FRESHWATER SNAIL DIVERSITY IN RELATION TO SCHISTOSOMIASIS IN THE MIDDLE ZAMBEZI BASIN IN LUSAKA PROVINCE

THESIS

BY

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DECLARATION

I, Patricia Mubita, hereby declare that this dissertation represents my own work and that it has not been previously submitted for a degree at this or any other University.

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SIGNATURE

12/09/08

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APPROVAL

This dissertation of PATRICIA MUBITA is approved as fulfilling part of the requirements for the award of the degree of Master of Science in Biosystematics and Ecology by the University of Zambia.

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ABSTRACT

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This study was carried out at the Middle Zambezi basin in Lusaka province to determine freshwater snail diversity, its relationship to the presence of schistosomiasis and the impact of prevailing environmental factors on snail diversity. The study was carried out during the cool-dry season (July), hot-dry season (October) and hot-wet season (February), from July 2006 to February 2007. Twenty 400 metre transects were established on three rivers, ten streams and one reservoir. Snails were sampled every four metres on each transect using a scoop net. Each transect was divided into five 80 metre segments. Environmental data consisting of physical, chemical and biotic data was obtained at the mid point of every segment. Physical data collected consisted of water depth, temperature and velocity. Chemical data collected consisted of conductivity, pH and calcium concentration. Biological data collected was vegetation by type and genera, and phytoplankton diversity.

Fifteen species of freshwater snails were collected from the study area. Pulmonate species consisted of *Bulinus globosus, Bulinus forskalli, Bulinus canescens, Biomphalaria pfeifferi, Segmentorbis angustus, Gyraulus costulatus, Lymnaea natalensis, Physa acuta* and *Ferrissia burnupi burnupi.* The prosobranch species consisted of *Bellamya cappillata, Cleopatra nsendwensis, Gabbiella kisalensis, Gabbiella sp, Lanistes ovum* and *Melanoides tuberculata.* Linear regression was used to determine the relationship between snail diversity (species richness) and the number of infected children at the schools in close proximity to the water sampling points. There was a significant relationship between snail diversity (in the presence of the intermediate snail

host) and schistosomiasis prevalence. Snail diversity explained 62% of the variance in schistosomiasis prevalence. Results of the study demonstrated that in the presence of high species diversity of snails, transmission of *Schistosoma haematobium* is lower.

Multiple regression analysis (Best Sub Set) revealed that water velocity was the most important factor affecting snail diversity in the rivers, while conductivity was the most important factor in the streams. Snail diversity was significantly different among the different vegetation types. All the floating vegetation types were associated with higher snail diversity whereas Phytoplankton diversity was not significantly associated with snail diversity.

DEDICATION

To the memory of my father.

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CHAPTER 1: INTRODUCTION

1.1 Schistosomiasis – background

Schistosomiasis, also known as bilharzia, is endemic to at least 76 tropical and sub-tropical countries. Close to 200 million people are actually infected, while more than 652 million people worldwide are at risk of schistosomiasis (Chitsulo, 2000). In Zambia, two million people are infected with schistosomiasis while three million are at risk of getting the disease. Like other trematodes, schistosomes require a molluscan intermediate host (Miyairi and Suzuki, 1913), in which they undergo part of their development. In Africa, the snail intermediate hosts of schistosomiasis belong to the family Planorbidae in the subclass Pulmonata. Species of *Biomphalaria* serve as hosts for the *Schistosoma mansoni* while species of *Bulinus* are hosts for *Schistosoma haematobium,* (Leiper, 1915). In Zambia the intermediate host for urinary Schistosomiasis is *Bulinus globosus* (Morelet, 1866), while *Biomphalaria pfeifferi* (Krauss 1848) is the intermediate host for intestinal schistosomiasis, (Hira, 1975; Mungomba, 1993).

Schistosomiasis is ranked as second most important human parasitic disease after malaria, (WHO, 2001). Schistosomiasis usually affects people who are often unable to avoid contact with water because of their profession or life styles, such as: fishing or agriculture personnel, or among people living in areas with lack of safe water for drinking, washing and bathing. Due to their low level of resistance and because of their intensive water contact when playing and swimming, children aged between seven and fifteen years are the most heavily infected, (WHO, 2001; Tucker, 1983; Ongom, 1970).

Although mortality rate due to Schistosomiasis infection is low and is mostly due to bladder cancer associated with urinary infection and to bleeding from varicose veins in the oesophagus associated with intestinal infection, the overall Schistosomiasis effect on the people's health and its insidious impact on the economy are enormous. Schistosomiasis can lead to anaemia, stunted growth and development, cognitive impairment (SHN, MOE, 2003) and reduces productivity and fertility in both infected adult males and females (WHO, 2005). The working ability of the people infected by schistosomiasis is severely reduced due to weakness and lethargy caused by the disease. According to the WHO (1965), it is estimated that schistosomiasis reduces productivity by as much as 33%.

1.2 Ecology and life cycle of Schistosomiasis

The ecology of schistosomiasis includes tropical rivers, streams, lakes and other water reservoirs. Transmission takes place when people get in contact with infected water. Intermediate snail hosts release cercariae, which are the infective forms of the parasite. Snail hosts are infected through urination and/or defecation into or near water by an infected person who releases schistosome eggs into the water. In water the eggs excreted hatch to release miracidia which actively swim through the water by means of cilia covering its body. Under optimal conditions, miracidia can survive for about eight to twelve hours, during this time it must find and penetrate the body of a suitable snail in order to develop further. Once inside the snail, the miracidium undergoes asexual reproduction to produce thousands of cercariae stage which comes out of the snail into the water, (Miyairi and Suzuki, 1913). The snail plays an important role in

cercariae stage. However, the life cycle of schistosomes can only be completed in specific species of snail intermediate hosts. The geographic distribution of schistosomiasis is therefore, closely linked with the distribution of specific freshwater snail species that are able to act as intermediate hosts, (Kristensen, 2005).

1.3 Freshwater snails

Freshwater snails are of the phylum Mollusca, class Gastropoda and subclasses Prosobranchiata and Pulmonata. Classification is based primarily on morphological characteristics, of the shell and radula. Anatomical characteristics of the reproductive systems are also used especially when distinction between species cannot be made based on morphological characteristics, (DBL, 1998). In recent years, the DNA approach is being used increasingly in the identification of snail species. The main morphological distinction between prosobranchs and pulmonates is the shell and the operculum. Prosobranchs are generally characterised by a large thick walled shell with an operculum whereas pulmonate snails usually have a smaller thin walled shell without an operculum (Brown and Kristensen, 1998).

1.4 Environmental factors affecting freshwater snails

The factors affecting freshwater snails can be considered in two categories, the abiotic and the biotic factors. The abiotic factors are the non-living part of the habitat. These are the physical and chemical properties of the water body. The parameters that will be considered under the abiotic factors are water temperature, water velocity, depth, calcium

levels, conductivity and pH. Biotic factors that will be studied are phytoplankton and macrophytes.

1.4.1 Abiotic factors

Among the physical properties, temperature is considered to be the most important factor impacting on snail populations in lakes, streams and reservoirs, (Appleton, 1978, Shehata, 1989). Temperature has an effect on the metabolic activity of the snail. Temperature can have both a direct as well as indirect effect on the snails. Directly, the effect is on egg production, hatching and the rate of growth, (DBL, 1989). The indirect effect is through the effect on the rate of photosynthesis and bacterial decomposition of organic matter which has a strong influence on the amount of dissolved oxygen. The amount of oxygen that dissolves in water reduces as temperature increases thereby affecting the snail metabolism.

Water velocity in rivers and streams is also an important factor on snail establishment. The effect of water velocity can be direct by exerting friction on the snail or indirect by determining the nature of the stream bottom or substrate, (Giller and Malmqvist, 1998). As the water velocity increases, friction also increases eventually dislodging the snail and carrying it away. The velocity tolerated varies from one snail species to another depending on the shape of the shell and the size of the foot. The intermediate hosts of schistosomiasis are generally found in water not exceeding $30cm^{-s}$ (Ndifon and Ukoli, 1989). The indirect effect of water velocity is its effect on the substratum of the water body. Fast flowing water does not allow detritus, which forms an important part of the food of some snail species to settle, hence limiting the food availability. The substratum

also affects plant growth. High water velocity can also directly inhibit vegetation which is used by snails for shelter and as egg laying sites.

The preferred depth is mainly decided by the mode of respiration by the snails. Pulmonate snails are generally confined to shallow water though some species can be found at considerable depths in lakes of up to five meters, (DBL, 1998; Utzinger and Tanner, 2000; Genner and Michel, 2003). In deep waters, the pulmonate snails are not able to go to the surface for air and therefore rely on cutaneous respiration. The preference for shallow depths is also related to the presence of aquatic vegetation, which is used for food, protection and as egg laying sites.

The chemical composition of water depends to a large extent on the nature of the soil of the catchment area and the composition of the inflowing water. Certain chemical factors display diurnal fluctuations, (oxygen and carbon dioxide), while some display seasonal fluctuations (conductivity and salinity) due to rainfall and evaporation. Correlation of individual chemical factors with presence or absence of snails is sometimes difficult, (Utzinger *et al.*, 1997; Erko *et al.*, 2006). Instead, the presence or absence of snails could be determined by interaction of environmental factors. In addition to this, the chemical factors in most water bodies do not exceed the tolerance limits for the snails.

Calcium, which makes up the major part of the shell of the snail, is obviously one of the most important chemical factors. The lower limit tolerated however varies according to the different species. Decreasing amounts of calcium are however correlated by a decrease in the number of species, (Williams, 1970). Calcium is usually present in water as calcium carbonate. Apart from the calcium carbonate, there are other dissolved salts in

the water, which might be of importance to snails, (Brown, 1994). The sum of the concentrations of individual ions and compounds dissolved in water determines total salinity whereas, conductivity is the concentration times the charges of individual dissolved ions. Conductivity often shows seasonal fluctuations due to evaporation and rainfall.

The amount of dissolved oxygen in a water body is of importance to snails, especially the prosobranch snails that are able to live at considerable depths and respire through gills. The oxygen content of water is closely linked to the carbon dioxide cycle. Increase in one leads to decrease of the other. The amount of oxygen dissolved in water and the oxygen requirements by the snails is determined by temperature. Higher temperature leads to lower dissolved oxygen while at the same time increasing the oxygen demand by the snails since it increases the rate of metabolism, (Madsen 1985).

1.4.2 Biotic factors

Among the biotic factors determining snail distribution and abundance, food is probably one of the most important. Most snails feed on periphyton and detritus, which comprises of macro detritus (decaying parts of aquatic plants) and micro detritus (finer organic particles). Of the periphyton, algae are the most common diet component. Green algae and diatoms are particularly preferred, (Madsen, 1985). Members of Ampullariidae are the only freshwater snails that feed on fresh plant leaves of aquatic macrophytes. Due to their large size, they are able to make a significant reduction to aquatic plants and have been used in some biological control programmes of aquatic weeds. For the pulmonate snail species, association with aquatic macrophytes is mostly for shelter from predators

and as egg laying sites. Pulmonates are more associated with the aquatic macrophytes than the prosobranchs, (Moyo, 2002).

1.5 Freshwater snail species diversity and schistosomiasis

Snail species diversity can be quantified in a number of ways. The two main factors taken into consideration when measuring snail diversity are species richness and species evenness, (Magurran, 1988). Species richness is a measure of the number of different kinds of species present. However species diversity depends not only on richness but also on evenness. Evenness is a measure of the relative abundance of the different species making up the richness of an area, (Whitaker, 1972).

Schistosomes are only able to develop in particular species of freshwater snails that are able to act as intermediate hosts. Therefore the distribution of schistosomiasis follows the presence of particular freshwater snails in which the schistosomes are able to develop. According to Chernin and Perlstein (1971), in the presence of non-intermediate host snails, the miracidia might not be so discerning and penetrate or attempt to penetrate the non-host snails. If the miracidia penetrate successfully into the non-host snail, they are killed by the internal defense system of the snail. Combes and Mone (1987), proposed that even attempts to penetrate are detrimental to the miracidia since they exhaust their energy reserves and penetration enzymes. The internal defense system of the host snail could also be stimulated by substances adhering to the miracidial surface after contact with non-host snails. This is known as the decoy effect. The number of miracidia that successfully develop into cercariae is therefore, considerably reduced in the presence of non-host snails, leading to reduced infection risk (Babiker *et al.*, 1984). High snail

species diversity may therefore help to keep the prevalence of schistosomiasis low through the aforementioned biological control.

Communities with high snail species diversity tend to have lower average species density. Many species will therefore be below their threshold density preventing one particular species from dominating. Lower snail diversity may lead to intermediate host snails becoming dominant at the expense of competitor and predator snails. According to the principle of competitive exclusion, (Gause, 1934) two species with identical ecological requirements cannot live for a long period in the same environment. A number of cases of what appears to be competitive displacement have been recorded (Pointier et al., 1991; Pointier and Guyard, 1992). The two families that have mostly been used in such exercises have been Thiaridae and Ampullariidae, (Mkoji *et al.*, 1998). Competitor and predator snails help to keep the population of intermediate hosts of schistosomiasis low. Competitor snails regulate the populations by competing for the available resources and space with the intermediate host snails. Predator snails do this mostly by preying on the eggs and neonates of the intermediate host snails.

According to Leps *et al.*, (2001), highly diverse communities are more stable and more resistant to changes. This stability provides a disease regulating effect, if any of the species are involved in the life cycle of an infectious disease and occupy an ecological niche that prevents the proliferation of a species involved in infectious disease transmission, (Patz and Confalonieri, in press). Disease agents with much of their life cycle occurring external to the human host, such as schistosomiasis are subject to environmental conditions. Such diseases are therefore, highly linked to ecosystem

conditions (Patz *et al.*, 2000). The relationship between schistosome parasites, their snail hosts and their ecosystems have co-evolved over a long period of time (Blair and Webster, 2006). The increasing disturbances to aquatic ecosystems by human activities such as dam construction, establishing of irrigation systems and pollution disturb this balance (DBL 1989). On a smaller scale, this includes enrichment of the water through introduction of organic wastes, such as food particles when cleaning utensils and runoff of fertilizers from small gardens near water bodies. Enriched water bodies result in prolific growth of vegetation, which is favourable for the pulmonate snails. Aquatic weeds and organic matter are usually characteristics associated with anthropogenic influences, (Fall *et al.*, 2004). Other activities such as washing clothes and application of pesticides might disturb snail diversity. The pulmonate snails are better able to survive in water with low Biochemical Oxygen Demand (BOD) as a result of pollution by sewage. This is due to their ability to take up oxygen from the air. Such disturbances lead to increased competitiveness of the pulmonate snails over the prosobranch snails.

