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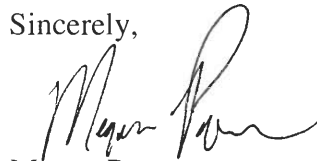
May 8, 2000

Dr. Joesph Troester, Research Hydrologist
Department of the Interior
United States Geological Survey
San Juan, Puerto Rico 009011

Dear Dr. Troester,

Enclosed is our report entitled Schistosomiasis in Natural Waters of Eastern Puerto Rico. It was written at the USGS during the period March 20 through May 8, 2000. Preliminary work was done in Worcester, Massachusetts, prior to our arrival in Puerto Rico. Copies of this report are simultaneously being submitted to Professors Menides and Woods for evaluation. Upon faculty review, the original copy of this report will be catalogued in the Gordon Library at Worcester Polytechnic Institute. We appreciate the time that you and everyone else at USGS have devoted to us.

Sincerely,



Megan Parsons



Lakee Smith



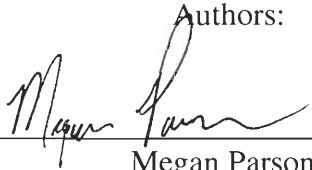
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Schistosomiasis in Natural Waters of Eastern Puerto Rico

An Interactive Qualifying Project Puerto Rico, Project Center

In Conjunction With
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May 9, 2000

This project report is submitted in partial fulfillment of the degree requirements of Worcester Polytechnic Institute. The views and opinions expressed herein are those of the authors and do not necessarily reflect the positions or opinions of the United States Geological Survey or Worcester Polytechnic Institute.

This report is the product of an education program, and is intended to serve as partial documentation for the evaluation of academic achievement. The reader should not construe this report as a working document.

Using studies documenting areas of high prevalence of the parasitic disease Schistosomiasis in Eastern Puerto Rico, we searched 18 sites for the disease's snail host *Biomphalaria glabrata*. No *B. glabrata* snails were found and populations of the competitive snails, *Thiara granifera* and *Marisa cornuarietis*, were documented. We believe that the population of *B. glabrata* is on the decline due, among other reasons, to these competitive snails, therefore decreasing the risk and human infection with *Schistosoma mansoni*. Further studies are recommended.

Authorship

This statement acknowledges that the project team members contributed equally to the following report. All members of the project team equally carried out the literature research, review, and discussion as well as the development of the methodology. All members of the project team formulated conclusions and recommendations.

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Executive Summary

The purposes of this project are to determine whether the eastern population of Puerto Rico is still in danger of infection with Schistosomiasis and to present possible reasons for the decline of *Biomphalaria glabrata* due to competitive snails, *Marisa cornuarietis* and *Thiara granifera*. Schistosomiasis, also known as Bilharzia, is a parasitic disease that can lead to chronic illness in human beings. This project, conducted by students of Worcester Polytechnic Institute in conjunction with the United States Department of the Interior, Geological Survey, assesses the malacologic survey of *Schistosoma mansoni*'s intermediate host *Biomphalaria glabrata*, and competitive snails in the natural waters of eastern Puerto Rico.

According to the World Health Organization, Schistosomiasis is currently widespread in seventy-four tropical, developing countries and approximately 600 million people run the risk of becoming infected. The parasite, *Schistosoma mansoni*, is a blood fluke and goes through a complex life cycle in fresh water beginning within the snail, *Biomphalaria glabrata*. The continuation of the cycle of the disease depends on the infection of a human host by contact with the parasite and by the human's passing of the infection back to the snail through urination or defecation into fresh waterways that *B. glabrata* claim as their home.

The project team members explored 14 established USGS sites and two non-USGS locations. These sites were chosen based on the location in or around an area of high prevalence according to studies done by Dr. George Hillyer (1999: 313-318). Out of the ten high prevalence areas that Hillyer listed, six municipalities were located in eastern Puerto Rico; Naguabo, Las Piedras, San Lorenzo, Loiza, Luquillo, and Rio Grande. We

visited sites in or close to all six municipalities. Each site was then explored to determine whether the snail *B. glabrata* was present and to gain a better understanding of the geographic make-up of the site. A detailed description was written about each site at the time of each visit, including plant life, water depth, the proximity to human contact, other types of snails or animal life present, and further observations. Samples of other snails present were taken to determine their species and whether they were possible competitors or predators of *B. glabrata*.

T. granifera snails were found at 12 of the 18 sites numbering in the thousands and in most instances covering the floor bed. *M. cornuarietis* snails were found at 3 different sites along the Rio Gurabo, and at Quebrada Salvatierra. It should be noted that although no snails were found at Lago Loiza and Rio Caguitas the water was too deep and muddy to be searched thoroughly. The other 2 sites without snails, Quebrada Guaba and Rio Sabana at Sabana were located in El Yunque National Caribbean Forest.

Harry and Aldrich first noted *T. granifera*'s presence in Puerto Rico in 1954 in the Comerio River. A decline in the population of *B. glabrata* was noted in Puerto Rico several years after this discovery. The mature *T. granifera* snail can easily withstand waters highly polluted with sewage treatment, mud, silt and detergents. It survives in large masses and can easily migrate, covering huge sections of waterways and invading the space of other snails such as *B. glabrata*. It has been observed that populations of *B. glabrata* snails have disappeared completely from sites colonized with *T. granifera* snails in just five years (Giboda, Malek & Correa, 1997: 564-568).

Giboda and his colleagues go on to report that *Marisa cornuarietis* is a predator of *B. glabrata*. This circular snail feeds on the eggs of the *Biomphalaria glabrata* snails,

which are on the vegetation it is consuming. *M. cornuarietis* was introduced in 1958 as a biological control in the island's 30 principle water reservoirs. Eighteen years after its introduction, only five reservoirs still contained *B. glabrata* snails (Giboda, *et.al*, 1997: 564).

With the results we have obtained, the project team hypothesizes that the absence of *B. glabrata* in the streams examined in Puerto Rico is due mostly to the presence of *T. granifera* and *M. cornuarietis* as well as to the rising levels of pollution in the waterways.

The cycle of the disease Schistosomiasis can only continue through the presence of *B. glabrata*, the intermediate host, which gets infected by human waste from infected individuals. If the population of *B. glabrata* and the incidences of Schistosomiasis are both low, the disease can eventually disappear. The IQP team recommends further studies on individuals already infected with Schistosomiasis. We also recommend other snail surveys to establish the population patterns of *B. glabrata*, *M. cornuarietis*, and *T. granifera*.

Chapter 1 Introduction

Schistosomiasis, also known as Bilharzia, is a parasitic disease that can lead to chronic illness in human beings. The parasite that causes this disease in Puerto Rico is called *Schistosoma mansoni*. This blood fluke goes through a complex life cycle in fresh water, where it lives in a snail. This is its intermediate host, *Biomphalaria glabrata*. The continuation of the cycle depends on the infection of a human host by contact with the parasite once it has been shed from the snail. This occurs when a person has contact with infected waterways and the parasite enters the body through the skin. The human then passes the infection back to the snail through eggs that are shed during urination or defecation into the fresh waterways that those snails claim as their home.

Theodore Bilharz discovered Bilharzia in Egypt in 1851. Since then many different species of schistosomes have been found, including *S. mansoni* which is the only form prevalent in Puerto Rico. *S. mansoni* is also known to inhabit Africa, St. Lucia, and other tropical climates. The different species of Schistosomes have demonstrated different egg shapes, different intermediate host snails, different adult forms, and different final hosts. According to the World Health Organization, Schistosomiasis is currently widespread in seventy-four tropical, developing countries and approximately 600 million people run the risk of becoming infected. The history of the disease in Puerto Rico shows a steady decline. An island wide survey of Puerto Rico showed a drop of infection from 20% to 5.3% in school aged children from 1927 to 1976 (WHO, 2000).

This project was performed at the United States Geological Survey in Guaynabo, Puerto Rico¹. Its goal is to perform a malacologic survey² of *B. glabrata* and competitive snails in the natural waters of Eastern Puerto Rico. The purpose of this project is to determine if the population of Eastern Puerto Rico is still in danger of infection with Schistosomiasis. It also presents possible reasons for the decline in *S. mansoni* and *B. glabrata*. Some of these hypothesized reasons include an increase in water pollution and competition caused by the snails *Marisa cornuarietis* and *Thiara granifera*.

The literature review contains relevant information from case studies, journal articles, books, medical organizations, and interviews. It consists of background information on the following: history of Schistosomiasis in Puerto Rico; calculating snail population densities; techniques for gathering cercariae and snail samples; infection control methods for schistosomes and their snail hosts; survival of the schistosome in the human host; the life cycle of *Schistosoma mansoni*; the development of immunity in the human hosts; and symptoms and cures in practice for Schistosomiasis.

The methodology includes training techniques that the project team used, materials needed for conducting the study, the collection of field data and the documentation of these data. The field data were collected at sites chosen based on the data the USGS had available, their locations within high prevalence municipalities, as determined by Dr. George Hillyer. Dr. Hillyer has over 35 years of experience with Schistosomiasis in Puerto Rico. At the time of this report he was Chancellor of the

¹ This report was prepared by members of the Worcester Polytechnic Institute Puerto Rico Project Center. The relationship of the Center to the United States Geological Survey and the relevance of the topic to the United States Geological Survey are presented in Appendix A.

² See Glossary.

University of Puerto Rico Medical School and was developing a vaccine for *Schistosomiasis mansoni* in Puerto Rico.

The IQP team visited eighteen sites in total, encompassing streams and tributaries of Rio Gurabo, Rio Grande de Loiza, and Rio Mameyes.

The results of our project will be of interest to the people of Puerto Rico, the medical field, the United States Geological Survey, and other researchers studying schistosomes and their snail hosts. If the disease is no longer a hazard to the people of Puerto Rico, they will be able to utilize fresh waterways again. This study, along with the recommended studies for the future will show whether the population of *B. glabrata* snails is decreasing and whether the disease is no longer a threat. This study provides information and significant data that the USGS and other organizations can use in future studies.

An Interactive Qualifying Project (IQP) is a project that links technological aspects to social aspects in scientific research. The project develops the students' ability to design their own educational programs, act as competent professionals, and understand the social implications of their work.

This project fulfills the requirements of an IQP by utilizing technology and previous research to collect data that can be used in deciding whether Schistosomiasis is still a threat to the people of Puerto Rico and by postulating the causes for its decline, and the decline in the population of *Biomphalaria glabrata*, over the last thirty years.

Chapter 2 Literature Review

Reader's Guide to the Literature Review

In order for the reader to gain a basic understanding of the background and goals of our project, we have extensively reviewed literature in many areas. This review is a culmination of background information on Schistosomiasis, *Schistosoma mansoni*, and *Biomphalaria glabrata* in Puerto Rico. Topics reviewed include a background of the disease's symptoms and treatments, the life cycle of *S. mansoni*, techniques for detecting the parasite in waterways, and techniques for calculating the population densities of snails. We also discuss case studies dealing with different collection techniques and devices and the current prevalence of the disease in Puerto Rico.

Schistosomiasis

Schistosomiasis, also known as Bilharzia, is a parasitic disease that leads to chronic illness. Sufferers are severely weakened by the disease and may develop a rash or itchy skin within days of becoming infected. Fever, chills, cough, and muscle aches may begin within one or two months of infection. However, most people have no symptoms at this early stage of infection.

The parasites, also known as schistosomes, are called blood flukes because they live in the blood stream of their hosts. The schistosomes lay eggs which travel to the liver or pass into the intestine or bladder (CDC, 1998). In rare instances, eggs are found in the spinal cord or brain and can cause spinal cord inflammation, seizures, or paralysis. For people who are repeatedly infected or have been infected for an extended period of

time, the parasite can cause damage to the liver, intestine, lungs and bladder (CDC, 1998).

There are multiple forms of schistosomes. The main forms found in humans are caused by five species of the blood fluke. Intestinal Schistosomiasis is caused by the species *Schistosoma mansoni*. This is the prevalent form in Puerto Rico, as well as in fifty-two other countries and territories of Africa, the Caribbean, the Eastern Mediterranean and South America (WHO, 2000). Other forms of intestinal Schistosomiasis are caused by *S. japonicum* and *S. mekongi*, and are found in seven Asian countries and the Pacific Region. In Africa, *S. intercalatum* is prevalent as well as urinary schistosomiasis caused by *S. haematobium* (WHO, 2000).

According to the World Health Organization, Schistosomiasis is common in seventy-four tropical developing countries, and approximately 600 million people run the risk of becoming infected. It is estimated that 200 million of these people have already acquired the disease. The World Health Organization's Division of Control of Tropical Diseases reports that factors contributing to the risk of infection include the lack of awareness of the risks, the inadequacy of public health facilities, poverty, and unsanitary living conditions. The disease is also associated with inadequate water supply, poor sanitation of the water, and unplanned water resource development (WHO, 2000).

Schistosomiasis mainly affects people who come into prolonged contact with fresh waterways. Such waterways include lakes, rivers, ponds, and streams. According to the Centers For Disease Control, contamination of the fresh water with schistosome eggs occurs when infected people urinate or defecate in the water (CDC, 1998). Human

infections can occur when the water is used for swimming, hygiene, fishing and irrigation (WHO, 2000).

