acinetobacter*, *Aeromonas*, *Vibrio*, *Citrobacter*, *Enterobacter*, *Moraxella*, and *Flavobacterium* [29]. Our data on the incidence of high bacterial density snails may reflect the incidence of opportunistic infections in natural snail populations. These findings have great relevance to the control of schistosomiasis. The keys to the control of schistosomiasis are ecological, not medical [5]. By understanding the natural patterns of stress and disease in vector snail populations we may come to a better understanding of applied ecological tactics for snail control. Naturally diseased snails may provide reservoirs for the isolation of potential biocontrol agents for use against snail populations.

In several of the antibiotic experiments, the removal of a specific population by an antibiotic resulted in growth of the remaining, unaffected bacteria to higher levels than were seen in control snails. This finding suggests that some bacterial populations may exert powerful influences over their co-inhabitants in the snails, and thus over the composition of the entire bacterial community. This phenomenon is reminiscent of the concept of "keystone species" as presented by Connell [7]. The finding also supplies a justification for our analysis only of predominant bacterial species. These species may significantly influence the bacterial community. Thus even when only two sets of data—total counts and predominant counts—are collected, interesting patterns in the ecology of bacterial communities can be revealed. Our perturbation experiments suggest relatively simple and straightforward methodologies for determining potential in situ growth rates in bacterial populations that do not involve removal, direct manipulation, or enclosure of the cell population. Use of antibiotics revealed in situ shifts in the patterns of dominance of different bacterial populations.

Finally we wish to state that invertebrate hosts such as *B. glabrata* are useful tools for the microbial ecologist: they are easily cultured, a diversity of strains can be obtained, the bacteria are contained in their natural habitats even in the laboratory, and numerous replicate communities are available for experimental analysis. Our specific studies could be extended into several specialized areas. Most importantly, the connection between ecology and pathology should be more firmly established. One way to do this would be to study the structure and dynamics of bacterial communities in schistosome-infected *B. glabrata*. Another would be to specify the location of opportunistic bacteria in different snail tissue and organs, perhaps by employing fluorescent antibodies and histochemical techniques on healthy, stressed, moribund, and infected snails.

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