Snail surveys carried out by Simoonga (2006) in the Middle Zambezi River basin revealed varying levels of freshwater snail diversity in the different water bodies. A total of fifteen species of freshwater snails were sampled from the Middle Zambezi River basin. Sixty eight percent were pulmonates. These were *Biomphalaria pfeifferi, Bulinus canescens, Bulinus forskalli, Bulinus globosus, Ferrisia burnupi, Gyrualus costulatus, Lymnaea natalensis, Physa acuta, Segmentorbis angustus* and one species from the *Tropicus truncatus* group. The prosobranchs species were *Bellamya capillata, Cleopatra nsedwensis, Gabiella kisalensis, Lanistes ovum* and *Melanoides tuberculata.*

Many studies have been carried out on the physical, chemical and biological factors determining distribution and abundance of individual snail species. In Zambia these studies have concentrated on the intermediate hosts of schistosomiasis. No study has been conducted on the factors determining diversity of snail species (species richness) and how this relates to schistosomiasis prevalence in Zambia.

This study makes use of schistosomiasis prevalence data from an earlier study conducted in the same area by Simoonga (2006). The schistosomiasis prevalence data will be used to determine the relationship with snail diversity. Prevalence levels of schistosomiasis ranging from 0-36 percent were recorded from the twenty schools in the area. Snail diversity at the water bodies in close proximity to the twenty schools will be established in order to determine the relationship between snail species richness and schistosomiasis. Some studies have related the increase in schistosomiasis cases in water development projects to the increase in establishment and abundance of intermediate host snails (Diaw, 2002; Hira, 1969). Comprehensive knowledge about the degree of diversity of the freshwater snails is therefore, important for understanding the distribution of schistosomiasis and its control

1.6 Objectives of the study

1.6.1 General objective

1. To determine freshwater snail diversity and its relationship to the prevalence of schistosomiasis, in part of the Middle Zambezi river basin.

1.6.2 Specific objectives

1. To determine snail abundance and diversity in different seasons and sites of the study area.

2. To investigate the influence of some abiotic and biotic parameters on the degree of snail diversity.

1.7 Hypotheses

- In the presence of non intermediate host snails, (high snail species diversity) transmission of schistosomiasis is lower.
- Species diversity of freshwater snails is dependent on physical and chemical parameters, and presence of aquatic macrophytes and phytoplankton.

1.8 Justification of the study

No study has been conducted on the factors determining diversity of snail species and how this relates to schistosomiasis prevalence in Zambia. A comprehensive survey on the degree of diversity of freshwater snails is therefore important for understanding the distribution of schistosomiasis and its control.

CHAPTER 2: LITERATURE REVIEW

2.1 Habitat factors impacting on snail distribution and diversity.

2.1.1 Physical factors

Studies trying to co-relate snail abundance to physical parameters have produced varying results. A review by Appleton (1978) has indicated that in lakes and reservoirs, the most important factor affecting snail establishment and distribution is water temperature. The favourable temperature varies with the species and life stage. Generally most snails can tolerate temperatures ranging from 10 to 36^0C. Sturrock (1966), reported that the optimum water temperature for a rapid population expansion of *B. pfeifferi* was around 25^0C. He also stated that the maximum temperature tolerated by B. pfeifferi was about 32^0C. In a field study on seasonal fluctuation in the population density of *Biomphalaria pfeifferi* and *Bulinus globosus* in Zambia, Shehata (1989) reported that the population of the two snail species is most dense during the cool dry season (July - August) and lowest during the hot-wet season (November – March).

The review by Appleton (1978) also established that water velocity plays a major role in determining snail abundances in streams and rivers. According to Brown (1994), water velocity has an impact in certain stretches of streams and rivers, determining the distribution of snails. The upper limit tolerance range for snail species living in a small stream was 30 cm s^{-1} for *Bulinus globosus, Biomphalaria pfeifferi* and *Melanoides tuberculata* and 320cm s^{-1} for *Burnupia.* Ndifon and Ukoli (1989), also reported the absence of *B. pfeifferi* in water bodies with velocity exceeding 30 cm s^{-1}, optimum velocity was 13 cm s^{-1}. According to Brown and Kristensen (1998), Viviparidae

(*Bellamya capillata*) and Ampullariidae (*Lanistes ovum*) also prefer water bodies with low velocity such as slowly moving streams and rivers and lentic water bodies such as lakes and swamps. In a study conducted by Fall *et al.*, (2004) in the Senegal River delta, various snail species were correlated with the water velocity. Snail density increased down stream as water velocity decreased.

Generally, pulmonate snails are confined to shallow water where they have a positive association with submerged macrophytes (Thomas and Tait, 1984). Ndifon and Ukoli (1989), reported that *B. pfeifferi* preferred depths from two to seven centimeters. Utzinger and Tanner (2000), showed distinct microhabitat preferences by *Biomphalaria pfeifferi* and *Lymnaea natalensis* Krauss, 1848. *B. pfeifferi* preferred shallower water compared to *L. natalensis*. Areas close to the shoreline and substrates with plant detritus were preferred.

2.1.2 Chemical factors

With regard to chemical ecology, snails have a wide range of tolerance (Klump and Chu, 1977). Some studies have reported significant associations with some chemical factors. Alves (1958), attempted to correlate the presence or absence of intermediate host snails with chemical water composition, and observed that snail-free environments tended to have low calcium concentrations. In South Africa, Schutte and Frank (1964) observed that snails were found in 80% of very hard water (calcium concentration over 150mg/l) but in only 30% of the very soft water (calcium concentration 0-10 mg/l).

Williams (1970) confirmed these findings in Zimbabwe where he found that *B. pfeifferi* was restricted to water with a minimum calcium concentration of 5 mg/l Ca^{2+}. According to Nduku and Harrison (1976), in calcium bicarbonate cultures, a concentration of 2 mg /1 Ca^{2+} was shown to be the lower limit for the survival of laboratory populations but a concentration of 4 mg l^{-1} Ca^{2+} was needed for a population to thrive. However, *B. globosus* was found to be more tolerant of low calcium concentrations, both in field and laboratory studies, (Williams, 1970).

McCelland and Jordan (1962) reported the absence of snails in the Bukoba area of West Lake region of Tanzania where low conductivity ranging between 8–19.7 μS cm^{-1} was recorded. Polderman *et al.*, (1985) found *B. pfeifferi* in waters with conductivities as low as 28 µS cm-^{1}, but noticed its absence where conductivities were only 10-12 µS cm-^{1}. Schutte and Frank (1964), found a correlation between calcium concentration and conductivity, with the hardest waters having high conductivities. The concentration of calcium relative to other ions has also been investigated as a possible influence on snail distribution.

According to WHO (1957), snails have been found in natural water bodies where the pH ranges from as low as 4.8 to as high as 9.8. However, a pH of 7-8 has been reported to be the most favorable for snails.

In a study conducted by Canete *et al.*, (2004), nitrite concentration was found to have opposing effects on the Lymneids, *Pseudosuccinea columella* and *Fossaria cubensis.* The effect was negative on *Pseudosuccinea columella* but positive on *Fossaria cubensis.* According to Giovanelli *et al.*, (2005), Chloride concentration and fecal coliforms were

most important factors explaining abundance of *M. tuberculata*, while alkalinity and
an velocity was correlated with abundance of *Physa marmorata* in lotic environments.

.3 Biotic factors

monate snails generally have a greater affinity for shallow water and vegetation while
osobranch snails are able to live in deeper water and are generally found on the bottom
the water body not attached to vegetation (Moyo, 2002). In a study conducted by Fall
al., (2004), *Biomphalaria sudanica* of Lake Ziway was found to show preference for
me aquatic weeds such as *Ceratophyllum demersum*, *Nymphaea* species, *Ludwigia*
yssinica and *Potamogeton* species. According to Thomas (1987), the association of
ails with certain aquatic plants may indicate the deliberate selection of microhabitats
ith higher oxygen tension, lower temperatures and more neutral pH than recorded in the
abitats as a whole. Aquatic macrophytes also reduce the flow of the water and facilitate
e sedimentation process (Fall *et al.*, 2004). Snails also equally require plants both as
urce of food and resting substrata.

ails are mostly primary consumers. Madsen (1992) observed a great similarity in the
oice of food by pulmonates. The food consisted of fine detritus, epiphytic algae and
caying macrophytes. No fragments of fresh macrophytes were found and animal
mains were rare. The Thiarids had a similar choice of food except for one site where
leopatra displayed a preference for green algae. The Ampullariid species are also able
feed on the food preferred by the Thiarids and Pulmonates. Their principal diet

components however are macrophytes. A study by Pointier and Guyard (1992), established that the food for snails consists of algae, diatoms and detritus.

2.2 Snail diversity and schistosomiasis

2.2.1 Miracidia interception, the Decoy effect

High diversity of freshwater snails has been shown to reduce the infection rate of host snails (Chernin and Perlstein, 1971). In a laboratory study Laracuente *et al.* (1979), looked at various species of aquatic snails which were used as decoys to intercept schistosome miracidia. Thus disturbing the miracidia from reaching the snail which normally serve as their intermediate hosts. Presence of five decoy snails for each target snail caused a reduction in experimental infection levels from 90% to 25%. Increasing the ratio to ten decoy snails per target snail further reduced the infection levels to 1%. Laracuente *et al.*, (1979) also conducted field trials in two ponds in which *Marisa cornuarietis* reduced infections at a ratio of six decoys to one target snail. Yousif *et al.*, (1999), recorded significant reduction in infection rate of *Biomphalaria alexandrina* in the presence of non-target snails. The degree of reduction depended on the level of mixing between the target and non-target snails. A higher reducing effect was noted with *Planorbis planorbis* than *Melanoides tuberculata.* Sapp and Loker (2000), showed that plasma of incompatible snail species reduced the viability of both sporocysts and rediae that manage to penetrate the non-host snail.

2.2.2 Competition and predation

In addition to reducing infection rates, high diversity of freshwater snails ensures presence of snail species acting as predators and or competitors (Cowie, 2001). Various freshwater snails have been investigated in attempts to find out their ability to control the snail vectors of schistosomes. The mechanism involved is out-competing them and or preying on them (especially on eggs and juveniles). These introductions have been predominantly Ampullariidae (*Pomacea glauca* (Linnaeus), and especially *Marisa cornuarietis* and Thiaridae (*M. tuberculata* and to a lesser extent *Tarebia granifera* (Lamarck).

The Ampullarids incorporate both predation and competition. Mkoji *et al.*. (1998) examined the ability of the ampullariid snail *Pila ovata* (Oliver, 1804) to control laboratory populations of *B. pfeifferi*. The results of the experiments showed that adult *P. ovata* attacked egg masses and neonates of up to 2.5 mm shell diameter of *Biomphalaria pfeifferi* but did not have any effect on the adults. Jobin *et al.*, (1977) established that *Marisa cornuarietis* is able to feed on the eggs and young of *B. pfeifferi*. In addition to this it also feeds on the aquatic macrophytes, which the pulmonate snail prefers for shelter and egg laying.

Exploitation competition has been suggested as the mechanism by which Thiarids compete with pulmonate snails. This is by feeding on the same food types as the pulmonate snails. The food consists of algae, diatoms and detritus (Pointier and Guyard, 1992). A study conducted by Okere and Odaibo (2005) showed that *Indoplanorbis exustus* (Deshayes, 1834) had a significant impact on the growth rates and fecundity of

B. pfeifferi. The exact mechanism of this reaction has been proposed to be beyond just competition for food. Proposed mechanisms include removal of essential ions needed by *B. pfeiferri* and secretion of growth limiting factors by *I. exustus*. Competitive interaction between *M. tuberculata* and *Biomphalaria* has been an important factor in the success of biological control programs in the French Antilles. The same was also reported in Brazil in the state of Minas Gerias. *M. tuberculata* was able to exclude populations of *Biomphalaria glabrata* (Say, 1818) and *Biomphalaria straminea* (Dunker, 1848) after its introduction in a lake. The competitive interaction that exists between *M. tuberculata* and the pulmonate species is related to the different life strategies employed by these species. *M. tuberculata* is a K strategist and therefore has low reproduction rates, low mortality rates and a long life span, (Pointier *et al.*, 1991). In permanent and stable habitats *M. tuberculata* would therefore have a competitive advantage over the pulmonate species, (Pointier and Guyard, 1992).

A number of studies on the other hand have shown that the Thiarid *M. tuberculata* is able to co exist with the intermediate host snails of schistosomiasis. According to Mkoji *et al.*, (1998) no negative influence of *M. tuberculata* on *Biomphalaria* was found in Kenya. Similarly, Giovanelli *et al.*, (2005) found no evidence of competition between *M. tuberculata* and a number of freshwater snails, which included *Biomphalaria.* This was attributed to the populations of both species being kept at low densities and their ability to utilize different microhabitats within the same water body.

2.2.3 Disturbance of freshwater snail habitats

Prior to the creation of Lake Kariba on the Zambezi River, schistosomiasis incidence was very low while the intermediate host snails were not present in the area. A few years after the creation of the lake, both *B. globosus* and *B. pfeifferi* were found in habitats along the shoreline of Lake Kariba (Hira, 1969). Disruption of aquatic ecosystems due to human activities may result in conditions more favourable for pulmonate snails than prosobranch snails (Lange, 2002). The reduction in snail diversity leads to increased numbers of the pulmonate snails, which include the intermediate host snails for schistosomiasis. According to Diaw, (2002), a high increase in Schistosomiasis cases was recorded in Senegal following damming on the River Senegal which affected the flow regime of the water in the river. This was due to prevention of intrusion of sea water into the Senegal River resulting in the regularization of the level of water and the pH of the water becoming more alkaline, 7-8. This combination provided for a more favourable environment for snails leading to rapid proliferation of *B. pfeifferi* and *Lymnaea natalensis Krauss, 1848. Bulinus globosus* was also reported to have colonized more sites and with higher densities.