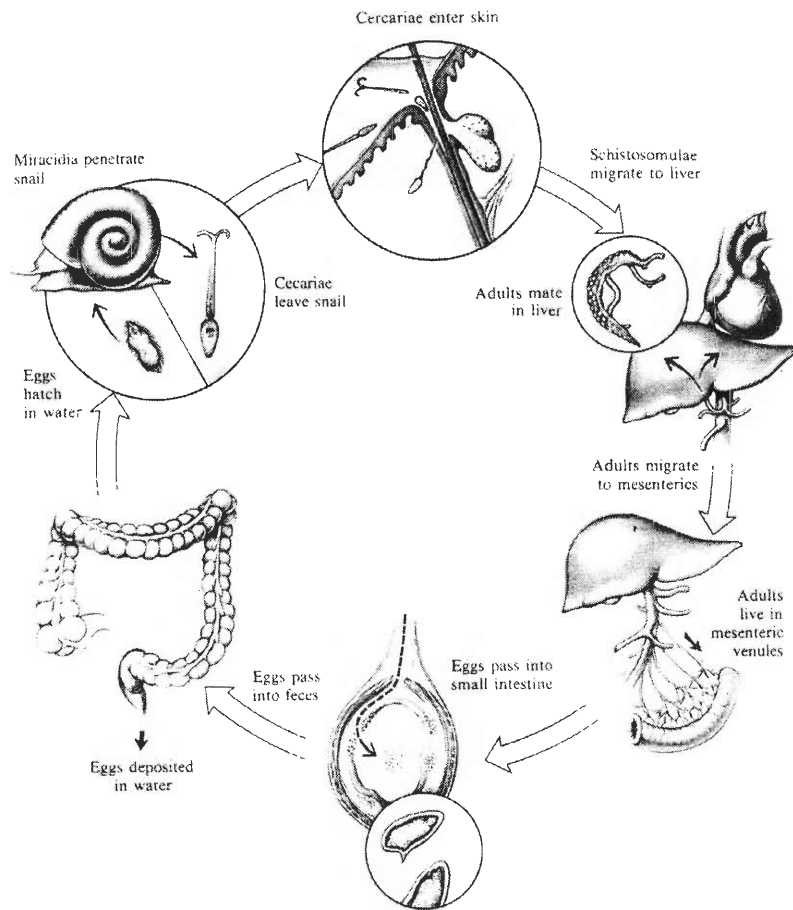
History of Discovery

Schistosomiasis parasites have been known to exist since the time of the Egyptian Pharaohs. In 1851, Theodore Bilharz first discovered *S. haematobium* worms in the intestinal veins of a post-mortem patient in Cairo (Jordan, 1982: 1). Many years later it was revealed that there were two different types of parasites infecting the men in Egypt. The first involved the bladder and was called *S. haematobium*. The other had effects on the intestine, and was named *S. mansoni* (Jordan, 1982: 1). Later, in 1915, the work of Leiper provided the final proof of the existence of two distinct species. He showed that the two strains of parasites had different egg shapes, inhabited specific intermediate host snails, and were not the same in their adult form. Baelz later discovered a third intestinal species named *S. japonicum*. Since that discovery, several additional species have been found, but most do not prefer humans as their final host (Jordan, 1982: 2).

The Life-Cycle of the Parasites

Peter Jordan describes the complex life cycle of the parasitic and free-living stages that occur in Schistosomiasis. These include: the egg, the miracidium, the first-stage (mother) sporocyst, the second-stage (daughter) sporocyst, the cercariae, the schistosomulum, and the adult schistosome. Below are Hillyer's illustration and a summary of Jordan's discussion of the life cycle (1982: 51-52).

Figure 2.1: The Life Cycle of Schistosomiasis



Source: Muro, Antonio, Vicente Ramajo, Julio Lopez, Fernando Simon & George Hillyer (1996). *Fasciola hepatica*: Vaccination of rabbits with native and recombinant antigens Related to fatty acid binding proteins, Veterinary Parasitology, Volume 69, 219-229.

Miracidium

The miracidium is the embryo that develops over six days. The eggs that are passed from a body during defecation or urination contain miracidiae, which are visible through the egg. The egg hatches in an environment of freshwater, warmth, and light. The miracidiae from the schistosomes are similar in behavior and makeup. One of the

essential parts of the miracidiae is the epidermal plate that is arranged in four tiers and covered with cilia all of equal length. The cilia are the tiny hairs that allow the miracidiae to swim rapidly in straight lines. Miracidiae swim freely for eight to twelve hours looking for a snail intermediate host, their rate increases when stimulated by the chemical substances emitted by snails (Jordan, 1982: 51-52).

The number of snails that become infected is directly proportional to the number of miracidiae to which the snail is exposed. If *Biomphalaria glabrata* is exposed to a sufficient number of miracidiae, one hundred percent infection will be achieved. The miracidiae penetrate when the larva attaches to the body surface of the snail (Jordan, 1982: 51-52).

Upon the penetration of miracidiae, the cilia disappear. Within a few days a mother sporocyst develops close to the point of penetration. This process takes place only if the miracidiae enter the correct species of snail; otherwise they are destroyed by defense mechanisms of the snail (Jordan, 1982: 51-52).

At the forty-eight hour mark the muscle layers and some glands degenerate after penetration. The mother sporocyst at ninety-six hours is an elongated sac filled with vacuoles and germinal cells. At approximately eight days, the mother sporocyst has a globular shape and germ balls attached. The germ balls fall off and develop into the daughter sporocyst. They migrate to other parts of the snail's body, including the liver. At the liver, further production of the germ balls occurs and the final larval form of the cercariae is produced (Jordan, 1982: 51-52).

Cercariae

The cercariae is produced from the asexual reproduction of the mother and daughter sporocysts from a single miracidium. It takes about four to five weeks for the cercariae to mature from the time the miracidiae is penetrated. When the cercariae mature and escape from the snail, they are in the free-swimming stage and they usually swim near the surface of the water. All of the cercariae produced from one miracidium are of the same sex. Cercariae are less than 1mm long and contain an oral sucker that secretes sticky mucus for attachment to the skin during penetration (Jordan, 1982: 51-52).

Production of Cercariae

Light is the primary stimulus that causes the release of the cercariae, usually at moderate to high temperatures. There are a small number of cercariae shed in the dark. The patterns of cercarial output in *S. mansoni* are peak between nine in the morning and five in the evening. Most of the cercarial output of the day is during that time span. In the field, the pattern of output of *S. mansoni* from *Biomphalaria glabrata* corresponds to the changes in temperature and intensity of illumination. Starting at 9 a.m., large outputs of cercariae are manufactured and the output rises to a peak at 3 p.m. (Jordan, 1982: 51-52).

The number of cercariae produced depends on the susceptibility of the snails and varies from day to day. The most important factor in the production of cercariae is the size of the snail. The output from *Biomphalaria glabrata* is very high. They produce about 1,000 to 3,000 cercariae each day, and more than 10,000 during the time of an infection (Jordan, 1982: 51-52).

Cercariae Behavior

Cercariae live for approximately forty-eight hours. They do not feed on anything because they have large glycogen reserves. The glycogen reserves provide the energy the cercariae need to swim. Glucose found in water can be used as a supplement for energy in addition to the glycogen. Cercariae become tired in fast, turbulent water, and as a result their survival rate decreases (Jordan, 1982: 51-52).

A study done by Rowan shows that the peak cercariae density in flowing water is between 11:00 and Noon. The density diminishes after 5 p.m. and is reduced to none by 9 a.m. In ponds, Rowan finds that from 10 a.m. to 4 p.m. the density increases and from 4 p.m. to 10 p.m. it decreases to zero (1965: 67-68).

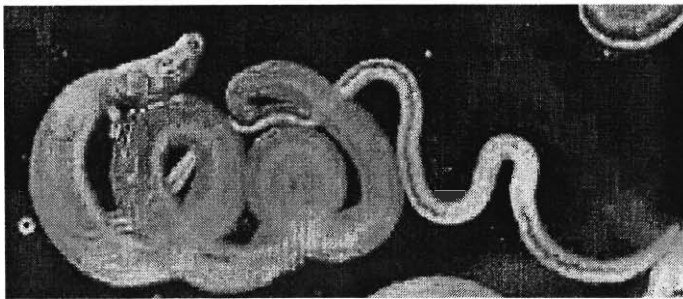
Rowan also found that under average field conditions *S. mansoni* cercariae in streams are found in equal density at any depth from zero to 50 centimeters. Also, he noted that the densities along the edges of a stream seem more sporadic and unreliable while the densities from the center tend to be more consistent (1965: 67-68).

Schistosomulum

Once the cercariae penetrate the skin they turn into schistosomulae. The schistosomulum is tailless and wormlike in appearance. After penetration, the schistosomulae pass through the layers of skin until they find blood vessels. They travel through the blood vessels until they reach the heart. Once they leave the heart, the schistosomulae travel to the liver and the hepatic portal vessels of the liver. The

schistosomulae mature into adult worms and lay eggs. The eggs are released into the urine and feces. It takes at least forty to fifty days until the adult worm lays eggs. If an infected person defecates or urinates in fresh water, the cycle repeats itself (Jordan, 1982: 51-52).

Figure 2.2: The Adult Schistosome Worm



Source: World Health Organization (WHO), <http://www.who.int/ctd/>. 1/20/00

Schistosomes

The species of schistosomes that interact with humans have similar basic life cycles. However, they show differences in the shapes of their eggs and their adult forms as well as differences in the larvae that hatch from the eggs (Jordan, 1982: 3). They also show a variance in the particular types of snails they use as intermediate hosts, as well as their ability to infect other mammalian hosts. All of the adult schistosomes are similar to elongated roundworms. This allows them to move within the blood vessels of their host (File, 1998: 671).

S. Mansoni

According to Jordan, the adult male worms of *S. mansoni* measure 6-13 mm in length while the females measure 7-17mm. The mature worms are usually found in the lower intestinal veins and the eggs pass into the lumen³ of the bowel and are discharged in the fecal matter (1982: 4).

Jordan goes on to report that the posterior caecum, a part the intestine⁴, of the male worm occupies two-thirds of the body length. The male has 4 to 13 testes. The female ovary is situated toward the head of the worm and the small uterus is on the anterior side, occupying two-thirds of the body and usually contains one or two eggs at a time. A single female produces from 100 to 300 eggs per day (1982: 5).

Snails

Emile Malik states that snails are made of an external shell and a body. The external shell of all freshwater snails is made of calcium carbonate. After a period of time, the external shell is shed. The shell can have several different features or textures. Larger features like grooves or spines are called sculpture. The apex of the sculpture is used to determine the species. Although determining the species by examining the apex is a useful practice, over time erosion occurs, so young or very well preserved shells must be used to determine the species (1985: 5).

The bodies of snails, according to Malik, are attached to the shell by a columellar retractor muscle, which runs from epithelial cells next to the shell into the head and foot.

³ See Glossary.

⁴ See Glossary.

The snail can retract its head and foot inside the shell by contracting the columellar retractor muscle. Some snails have an operculum or “trapdoor” that completely shuts the opening of the shell (1985: 7-12).

The general shape of a snail is determined by the position of the coils in respect to each other and the rate at which the diameter increases. If the coils, or whorls, are located outside of each other as in *Biomphalaria glabrata*, the shell will be flat, or discoidal or planispiral. The size of the *Biomphalaria glabrata* shell ranges from 15 to 30 mm diameter and 5 to 8 mm width. The average shell has about 5 to 6.5 whorls (1985: 7-12).

Biomphalaria glabrata

The intermediate hosts of *S. mansoni* are classified under the genus *Biomphalaria* and the sub-family *Planorbinae*. All snail hosts that transmit *S. mansoni* are classified under the genus *Biomphalaria*. The snail hosts of *S. mansoni* from Puerto Rico are classified as the species *glabrata* (Jordan, 1982: 16 -17).

The *Biomphalaria* intermediate host snails live throughout the world. They are located in Africa, Arabia, the Caribbean and South America. *Biomphalaria glabrata* is considered the most efficient carrier of *S. mansoni* (Jordan, 1982: 17).

Ecology

Jordan tells us that the *S. mansoni* intermediate hosts are found in a variety of habitat types, such as man-made and natural lakes, rivers, streams, swamps, marshes and

small ponds. The snail hosts can survive in a variety of environmental conditions. The habitat can consist of shallow water in streams with a sufficient amount of organic matter, little turbidity, vegetation, abundant microscopic plant organisms, and moderate light penetration. The pH of the freshwater varies between 5.3 and 9, and the water temperature ranges between 18 degrees Celsius and 30 degrees Celsius. The stream gradient can also limit *Biomphalaria glabrata*, but most of the time shallow areas of water will occur that are able to support high densities of snails (1982: 33-34).

Chemical and Physical Factors

Snails are able to tolerate a wide range of chemical and physical conditions, which makes it very difficult to predict colonization through the analysis of the water content. However, results have been obtained by measuring the salinity, which is the total concentration of electrolytes (Jordan, 1982: 32-34).

Jordan reports that studies in Puerto Rico have shown some waters with less than 150 mg per liter of dissolved solids, represented with a conductivity of about 222 micromhos, were free of colonies of *Biomphalaria glabrata*. This is due to a low number of electrolytes. If the number of electrolytes is too low, it is hard to maintain the proper salt balance. Low pH may also be harmful because the mucus on the snails' skin surfaces may coagulate⁵ (1982: 34-35).

Climatic Factors

One of the most important climatic factors that contribute to the life of snails is rainfall. The rainfall cycles help determine seasonal fluctuations in snail population

⁵ See Glossary.

density. There is a severe decrease of the snail density during rainfall and an upsurge in the population in the drier months that follow. The numbers of snails that inhabit the water are optimal at warm temperatures. Cold weather can stop or decrease the propagation of a snail population. Once temperatures are elevated again, the number of snails will increase (Jordan, 1982: 37).

Snails, otherwise known as pulmonates, are animals that feed continuously as they move. Most of the aquatic habitats contain decaying vegetable matter, and an abundance of organic microscopic plant organisms that provide food for the snails. The amount of food and vegetation remains high in most aquatic systems as long as the habitat does not desiccate or dry out. However, snails are not as tolerant to desiccation if they are infected with schistosomes. In *Biomphalaria glabrata* desiccation causes cercariae and sporocysts of *S. mansoni* to start to degenerate in about three weeks. Ultimately, desiccation kills snails and their eggs and is used as control method in certain circumstances (Jordan, 1982: 41-43).

Life-Cycle

Biomphalaria glabrata are hermaphroditic and capable of self-fertilization. The ova or eggs of the snails are laid in water in wide range of temperatures from 20° Celsius to 30° Celsius. The snails that lay eggs inside the body can produce the eggs all year long (Jordan, 1982: 42).

Competitive and Predatory Snails

According to Chaniotis and his colleagues, *Thiara granifera* also referred to, as *Tarebia granifera* is a widely distributed operculate snail. Harry and Aldrich first noted its presence in Puerto Rico in 1954 from the Comerio River. Its circumstances of introduction to the island are unknown. It accommodates a large variety of fresh water habitats, including high velocity and placid streams. Habitat temperatures range from twenty-two degrees Celsius to thirty-six degrees Celsius and pH lies between 7.1 and 8.5. Waters highly polluted with sewage treatment, mud, silt and detergents are easily withstood by the mature 6mm (on average) conical snail (Chaniotis, Butler, Ferguson, & Jobin, 1980: 81).

A current study done by Giboda, Malek and Correa in 1997 supports a theory that this snail is a competitive species of *B. glabrata*. It survives in large masses and can easily migrate, covering huge sections of waterways and invading the space of other snails such as *B. glabrata*. Butlers and others did the first study on the comparison of Thiariid snails and *B. glabrata* from 1964-1969. This study showed that the population of *B. glabrata* snails disappeared completely from the sites colonized with *T. granifera* snails in five years (Giboda, Malek, & Correa, 1997:564-567).

The same study also noted that *Marisa cornuarietis* is a predator of *B. glabrata*. This circular snail feeds on the eggs of the *Biomphalaria glabrata* snails, which are on the vegetation it is consuming. It was introduced in 1958 in the island's 30 principle water reservoirs as a biological control. Eighteen years after its introduction, only five reservoirs still contained *B. glabrata* snails (Giboda, Malek, & Correa, 1997:564-567).

Extent of Schistosomiasis in Puerto Rico

According to studies done by George V. Hillyer and Maricelis Soler De Galanes, the exact current prevalence of infection with *S. mansoni* in Puerto Rico is unknown.

There are no current efforts to control Schistosomiasis being sponsored by the government currently (1999: 828). Because Schistosomiasis is not a reportable disease, many infected persons are not identified and thus are not treated (File, 1998: 671).

According to studies performed on blood serum by Hillyer and Soler De Galanes, there appears to be an age-specific decrease in seroprevalence⁶, suggesting that the infected population is an aging one and reinforcing the current hypothesis that there are no new infections (1999: 828). Hillyer, Tsang, Vivas-Gonzales, Noh, Ahn & Vorndam report additional studies, that support the case for diminished infection levels of *Schistosomiasis mansoni* in the human population of Puerto Rico (1999: 313-317).

Age-Specific Decrease in Prevalence

Results of the study done by Hillyer and his colleagues show support for the concept that there has been little transmission of Schistosomiasis in Puerto Rico during the first half of the 90's. Physicians of the island also report virtually no new infections during the past three years. Two reasons have been proposed for this diminished infection level. The first attributes the decreased level to the pollution of waterways in Puerto Rico. A recent island-wide study showed that four out of ever five sites tested for fecal coliforms and streptococci were too polluted for recreational use. The second proposed reason is that there has been an increase in the standard of living in Puerto Rico (1999: 313-317).

Findings of the parasites in feces also support the serology. The Central Laboratory of the Puerto Rico Medical Center processes approximately 5,000-6,000 stool samples yearly for the detection of eggs and parasites. The method used is a concentration filtration technique that processes close to one gram of feces. This is read qualitatively. Patient samples are gathered island-wide. Diagnosis of Schistosomiasis by stool examination has been extremely low: one *S. mansoni* egg in the last three months of 1993, two in 1994, one in 1995, and none from 1996-1998 (Hillyer & Soler De Galanes, 1999: 829). According to File, although instances of recent, active transmission of Schistosomiasis in Puerto Rico are seldom reported, the infection still most likely exists to some extent. Recent studies tell us that many adults still show positive blood serum results, indicating that they have *S. mansoni* (1998: 671).

File goes on to say that the many changes in life styles, water contact, sewage disposal, and the environment have occurred on the island in the last twenty-five years. Most of these changes are unfavorable for the transmission of the parasite. Apparently, transmission is intermittent or is at a low level of intensity with most infections consisting of few worms in otherwise healthy persons. This is why many infections may go undetected (1998: 671).

High and Low Prevalence Municipalities in Puerto Rico

Studies done by Hillyer and his colleagues have shown the areas of high schistosomiasis prevalence in the past in Puerto Rico to be Jayuya, Utuado, Naguabo, Las Piedras, San Lorenzo, Cidra, Loiza, Luquillo, Ciales, and Rio Grande. Jayuya was shown to have the highest positivity at 38 percent, while the municipality of Rio Grande had a

⁶ See Glossary.

prevalence of only 25 percent. The 10 municipalities showing the lowest prevalence in ascending order from 1.35 to 5 percent included Ponce, Moca, Corozal, Manati, San Sebastian, Rincon, Arecibo, Coamo, Mayaguez, and Barceloneta (1999: 313-314). Most of the high prevalence regions are located east of a north/south line from San Juan, according to Hillyer and Soler De Galanes (1999: 827).

A High prevalence municipalities

Map of Puerto Rico showing municipalities with high prevalence of dengue. The highlighted municipalities are Lotza, Río Grande, Quírico, Naguabo, Las Pailas, and San Lorenzo.

B Low prevalence municipalities

Map of Puerto Rico showing municipalities with low prevalence of dengue. The highlighted municipalities are Hoca, San Sebastián, Mayaguez, Ponce, Coamo, Dorozal, Arecibo, Barceloneta, and Manati.

20

Trace elements

In a study done by James W. Crooks in Puerto Rico, it was shown that several trace elements could have an effect on the population of *Biomphalaria glabrata*. This was shown through a comparison of streams and ponds that have a high snail population to one that did not have any snails. The stream that did not show a snail population was low in silica, magnesium, sodium and potassium in comparison to water in other streams in Puerto Rico. It is possible that the amounts of these elements are insufficient to meet the needs of the snail or the plant food upon which it depends. Researchers have also attributed anomalies in shell development to deficiencies of these minerals (1971: 240).

Control of Schistosomiasis

According to the World Health Organization's Division of Control of Tropical Diseases, several tools and strategies for the control of Schistosomiasis exist. One of these tools is education. Health education is meant to increase knowledge of the use of water, and to prevent urination and defecation into fresh water. It is also aimed at getting citizens to comply with medical treatments if infected and at making sure that communities know they can play an active role in the control of Schistosomiasis. The requirement of sanitation facilities and safe water supplies is another tool expected to reduce the prevalence of the disease. A third tool that can be utilized is the use of molluscicides, which reduce the host snail population (WHO, 2000). Hillyer and De Galanes report that this method was used in Puerto Rico, but was cancelled in 1980. They go on to say that the commonwealth of Puerto Rico currently has no control effort

against Schistosomiasis. There is no organized chemotherapy for the treatment of infected patients either (1999: 829).

Currently Hillyer and De Galanes think that Puerto Rico is leaning towards an increase in economic advancement and better living conditions. This is shown by an increase in the urbanization of areas where natural waters existed. The urbanization causes streams to dry up due to canal formation. The increase in population also encourages more clean water to be piped into houses, and less need for water contact in the streams near residential areas (1999: 829).

Chemotherapy Strategies for Treatment of Schistosomiasis

Two types of drugs are usually used to treat patients diagnosed with Schistosomiasis. The first is a drug called praziquantel. This drug treats all species of Schistosomiasis at a dosage of 40-mg/kg body weight (WHO, 2000). Although praziquantel has been shown recently by Borinquen Laboratories in Caguas Puerto Rico to decrease the percentages of egg reactors and effectively control schistosomiasis cases, some concern for a praziquantel-tolerant *S. mansoni* strain exists (Stelma, 1997: 304). The other drug currently used for chemotherapy treatment is oaxmniquine, which showed a higher cure rate than praziquantel in a recent study performed by Stelma. According to the World Health Organization, oaxmniquine is highly effective for *S. mansoni*, and dosage varies between 15 and 60 mg/kg. While both drugs show a reduction in egg counts, it is still not known if one is slightly better than the other (Stelma, 1997: 304). Both are safe and are taken in pill form for a short period of time. Both are capable of curing the disease if taken correctly (CDC, 1998).

Past Control Methods

According to Aponte, Henry, and Jobin, a control program for Schistosomiasis began in Puerto Rico in 1953. This control effort used all available control techniques including limited chemotherapy with chemical, biological, and environmental means of snail control, sanitation and health education activities. The main techniques were control of the snail population and an increase in improved water supply (1979: 515-525).

Some of the chemicals used as molluscicides included Faudin, which was is continued in 1957 due to drug-related deaths, sodium pentachlorophenate, acrolein, bayluscide, and niclosamide. Biological control began in 1958 with the ampullarid snail, *Marisa cornuarietis* that eats egg masses of *B. glabrata* (Aponte *et al*, 1979: 515-525).

In addition to these control methods, the Aqueduct and Sewer Authority began a program in 1945 to improve large urban water systems with chemical coagulation, sand filtration, chlorination, fluoridation, and rural water supplies that merely stored water from a protected source and delivered it directly to the homes in a piped but untreated system. The rural water supplies served about 300,000 people in 1953 when the Bilharzia control program started and expanded to over one million people by 1978 (Aponte *et al*, 1979: 515-525). These all contributed to cleaner water.

From the beginning of the control program in 1953 to its end in 1978, approximately 8.3 million dollars was spent by the Health Department. This did not include epidemiological evaluation or scientific consultation, significant additional costs items that were covered by the Centers for Disease Control. Throughout these studies it

was shown that there was a significant decline of Schistosomiasis in Puerto Rico over a thirteen-year period (Aponte *et al*, 1979: 515-525).

Possible New Cases

In September of 1998, hurricane Georges devastated all of Puerto Rico. It affected the electric and water distribution systems island wide (Hillyer and De Galanes, 1999: 829). With the absence of water in their homes, a portion of the population resorted to bathing and washing in nearby streams, increasing the human water contact (Hillyer and De Galanes, 1999: 829). This was observed quite vividly in the municipalities east and northeast of San Juan and in the mountain regions. According to Hillyer and De Galanes, this increased water contact may lead to a short-term increase of cases of Schistosomiasis within family groups (1999: 829). The hope is that these cases, when identified, will be treated, thereby continuing the decrease of human schistosome infections in Puerto Rico.

Snail Collection

Collecting and observing snails was a major portion of this project and will be in future studies. This section, adapted from Malek (1985: 230-233), suggests the following items for snail collection: bottles of 70 percent alcohol, insect repellent, forceps, a scoop net, pH measuring paper of instruments, specimen bottles, labels, diers, maps, menthol, jars with screen mesh caps, fixative, hip wading boots, a thermometer, a field notebook, and cardboard boxes with perforated tops (1985: 230-233).

It would be unwise to touch plant life, water, or snails to bare skin. Rubber gloves are not advised because they cause perspiration and may tear. Long forceps would be the best replacement. Massaging rubbing alcohol on arms and hands before collecting and in the event of contact with anything possibly carrying the parasite will lower chances of infection. Wiping an area of the skin dry with a cloth will also be effective (Malek, 1985: 230-233).

Surveying an area

Areas that are to be surveyed should be thoroughly examined. All bodies of water in the survey area must be observed, such as: seepages, side pools, borrow pits, and main and natural watercourses. The wet and dry seasons of the area should be noted as well as flood periods and temperature fluctuations (Malek, 1985: 230-233).

Snail Samples

Any snail samples collected should be clearly labeled for each site, dated, and kept separate from the others. They should be kept in moist vegetation, not water. Keeping them cool and preventing them from being in direct sunlight are critical (Malek, 1985: 230-233).

Snail Collection Techniques

Olivier mentions several techniques for calculating an estimation of a snail population. One method, not easily used for a quantitative study, is counting the snails found on palm leaves immersed in the water. Another technique estimates the density by

calculating the average number of snails collected by passes of a standard collection device over the site area. The standard collection device would be a sieve, net, or dredge (1956: 110).

Malek discusses two techniques that are suggested that can be used for quantitative studies on snail populations such as *Biomphalaria glabrata*. The first technique uses a scoop or sieve to do a marked number of passes on a clearly defined area. Each catch is carefully examined and counted. The second technique is a timed collection. A fixed number of collectors handpick snails with forceps for an interval of time in a clearly marked area. “The measure of snail density is the number of snails collected per man per unit of time” (Malek, 1985: 230-233). Olivier mainly discusses the timed sampling method and claims it to be the most effective (1956: 111).

Cercariometry

Theron states that cercariometry gathers information about the infection risk for a population, while snail sampling gathers information about the rate of deposition of schistosome eggs in the site (1986: 62).

According to Donald G. Sandt’s report, there are five existing techniques for detecting Schistosome cercariae in the field: direct filtration, mouse immersion, phototaxis, continuous centrifugation, and field snail shedding (1973: 27).