Genner and Michel (2003), revealed that increased levels of sediment input on Lake Malawi have been connected to increased densities of schistosomiasis intermediate hosts. Higher rates of erosion in the lake's catchment due to agricultural practices have led to the increased sediment input. This results in increased habitat that is favourable for the schistosome intermediate hosts.

CHAPTER 3: MATERIALS AND METHODS

3.1 Study area

This study was conducted in the Middle Zambezi river basin in Lusaka province, (figure 3.1) lying between latitudes 15.09^0S -16.07^0S and longitudes 28.22^0E – 30.41^0E. The study area consists of three catchment areas that drain into the Middle Zambezi basin: Lower Kafue, Chiawa and Lower Luangwa. The lower Kafue catchment area consisted of the Kafue River and its tributaries (Muchuto, Chilileka, Hachipilika, Siamikobo and Moobe streams) and Nanduba reserviour. The lower Luangwa catchment area consisted of the Luangwa River and its tributaries, (Kaulungu, which supports irrigation canals, Mwalilia, Rufunsa, Kaunga and Mphuka streams) The Chiawa catchment area consisted of the Zambezi River and its tributary, (Kabwadu stream).

3.2 Climate

In general, Zambia has a sub-tropical type of climate with three distinct seasons; the hot-wet, cool-dry and hot-dry. The hot-wet season (November to April), the cool-dry season (May to August) and the hot-dry season (September and October), (Archer, 1971). The country generally experiences four to eight dry months per year. The temperature ranges from 2.5^0C in the cool dry season to 37^0C in the hot dry season. The relative humidity ranges from about 75 to 85% in the hot wet season. The rainfall ranges from about 700mm in the south to 2000 mm in the north. The study area receives 700-800mm of rainfall annually.

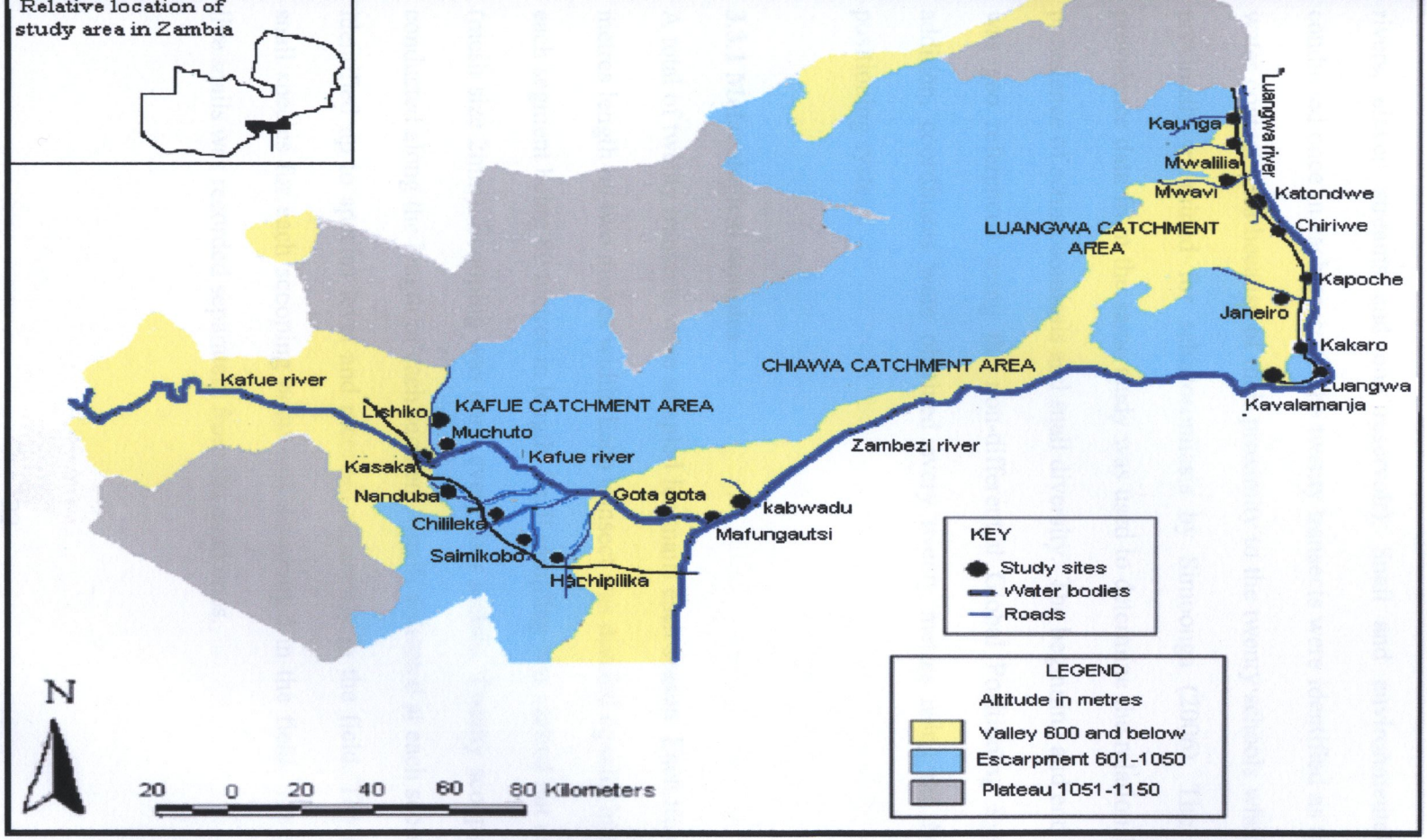


Figure 3.1. Map of the study area in the Middle Zambezi River basin in Lusaka province

3.3 Study design

A longitudinal study was carried out during the above-mentioned seasons in 2006 and 2007. Twenty transects, were established along the above mentioned water bodies (three rivers, eleven streams and one reservoir). Snail and environmental surveys were conducted once in each season. The twenty transects were identified as the major human water contact sites because of their proximity to the twenty schools where children were previously examined for schistosomiasis by Simoonga (2006). The schistosomiasis prevalence data from the same study was used to determine the relationship between the prevalence of schistosomiasis and snail diversity. The beginning and end of each transect was geo referenced using the non-differential Global Positioning System (GPS). In addition, coordinates were obtained every twenty metres using the Magellan Global positioning system.

3.3.1 Malacological samples

A total of twenty transects were sampled for snails each season. Each transect was of 400 metres length by two metres width. Each transect was divided equally into five segments, each segment being 80 metres in length. Snail sampling was carried out using a scoop net (mesh size 2mm). Scooping was done every four metres. Twenty scoops were therefore conducted along the length of each segment. Snails collected at each scooping point were identified up to species level and recorded separately in the field. The number of each snail species for each scooping point was determined in the field. The total number of live snails was recorded separately from the dead ones.

A sample of each snail species collected on each transect were preserved in 70% ethanol for further identification in the laboratory at University of Zambia using keys prepared by Danish Bilharziasis laboratory (Brown and Kristensen 1998). Confirmation of snail identification was performed by the Danish Bilharziasis laboratory at the Institute for Health Research and Development, Charlottenlund, Denmark (personal communication). Identification of snails that were not present in the keys was done by DBL – IHRD using DNA analysis.

3.3.2 Determination of environmental variables

Environmental parameters were determined from July 2006 to February 2007. Each 400 metres transect was divided into five segments, 80 metres in length by two metres width. Environmental parameters were taken on the mid point of each segment (Lloyd *et al.*, 2005). Two by two metres quadrants were established at the midpoint of each segment. Each quadrant was geo referenced using the hand held non-differential GPS. Selected physical and chemical parameters were measured by the same workers once during each season.

3.3.2.1 Abiotic parameters

The selected physical parameters that were measured are water depth, water temperature and water velocity. Water temperature at a depth of five centimeters was determined using a thermometer. Water depth was determined using a calibrated pole (Utzinger and Tanner, 2000). Water velocity was determined using a current meter.

The chosen chemical parameters that were measured are conductivity, calcium concentration, and pH. Conductivity and pH were determined in the field by portable water testers. Conductivity was and pH were determined using a the Whatmans portable water tester. Analysis of the water samples for calcium was done using the EDTA titration method, (Alpha, 1992).

3.3.2.2 Biotic parameters

The major vegetation within the quadrant was identified by type. The categories were emergent, floating and submerged. The major aquatic plants within the quadrant were identified up to genus level.

Phytoplankton was collected at the centre of each quadrant with a plankton net. Water samples for plankton identification were preserved using Lugols solution. Identification of Phytoplankton was conducted in the laboratory at the University of Zambia.

3.3.3 Use of schistosomiasis prevalence data

Secondary prevalence data on *Schistosoma haematobium* recorded by Simoonga (2006) in the same study area was used in this study to determine the relationship between the prevalence of urinary bilharziasis with snail diversity.

3.4 Data entry and analysis

The data collected was entered in spreadsheets using Microsoft Excel, 2001.

3.4.1 Relationship between snail diversity and schistosomiasis transmission

Total snail species on each transect was the summation of all the species from the 100 sampling points. Snail diversity for each survey site was quantified using Shannon-Weiner and Simpson's diversity indices in PC-ord computer program. Differences in snail diversity and snail species richness between seasons were determined using One-Way ANOVA in the StatistiX computer program. Bonferroni means comparison test was used to compare the means. To determine the relationship between schistosomiasis prevalence and snail species richness, the *Schistosoma haematobium* prevalence data from the twenty schools was transformed using angular transformation. The prevalence data, which was in proportion form, was therefore transformed to normally distributed data. The relationship between the transformed prevalence data and snail species richness was then determined using Regression analysis in StatistiX computer program. Significance was determined at $P \leq 0.05$.

3.4.2 Environmental variables influencing freshwater snail diversity

StatistiX computer program was used to determine the environmental variables influencing freshwater snail diversity. The average value for each of the abiotic factors from the five sections on each survey site was computed together with the standard error. Differences between survey sites and seasons was determined using One-Way Anova. Best Subset Regression was then used to determine the effect of interactions of physical and chemical factors on snail diversity.

One-Way ANOVA (Analysis of Variance) was used to determine if there were significant differences in snail diversity, Pulmonate and Prosobranch snail diversity among the different aquatic vegetation types. Comparison of means was carried out using Bonferroni means comparison test. Association between snail diversity and phytoplankton diversity was determined using Chi-square test of association.

Significance for all the statistical analyses was interpreted at 0.05.

CHAPTER 4: RESULTS

4.1 Snail species diversity and abundance

The snails sampled belonged to eight families, twelve genera and fifteen species (Figure 4.1.1). The number of families belonging to Order Prosobranchia was equal to the number belonging to Pulmonata. Pulmonata was however more diverse at genus level. The study area had seven genera belonging to Pulmonata while five genera belonged to Prosobranchia. The most diverse family was Planorbidae, which has two sub-families: Planorbinae and Bulininae. Family Planorbinae was more diverse (three genera), than Family Bulininae with only one genus, *Bulinus*. *Bulinus* was the most diverse genus with three species. Two species each were recorded belonging to families Thiaridae and Bithynidae. Families Ampullariidae, Viviparidae, Ancylidae, Lymneidae and Physidae were represented by a single species each.

Pulmonate and prosobranch snails made up 62.5% and 37.5%, respectively, of the total snails sampled in the study area. The prosobranchs were mainly confined to the big rivers, such as the Zambezi, Kafue and Luangwa. Out of the 11 streams and one reservoir sampled, only three streams (Kabwadu, Mwalilia and Muchuto) supported prosobranchs (Table 4.1.1). This includes the Kabwadu stream, a tributary of the Zambezi that receives a backflow from the Zambezi River when the water level rises in the Zambezi River. The snail species found in the Kabwadu stream could therefore be assumed to originate from the Zambezi River. Total snail species diversity on the stream transects was equal to the river transects (13). The stream transects however had a marginally higher proportion of pulmonates (69%) than the rivers (62%). Average snail species diversity on the stream

transects was lower (three) than the river transects (five) whereas the average number of snails on the stream transects was much higher (228) than on the river transects (85).