Direct filtration utilizes a pump and filter. The filter catches the cercariae. The field team calculates the density of the cercariae by use of a turbidity function. Direct filtration appears to be the most commonly used technique (Sandt, 1973: 35-36).

Mouse immersion or mouse floatation involves the placing of mice in the water of the test site to infect the mice with the schistosome. Several weeks later after the cercariae have grown into the adult blood flukes the mouse will be dissected to examine the parasite. This is the only method enabling the differentiation of cercariae when a few species of schistosome populate a single area (Theron, 1986: 61).

Phototaxis uses an apparatus that attracts the *Schistosoma mansoni* through phototactic response. The use of flood lamps and heavy equipment makes this technique expensive. However, it appears to have a higher efficiency than other techniques because turbidity is not a large factor (Rowan, 1965: 66).

Sandt states that during continuous centrifugation the water sample is run through the centrifuge to separate the cercariae from the water. The cercariae are then stained and observed under a microscope (1973: 36).

According to Rowan, field snail shedding is used to determine whether a snail is infected and the extent of the infection without killing the snail. The snail is put into a jar with water. The jar is then placed in direct sunlight. The light, water, and warmth encourage cercariae shedding (1965: 68).

Of these techniques, Sandt prefers direct filtration and Theron (1986: 61) claims that “direct filtration techniques seem the most efficient and successful.” Given the volume of filtered water, direct comparison of cercarial densities can be calculated (1973: 27).

Theron also states some benefits of direct filtration: that cercariae can be counted in the field or at a lab, that the direct filtration apparatus is uncomplicated and effortless

to use, and that the amount of cercariae collected is almost as high as eighty percent (1986: 62).

The main problems with any of these techniques involve turbidity. Rainfall, human activity, and biological organisms affect turbidity. They also affect the accuracy in the sample size (Sandt, 1973: 27).

Theron says that when direct filtration was first put into use the main drawback was the filters. The early filters were Millipore, made of paper or glass, and weren't sufficiently porous. They would quickly clog which would decrease the volume of filtered water (1986: 61).

The filter problem has been lessened by the use of a prefiltration column, a device that is designed with a set of sieves of decreasing pore size. The pore size is between 25 and 40 micrometers. Each sieve uses a monofilament polyamide filter. For the greatest efficiency, water filtration should take place during the hours of peak cercarial emission (Theron, 1986: 62).

Chapter 3 Methodology

The methodology is a description of the procedure that our team followed while completing the project on Schistosomiasis in the natural waters of eastern Puerto Rico. The project was completed at the United States Geological Survey office in Guaynabo, Puerto Rico in association with the WPI Interdisciplinary and Global Studies Division project center in San Juan, Puerto Rico.

The goal was to perform a malacologic survey of the intermediate host *Biomphalaria glabrata* and possible competitive snails in the natural waters of eastern Puerto Rico. The *B. glabrata* snail plays a critical role in the continuation of the cycle of Schistosomiasis. Sixteen different USGS sites and two non-USGS sites were searched. We found populations of *Thiara granifera* and *Marisa cornuarietis* but no *Biomphalaria glabrata* snails.

Preparation

Upon arrival at the United States Geological Survey, we began training in the use of the water quality database. This database lists several characteristics of water at all of the established USGS sites. Examples of these characteristics include temperature, pH, and measurements of organic compounds. Records are kept of these measurements in the computer database, which is updated frequently. The same information is published annually in a Water Resources Data Book.

Based on prior research at WPI of what would be needed to conduct the study, we requested that the following equipment be ordered for our fieldwork:

- Protective Hip Wader Boots
- Five Gallon Bucket

- Rolls of Flagging Tape
- Pocket Notebooks
- Thermometers
- Dip Nets
- Forceps
- Funnels
- 20-45µm Whatman Filter Paper
- Petri Dishes
- Iodine

We ordered the funnels, filter paper, petri dishes, and iodine to filter the water for cercariae if *B. glabrata* snails were found.

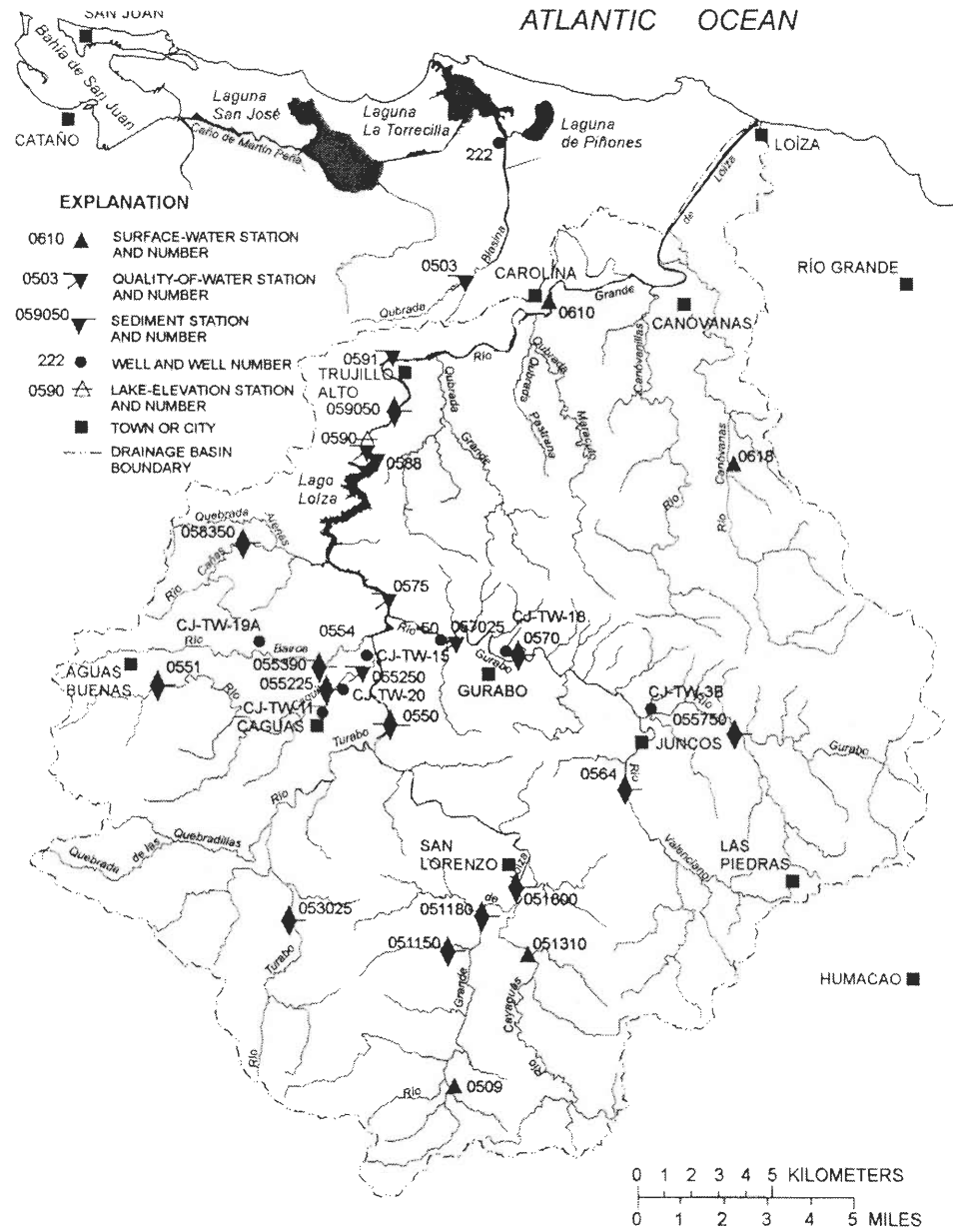
Data Acquisition

The goal of the project was to perform a malacologic survey of the intermediate host *Biomphalaria glabrata* and possible competitive snails in the natural waters of eastern Puerto Rico. To accomplish this, sites were chosen based on accessibility and the location in or around an area of high prevalence according to studies done by Dr. George Hillyer (1999: 313-318). Out of the ten high prevalence areas that Hillyer listed, six sites were located in eastern Puerto Rico: Naguabo, Las Piedras, San Lorenzo, Loiza, Luquillo, and Rio Grande. The following maps show the 16 established USGS sites and two non-USGS locations in or close to all six municipalities we explored.

Map of the Cordillera Occidental region in Puerto Rico, showing the distribution of 15 bird species. The map includes major rivers (Río Grande, Río Cuyuy, Río Fajardo, Río San Juan, Río Portugués, Río San Pedro, Río San Juan, Río Portugués), towns (Loíza, Canóvanas, Río Grande, Luquillo, Fajardo, Ceiba, Naguabo, Humacao), and various bird species codes (RE-2A, RM-2, RS-02, RP-04, RF-12, RF-04, CEIBA, ARR-1, NAGUABO, LAS PIEDRAS, HUMACAO). The map also shows the Atlantic Ocean to the north and east.

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Figure 3.2: Rio Grande de Loiza Basin



Source: Diaz, Pedro L., Zaida Aquino, Carlos Figueroa-Alamo, Ricardo J. Vachier, & Ana V. Sanchez. Water Resources Data Puerto Rico and the U.S. Virgin Islands Water Year 1998. U.S. Department of the Interior. U.S. Geological Survey. 1998.

Each site was then explored to determine whether the snail *B. glabrata* was present and to gain a better understanding of the geographic make-up of each site. All three of the group members put on hip wader boots, gloves, and carried nets to the water.

We were able to examine the waterways thoroughly by using the hip wader boots to walk around in the streams to find snails. We then proceeded to enter the streams and look for *B. glabrata* snails. A sample was taken from any group of snails discovered at the sites. The snails were later identified by descriptions, pictures gathered from publications and books, and by Carlos Conde (a biologist at the USGS), and by Dr. George Hillyer (a parasitologist and chancellor of University of Puerto Rico). A detailed description was written about each site at the time of each visit, including plant life, water depth, the proximity to human contact, types of snails or animal life present, and further observations. Snail samples were taken to determine their species. Populations of *M. Cornuarietis* were counted by marking off an area of the streams containing it. This area was approximately 100 feet in length and snails were counted by at least two people in each 10-foot section. This information is presented in the Sites Analysis section of Chapter 4. The following Table, 3.1, lists the sites that were investigated:

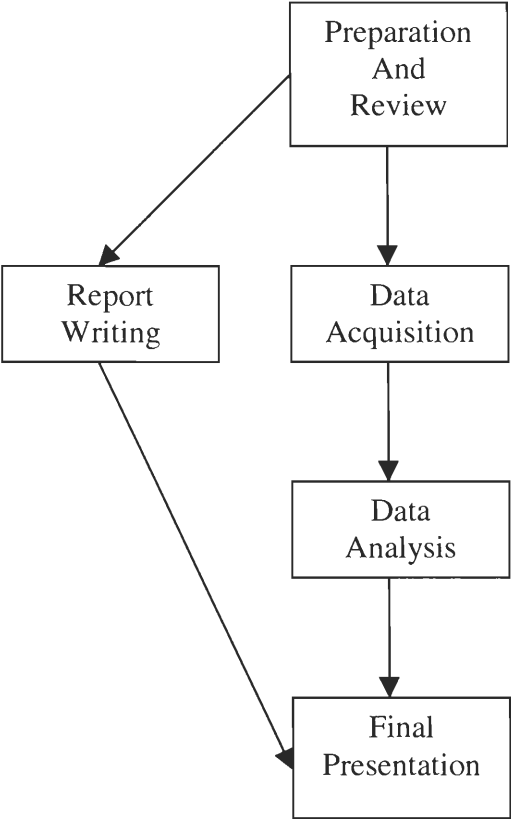
Table 3.1: Sites Visited

Site Name	USGS Site Number
Quebrada Blasina near Carolina	50050300
Quebrada Salvatierra near San Lorenzo	50051180
Río Grande de Loíza at Caguas	50055000
Río Caguitas at Villa Blanca at Caguas	50055225
Río Caguitas at Highway 30 at Caguas	50055250
Río Gurabo below El Mangó	50055750
Río Valenciano near Juncos	50056400
Río Gurabo at Gurabo	50057000
Río Gurabo near Gurabo	50057025
Lago Loiza at Damnsite near Trujillo Alto	50059000
Río Canóvanas near Campo Rico	50061800
Río Espíritu Santo near Río Grande	50063800
Río Grande near El Verde	50064200
Río Mameyes near Sabana	50065500
Río Sabana at Sabana	50067000
Quebrada Guabá near Naguabo	50074950
Highway 181	Non-USGS site
Highway 184 at Cayey	Non-USGS site

Table 3.2: Task Chart

	Week 1		Week 2		Week 3		Week 4		Week 5		Week 6		Week 7		Week 8	
Preparation and Review																
Data Acquisition																
Data Analysis																
Report Writing																
Final Presentation																

Figure 3.3: Flow Chart of Methodology



Chapter 4 Data Analysis and Results

In this chapter we discuss the data acquired from each site visited, along with the analysis of these data. Each stream is listed and described in a short paragraph.

Site Analysis

Monday April 3, 2000

Rio Mameyes near Sabana (Picture not available)

The Rio Mameyes near Sabana USGS hydrologic unit is located on the left bank, at the bridge on Highway 988. All three group members about 200 feet downstream entered the small stream from the bridge at approximately 12:30 pm. The stream was slightly polluted: beer cans, tires, and other foreign household objects were located in the water. One team member proceeded to travel upstream and the two other members went downstream. Approximately $\frac{1}{4}$ mile of the stream was searched for *Biomphalaria glabrata*. The stream had several large rocks, small fish and a population of black snails with a conical shape identified as *Thiara granifera*. No *B. glabrata* snails were found.