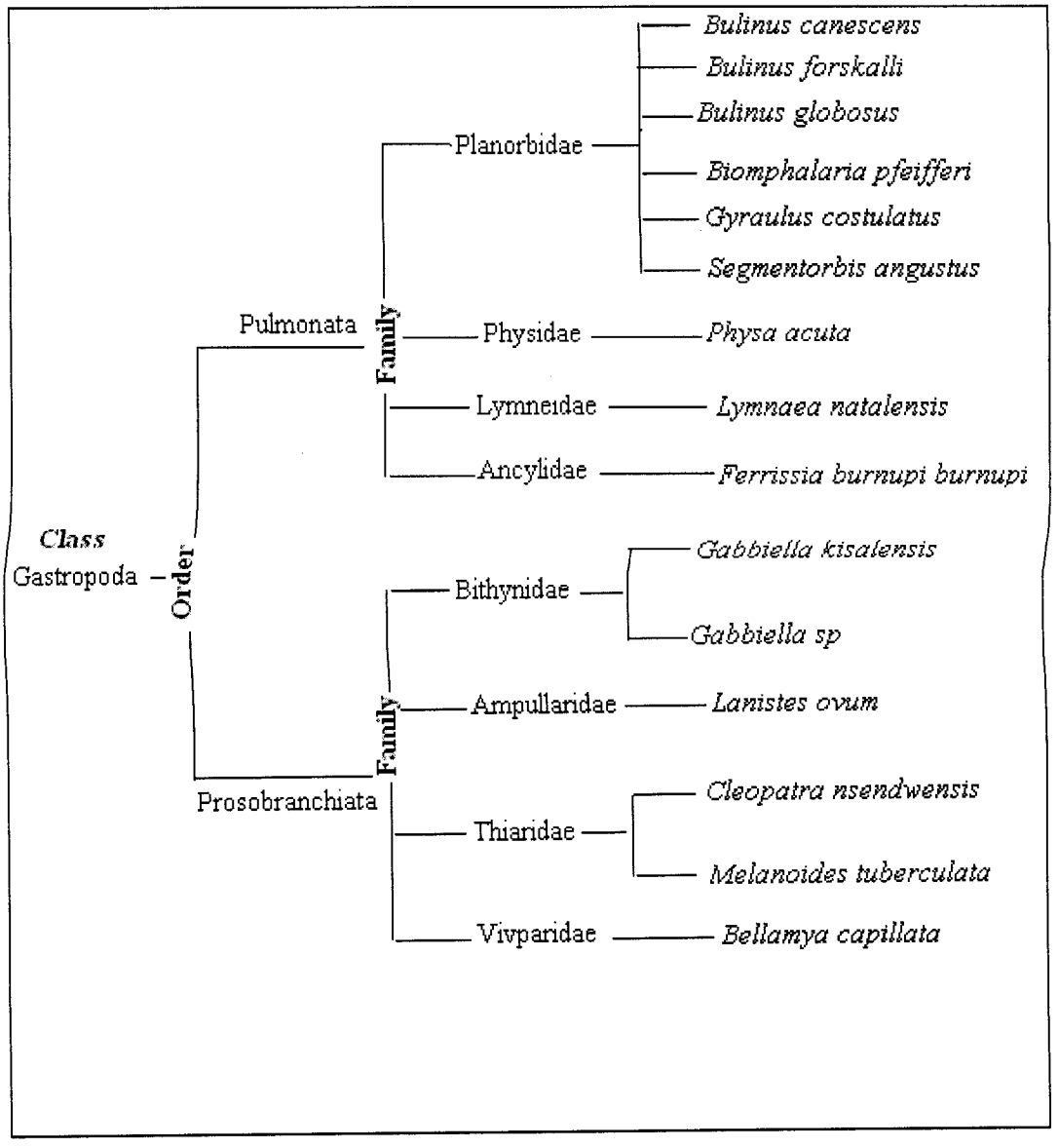


Figure 4.1.1 Taxonomic arrangement of freshwater snails recorded on survey transects during 2006 and 2007.

Table 4.1.1 Number of snails collected at the twenty water bodies in the Middle Zambezi River basin during 2008-2009

Transect	Pulmonate snails									Prosobranch snails						
	Intermediate hosts		Non intermediate hosts							Non intermediate hosts						
Streams	*Biomphalaria pfeifferi*	*Bulinus globosus*	*Bulinus canescens*	*Bulinus forskali*	*Ferrisia burnupi*	*Gyraulus costulatus*	*Lymnaea natalensis*	*Physa acuta*	*Segmentorbis angustus*	*Bellamya capillata*	*Cleopatra nsendwensis*	*Gabbiella kisalensis*	*Gabbiella sp*	*Lanistes ovum*	*Melanoides tuberculata*	Total # of snails
Mwalilia	50	184	-	60	-	89	10	-	-	-	-	627	-	2	-	1022
Mwavi	239	11	-	-	-	4	21	-	379	-	-	-	-	-	-	654
Chilileka	-	1	1	-	222	-	-	-	-	-	-	-	-	-	-	224
Katondwe	19	126	-	6	50	-	10	-	-	-	-	-	-	-	-	211
Hachipilika	-	-	47	39	58	7	-	-	-	-	-	-	-	-	-	151
Muchuto	-	24	-	-	70	-	-	-	-	-	-	-	-	-	38	132
Lishiko	-	78	-	6	-	-	-	-	-	-	-	-	-	-	-	84
Kabwadu	-	3	-	4	-	-	3	37	-	-	10	-	-	-	-	57
Siamikobo	-	-	3	-	-	-	-	-	-	-	-	-	-	-	-	3
Janeiro	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Kaunga	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Dam																
Nanduba	-	194	-	-	-	-	-	-	-	-	-	-	-	-	-	194
Rivers																
Kakaro	-	-	-	-	197	-	-	-	-	-	-	-	-	-	4	201
Kasaka	1	2	-	-	34	-	124	-	1	9	-	-	-	3	-	174
Luangwa	-	5	-	-	5	-	37	47	1	-	11	-	-	5	-	111
Gota Gota	-	-	-	-	3	1	56	-	1	-	-	-	-	1	28	90
Kavalamanja	-	1	-	7	-	2	9	12	5	-	18	-	2	9	3	68
Mafungausti	4	-	-	-	1	7	18	-	-	-	7	-	-	1	-	38
Chiriwe	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Kapoche	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
TOTAL	**313**	**629**	**51**	**122**	**640**	**110**	**278**	**96**	**387**	**9**	**46**	**627**	**2**	**21**	**73**	**3282**

The two snail species that act as Schistosomiaisis intermediate hosts were both present in the study area. *Bulinus globosus,* the intermediate host for urinary schistosomiasis was more widely distributed than *Biomphalaria pfeifferi* the intermediate host for intestinal schistosomiasis. *B. globosus* was also the most widely distributed and was present at 11 survey sites followed by *Ferrisia burnupu burnupi* (10 sites) and *Lymnaea natalensis* (9 sites). The most abundant snail species in the study area was *F. burnupu burnupi* (640) followed by *B. globosus* (629) and *Gabiella kisalensis* (627). 13 non-host snails were found in the study area, (Table 4.1.1).

4.1.1 Variation of snail diversity in different seasons and sites

Host and non-host snails were present throughout the study period at 11 out the 20 survey sites (Figure 4.1.1). Highest snail diversity (10 snail species) was found in the Chiawa catchment area followed by Luangwa, then Kafue catchment areas with seven and six snail species respectively. Snails were found at all the Chiawa and Kafue catchment sites, while four sites in the Luangwa catchment area (Janeiro, Kaunga, Chiriwe and Kapoche) did not have any snails throughout the study period. Snail diversity at the river sites was higher than the stream sites in the Chiawa and Kafue catchment areas.

Snail species diversity per survey site ranged from 0-10. There were significant differences in snail species diversity among the survey sites ($F = 4.3$, $p = 0.0001$) although there were no pair wise differences between the means. The Shannon-Weiner (H) and Simpson's (D) diversity indices were similarly significantly different across survey sites. The mean of the diversity indices on survey sites that had snails (1.4 ± 0.63), (0.6 ± 0.2) were significantly higher than on sites with no snails ($F = 26.8$, $p = 0.0001$), ($F = 42.2$, $p = 0.0001$) respectively.

Seasonal variation in snail species diversity was not significant. In contrast, there were significant differences in the diversity indices across the seasons. The mean Shannon-Weiner (H) and Simpson's (D) indices were significantly higher during the hot-dry and cool-dry seasons (1.7 ± 0.3), (0.7 ± 0.1) than the hot-wet season (0.9 ± 0.3), ($F = 4.23$, $p = 0.02$), (0.4 ± 0.1), ($F = 5.81$, $p = 0.006$) respectively.

There were no significant differences in both snail species richness and the diversity indices among the catchments. The variance within the catchments was much higher than among the catchments for both snail species richness and the diversity indices.

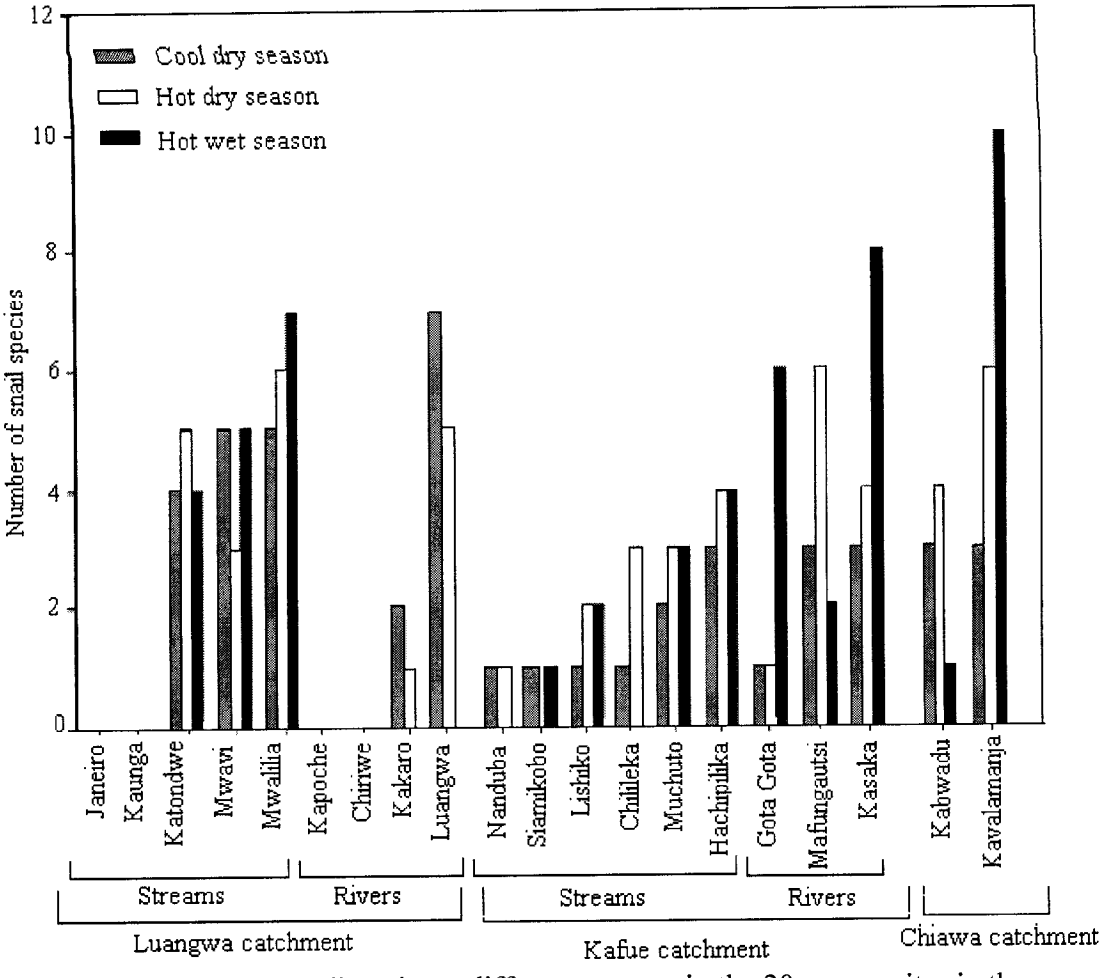


Figure 4.1.2 Snail species diversity at different seasons in the 20 survey sites in the Middle Zambezi River basin in Zambia.

Disaggregating the data according to water body type (streams and rivers) shows that snail species richness on each river transect was characterised by large seasonal fluctuations (Figure 4.1.3) compared to the stream transects (Figure 4.1.4). The variation in snail species richness and the diversity indices on the river transects were however not statistically significant across the seasons. There were significant differences in species richness ($F = 2.66$, $p = 0.05$) but not the diversity indices across the river transects.

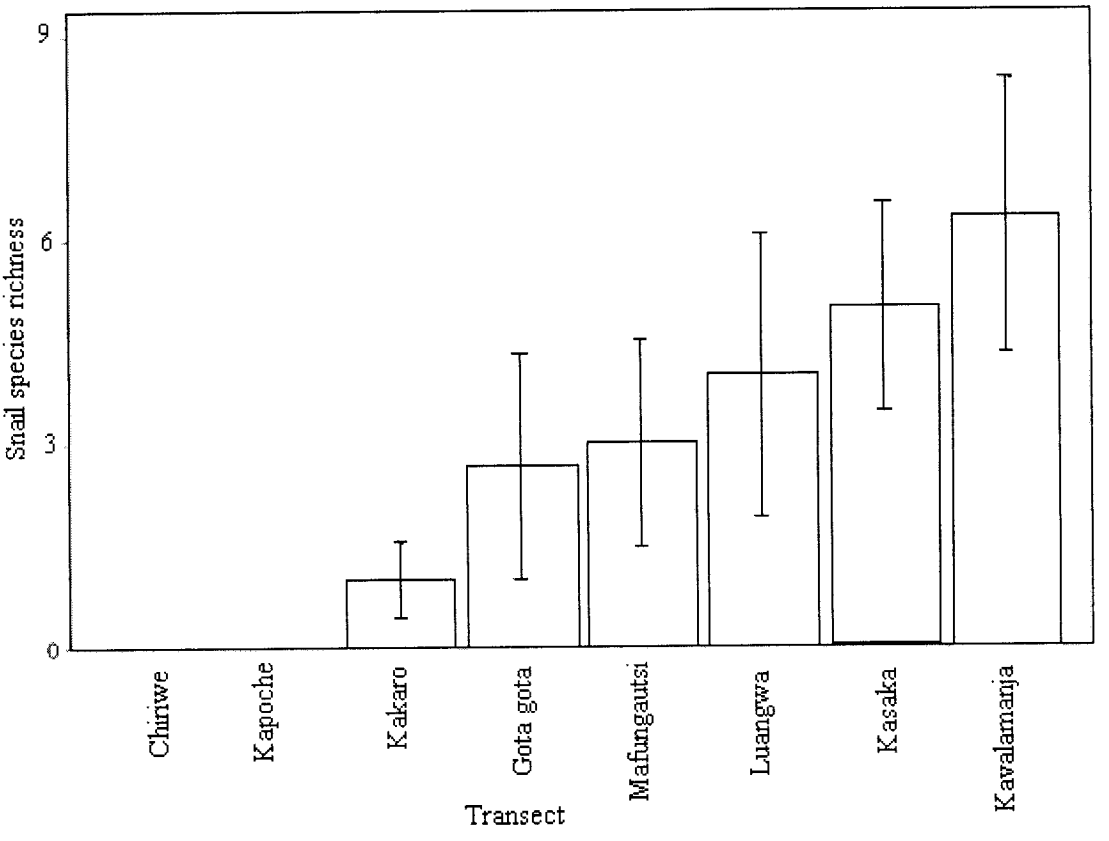


Figure 4.1.3 Mean snail species richness on the river transects during 2006-2007 (bars represent standard error (± SE)

Snail species richness on the stream transects ranged from 0-8. There were significant differences in snail species richness among the stream transects ($F = 14.33$, $p = 0.0001$). There were similarly significant differences in the diversity indices across the stream transects. The Shannon-Weiner (H) and Simpson's (D) diversity indices were higher on sites with snails (1.5 ± 0.6), (0.6 ± 0.2) than those without snails ($F = 14.6$, $p = 0.005$), ($F = 25.5$, $p = 0.0001$) respectively. However, there were no significant differences in both snail species richness and the diversity indices across the seasons.