Monday April 3, 2000

Rio Sabana at Sabana (Picture not available)

The Rio Sabana at Sabana hydrologic unit is located on the right bank along Highway 988 by the El Yunque Caribbean National Forest. The team members entered at approximately 1:30 pm. There was not any visible pollution in the water. Team members searched approximately $\frac{1}{4}$ mile up and downstream. The water was clear, had a

small amount of algae, and several large rocks that covered the floor bed. No snails were found.

Monday April 3, 2000

Quebrada Guaba near Naguabo (Picture not available)

The Quebrada Guaba near Naguabo hydrologic unit is located on the right bank, off Highway 191 at El Yunque Caribbean National Forest. The stream, located about 20 feet down a small hill, was entered at approximately 2:15 pm. The stream was very shallow (a few inches deep) and very sandy. There were high walls of dirt and grass that surrounded the narrow stream. There was a giant log, and few large rocks located in the water. A small waterfall was located approximately 40 feet downstream. There were worms and mosquitoes in the water. Team members searched approximately 100 feet up and downstream. No snails were found.

Tuesday April 4, 2000

Figure 4.1: Highway 184 at Cayey



The stream, located along Highway 184 at Cayey, was searched at approximately 11:00 am. It was a small waterway with several large rocks and an abundance of orange clay. There was a good deal of pollution including light bulbs, trash, and other debris. A crab, water bug, and a few fish were spotted. The small fish were dark green and approximately 3 inches long. There were green algae present. Many *T. granifera* snails were located on the rocks that covered the floor bed. Team members searched approximately ¼ mile up and downstream. No *B. glabrata* snails were found.

Tuesday April 4, 2000

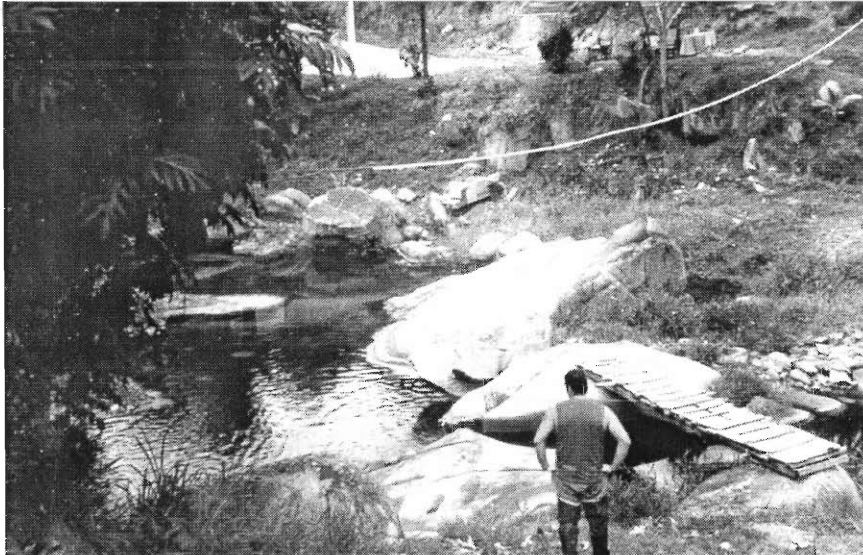
Figure 4.2: Highway 181



The stream, located along Highway 181, was searched at approximately 12:00 noon. The stream was small and very shallow. The deepest section was about one foot. The water was very clear and the floor bed was sandy and covered with small pebbles. There were not a lot of algae present in the water. There were small fish, water bugs, and very few *T. granifera* snails. Team members searched approximately ¼ mile up and downstream. No *B. glabrata* snails were found.

Tuesday April 4, 2000

Figure 4.3: Quebrada Salvatierra near San Lorenzo



The Quebrada Salvatierra near San Lorenzo hydrologic unit is located on the left downstream side of the bridge on Highway 181. The stream was searched at approximately 12:30 pm. There were large rocks protruding about 4 feet out of the water from the edge to the center of the stream. There was a combination of some deep places with standing water approximately 3 feet deep and some places further downstream with flowing water approximately 1 foot deep. A variety of small and large colorful fish were swimming throughout the water. Algae were present along the edges of the rocks and the banks. Five *Marisa cornuarietis* snails were found in a 1000 square foot area (100 ft X 10 ft). These snails were found in areas of standing water and green moss about 4 inches thick. *T. granifera* snails were found there also. This site had roosters crowing and small children playing in nearby houses, located on both sides of the bank. Team members searched approximately ½ mile up and downstream. No *B. glabrata* snails were found.

Wednesday April 5, 2000

Rio Caguitas at Highway 30 at Caguas and Rio Caguitas at Villa Blanca at Caguas (Picture not available)

The Rio Caguitas at Highway 30 and Villa Blanca at Caguas, located less than a half mile apart, were searched starting at 10:45 am. The water was shallow under the Highway 30 bridge and deep otherwise. The water was very muddy and had a small amount of algae. There were many birds and small fish and a strong odor of raw sewage around the area. Team members searched approximately 100 feet along the bank on land. No snails were found.

Wednesday April 5, 2000

Rio Gurabo at Gurabo (Picture not available)

The Rio Gurabo at Gurabo hydrologic unit is located on the left bank at the bridge on Highway 181. The stream was searched at approximately 11:10 am. The water was deep and not able to be searched thoroughly. The outer edges underneath the bridge were scanned for snails. One *M. cornuarietis* shell and many *T. granifera* snails were found; however, no *B. glabrata* snails were found. The water had a green color and there were men fishing on the left edge of the bank. Team members searched approximately 50 feet along the edges of the bank.

Wednesday April 5, 2000

Figure 4.4: Rio Gurabo near Gurabo



The Rio Gurabo near Gurabo area is located at the bridge on highway 941. The stream was searched at approximately 11:45 am. The water was deep and not able to be searched thoroughly. However, 13 *M. cornuarietis* snails in a 300 square foot area (100 ft X 3 ft) were found living along the edge. The water had a dark green color and tall grass was located approximately two feet from the edge of the right water edge. A thick bed of mud surrounded the edges of the banks, which prevented travel along the edges. There were several cows and bulls located in the green pastures adjacent to the water. Some *T. granifera* snails were found also. Team members searched approximately $\frac{3}{4}$ mile up and downstream. No *B. glabrata* snails were found.

Wednesday April 5, 2000

Rio Grande de Loiza at Caguas (Picture not available)

The Rio Grande de Loiza at Caguas hydrologic unit is located on the right bank 250 feet upstream from the bridge on highway 189. The stream was searched at approximately 12:33 pm. The area was too deep to be searched anywhere except the outermost edges. The water had a dark brown, muddy color. There were several large rocks protruding about 3 feet out of the water. Many *T. granifera* snails were found. Team members searched approximately 100 feet along the outer edges. No *B. glabrata* snails were found.

Monday April 10, 2000

Figure 4.5: Quebrada Blasina near Carolina



The Quebrada Blasina is located at the bridge on Highway 3. The stream was searched at approximately 9:50 am. The water was very shallow (less than 2 feet) and many large rocks covered the floor bed. There was a large amount of green algae and moss located

close to the edges. There was a good deal of pollution in the grass along the side of the river and a little pollution in the water. Many *T. granifera* snails were found. Team members searched approximately 100 feet up and downstream. No *B. glabrata* snails were found.

Monday April 10, 2000

Figure 4.6: Lago Loiza at Damsite



The Lago Loiza is located near the pumpsite at the damnsite. The lake was searched at approximately 11:20 am. The large area was very marshy with much plant life growing on top of the water. The water was not entered because of its deep depth. All observations were obtained from the land along the bank. Team members searched approximately 100 feet along the edges of the bank. There were no snails found.

Monday April 10, 2000

Rio Canovanas near Campo Rico (Picture not available)

The Rio Canovanas hydrologic unit is located at the upstream side of bridge located northeast of the junction of Highways 185 and 186. The stream was searched at approximately 1:15 pm. The water was shallow with many large rocks that covered the floor. There was a little pollution in and around the stream. There were houses and small animals nearby. Many *T. granifera* snails were found. Team members searched approximately ¼ mile up and downstream. No *B. glabrata* snails were found.

Wednesday April 12, 2000

Rio Grande near El Verde (Picture not available)

The Rio Grande near El Verde hydrologic unit is located on the left bank 250 feet on the upstream side of the bridge at Highway 960. The stream was searched at approximately 8:50 am. The water was shallow and had a little pollution. Many rocks covered the floor bed. On most of the rocks there were *T. granifera* snails. There was little vegetation and a few fish. The area was very quiet and no houses were nearby. Team members searched approximately ¼ mile up and downstream. No *B. glabrata* snails were found.

Wednesday April 12, 2000

Rio Espiritu Santo near Rio Grande (Picture not available)

The Rio Espiritu Santo near Rio Grande hydrologic unit is located on the downstream side of the bridge on Highway 966. The stream was searched at approximately 9:55 am. The water was shallow with a few scattered large rocks that protruded approximately 2

feet above the water. There were thick clumps of moss that *T. granifera* was noticed on. There was a good deal of pollution along the sides and houses nearby. Team members searched approximately ¼ mile up and downstream. No *B. glabrata* snails were found.

Wednesday April 12, 2000

Figure 4.7: Rio Valenciano near Juncos



The Rio Valenciano hydrologic unit is located on the left bank at Highway 919. The stream was searched at approximately 11:00 am. The bed floor was made up of sand and the water was very shallow, approximately 1 foot. In the center of the stream, several large rocks protruded about 6 feet out of the water. There were many tadpoles swimming throughout the stream and ducks frolicking nearby. Team members searched approximately ¼ mile up and downstream. No snails were found.

Wednesday April 12, 2000

Figure 4.8: Rio Gurabo below El Mango



The Rio Gurabo below El Mango is located on Route 191. The stream was searched at approximately 12:30 pm. The water was shallow and the floor was covered with rocks. The water was clear and had no pollution in the water. Three *M. cornuarietis* snails were in a 300 square foot area (100 ft X 3 ft) found along the left edge of water. The snails were located under the plant life along the edge of the water. Team members searched approximately ½ mile up and downstream.

Table 4.1: Snails Found at Sites

	<i>M. cornuarietis</i>	<i>T. granifera</i>	<i>B. glabrata</i>	none
Rio Mameyes near Sabana		+		
Highway 184 at Cayey		+		
Highway 181		+		
Quebrada Salvatierra near San Lorenzo	+	+		
Rio Gurabo at Gurabo	+	+		
Rio Gurabo near Gurabo	+	+		
Rio Gurabo below El Mango	+	+		
Rio Grande de Loiza at Caguas		+		
Quebrada Blasina near Carolina		+		
Rio Canovanas near Campo Rico		+		
Rio Grande near El Verde		+		
Rio Espiritu Santo near Rio Grande		+		
Rio Sabana at Sabana				+
Quebrada Guaba near Naguabo				+
Rio Caguitas at Highway 30 at Caguas				+
Rio Caguitas at Villa Blanca at Caguas				+
Lago Loiza at Damsite				+
Rio Valenciano near Juncos				+

The preceding table shows the distribution of the competitive snails observed at each site. *T. granifera* snails were found at 12 of the 18 sites numbering in the thousands and in most instances covering the floor bed. *M. cornuarietis* snails were found at 3 different sites along Rio Gurabo, and at Quebrada Salvatierra. It should be noted that although no snails were found at Lago Loiza and Rio Caguitas the water was too deep and muddy to be searched thoroughly. The other 2 sites without snails, Quebrada Guaba and Rio Sabana at Sabana were located in El Yunque National Caribbean Forest.