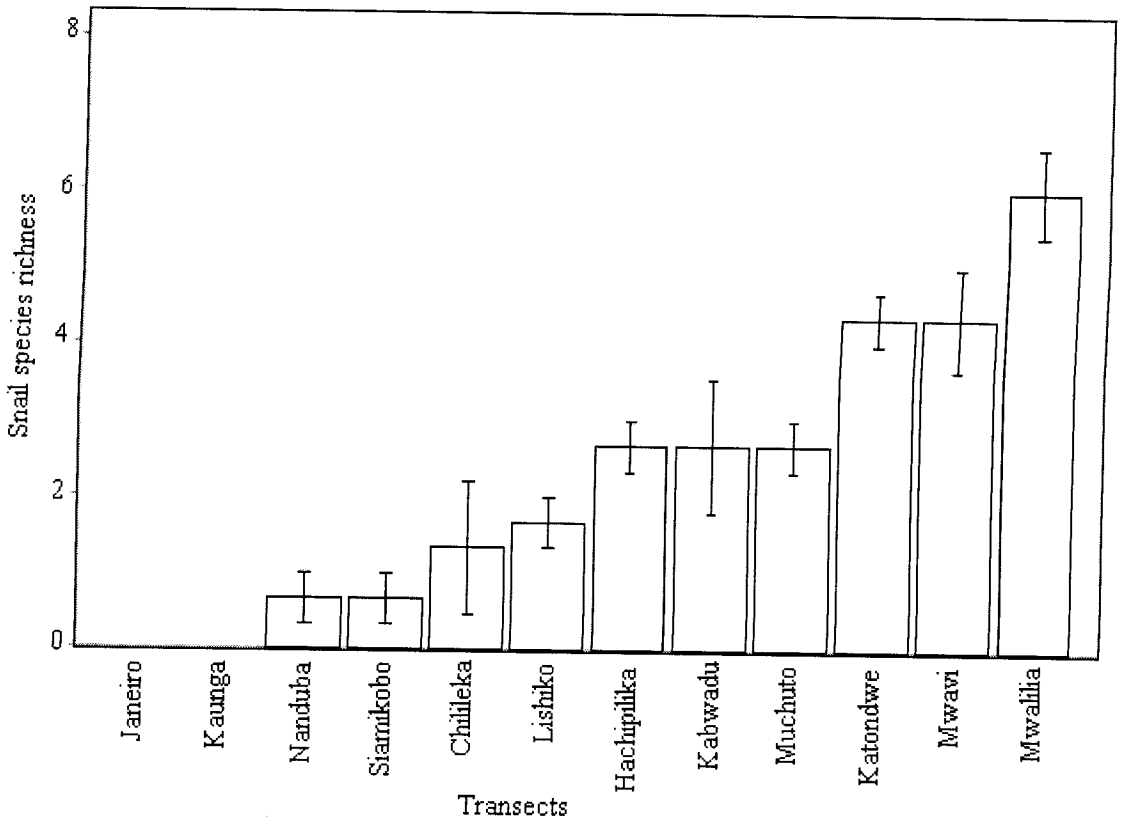


Figure 4.1.4 Mean snail species richness on the stream transects during 2006-2007 (bars represent standard error (± SE)

4.2 Habitat characteristics at snail survey sites

4.2.1 Physical factors

The results of physical parameters of water from snail habitats are summarized in Table 4.2.1.

4.2.1.1 Water velocity

Water velocity ranged from 0.88cm^{-s} to 58.06cm^{-s} over the study period. Water velocity was highest during the hot wet season due to the rain. Mean water velocity during the hot-wet season (22.4cm^{-s}) was higher than during the cool-dry and hot-dry seasons (11.9cm^{-s}) ($F = 3.72$, $p = 0.03$).

Table 4.2.1 Seasonal mean (±SE) values for water depth, velocity and temperature at snail survey sites in the Middle Zambezi River basin.

	Hot wet season			Cool dry season			Hot dry season		
Transect	Depth (cm)	Velocity (cm^{-s})	Temperature (^0C)	Depth (cm)	Velocity(cm^{-s})	Temperature (^0C)	Depth (cm)	Velocity (cm^{-s})	Temperature (^0C)
Luangwa catchment									
Janeiro(s)	20.8 ±1.7	43.65 ±1.4	36.14 ±0.28	-*	-	-	-	-	-
Katondwe(s)	19.8 ±1.6	12.61 ±4.1	27.7 ±0.07	21.44 ±4.9	13.86 ±8.5	22.4 ±0.4	18 ±4.6	8.35 ±5.1	30.22 ±0.11
Kaunga(s)	17.4 ±2.7	39.51 ±1.6	38.64 ±0.20	55.28 ±9.5	0	22.5 ±1.66	-	-	-*
Mwalilia(s)	26 ±7.5	6.92 ±4.2	30.88 ±0.68	24.98 ±5.9	12.2 ±3.2	17.6 ±0.2	20.82 ±5.6	5.23 ±1.6	31.18 ±0.24
Mwavi(s)	27.2 ±6.9	15.82 ±4.0	25.6 ±0.4	49.08 ±5.4	22.45 ±6.6	25.7 ±1.04	22.4 ±1.4	9.15 ±5.8	26.3 ±0.15
Chiriwe(r)	97.2 ±2.7	27.59 ±0.98	32.66 ±0.32	28.38 ±5.1	16.97 ±10.4	22.2 ±0.2	26.1 ±4.9	15.85 ±9.7	32.94 ±0.27
Kakaro(r)	67.6 ±1.3	39.88 ±3.1	28.6 ±0.24	10.34 ±1.9	24.89 ±1.9	24.6 ±0.4	7.9 ±1.2	22.58 ±1.5	29.88 ±0.22
Kapoche(r)	97.6 ±14.5	44.24 ±11.0	33.04 ±0.48	44.7 ±7.8	42.05 ±7.1	24 ±0.5	49.84 ±1.8	18.03 ±1.2	28.7 ±0.3
Luangwa(r)	82.2 ±18	0	30 ±0	54.16 ±3.2	0	21.6 ±0.24	48.98 ±4.0	0	30.86 ±0.1
Kafue catchment									
Chilileka(s)	29.8 ±7.5	22.97 ±11.0	30.3 ±0.64	22.26 ±10.1	15.66 ±3.9	21.7 ±0.44	19.24 ±8.9	4 ±1.8	30.8 ±0.49
Hachipilika(s)	15.2 ±1.1	58.06 ±12.5	33.8 ±0.22	28.38 ±1.8	30.65 ±5.0	22 ±0.16	17.08 ±3.4	8.95 ±1.2	27.25 ±0.75
Lishiko(s)	47.6 ±13.8	20.11 ±12.3	28.2 ±0.12	43.4 ±13.7	16.5 ±10.1	20.3 ±0.2	15.7 ±2.1	0	28.1 ±0.15
Muchuto(s)	25.4 ±5.1	32.95 ±8.3	28.1 ±0.29	15.04 ±3.8	27.23 ±4.3	21.1 ±0.93	24.45 ±6.2	0	31.25 ±1.5
Nanduba(rs)	12.4 ±2.3	0	31.1 ±0.1	18.72 ±6.4	0	21.3 ±0.26	14.32 ±3.0	0	29.9 ±1.0
Siamikobo(s)	16.2 ±1.5	37.77 ±4.9	29.74 ±0.49	20.64 ±2.3	1.74 ±1.1	18.5 ±0.95	-	-	-*
Gota Gota(r)	228.4 ±71.1	19.43 ±8.1	28.98 ±0.3	223.36 ±75.8	21.48 ±8.8	20.5 ±0.22	208.16 ±82.8	10.52 ±5.2	26.2 ±0.2
Kasaka(r)	272.8 ±93.2	10.04 ±4.1	29.1 ±0.24	302.58 ±13.0	15.14 ±0.8	20.54 ±0.13	254.4 ±87.9	9.25 ±3.8	29.1 ±0.3
Mafungautsi(r)	201.6 ±61.6	26.44 ±8.4	29.28 ±0.35	222.4 ±54.9	14.84 ±7.0	23.7 ±0.82	201.3 ±70	20.48 ±8.4	26.9 ±0.1
Chiawa catchment									
Kabwadu(s)	11.6 ±1.9	0.88 ±0.88	38.62 ±0.51	28.1 ±3.1	0	23.7 ±0.34	17.16 ±3.3	0	35.2 ±1.9
Kavalamanja(r)	36.2 ±3.5	0	30 ±0	52.38 ±6.1	0	24.8 ±0.58	45.6 ±5.7	0	31.5 ±0.22

* -Dry (s) stream, (r) river, (rs) reservoir

Water velocity was significantly different across the study sites ($F = 2.7$, $p = 0.005$) (Appendix B). Disaggregating the data according to water body type revealed that there were significant differences in water velocity ($F = 31.54$, $p = 0.001$) across the river survey sites (Appendix C) but not the streams.

4.2.1.2 Water depth

Seasonal water depth for the period 2006 –2007 ranged from 7.9cm to 302.58cm. Comparison of the data statistically shows that water depth differs significantly among the survey sites $F = 37.2$, $p = 0.000$) (Appendix B) but not the seasons. Disaggregating the data according to water bodies (streams and rivers) revealed that water velocity was significantly different across the river survey sites (Appendix A) but not the seasons. Survey sites on the Kafue catchment were deeper than those on the Luangwa and Chiawa catchments. There were no significant differences among the stream survey sites. However, the mean depth during the cool-dry and hot-wet seasons (24cm ±4.8) was greater than during the hot-dry season, (13.8cm ±4.8), ($F = 3.72$, $p = 0.03$).

4.2.1.3 Water temperature

Seasonal water temperature values were between 17.6^0C and 38.6^0C. Statistical analysis of the data shows that there were no significant differences in water temperature among the survey sites. Water temperature was however significantly different across the seasons. Mean water temperature during the cool-dry season (22^0C) was lower than the hot-wet and hot-dry seasons (30.57^0C), ($F = 56.67$, $p = 0.001$). Analysing the data from the rivers and streams separately revealed that on the river transects, mean water

temperature during the hot-wet and hot-dry seasons (29.9^{0}C ±1.0) was equally higher than during the cool-dry season (22.5^{0}C ±1.00, (F = 34.6, p = 0.001). However, on the stream transects, mean water temperature during the hot-wet season (31.6^{0}C ±3.8) was significantly higher than the cool-dry season (19.7^{0}C ±3.8) while the hot-dry season (22.7^{0}C ±3.8) was intermediate.

4.2.2 Chemical factors

Calcium concentration, conductivity and pH of the representative water samples from the water bodies in the study area are presented in Table 4.2.2.

4.2.2.1 Calcium

Calcium concentration in the water samples from the different water bodies in the study area ranged between 12.3 mg/l and 126.3 mg/l. Analysis of variance carried out on the data shows that there were no significant differences among the survey sites while there were significant differences in calcium concentration among the seasons. Mean calcium concentration during the hot- wet season (47.8 ±5.9) was higher than during the cool-dry and hot-dry seasons (24 ±5.9) (F = 10.91, p = 0.0001). Analysing the data from the rivers and streams separately shows that mean calcium concentration in the rivers and the streams underwent similar significant seasonal variation. On the river survey sites, mean calcium concentration during the hot-wet season (44 ± 5.7), was higher than the cool-dry and hot-dry seasons, (23 ±5.7), (F = 9.27, p = 0.001). In the streams, mean calcium concentration was higher during the hot-wet season (50.4 ±9.2) than the hot-dry season (20.3 ±9.2) while the cold dry season was intermediate between the two (29.2 ±9.2), (F = 5.62, p = 0.008).

4.2.2.2 Conductivity

Conductivity values ranged between 174.8 and 914 μmhos. Comparison of the data statistically shows that there were significant differences in conductivity among the survey sites in the study area, ($F = 21.2$, $p = 0.000$) (Appendix B) but not the seasons. Disaggregating the data according to water body type revealed that differences in conductivity across the stream and river transects (Appendix A) were equally significant, ($F= 8.4$, $p = 0.001$), ($F = 3.5$, $p = 0.02$), respectively but not among the seasons.

4.2.2.3 pH

The water from Katondwe stream (pH 6.18-6.7) and Nanduba reservoir (pH 6.15 and 6.9) was slightly acidic, whereas the water from the other 18 water bodies was alkaline. The pH ranged between 7.2 and 8.87. There were no significant differences in pH across the study sites and seasons. When the data from the streams and rivers were analysed separately, there were significant differences among the stream transects, ($F = 6.8$, $p = 0.0001$), and across the river transects, ($F = 4.26$, $p = 0.008$) (Appendix A).