Chapter 5 Conclusions and Recommendations

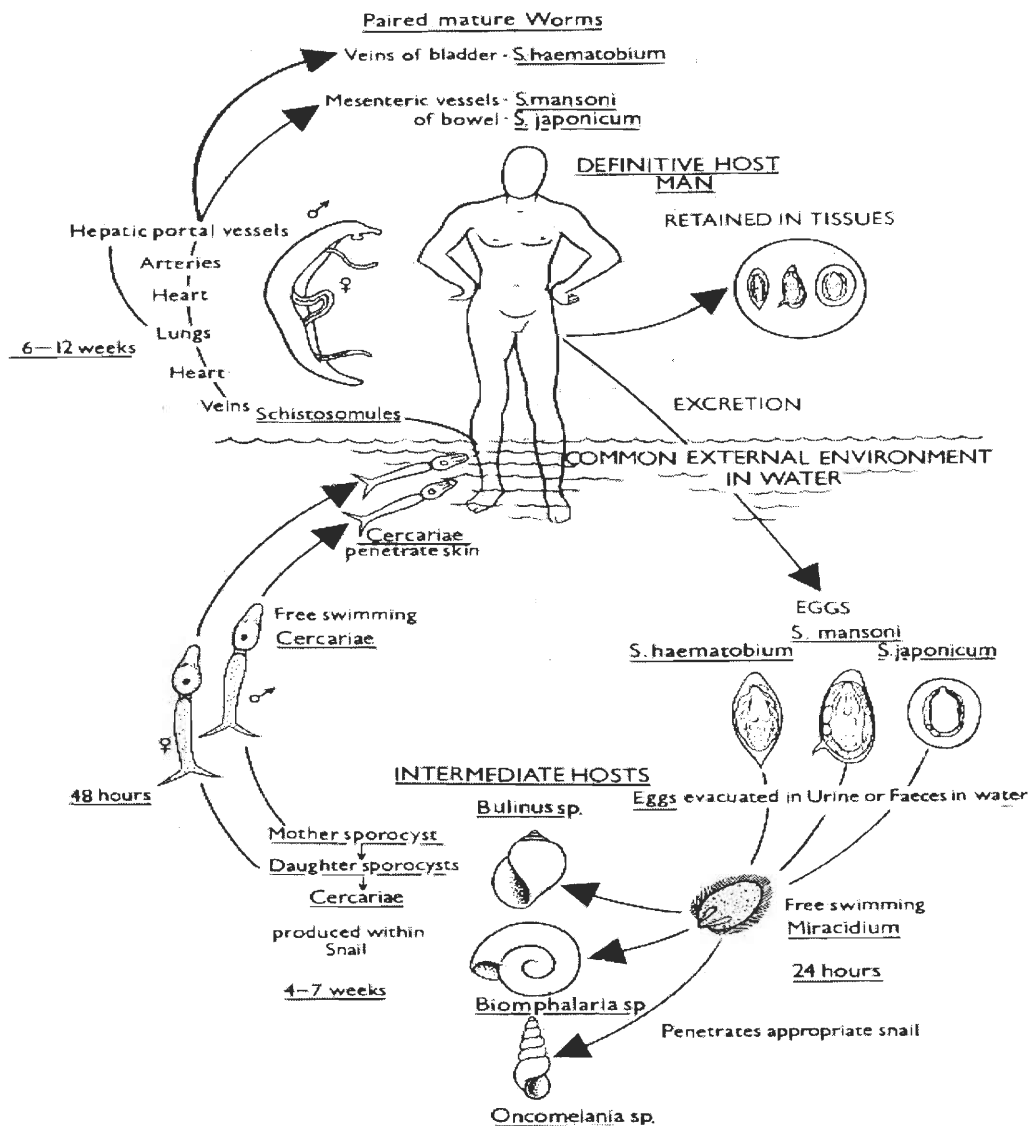
The conclusions of this report have been drawn by the project group with the guidance of the USGS staff and the opinions of professionals who have written in scientific journals. The recommendations that follow represent the opinions of the IQP project team and do not necessarily reflect those of any other agency involved with this study. Topics discussed in this section of the report are: the reasons for decrease in the prevalence of Schistosomiasis in Puerto Rico, the absence of *B. glabrata* and the presence of other snails at the sites visited, the probable low risk level of Schistosomiasis infection for the citizens of Puerto Rico, and the recommendations for future studies of Schistosomiasis in Puerto Rico.

Decrease in the Prevalence of Schistosomiasis

Dr. George Hillyer, an expert on Schistosomiasis, believes that a parasitic disease, such as Schistosomiasis, which follows a very complex life cycle, can be stopped once a “breakpoint” is reached. A “breakpoint” can occur when the cycle no longer takes place and may be caused by lowering the probabilities of the necessary events in the cycle. The Schistosomiasis cycle, as shown below in figure 5.1, begins with an infected human. The parasite matures and lays eggs inside the human’s body, which are deposited into fresh water when the infected person urinates or defecates into that water. The eggs then hatch in the water and the newly formed miracidiae have twenty-four hours to find the host snail, *Biomphalaria glabrata*, to infect. The miracidiae then develop within the snail for four to seven weeks and upon maturation are shed into the water as cercariae. These cercariae then have approximately forty-eight hours to find a human host to infect. If a

host is found (a human who has contact with this water), the cycle then continues with the maturation of adult worms in the human body.

Figure 5.1: The Life Cycle of Schistosomiasis



Source: Jordan, Peter and Gerald Webbe. Schistosomiasis. William Heinemann Medical Books, LTD, 1982.

Schistosomiasis mansoni is the strain of Schistosomiasis found in Puerto Rico and the host snail in Puerto Rico is *Biomphalaria glabrata*, shown in the middle of the intermediate hosts in Figure 5.1. The first two *Schistosomiasis mansoni* cases in Puerto Rico were first reported there in 1904 by Gonzalez-Martinez. In 1927, the prevalence of

the disease was 20% and decreased to 9.9% by 1945. These percentages are taken from a pool of people, from high prevalence municipalities in Puerto Rico, who gave stool samples. In 1953, White and others performed an island-wide stool examination in school-aged children 5-18 years old. The results showed a prevalence of 10% (Hillyer, 1997: 107). In 1963, an island-wide immunodiagnostic survey was started among 5th graders. The test showed a decrease from 24% in 1963, to 14% in 1969 and finally 5.3% in 1976 (Doumenge *et al.*, 1987: 372). No systematic prevalence data has been taken since the mid 1970s when all control methods were discontinued due to the decrease in the prevalence of the disease (Hillyer, 1997: 107).

We believe that the breaking of the cycle of Schistosomiasis has occurred in Puerto Rico over the last 30 years due to several reasons:

- The apparent decrease in the population of *Biomphalaria glabrata*, the host snails, due to an increase in population of other snails, *Thiara granifera* and *Marisa cornuarietis*, which may be *B. glabrata*'s natural enemy
- The increase in water pollution, leading to a decrease in human contact with possibly infected waters, and resulting in a habitat possibly not suited for the host snail
- The increase in sanitation, and more clean water being piped into houses
- The testing and treating of families by physicians for parasite infections each year
- Older generations show higher seroprevalence than younger generations, who have more contact with streams and other waterways

- The educational programs and control methods of the past have increased the overall awareness of Schistosomiasis

Absence of *Biomphalaria glabrata*

Over a period of approximately two weeks, April 3 to April 12, the project team explored 18 sites across the eastern region of the island. At these sites we performed malacologic surveys and noted the approximate number of *M. cornuarietis*, and *T. granifera* snails found at each. The following table shows the distribution of the snails observed at each site:

Table 5.1: Distribution of Snails Observed at Sites

Water bodies	<i>M. cornuarietis</i>	<i>T. granifera</i>	<i>B. glabrata</i>	No Snails
(N = 18)				
Rio Mameyes near Sabana		+		
Highway 184 at Cayey		+		
Highway 181		+		
Quebrada Salvatierra near San Lorenzo	+	+		
Rio Gurabo at Gurabo	+	+		
Rio Gurabo near Gurabo	+	+		
Rio Gurabo below El Mango	+	+		
Rio Grande de Loiza at Caguas		+		
Quebrada Blasina near Carolina		+		
Rio Canovanas near Campo Rico		+		
Rio Grande near El Verde		+		
Rio Espiritu Santo near Rio Grande		+		
Rio Sabana at Sabana				+
Quebrada Guaba near Naguabo				+
Rio Caguaitas at Highway 30 at Caguas				+
Rio Caguaitas at Villa Blanca at Caguas				+
Lago Loiza at Damsite				+
Rio Valenciano near Juncos				+

T. granifera snails were found at 12 of the 18 sites numbering in the thousands and in most instances covering the floor bed. *M. cornuarietis* snails were found at 3 different sites. These were along Rio Gurabo (fourteen *M. cornuarietis* snails found) and at Quebrada Salvatierra (five *M. cornuarietis* snails found). No *B. glabrata* snails were found. It should be noted that although no snails were found at Lago Loiza and Rio

Caguitas, the water was too deep and muddy to be searched thoroughly. Our team concentrated on searching the edges of these locations, looking under plant life and in the shallower areas.

The sites without any snails at all, Quebrada Guaba and Rio Sabana at Sabana, were located in El Yunque National Caribbean Forest. Those sites did not have trash or foreign household objects and they were not easily accessible to people as they are located on service roads and areas only open to government officials.

A similar malacologic survey performed by Giboda, Maled, and Correa in 1995 showed similar results, which are shown in the following table:

Table 5.2: Malacologic Survey

Water bodies	<i>M. cornuarietis</i>	<i>T. granifera</i>	<i>B. glabrata</i>
Rivers (n = 10)			
Rio Grande de Loiza		+	
Rio Caguitas	+	+	
Rio Grande Arecibo		+	
Rio Yunes		+	
Rio Culebrinas		+	
Rio Grande de Anasco		+	
Rio Guanajibo		+	
Canal de Guamani			+
Rio Grande de Patillas	+	+	
Rio Jayuya		+	
Lakes (n = 8)			
Cidra		+	
Dos Bocas		+	
Coamo		+	
Guayabal	+		
Toa Vaca		+	
Guajataca		+	
Lucchetti/Yauco		+	
Patillas		+	
Streams (n = 4)			
Parcelas de Boqueron	+	+	
Publito de Rio		+	
Bariio Negra-Corozal		+	
San Lorenzo-Hogar Crea		+	

Source: Giboda, Maled, and Correa (1997). Human Schistosomiasis in Puerto Rico: reduced prevalence rate and absence of *Biomphalaria glabrata*, American Journal of Tropical Medicine and Hygiene, Volume 57, 566.

In the Giboda et al survey, *T. granifera* snails were found at 20 of 22 locations. *M. cornuarietis* snails were found at 4 of 22 locations and *B. glabrata* snails were found at only 1 location. In the two studies, the 1997 Giboda et al study, and the one that our team conducted in 2000, one dead specimen of *B. glabrata* was found at the Canal de Guamani.

There are many reasons that could account for the low number of *B. glabrata* snails. One of those reasons could be the population of *T. granifera* and *M. cornuarietis*. *Thiara granifera*'s presence in Puerto Rico was first noted in 1954. A decline in the population of *B. glabrata* was noted several years after this discovery. Mature *T. granifera* snails can easily withstand waters highly polluted with sewage treatment, mud, silt and detergents. They survive in large masses and can easily cover huge sections of waterways invading the space of other snails such as *B. glabrata*. Populations of *B. glabrata* snails have disappeared completely from sites colonized with *T. granifera* snails in just five years (Giboda, Malek & Correa, 1997: 564-568). Another study states that *T. granifera* snails are capable of maintaining very high population densities and have the same diet as *B. glabrata* (Madsen, 1990: 237-240).

Marisa cornuarietis is a predator of *B. glabrata*. This circular snail feeds on the eggs of the *B. glabrata* snails. In 1958 as a biological control of *B. glabrata*, it was introduced into 30 of the island's principle water reservoirs. Eighteen years later, in 1976, only five reservoirs still contained *B. glabrata* snails (Giboda, et.al, 1997: 564).

While these studies have showed a correlation between the decrease in *Biomphalaria glabrata* and the spread of *Thiara granifera* and *Marisa cornuarietis*, Dr. George Hillyer states that if *B. glabrata* snails are present, they can still transmit

cercariae even with the other competitive snails nearby (Hillyer, 2000). If the *B. glabrata* population continues to decrease, infection will eventually no longer be possible.

Although we are not sure whether *Biomphalaria glabrata* snails previously existed in the sites visited, we hypothesize that new colonies of *B. glabrata* would not be able to survive due to the presence of the competing snails, *T. granifera* and *M. cornuarietis*.

Increase in Water Pollution

Hillyer also stated that water pollution might be a reason that the *B. glabrata* population seems to be decreasing (Hillyer 2000) and we concur with this hypothesis.

While exploring many of the sites, we encountered a great deal of pollutants in the waterways. It is possible that this pollution prevents the *B. glabrata* snails from populating the waterways and decreases the amount of human contact with water, thus decreasing the incidence of Schistosomiasis. The article, *Paradise Lost: An Introduction to the Geography of Water Pollution in Puerto Rico*, documented a study to evaluate water pollution across the island of Puerto Rico. Hunter and Arbona stated that 54 of 67 (81%) of rivers sampled exceeded maximal contaminant levels for fecal coliforms. Fecal coliforms are the indicators of fecal contamination, or human waste. All of the rivers (100%) were also found to be in violation of drinking water standards (1995: 1334-1335).

Hunter and Arbona state that toxic chemicals and hazards have increased instead of diminishing in the past decades and the success of industrialization has lessened the supply of clean public water. There are several causes of pollution in the water including

raw sewage, solid waste, toxic waste, industrial dumpsites, and uses of fertilizers, pesticides and herbicides. In 1988, 397 tons of toxic wastes were discharged to streams and surface waters (1995: 1352-1353).

Hunter and Arbona also stated that 51% (by length) of rivers are moderately to severely contaminated and 49% are inadvisable for swimming and fishing. These statistics are similar for lakes, lagoons, estuaries and the coastline (1995: 1351)

Sanitation Habits

Another impact on the reinfection of the snails is that the urbanization of Puerto Rico has improved sanitation habits. According Hillyer, it is very uncommon for someone to defecate or urinate in the water and even more rare for that to be in the proximity of *Biomphalaria glabrata* snails (4/25/00 :Interview).

Age-Specific Decrease in Prevalence

Most of the people who are in contact with the waterways are of a young age and Hillyer's recent studies show only one person under the age of 30 that is infected.

Hillyer has noted that the population of Puerto Rico infected with *Schistosoma mansoni* consists mostly of people older than 30 years of age. A 1999 article of Hillyer's states that from 1993 to 1998 between five thousand and six thousand stool samples were processed at the Puerto Rico Medical Center for ova and parasites. Over the course of these years only four people were diagnosed with *Shistosomiasis mansoni*. None were diagnosed with the disease during 1996 to 1998.

Treatment

We believe that because of the apparent decrease in *B. glabrata*'s population the dangers of getting infected have been greatly decreased. Hillyer also states that when someone is found infected he/she is treated, thus lowering the chances of the reinfection among the snails.

Final Conclusion

Based on these factors--absence of *B. glabrata*, an increase in water pollution, better sanitation habits, an age-specific decrease in prevalence, education and medical treatment--the project team believes that the public of Puerto Rico is at a very low risk of further infections with Schistosomiasis. If the public continues to be aware of the areas where *B. glabrata* is located and is aware of the treatment available for infection, the cycle should be completely broken in Puerto Rico within the next few years. This would mean that Schistosomiasis would not be a threat in the future and that the waters would be safe to go into again. It is our hope that Hillyer's future publications and other future studies will support this hypothesis. We also hope that other high prevalence areas of the world will be able to benefit and learn from the history of Schistosomiasis in Puerto Rico. If people in these areas are informed about how to break the cycle and that natural competitors exist for the reduction of the population of host snails, they may be able to apply this knowledge to their situations.

Recommendations

The recommendations of this project are solely the ideas of the project team. These recommendations are based on our conclusions and research. This section describes a proposed project and recommends actions to be taken by the USGS.

While the project team found no *B. glabrata* snails at the sites searched, it is still thought that some are present in other locations. While speaking with Dr. Hillyer, we were informed that Sharon File of the University of Puerto Rico has recently spotted *B. glabrata* snails in streams other than those we have searched. File has also published literature on the incidence of Schistosomiasis in Puerto Rico. While no new infections have been reported, the possibility of infection still exists at some level as long as the host snails are present.

For this reason, we propose that future studies be conducted to monitor the population density of *B. glabrata*, *T. granifera*, and *M. cornaurietis* snails. We have several suggestions on how this should be done based on our experience, as well as on published literature, case studies, and the advice of experts such as Dr. Hillyer in this area of research.

Recommendations for Future Projects

We recommend that a large-scale study be done in Puerto Rico to determine whether Schistosomiasis is still a danger and to further investigate the reasons for the decrease in the *B. glabrata* population. This study should be both qualitative and quantitative in nature to provide a complete account of the matter.

We propose that this study be conducted over a period of approximately five years. During this time, the population densities of the three aforementioned snails would be carefully monitored and studied in a qualitative manner. Correlations would be made between *T. granifera* and *M. Cornuarietis* populations and any *B. glabrata* colonies. We also recommended that project teams examine the water quality data available for the sites where *B. glabrata* is found to determine whether any correlations could be made to prove why the intermediate host seems to be disappearing and whether the snail will eventually become extinct on the island. If water quality data were collected at each site concurrently with observations on the snail populations, conclusions could be drawn regarding pollutants and any change in the snail densities.

The first step in this recommended study would be to target the exact locations where *B. glabrata* snails have been found recently. This could be done with the help of Sharon File, George Hillyer, several hydrologists at the USGS, and other researchers doing work in or around streams. Hillyer mentioned, in our interview, that *B. glabrata* snails are known to be present in one part of the Lago Caonillas and in some streams in the vicinity of San Lorenzo. Also, researchers should be thoroughly trained in detecting the species of snails in question as well as in the field techniques they will be using before research begins. Once suspected areas have been identified, each site should be visited for a pre-study exploration. During this time, researchers should become familiar with the equipment they will be using, as well as with the water and the geography of the land around it that they will be studying. In this manner, project teams could note which streams actually have snails and which type(s) are present.

At each site visited, every researcher should write a detailed description. Details should include date and time of visit, previous and present weather conditions, vegetation present, wildlife, fish, stream depth, approximate length searched, flow conditions of the waterway, type of waterway (i.e. stream, pond, lake), temperature and pH of the water, and a description of the site location so that it can be easily re-visited. Descriptions should then be compiled with photos taken of the location.

Note should be made if only one type of snail is present, but the sites of particular interest are those with all three types or a combination of the competitive snails and the intermediate host since these are the areas where studies of the effects of competition can be made. We recommend that the sites be visited on a monthly basis. During these visits, the number of each type of snail should be recorded. To provide such quantitative data we recommend following a study by Sturrock.

In 1967-1969 R.F. Sturrock conducted a 2-½ year field study on the “Transmission of *Schistosoma mansoni* and on the Bionomics of its intermediate host, *Biomphalaria glabrata*, on St. Lucia, West Indies.” In this extensive study, Sturrock surveyed twenty-seven sites in St. Lucia including ponds, marshes, streams and banana drains. These areas were sampled for *B. glabrata* every two weeks. Different techniques for counting and collecting the snails were used for the different kinds of sites.

For example, Sturrock used a standard scoop to search a fixed area for ponds and streams and a mud-corer technique was used for the banana drains. After the snails were collected they were washed and measured in the laboratory. All snails were returned to their respective habitats within 30 hours of being collected. In order to check the infection rates, Sturrock re-collected snails and performed a qualitative study in the lab.

He used the mouse immersion technique⁷ to determine the amount of cercariae in the water.

The next step would involve determining whether the *Biomphalaria glabrata* snails were infected with human cercariae. Besides the mouse immersion technique, there are several other methods of counting cercariae, which are referred to in the literature review of this report. Based on our experience and the advice of Hillyer, we would suggest that either snail shedding or direct filtration be used and that the researchers also be trained in detecting the human schistosome.

We also recommend that accurate and detailed records of all findings be recorded and compiled into a database. In this way, findings from each visit can be compared and correlations can be made over the five-year period. Water quality data from the sites available could also be correlated with the findings. From these studies, our conclusions can be further explored and the decline in the prevalence of Schistosomiasis could be better explained.

Recommendations for the USGS

We would like to see the USGS provide a brief description of the snails present in the waterways where they collect water quality data. The description would state what snails were present and an estimate of the quantity. We would also like to suggest that members of USGS who are often in the waters of Puerto Rico have parasitology tests done at least once or twice a year.

⁷ Cercariametry techniques are discussed in detail in Chapter 2 Literature Review

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Appendix A: Mission and Organization of the United States Geological Survey

Adapted from: The Effects of Urbanization on the Hydrologic Response to Rainfall. written by Nicholas Amaral, Ravi Misra, Samuel Bullock, and Adam Fairbanks, submitted May 7, 1999 and Sediment from Landslides in Puerto Rico's Water. written by Tim Doherty, Steve Manning, and Chris Seveney, submitted May 7, 1996.

The United States Geological Survey (USGS) was established 1879 by an act of Congress as an agency of the Department of the Interior. The act decreed that the USGS was to provide a permanent federal agency to conduct the systematic and scientific classification of the public lands and examination of the geological structure, mineral resources, and products of national domain. Hence, the USGS was primarily defined as a scientific fact-finding and research organization. It is now the main source of scientific and technical expertise in the earth sciences within the Department of the Interior and the Federal Government.

The headquarters of the USGS is located at Reston, Virginia. The agency employs approximately 8,600 permanent scientific, technical, administrative, and clerical personnel, organized into five sectors. Three of these are major program divisions (Geologic, National Mapping, and Water Resources), and the other two are supportive divisions (Administrative and Information Systems). Each division has its own set of responsibilities to support the overall agency mission.

As described in Houseknecht (1993), the USGS is divided into several divisions:

- The Geological Division provides geologic, geophysical, and geochemical information on land resources, energy and mineral resources, and geologic hazards of the Nation and its territories.

- The National Mapping Division provides geographic and cartographic information, maps, and technical assistance and conducts related research responsive to national needs.
- The Water Resource Division provides information on the occurrence, quantity, quality, distribution, and movement of surface and underground waters that constitute the Nation's water resources.
- The Administrative Division provides finance, personnel, contract negotiation and administration, property and space management, organization and methods, management analysis, and other administrative services to the USGS as a whole.
- The Information Systems Division provides guidance and advice to the Survey and to the Department of the Interior on all matters relating to USGS information technology and automated data processing.

The mission of the USGS, which during its first century focused on surveying the nation's lands and assessing the resources the nation needed to expand, has evolved to include the analysis of the earth's hazards and resources to assure sustained global health and prosperity. The USGS also provides unbiased earth-science information of value to current and future generations. Through collaborative scientific research, information is acquired on the past, present, and future conditions of the earth's environment, hazards, and resources. This essential information is communicated in forms that are effective for users and those concerned with the earth and its assets.

The USGS budget for Federal appropriations is the primary means by which the agency presents its mission and priorities to the Department of the Interior, the Office of Management and Budget, Congress, and agency employees. The budget of the USGS

ideally should represent the mission and priorities of the agency and facilitate activities that contribute to the achievement of mission goals. The goals of the USGS, as stated in Houseknecht (1993) are:

- To enhance understanding of earth systems and their mutual interactions so that knowledge is available to address emerging and future societal needs.
- To evaluate the influence of the earth systems and human activities on the global environment so that scientifically sound decisions can be made to avoid harmful perturbations of the earth's geosphere and biosphere.
- To characterize, assess, and predict chronic and catastrophic hydrologic and geologic hazards (e.g., natural and synthetic pollutants, floods, and earthquakes) for sustained global health and welfare.
- To provide earth-science data information that enhances the availability of sufficient resources (land, water, mineral, and energy) to support the Nation's infrastructure for the prosperity of future generations.
- To develop and implement state-of-the-art earth-science data and information systems (e.g., the National Spatial Data Infrastructure) coordinated through Federal, State, local, academic, and private partnerships for the enhanced availability of spatial and other types of data.
- To educate society on the earth's systems, environment, hazards, and resources so that the earth-science information will be fundamentally intertwined with public policy.

The objective of the USGS in Puerto Rico is to develop programs that are consistent with the vision and mission set by the USGS, attractive to the Department of

the Interior, Office of Management and Budget, the President and Congress, and that meet the needs of the public.

The USGS in Puerto Rico follows the goals and mission stated by national headquarters. The district chief, Rafael W. Rodriguez Cruzado, oversees the daily operation of the agency. Maria M. Irizarry, the Associate District Chief, oversees all operations and research including the Water Energy and Biogeochemical Budget (WEBB) project.

We will be in the Water Resources Division. Joseph W. Troester, our liason, is a Hydrologist within this division and our project coordinator.

The IQP with the USGS is related to the agency's mission, because it researches a danger to USGS hydrologists and a biological element of Puerto Rico's waters. Our study will be used as a reference for further studies and it presents supportive evidence for the decrease of Schistosomiasis in Puerto Rico.

Appendix B: Water Quality Data

Water Quality Data for site 50050300 Quebrada Blasina near Carolina

Date	ANC Water Unfiltered Fet Field (mg/L as CaCO ₃)	Specific Conductance (micromhos/cm)	Dissolved Solids, Sum of Constituents (mg/L)	Discharge, Inst. Cubic Feet per Second
February 19, 1997	200	495		7.6
June 6, 1997	190	625	160	2.7
September 10, 1997	210	505	253	4.2
October 28, 1997	205	520	306	4.2
March 25, 1998	210	461		3.8
June 9, 1998	170	435	236	5.5
September 8, 1998	210	470	277	11
December 16, 1998	221	529		12
March 1, 1999	188	519	311	6.6
June 10, 1999	175	505		21
September 28, 1999	189	340	289	66
December 3, 1999	169	479	224	26

Date	Fecal Streptococci (Cols. Per 100 ML)	Dissolved Calcium (mg/L as Ca)	Dissolved Magnesium (mg/L as Mg)	Dissolved Sodium (mg/L as Na)
February 19, 1997	310000			
June 6, 1997	71000	6.3	3	7.4
September 10, 1997	1600	49	8	29
October 28, 1997	3400	60	9.1	29
March 25, 1998	22000			
June 9, 1998	45000	46	5.2	15
September 8, 1998	700000	44	7.8	26
December 16, 1998	66000	58	10	29
March 1, 1999	23000			
June 10, 1999	28000	56	10	30
September 28, 1999	>1000000	36	5.7	17
December 3, 1999	7900			

Date	Ph Water Whole Field (Standard Units)	Water Temperature	Turbidity (NTU)	Dissolved Oxygen
February 19, 1997	6.5	24.5	2.2	5.9
June 6, 1997	7	28	1.2	3.6
September 10, 1997	7.3	28.5	3.2	5.4
October 28, 1997	7.4	26.5	1.5	6.7
March 25, 1998	7.1	26.5	1.2	3
June 9, 1998	7.2	27.5	2.3	4.6
September 8, 1998	7	28	62	2.8
December 16, 1998	7.1	26.4	10	3.2
March 1, 1999	7.9	24.7	14	6.1
June 10, 1999	7.8	28.4	2	5.8
September 28, 1999	7.8	26	190	5.2
December 3, 1999	7.3	25.5		5.4

Date	Dissolved Potassium (mg/L as K)	Dissolved Chloride (mg/L as Cl)	Organic Nitrogen Total (mg/L as N)	Fecal Coliform .45 UM-MF (cols./100 mL)
February 19, 1997			0.31	49000
June 6, 1997	0.62	9.6	0.45	32000
September 10, 1997	4.5	33	0.5	5500
October 28, 1997	3.3	42	0.2	K6900
March 25, 1998			0.2	K17000
June 9, 1998	0.73	7.8	0.5	24000
September 8, 1998	4.2	35	0.9	600000
December 16, 1998	3.5	39	0.5	2200000
March 1, 1999				46000
June 10, 1999	3	41		22000
September 28, 1999	3.6	22		>600000
December 3, 1999				34000

Water Quality Data for site 50055000 Rio Grande de Loiza at Caguas

Date	ANC Water Unfiltered Fet Field (mg/L as CaCO3)	Specific Conductance (micromhos/cm)	Dissolved Solids, Sum of Constituents (mg/L)	Discharge, Inst. Cubic Feet per Second
March 4, 1997	84	258		103
April 2, 1997				4270
April 2, 1997				4510
April 3, 1997				2850
April 3, 1997				1240
April 3, 1997				75
May 20, 1997	86	272	171	45
June 3, 1997				258
July 21, 1997				1050
July 22, 1997				1100
July 28, 1997				902
August 22, 1997				2210
August 22, 1997				3130
August 25, 1997				1030
August 28, 1997				225
September 4, 1997	85	239	156	95
October 15, 1997	39	152	96	1010
October 17, 1997				1230
November 10, 1997				2200
January 6, 1998				5740
January 7, 1998				2820
February 5, 1998				1500
February 5, 1998				6550
February 5, 1998				2780
February 5, 1998				2660
February 9, 1998				1340
March 7, 1998				3140
March 10, 1998	82	238		161
March 20, 1998				1710
May 11, 1998				78
June 2, 1998	78	271	154	70
August 25, 1998				1990
September 4, 1998	79	202	135	489
September 8, 1998				841
September 21, 1998				20800
December 18, 1998	82	265	165	189
March 18, 1999	103	314		67
May 26, 1999	107	333	207	33
September 30, 1999	75	225	143	157
December 8, 1999	75	215		305