Table 4.2.2 Seasonal mean values for pH, conductivity and calcium at snail survey sites in the Middle Zambezi River basin

	Hot wet season			Cold dry season			Hot dry season		
Transect	pH	Calcium (mg/l)	Conductivity(µS)	pH	Conductivity(µS)	Calcium (mg/l)	pH	Conductivity (µS)	Calcium (mg/l)
Luangwa catchment									
Janeiro(s)	7.92 ±0.09	37.58 ±2.5	184 ±2.6	-	-	-	-	-	-*
Katondwe(s)	6.18 ±0.17	22.3 ±1.9	305 ±6.4	6.64 ±0.09	310 ±17.6	27.2 ±3.1	6.7 ±0.04	310 ±9.3	22.4 ±0.7
Kaunga(s)	8.06 ±0.04	32.89 ±0.7	368 ± 2	7.73 ±0.03	425 ±5	32 ±6.2	-	-	-
Mwalilia(s)	7.78 ±0.14	65.29 ±1.5	904 ±10.8	7.98 ±0.07	830 ±8.4	31 ±4.6	7.9 ±0.05	914 ±4	40.1 ±0.9
Mwavi(s)	7.24 ±0.02	126.25 ±3.1	692 ±5.8	7.56 ±0.07	738 ±23.3	31.52 ±7.8	7.66 ±0.08	770 ±4.5	53.6 ±1.1
Chiriwe(r)	7.36 ±0.12	27.56 ±1.5	206 ±4	7.74 ±0.02	270 ±6.3	23.04 ±0.8	7.56 ±0.02	284 ±2.5	24.8 ±0.4
Kakaro(r)	8.2 ±0.11	28.56 ±1.7	184 ±6.6	8.08 ±0.05	258 ±5.8	12.8 ±0.4	8.86 ±0.02	282 ±2	28.06 ±2.0
Kapoche(r)	7.84 ±0.16	40.75 ±0.67	218 ±11.5	7.9 ±0.03	266 ±8.2	18.1 ±2.5	7.98 ±0.04	260 ±0	19.2 ±0.9
Luangwa(r)	7.94 ±0.07	30.06 ±1.2	200 ±4.5	8.06 ±0.04	212 ±2	14.4 ±0.5	8.24 ±0.06	226 ±2.5	27.57 ±0.25
Kafue catchment									
Chilileka(s)	7.8 ±0.07	45.29 ±1.2	422 ±3.7	7.86 ±0.09	632 ±8	59.36 ±3.1	8.78 ±0.1	621 ±20.2	26.56 ±1.3
Hachipilika(s)	8.22 ±0.06	84.67 ±4.9	578 ±4.9	8.28 ±0.08	596 ±2.5	33.28 ±0.8	8.7 ±0.21	490 ±12.3	19.2 ±3.8
Lishiko(s)	7.24 ±0.07	16.43 ±0.83	196 ±9.3	7.2 ±0.08	184 ±2.5	12.3 ±1.3	7.92 ±0.16	210 ±10	15.8 ±0.1
Muchuto(s)	7.7 ±0.05	34.08 ±1.0	254 ±9.3	7.86 ±0.04	266 ±6	12.96 ±1.3	8.87 ±0.54	283 ±42.6	16.96 ±2.4
Nanduba(rs)	6.9 ±0.04	33.46 ±3.8	342 ±5.3	7.14 ±0.04	174.8 ±1.3	30.72 ±1.9	6.15 ±0.06	437 ±27	26.6 ±1.2
Siamikobo(s)	8.08 ±0.07	44.08 ±1.8	440 ±13.8	8.04 ±0.04	590 ±36.7	63.36 ±1.2	-	-	-
Gota Gota(r)	7.48 ±0.13	69.64 +7	318 ±11.6	7.8 ±0.03	254 ±5.1	29.2 ±1.0	7.84 ±0.22	233 ±11.8	27.52 ±3.7
Kasaka(r)	7.8 ±0.09	67.2 ±1.92	336 ±6	7.78 ±0.1	290 ±4.5	28.08 ±1.9	7.76 ±0.09	360 ±3.2	25.2 ±0.6
Mafungautsi(r)	7.56 ±0.12	65.13 ±1.9	344 ±13.3	7.82 ±0.04	278 ±3.7	29.2 ±0.3	8.22 ±0.04	225 ±1.5	24.8 ±5.3
Chiawa catchment									
Kabwadu(s)	7.88 ±0.04	64.13 ±18.7	482 ±52.1	8.1 ±0.09	212.6 ±1.9	20.8 ±0.9	8.52 ±0.09	186.4 ±2.8	21.87 ±0.8
Kavalamanja(r)	7.98 ±0.04	35.57 ±3.6	196 ±6	7.96 ±0.03	176 ±2.5	15.04 ±1.7	8.12 ±0.05	206 ±2.5	20.8 ±0

* - Dry (s) stream, (r) river, (rs) reservoir

4.2.3 Vegetation

The major vegetation type recorded at the study sites was emergent vegetation followed by floating vegetation, (Table 4.2.3). The common genera in emergent vegetation were *Phragmites* (common reed), *Vossia* (Hippo grass), *Typha* (Cattails) and *Cyperus* (Sedge). While those in floating vegetation were *Eichornia* (Water hyacinth), *Nymphaea* (Water lily) and *Azolla* (Water fern).

Table 4.2.3 Common plant genera recorded on the twenty transects in the Middle Zambezi River Basin.

Transects	Vegetation Type/ Dominant Plants		
	Emergent	Floating	Submerged
Luangwa catchment			
Janeiro(s)	*Phragmites*		
Katondwe(s)		*Nymphaea*	
Kaunga(s)	*Phragmites*		
Mwalilia(s)	*Cyperus*	*Nymphaea*	
Mwavi(s)	*Typha*	*Azolla*	
Chiriwe(r)	*Phragmites*		
Kakaro(r)	*Phragmites*		
Kapoche(r)	*Phragmites*		
Luangwa(r)		*Eichhornia*	
Kafue catchment			
Chilileka(s)	*Phragmites*		
Hachipilika(s)	*Phragmites*		
Lishiko(s)		*Nymphaea*	
Muchuto(s)	Bare		
Siamikobo(s)	*Phragmites*		
Nanduba(rs)	*Bare*		
Gota Gota(r)	*Vossia*	*Eichhornia*	
Kasaka(r)	*Vossia*	*Eichhornia*	*Ceratophylum*
Mafungautsi(r)	*Vossia*	*Eichhornia*	
Chiawa catchment			
Kabwadu(s)	*Phragmites*		
Kavalamanja(r)	*Vossia*	*Eichhornia*	

(s) = stream, (rs) = reservoir, (r) = river

4.2.3.1 Emergent vegetation

Emergent vegetation was found on all river and most of the stream transects. The Kafue and Zambezi rivers had *Vossia* as the common genus in the emergent vegetation, while *Phragmites* was the common emergent plant on the Luangwa River transects and five streams, (Kaunga, Janeiro, Chilileka, Siamikobo and Hachipilika).

The degree of coverage varied in the different water bodies. *Vossia* on the Kafue and Zambezi Rivers covered up to five metres of water from the riverbank and up to four metres deep, while *Phragmites* on the Luangwa River extended up to two meters from the riverbank. On the streams *Phragmites* was restricted to the stream edges. On the other hand, *Typha* and *Cyperus* on the Mwavi and Mwalilia streams respectively covered the entire stream in some places.

4.2.3.2 Floating vegetation

Floating vegetation was present on 45 percent of the water bodies. All these water bodies had snails in all seasons. *Eichhornia* dominated the floating vegetation. *Eichhornia* was found on the Kafue and Zambezi River transects and was often associated with *Vossia* but the former occurred in deeper water beyond the emergent *Vossia* vegetation. *Nymphaea* was only found on the streams. It was commonly found on pools. *Azolla* was only found on the Mwavi transect on areas where the stream forms pools.

4.2.4 Phytoplankton

The phytoplankton sampled belonged to five classes; Cyanophyceae, Dinophyceae, Diatomophyceae, Chlorophyceae and Euglenophyceae. However only Diatomophyceae

(diatoms) and Chlorophyceae (green algae) form part of the food for snails. Phytoplankton will henceforth refer to diatoms and green algae. The green algae consisted of 15 genera while the diatom genera were 13. There were significant differences in the phytoplankton diversity among the survey sites, ($F = 4.56$, $p = 0.000$) but not the seasons. Chiawa catchment had the highest mean phytoplankton genera (7.5) followed by Kafue (4.3) then Luangwa (1.5), ($F = 15.6$, $p = 0.000$).

The mean green algae genera on the Chiawa and Kafue catchments (2.9) were significantly higher than on the Luangwa catchment (0.7), ($F= 10.75$, $p = 0.0001$). Mean diatom genera on the Chiawa catchment were similarly higher (3.8) than on the Luangwa catchment (0.8) while the Kafue catchment was intermediate (2), ($F = 7.39$, $p = 0.0014$).

The stream transects more green algae genera (14) than the rivers (7). The diatom genera were similarly more on the stream transects (13) than the river transects (6). Survey sites on the Luangwa River had comparatively lower diversity of phytoplankton. The only exception was the Luangwa transect which is near the confluence with the Zambezi river. Phytoplankton richness were significantly different on the stream transects, ($F = 5.41$, $p = 0.0003$) and also on the river transects ($F = 2.65$, $p = 0.05$). However there were no significant differences among the three seasons.

4.3 Effect of habitat factors on snail species diversity and abundance

4.3.1 Abiotic factors

The effect of all six factors was examined simultaneously by means of multiple regression analysis (Best sub set) using both physical and chemical factors as

independent variates. The analysis showed that water temperature, depth, pH and calcium concentration played an insignificant part in the regression. However, water velocity played a significant part in determining the value of R^2 (Coefficient of determination) explaining 65% of the variance in snail diversity in the rivers ($p = 0.009$), while conductivity explained 42% of the variance in snail diversity in the streams, ($p = 0.02$).

4.3.2 Biotic factors

There were significant differences in snail diversity among types of emergent vegetation. Mean snail species richness on *Vossia* dominated transects (7.3 ± 0.85), was significantly higher than on transects dominated by other emergent plants (2.8 ± 0.85), ($F = 11$, $p = 0.004$). The means of Pulmonate snails on transects with *Typha, Vossia* and *Cyperus* (3.7 ± 0.71), were significantly higher than on transects with *Phragmites*, (1.3 ± 0.71), ($F = 8.15$, $p = 0.011$) whereas mean Prosobranch snail species diversity on transects with *Vossia* (2.5 ± 0.44), were significantly higher than transects with the other emergent vegetation types, (0.44 ± 0.44), ($F = 5.19$, $p = 0.008$).

Mean snail species richness were statistically higher on transects with floating vegetation (6.1 ± 0.85) than those without, (1.7 ± 0.85), ($F = 8.92$, $p = 0.001$). The mean of pulmonate snails on transects where floating vegetation was present were similarly significantly different (4.6 ± 0.61) from those without floating vegetation (1.5 ± 0.61) ($F = 23.19$, $p = 0.0001$). Means of Prosobranch snails were however only significantly higher on sites with *Eichhornia* (2.4 ± 0.25) compared with sites where the floating vegetation was dominated by other plants (0.33 ± 0.25), ($F = 33.79$, $p = 0.0001$). In spite

of the association, only pulmonate snails were actually found resting on *Eichornia* while the prosobranchs snails were found on the substrate. *L. natalensis* was the most dominant snail species found on *Eichhornia.* *B. globosus* was found on all the streams which had *Nymphaea.*

No association was found between snail species diversity and phytoplankton ($X^2 = 39.42$, $p = 0.002$). Similarly, no association was found between green algae diversity and snail species richness ($X^2 = 28.96$, $p = 0.035$) and diatom diversity and snail species diversity ($X^2 = 40.59$, $p = 0.001$).

4.4 The relationship between snail diversity and Schistosomiasis prevalence

To study the relationship between snail diversity and schistosomiasis prevalence in the Middle Zambezi River basin in Zambia, parasitological data on the prevalence of *Schistosoma haematobium* at the 20 schools in the study area was taken from the bilharziasis survey conducted by Simoonga (2006) as shown in Table 4.4.2.

The twenty survey sites (Table 4.4.2) were divided into four categories based on the presence of snail species diversity. Survey sites that did not have any snails through out the study period were given a category of zero snail species diversity. Survey sites with 1-3 snail species were regarded as having low snail species diversity. Sites with 4-6 and 7-10 snail species were designated as medium and high snail species diversity respectively.

Table 4.4.1 Snail species collected at the twenty water bodies in the Middle Zambezi River basin during 2006-2007

Transect	Pulmonate snails									Prosobranch snails						
	Intermediate hosts		Non intermediate hosts							Non intermediate hosts						
Streams	*Biomphalaria pfeifferi*	*Bulinus globosus*	*Bulinus canescens*	*Bulinus forskali*	*Ferrisia burnupi*	*Gyraulus costulatus*	*Lymnaea natalensis*	*Physa acuta*	*Segmentorbis angustus*	*Bellamya capillata*	*Cleopatra nsendwensis*	*Gabbiella kisalensis*	*Gabbiella sp*	*Lanistes ovum*	*Melanoides tuberculata*	Species diversity
Mwalilia	+	+	-	+	+	+	+	-	-	-	-	+	-	+	-	8
Katondwe	+	+	-	+	+	-	+	-	-	-	-	-	-	-	-	5
Mwavi	+	+	-	-	-	+	+	-	+	-	-	-	-	-	-	5
Kabwadu	-	+	-	+	-	-	+	+	-	-	+	-	-	-	-	4
Hachipilika	-	-	+	+	+	+	-	-	-	-	-	-	-	-	-	4
Chilileka	-	+	+	-	+	-	-	-	-	-	-	-	-	-	-	3
Muchuto	-	+	-	-	+	-	-	-	-	-	-	-	-	-	+	3
Lishiko	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	2
Nanduba	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	1
Siamikobo	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	1
Janeiro	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Kaunga	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Rivers																
Kavalamanja	-	+	-	+	-	+	+	+	+	-	+	-	+	+	+	10
Kasaka	+	+	-	-	+	-	+	-	+	+	-	-	-	+	-	7
Luangwa	-	+	-	-	+	-	+	+	+	-	+	-	-	+	-	7
Mafungausti	+	-	-	-	+	+	+	-	-	-	+	-	-	+	-	6
Gota Gota	-	-	-	-	+	+	+	-	+	-	-	-	-	+	+	6
Kakaro	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	2
Chiriwe	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0
Kapoche	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0

+ Snail species present
- Snail species absent

According to Table 4.4.2, six sites (Muchuto, Chilileka, Lishiko, Kakaro, Nanduba and Siamikobo) had low snail species richness, while another six sites (Mwavi, Katondwe, Gota Gota, Mafungautsi, Kabwadu and Hachipilika) had medium snail species richness. Four sites (Kavalamanja, Mwalilia, Kasaka and Luangwa) had high snail species diversity.

Assuming that the total cases of *Schistosoma haematobium* can be represented as a unit, the number of infected children in each school might represent the transmission potential (%). The transmission potential is therefore obtained by dividing the number of infected children in each school by the total number of *Schistosoma haematobium* cases for the study area.

The calculated Schistosomiasis transmission potential from water bodies of high snail species richness was 5.39% of the total bilharzia cases, whereas it was 30.11% and 51.1% for water bodies of medium and low snail species diversity respectively. The study showed that schools located near water bodies with high snail species diversity had less bilharzia cases than those near water bodies with low snail species diversity.

Table 4.4.2 Schistosomiasis transmission potential at the twenty survey sites in the Middle Zambezi River basin according to snail species richness

Name of waterbody	No. of snail species at survey site	Level of snail species richness	School close to waterbody	Number of Children examined	Number of Positive children	Schistosomiaisis Transmission Potential %*
Luangwa river	0	zero	Chiriwe	102	9	4.84
Luangwa river			Kapoche	102	4	2.16
Rufunsa stream			Janeiro	104	1	0.54
Kaunga stream			Kaunga	81	11	5.86
Sub total						**13.4**
Muchuto stream	1-3	Low	Muchuto	94	8	4.32
Moobe stream			Chilileka	94	16	8.6
Luangwa river			Kakaro	91	2	1.08
Muchuto stream			Lishiko	99	33	17.74
Nanduba dam			Nanduba	97	35	18.82
Siamikobo stream			Siamikobo	91	1	0.54
Sub total						**51.1**
Kafue river	4-6	Medium	Gota Gota	102	3	1.6
Kafue river			Mafungautsi	92	2	1.08
Kaulungu stream			Mwavi	95	28	15.05
Mphuka stream			Katondwe	102	17	9.14
Kabwadu stream			Kabwadu	83	2	1.08
Hachipilka stream			Hachipilika	97	4	2.16
Sub total						**30.11**
Zambezi river	7-10	High	Kavalamanja	102	0	0
Mwalilia stream			Mwalilia	100	6	3.23
Kafue river			Kasaka	98	2	1.08
Luangwa river			Luangwa	85	2	1.08
Sub total						**5.39**
TOTAL						**100**

Source: Simoonga, 2006.

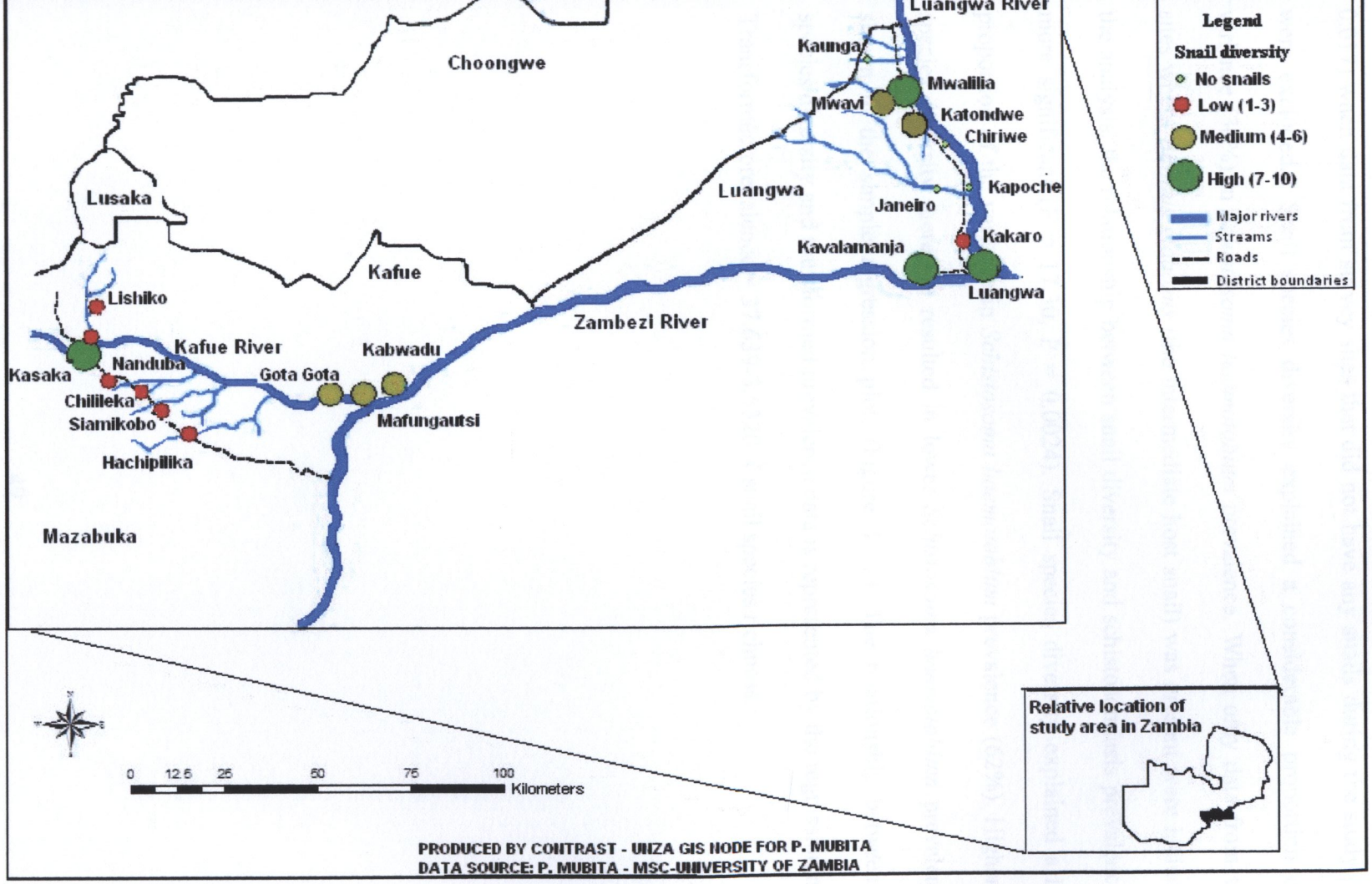
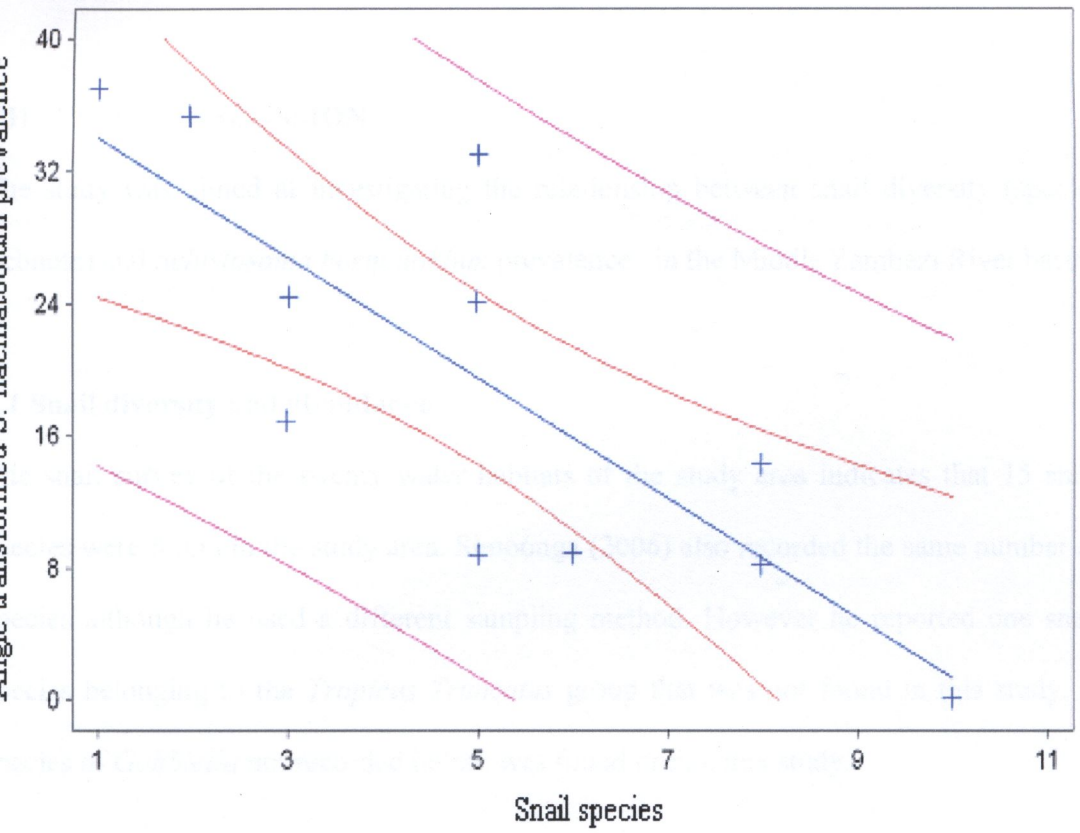


Figure 4.4.1. Map showing distribution of snail diversity at the survey sites in the Middle Zambezi River Basin, 2006-2007.

The regression analysis showed that there is a significant relationship between snail species diversity and angle transformed schistosomiasis prevalence data ($F = 7.39$, $p = 0.017$) when data from survey sites that did not have any snails during the study period were excluded. Snail species diversity explained a considerable proportion of the variance (30%) in *Schistosoma haematobium* prevalence. When only data from survey sites where *Bulinus globosus* (the intermediate host snail) was present were included in the analysis, the relationship between snail diversity and schistosomiasis prevalence was more significant ($F = 17.30$, $P = 0.0024$). Snail species diversity explained a higher proportion of the variance in *Schistosoma haematobium* prevalence (62%). Higher snail species diversity therefore resulted in lower *Schistosoma haematobium* prevalence as shown in the simple regression plot, (Figure 4.4.2). The relationship between snail species diversity and transformed prevalence data is represented by the regression model; Transformed prevalence = 37.639-3.6320 X snail species richness.



gure 4.4.2 Regression plot of transformed *Schistosoma haematobium* data against snail species chness showing the confidence and prediction intervals

CHAPTER 5: DISCUSSION

The study was aimed at investigating the relationship between snail diversity (species richness) and *Schistosoma haematobium* prevalence in the Middle Zambezi River basin.

5.1 Snail diversity and abundance

The snail survey of the twenty water habitats of the study area indicates that 15 snail species were found in the study area. Simoonga (2006) also recorded the same number of species although he used a different sampling method. However he reported one snail species belonging to the *Tropicus Truncatus* group that was not found in this study. A species of *Gabbiella* not recorded before was found during this study.

The snails belonging to genus *Bulinus* were the most widely distributed in the study area. *B. gobosus* and *B. forskalli* were present on all three catchment areas. Numbers of these species in the rivers were low compared to the streams. A previous study by Simoonga (2006), in the same area we conducted our study, revealed comparatively higher numbers of *B. globosus* on the Kasaka, Chilileka and Kabwadu transects. Higher numbers of *B. globosus* at the Kasaka transect was due to the presence of pools off the main course of the river. Such pools were not encountered during this study possibly due to the establishment of a harbour on the transect. Submerged vegetation such as *Lagarosiphon* and *Chara*, which was recorded by Simoonga (2006) at Kabwadu and Chilileka respectively, were not present during this study. The change of substrate from firm mud to sand could have led to the reduced abundance of *B. globosus*, which prefers muddy substrates, (Madsen, 1985; Coulibaly and Madsen, 1990). *Bulinus canescens* on the other

hand had the most restricted distribution among the pulmonates. Apart from the Hachipilika stream where it is relatively abundant, a few snails were found on two other streams (Chilileka and Siamikobo) within 40 kilometres distance from Hachipilika stream. According to Bronmark (1985), snail species dispersal can occur in water bodies in close proximity to each other. *B. canescens* appears to tolerate much higher water velocity than *B. globosus* and was found on a sandy substrate

Biomphalaria pfeifferi (the intermediate host snail for *Schistosoma mansoni*) had a very restricted distribution. This could account for the relatively lower prevalence of intestinal schistosomiasis in the study area, (Simoonga 2006). It was only present on the Kafue River on two transects in very low numbers on the floating macrophyte, *Eichhornia.* Higher numbers of *B. pfeifferi* were recorded on the Luangwa catchment, specifically on the perennial streams, Katondwe, Mwalilia and Mwavi. In spite of the high numbers on the Mwavi survey site, none of the children at Mwavi basic school was infected with *Schistosoma masoni,* (Simoonga 2006). The comparatively higher numbers of *B. pfeifferi* on the Mwavi transect could be attributed to the favourable temperature and high calcium concentration. *B. pfeifferi* prefers medium (20-200mg/l) to hard waters (>200 mg/l), (Williams, 1970). Generally members of Planorbinae have been reported to have wide tolerance of conductivity (120-3000µS/cm), (Yacoubi *et al.*, 2007; Jordan and Webbe, 1982). The temperature at the Mwavi transect had a very narrow range, 25.3 – 26.3^{0}C over the three seasons. Sturrock (1966) reported that 25^{0}C was the optimal temperature for a rapid expansion of B. pfeifferi in Tanzania. The other member of sub family Planorbinae, *Segmentorbis angustus* was also present in even higher numbers on the

Mwavi transect but only as a single specimen on two other transects. The ecological requirements of *S. angustus* are likely to be similar to those for *B. pfeifferi*. The Mwavi transect apparently has optimal conditions for the Planorbinae.

The distribution of *Lymnaea natalensis* was similar to that of *B. pfeifferi*. It was only found on the rivers and the three perennial streams (Katondwe, Mwalilia and Mwavi) on the Luangwa catchment. On the rivers *Lymnaea natalensis* was always found in association with *Eichhornia*. Presence of *Eichhornia* could therefore be used as an indicator for presence of *L. natalensis*. It has also been reported that Lymneids prefer environments with slow flow or stagnant water with a relatively high water column, (Giovannelli *et al.*, 2005; Hanely and Ultsch, 1999). Since *L. natalensis* is found in association with *Eichhornia*, it is protected from the effect of water velocity on the rivers. The three perennial streams on the Luangwa catchment are characterised by relatively low water velocity and presence of pools.