Date	Fecal Streptococci (Cols. Per 100 mL)	Dissolved Calcium (mg/L as Ca)	Dissolved Magnesium (mg/L as Mg)	Dissolved Sodium (mg/L as Na)
March 4, 1997	750			
May 20, 1997	K110	20	6.6	21
September 4, 1997	240	18	5.9	18
October 15, 1997	22000	11	3.9	12
March 10, 1998	2000			
May 11, 1998	45			
June 2, 1998	K170	18	6.2	20
September 4, 1998	49000	14	5.9	17
December 18, 1998	3400	22	8.1	20
March 18, 1999	310			
May 26, 1999	80	26	8.7	28
September 30, 1999	2300	16	6	17
December 8, 1999	2500			

Date	Ph Water Whole Field (Standard Units)	Water Temperature (Degrees Celsius)	Turbidity (NTU)	Dissolved Oxygen
March 4, 1997	6.9	24.5	18	7
May 20, 1997	6.8	28.5	20	6.5
August 28, 1997	6.9	29.5	6.5	6.4
September 4, 1997	6.6	25.5	80	7.4
March 10, 1998	7	25	17	7.2
June 2, 1998	6.8	29.5	14	6.7
September 4, 1998	6.3	27.5	290	6.6
December 18, 1998	7.6	27	17	8
March 18, 1999	8	27.4	120	7.2
May 26, 1999	8	32.4	32	8.2
September 30, 1999	7.7	28	260	6.8
December 8, 1999	7.6	23		8.1

Date	Dissolved Potassium (mg/L as K)	Dissolved Chloride (mg/L as Cl)	Organic Total of Nitrogen (mg/L as N)	Fecal Coliform .45 UM-MF (cols per 100 mL)
March 4, 1997			0.24	9100
May 20, 1997	2.3	21	0.33	560
September 4, 1997	2.3	18	0.28	3900
October 15, 1997	2.1	13	0.41	31000
March 20, 1998				K16000
June 2, 1998	2.1	17	29	600
August 25, 1998				3400
September 4, 1998	2.2	14	178	
September 8, 1998				31000
December 18, 1998	2.1	20	84.2	25000
March 18, 1999				23000
May 26, 1999	2	17	18.2	1300
September 30, 1999	1.9	16	0.32	26000
December 8, 1999				K17000

Water Quality Data for site 50063800 Rio Espiritu Santo near Rio Grande

Date	ANC Water Unfiltered Fet Field (mg/L as CaCO ₃)	Specific Conductance (micromhos/cm)	Dissolved Solids, Sum of Constituents (mg/L)	Discharge, Inst. Cubic Feet per Second
February 19, 1997	34	107		47
June 6, 1997	31	92	236	13
September 10, 1997	20	68	100	27
November 28, 1997	40	115	80	17
March 25, 1998	44	115		24
June 9, 1998	33	89	64	17
August 24, 1998	82	31	66	506
December 16, 1998	39	123	85	66
March 1, 1999	51	142		17
June 14, 1999	38	120	74	17
September 28, 1999	10	47	28	158
December 9, 1999	33	87		88

Date	Ph Water Whole Field (Standard Units)	Water Temperature	Turbidity (NTU)	Dissolved Oxygen
February 19, 1997	6.5	21.3	3.2	8.6
June 6, 1997	6.8	26.5	2.8	7.8
September 10, 1997	6.5	25	2.6	7.7
November 28, 1997	7.4	27	0.4	7.3
March 25, 1998	6.8	24.5	2.1	8.2
June 9, 1998	6.7	26	2	8
August 24, 1998	6.2	24.5	71	8.1
December 16, 1998	7.3	23.5	12	8.2
March 1, 1999	8	25.5	2	7.6
June 14, 1999	7.4	29.7	4.2	7
September 28, 1999	7.1	23.7	25	8.3
December 9, 1999	7.6	23.1		7.7

Date	Fecal Coliform .45 UM-MF (cols per 100 mL)	Fecal Streptococci (Cols. Per 100 mL)	Dissolved Calcium (mg/L as Ca)	Dissolved Magnesium (mg/L as Mg)
February 19, 1997	2600	K1700		
June 6, 1997	330	510	66	11
September 10, 1997	1500	2200	9.5	4.9
November 28, 1997	310	360	8.6	4.3
March 25, 1998	4800	2700		
June 9, 1998	340	310	5.9	3
August 24, 1998	5800	K14000	1.8	0.92
December 16, 1998	20000	4700	11	5.3
March 1, 1999	290	99		
June 14, 1999	400	440	7.7	4
September 28, 1999	390	2500	2.6	1.2
December 9, 1999	2400	750		

Date	Dissolved Sodium (mg/L as Na)	Dissolved Potassium (mg/L as K)	Dissolved Chloride (mg/L as Cl)	Organic Total of Nitrogen (mg/L as N)
February 19, 1997				
June 6, 1997	38	3.5	50	<.20
September 10, 1997	9.6	5.5	15	<.20
November 28, 1997	8.4	0.73	10	0.23
March 25, 1998				
June 9, 1998	7	0.43	8.5	0.41
August 24, 1998	3.2	0.46	4.2	
December 16, 1998	10	0.97	12	1.4
March 1, 1999				
June 14, 1999	8.2	0.65	11	
September 28, 1999	3.9	0.5	5	0.42
December 9, 1999				

Water Quality Data for site 50055250 Rio Caguaitas at Highway 30 at Caguas

Date	ANC Water Unfiltered Fet Field (mg/L as CaCO ₃)	Specific Conductance (micromhos/cm)	Dissolved Solids, Sum of Constituents (mg/L)	Discharge, Inst. Cubic Feet per Second
February 10, 1997	190	499		19
May 16, 1997	160	509	307	14
September 2, 1997	180	514	314	9.8
October 21, 1997	200	563	350	14
February 20, 1998	180	551		10
May 26, 1998	140	432	249	12
June 2, 1998				5.1
August 17, 1998	190	583	357	5.8
September 18, 1998				13
December 15, 1998	164	540	314	42
February 23, 1999	172	616		14
May 25, 1999	164	641	363	9.7
September 29, 1999	140	417	162	24
December 2, 1999	120	318		66

Date	Ph Water Whole Field (Standard Units)	Water Temperature	Turbidity (NTU)	Dissolved Oxygen
February 10, 1997	6.7	22.5	14	4
May 16, 1997	6.8	26.7	72	3.5
September 2, 1997	6.9	27	5.9	3.9
October 21, 1997	7.2	26	5.2	4.2
February 20, 1998	7.1	24	2	4.9
May 26, 1998	7	28	62	3
June 2, 1998				
August 17, 1998	6.8	28.7	4.7	2.8
September 18, 1998				
December 15, 1998	7.1	26.5	4.3	4.8
February 23, 1999	7.8	26.5	10	5.6
May 25, 1999	7.8	35	10	3.8
September 29, 1999	7.9	27.2	0.5	6.8
December 2, 1999	7.7	25.5		6.6

Date	Fecal Coliform .45 UM-MF (cols per 100 mL)	Fecal Streptococci (Cols. Per 100 mL)	Dissolved Calcium (mg/L as Ca)	Dissolved Magnesium (mg/L as Mg)
February 10, 1997	K170000	27000		
May 16, 1997	47000	77000	43	13
September 2, 1997	8100	2900	44	13
October 21, 1997	44000	3000	54	17
February 20, 1998	24000	K1400		
May 26, 1998	58000	42000	35	11
June 2, 1998	30000	2800		
August 17, 1998	35000	K1500	52	16
September 18, 1998	K90000	9300		
December 15, 1998	320000	78000	48	16
February 23, 1999	63000	34000		
May 25, 1999	820000	350000	44	14
September 29, 1999	65000	5500	13	5.2
December 2, 1999	K130000	56000		

Date	Dissolved Sodium (mg/L as Na)	Dissolved Potassium (mg/L as K)	Dissolved Chloride (mg/L as Cl)	Organic Total of Nitrogen (mg/L as N)
February 10, 1997				0.6
May 16, 1997	34	3.4	36	1.5
September 2, 1997	31	3	39	0.71
October 21, 1997	30	3.4	40	0.2
February 20, 1998				0.4
May 26, 1998	30	3.5	32	0.4
June 2, 1998				
August 17, 1998	36	3.5	44	0
September 18, 1998				
December 15, 1998	32	4.2	40	0.7
February 23, 1999				0.6
May 25, 1999	55	4.5	59	1.5
September 29, 1999	14	3.8	14	0.26

Water Quality Data for site 50057025 Rio Gurabo near Gurabo

Date	ANC Water Unfiltered Fet Field (mg/L as CaCO ₃)	Specific Conductance (micromhos/cm)	Dissolved Solids, Sum of Constituents (mg/L)	Discharge, Inst. Cubic Feet per Second
February 20, 1997	160	490		E44
May 16, 1997	100	323	192	29
August 28, 1997	98	287	174	E44
October 15, 1997	56	200	116	
February 18, 1998	130	341		19
May 26, 1998	130	391	236	6.9
August 17, 1998	140	399	240	90
December 21, 1998	140	395	229	
February 22, 1999	149	422		
May 25, 1999	125	388	225	
September 29, 1999	57	179	195	
November 29, 1999	115	338		

Date	Ph Water Whole Field (Standard Units)	Water Temperature	Turbidity (NTU)	Dissolved Oxygen
February 20, 1997	6.6	24.5	2.7	2.8
May 16, 1997	6.5	27	12	3.8
August 28, 1997	6.6	30	17	2.3
October 15, 1997	6.6	26	88	5.7
February 18, 1998	7.1	26	10	4.4
May 26, 1998	7	30	10	5.3
August 17, 1998	6.5	29	25	4.2
December 21, 1998	7.5	27	4	6.4
February 22, 1999	7.5	26.4	8.5	2.3
May 25, 1999	7.6	29.9	15	2.7
September 29, 1999	7.4	26.1	37	4.9
November 29, 1999	7.4	26.5		4.6

Date	Fecal Coliform .45 UM-MF (cols per 100 mL)	Fecal Streptococci (Cols. Per 100 mL)	Dissolved Calcium (mg/L as Ca)	Dissolved Magnesium (mg/L as Mg)
February 20, 1997	41000	280		
May 16, 1997	2000	160	21	9.2
August 28, 1997	2900	510	20	8
October 15, 1997	46000	K14000	14	5.8
February 18, 1998	2000	380		
May 26, 1998	2100	K120	27	13
August 17, 1998	4900	K1900	29	13
December 21, 1998	K1300	K170	31	14
February 22, 1999	420	210		
May 25, 1999	480	40	22	13
September 29, 1999	21000	7700	38	14
November 29, 1999	K14000	5200		

Date	Dissolved Sodium (mg/L as Na)	Dissolved Potassium (mg/L as K)	Dissolved Chloride (mg/L as Cl)	Organic Total of Nitrogen (mg/L as N)
February 20, 1997				0.4
May 16, 1997	25	3.6	27	0.53
August 28, 1997	20	4.5	21	0.72
October 15, 1997	14	3.5	15	0.35
February 18, 1998				0.47
May 26, 1998	32	3.9	35	0.82
August 17, 1998	28	5	31	0.68
December 21, 1998	25	3.4	29	0.3
February 22, 1999				0.46
May 25, 1999	31	4.3	33	1.5
September 29, 1999	21	2.3	25	0.99
November 29, 1999				

Glossary

Bilharzia – See Schistosomiasis

***Biomphalaria glabrata* (*B. glabrata*)** – the intermediate snail host for *S. mansoni* in Puerto Rico

Blood fluke – a parasitic worm that lives in the blood of its hosts

Caecum - The blind pouch at the beginning of the large intestine into which the ileum opens from one side and which is continuous with the colon

Coagulate- To clot

Endemic disease- A disease that is commonly or constantly present in a population, usually at a relatively steady low frequency

Glucose- Sugar found especially in blood, plant, sap, and fruits

Glycogen- a highly branched polysaccharide containing glucose, which is used to store carbon and energy

Hepatic portal vessels- Vessels that carries blood between the intestinal capillaries and the liver

Lumen - the cavity of the intestine

Malacologic survey – surveying and documenting mollusks

***Marisa cornuarietis* (*M. cornuarietis*)** – A predator and competitor of *B. glabrata*. It will eat *B. glabrata*'s eggs and attacks young snails.

Morphology- Study of form and structure

Electrolytes- nonmetallic electric conductor

Serology- The branch of immunology that is concerned with in vitro reactions involving one or more serum constituents (e.g. antibodies & complement)

Schistosomiasis – a parasitic disease caused by a blood fluke. It is usually a danger in tropical regions where the sanitation habits are poor.

***Schistosoma mansoni* (*S. mansoni*)** – a type of blood fluke which causes the disease Schistosomiasis

Schistosomiasis mansoni – the strain of Schistosomiasis caused by *S. mansoni*

Thiara granifera (*T. granifera*) – A competitor snail of *B. glabrata* and a host for *P. westermangi* (a lung fluke)