Ferrisia (burnupi) burnupi, the most abundant snail, was widely distributed and present in both the streams and the rivers. *Ferrisia b. burnupi* was found on rotting leaves of grass, *Phragmites* and trees. Due to its small size, a leaf of the aquatic vegetation is able to support very high numbers of *Ferrisia b. burnupi.* During the rainy season when water velocity is high, the number of *Ferrisia b. burnupi* was very low due to high water current. *Giovanelli et al.*, (2005), reported similar findings on the ancilids.

The distribution of *Physa acuta* was restricted to the Zambezi River and transects that receive a back flow from it. *P. acuta* is an invasive snail that is spread mostly through human activities (Brown and Kristensen, 1998). It thrives well in polluted urban areas. This could explain its low presence in our study areas.

Gabbiella kisalensis was only found on one transect. It was the most abundant snail in the study area especially in dense stands of *Cyperus*. There was no evidence of competition with *B. globosus* in spite of the high number. Brown and Kristensen (1998), reported that *G. kisalensis* is the only species of *Gabbiella* that is found in Southern Africa. *Gabbiella sp* which might be a new species, was however recorded on the Zambezi River. Other than *G. Kisalensis*, *Melanoides tuberculata* was the only Prosobranch found in a stream. It was also the most widely distributed Prosobranch. *M. tuberculata* has been investigated in a number of trials as a possible competitor of schistosome intermediate snails. However, no evidence of competition was found during this study. *M. tuberculata* displayed a tendency to aggregate in three of the four transects where it was found. The tendency to aggregate would lower the chance of interaction with and hence competition with the intermediate hosts of schistosomiasis. Only one out of the four transects where *M. tuberculata* was found also supported *B. globosus*. Most of the *M. tuberculata* was found downstream of the *Bulinus globosus*.

5.2 Effect of environmental factors on snail diversity

Data collected from the waters of different streams and rivers in the study area indicate that although differences in water quality were detected in the different seasons, both physical and chemical factors in these habitats were within the tolerance range of either host and non-host snail. However, this does not imply that these abiotic characteristics have no impact on snail diversity and abundance. Investigating the effect of abiotic factors according to water body type revealed that factors affecting snail diversity in the streams and the rivers were different.

It was found that the most important factor affecting snail diversity in the rivers was water velocity. It was the only physical factor that correlated (negatively) with snail diversity. The results corroborate with a review by Appleton (1978), that water velocity is the most important factor in lotic water bodies. The water velocity tolerated varies according to the species. However, most of the snail species encountered in the study area prefer slow waters as observed in another study, (Brown and Kristensen, 1998). The morphology of the shell generally gives an indication of the water velocity a particular snail species can tolerate (Brown, 1980). High conical shapes like *M. tuberculata* are able to tolerate relatively higher velocities. High water velocity dislodges and sweeps away the snails making it impossible for establishment of snail colonies. In addition to the effect on the snail, high water velocity sweeps away floating and submerged vegetation (Macan, 1974). Only sturdy emergent types such as *Phragmites* are able to establish. High water velocity is associated with unstable substrate (Giller and Malmqvist, 1998).

Periphytic algae and detritus, which are important components of snail food, are not able to establish.

It was observed that in the streams, the most important factor influencing snail diversity was conductivity. It was the only factor that correlated (positively) with snail diversity. According to Brown (1994), conductivity is a measurement that expresses a complex of chemical and physical variables. It is no more than a guide to the factors, which actually influence an organism. This is due to the fact that conductivity measures the total dissolved chemical content. The differences in conductivity in the water bodies reflect a heterogeneous geological background. Streams flowing through catchments based on igneous rocks such as granites usually have low levels of dissolved salts while those flowing over sedimentary rocks such as sandstones and limestone have relatively high levels of dissolved salts (Brown, 1994). A positive correlation was observed between calcium concentration and conductivity, implying that calcium ions contribute to the variation in conductivity. Schutte and Frank (1964), found similar results in a study in Drakensburg. Increasing concentration of calcium was correlated with increasing conductivity. However, hardness of water with the highest conductivity was attributed to magnesium and not calcium. Snails were found in 80% of the water bodies with high conductivity but in only 30% of water bodies with low conductivity.

It has also been reported that conductivity affects snail distribution and abundance (Ofoezie, 1999; Akafor and Obiezue, 2004 and Owojori, 2006). Most snails are able to establish in waters of widely varying conductivity (Jordan and Webbe, 1982; Yacoubi *et al.*, 2007). Different species of snails have different levels of tolerance to conductivity,

(Brown, 1994). The upper limits reported were all much higher than the highest conductivity recorded during this study. However prosobranchs were reported to be more tolerant to high conductivity. Within the limits of tolerance, a small increase in conductivity results in increased growth and reproduction until a maximum is reached. Beyond this point, further increase in conductivity results in decreased growth and reproduction. The optimum range for growth in a study by Kefford and Nugegoda, (2005) was 500-1000 μS/cm. The values for conductivity in our study area did not go beyond 1000 μS/cm, this within the favourable range. Kariuki *et al.,* (2004) reported a negative relation between *Lanistes purpureus* and high conductivity; however, the actual conductivity was not indicated.

Abiotic factors impacting snail diversity varied with the seasons and water body type. In the rivers, the interaction of abiotic factors affected snail diversity only during the cool-dry season. In the streams on the other hand, the interaction of abiotic factors influenced snail diversity in all the three seasons. The diversity indices during the hot-wet season were lower than that observed during the cool-dry and hot-dry seasons. Snail species richness however did not vary significantly. The reduction in the diversity indices could be attributed to reduced evenness. Rainfall has been shown to affect snail populations in a number of studies (Owojori *et al.*, 2006; Ofoezie, 1999; Akafor and Obiezue, 2004 and Erko *et al.,* 2006).

As regards the influence of aquatic vegetation on snail diversity, the results of the study show that snails are significantly associated with aquatic vegetation. Vegetation provides

areas rich with adequate oxygen, egg laying surfaces, shelter from the sun, protection from predators, site for periphyton to attach (Kariuki *et al.*, 2004), and in the reduction of water velocity (Fall *et al.*, 2004; Boelee and Laamrani, 2004). Pulmonate snails were associated with all the floating vegetation found in the study area. Preference for a particular type of vegetation by snails differs according to their species. The most important floating vegetation in the rivers was *Eichhornia. Eichhornia* seems to play an important role in snail dispersal and translocation because it is not-rooted and tends to move with the water flow. This may explain the presence of snails found on the Luangwa transect, since all these snails were found on or close to *Eichhornia* vegetation. *L. natalensis* was the snail most commonly associated with *Eichhornia, B. globosus* was rarely found. *Eichhornia* therefore, might not lead to increased densities of *B. globosus* and hence schistosomiasis. *Eicchornia* vegetation is thus expected to result in increased prevalence of *L. natalensis* transmitted *Fasciola hepatica* infections (Phiri, 2007).

B. globosus was found on all the sites where *Nymphaea* vegetation was found. This confirms the findings by Kariuki *et al.*, (2004) who suggested that presence of *Nymphaea* could be used as an indicator for the presence of *Bulinus*. Presence of *Nymphaea* is indicative of good conditions for snail survival (DBL 1998). The emergent vegetation (*Phragmites* and *Vossia*) on the rivers was however not directly associated with the pulmonate snails since they were not actually found resting on them. The role of the emergent vegetation is indirect in that it shelters the floating vegetation. Only the emergent vegetation on the streams was directly associated with snails as they were actually observed resting on it.

No association was found between snail diversity and diversity of green algae and diatoms. Although green algae and diatoms have been reported as food for snails (Pointier and Guyard, 1992), they are apparently not the most important food. Their association with snails might be in the snail abundance and not their diversity. Madsen (1992), reported that the pulmonate snails and Thiarids prefer fine detritus, epiphytic algae and decaying macrophytes. Only one species of *Cleopatra* was found to prefer green algae to any other food items. These snails probably prefer the epiphytic algae to phytoplankton due to the fact that they are found attached to the algae and therefore, making it easier for them to feed on it.

5.3 The relationship between snail diversity and *Schistosoma haematobium* prevalence in schools

It is of interest to find that host and non-host snails for schistosomiasis were present through out the study period at 11 out of the 20 survey sites. Non-host snails were more numerous in most of the different habitats than both host snail species. Snail species diversity varied in the different water bodies, with a range of 0-10 species per study site.

The results of the study indicate that snail species diversity was responsible for keeping schistosomiasis transmission potential low in the study area. When the number of snail species at survey sites was high, that is, 7-10 snail species, the transmission potential was low (5.39%), while it was high (51.1%) when the number of snail species at survey sites was low, that is, 1-3 snail species. The regression analysis showed that there was a

significant relationship between snail species diversity and *Schistosoma haematobium* prevalence in the study area on survey sites where any snail species were present. The relationship is even more significant on survey sites where the intermediate host (*Bulinus globosus*) was present. The regression plot was negative implying that high snail species diversity results in lower schistosomiasis prevalence. Stensgaard (2005), found similar negative correlation between species diversity of freshwater gastropods and schistosomiasis prevalence in Uganda. The results validate the first and main hypothesis of the study, which states that, In the presence of non-host species, (high species diversity of freshwater snails) transmission of schistosomiasis is lower. *Babiker et al.*, (2004), have suggested that non-host snails not only reduce the prevalence but also the intensity, which results in a reduction in morbidity.

The relationship between urinary schistosomiasis prevalence and snail species diversity was insignificant when all the survey sites in the study area were included in the regression analysis. This was due to the influence of transects (Luangwa catchment) that had no snail species throughout the study period. However, during the parasitological surveys, the schools in proximity to these water bodies had children who were infected with urinary schistosomiasis. The two schools with the highest infections are near the transmission 'hot spot' in the Luangwa catchment (Appendix A). Therefore, the children could have been exposed to the parasite at some point. As the distance from the water bodies with infested snails increases, the prevalence recorded at schools whose nearby water bodies do not have any snails reduced (SHN, 2004). The low intensities of infections (Simoonga, 2006) found at these schools also prove that the infection is not

from a source that the children are frequently exposed to (Larson, 1994). This is due to the fact that one cercaria that penetrates a human being can only develop into one adult worm and no replication of the adults takes place within the human being.

In spite of the significant relationship between snail species diversity and schistosomiasis prevalence, there is still some unexplained variance. This can be attributed to factors such as water body size and water contact. The highest prevalence of schistosomiasis infected pupils were found at schools near streams. *Yousif et al.*, (1998) and Babiker (2004), showed that miracidia are able to locate the host snails more easily in smaller volumes of water than in larger volumes. Schools with the highest prevalence (Nanduba, Lishiko and Mwavi Basic Schools) are located near water bodies that are intensively utilized by the communities. The Nanduba reservoir on the Kafue catchment is the only water source in the area. Uses of this reservoir include fishing, bathing and drawing water for domestic use. This situation facilitates a high degree of exposure to cercariae. In contrast to other studies which reported high schistosomiasis prevalence but low snail shedding proportions (*Sturrock et al.*, 1990; Kariuki *et al.*, 2004), the study by Simoonga (2006) reported 97 percent shedding by the snails at the Nanduba reservoir. On the Luangwa catchment area, the Mwavi stream is extensively used for irrigation of banana gardens. The fertilizers applied to the banana gardens most likely help in enriching the water. This could be the reason for the presence of the dense aquatic macrophytes present on the stream, which provide a favourable habitat for the intermediate host snails.

CHAPTER 6: CONCLUSIONS AND RECOMMENDATIONS

6.1 Conclusions

There is a relationship between snail diversity (species diversity) and schistosomiasis prevalence. In the presence of high snail diversity, schistosomiasis prevalence is kept low.

Streams generally supported lower snail diversity and higher densities of pulmonate snails than the rivers. The lower snail diversity and water volumes and higher water contact found on the streams, makes the streams to be higher risk areas for schistosomiasis transmission when the intermediate hosts are present. Higher prevalence of schistosomiasis was associated with communities living near streams than rivers.

Water development projects such as reservoirs and irrigation canals lead to conditions favourable for pulmonate snail establishment.

Water velocity and conductivity are the most important abiotic factors influencing snail diversity in the Middle Zambezi River basin.

Vegetation plays an important role in snail distribution. Floating vegetation such as *Eichhornia* assists in the transportation of pulmonate snails and therefore, might facilitate translocation of snails to previously uncolonised areas. The floating macrophyte *Nymphaea* is highly preferred by *B. globosus*, the intermediate host for schistosomiasis.

6.2 Recommendations

1. Snail surveys to determine snail diversity should be carried out prior to establishing water development projects such as dams and irrigation schemes. This is in order to determine the impact of such projects on snail diversity and consequently prevalence of schistosomiasis. This should be coupled with health education to the local communities, improved water supply and sanitation.
2. Minimise activities that disturb freshwater ecosystems and snail diversity such as molluscicides and pollutants.
3. More studies need to be carried out on the rivers to determine the abiotic factors influencing seasonal variation of snail diversity.

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APPENDICES

APPENDIX A: Means (± SE) of conductivity, Depth, pH and water velocity in the rivers of the Middle Zambezi River basin during 2006-2007.

nsect	Conductivity	Depth	pH	Water velocity
riwe	253.3 ±24ab	50.6 ±23.2b	7.6±0.09b	20.1abc
ta Gota	268.4 ±25.5ab	220 ±6.1a	7.7±0.1b	17.1 abc
karo	248 ±32ab	34.5 ±17.5b	8.4 ±0.25a	29.1 ab
poche	264 ±2ab	64 ±16.8b	7.9±0.06ab	34.5 a
saka	328.7 ±20.5a	299.9 ±36.7a	7.8±0ab	11.5 bc
avalamanja	192.7 ±8.8b	44.8 ±4.7b	8.1±0.03ab	0.0 c
angwa	212.7 ±7.5ab	61.8 ±10.3b	8.1±0.09ab	0.0 c
afungautsi	282.3 ±34.4ab	208.4 ±7a	7.9±0.18ab	20.5 abc

Means in the same column followed by the same letter(s) are not significantly different at 5% level using Bonferroni comparison of means test

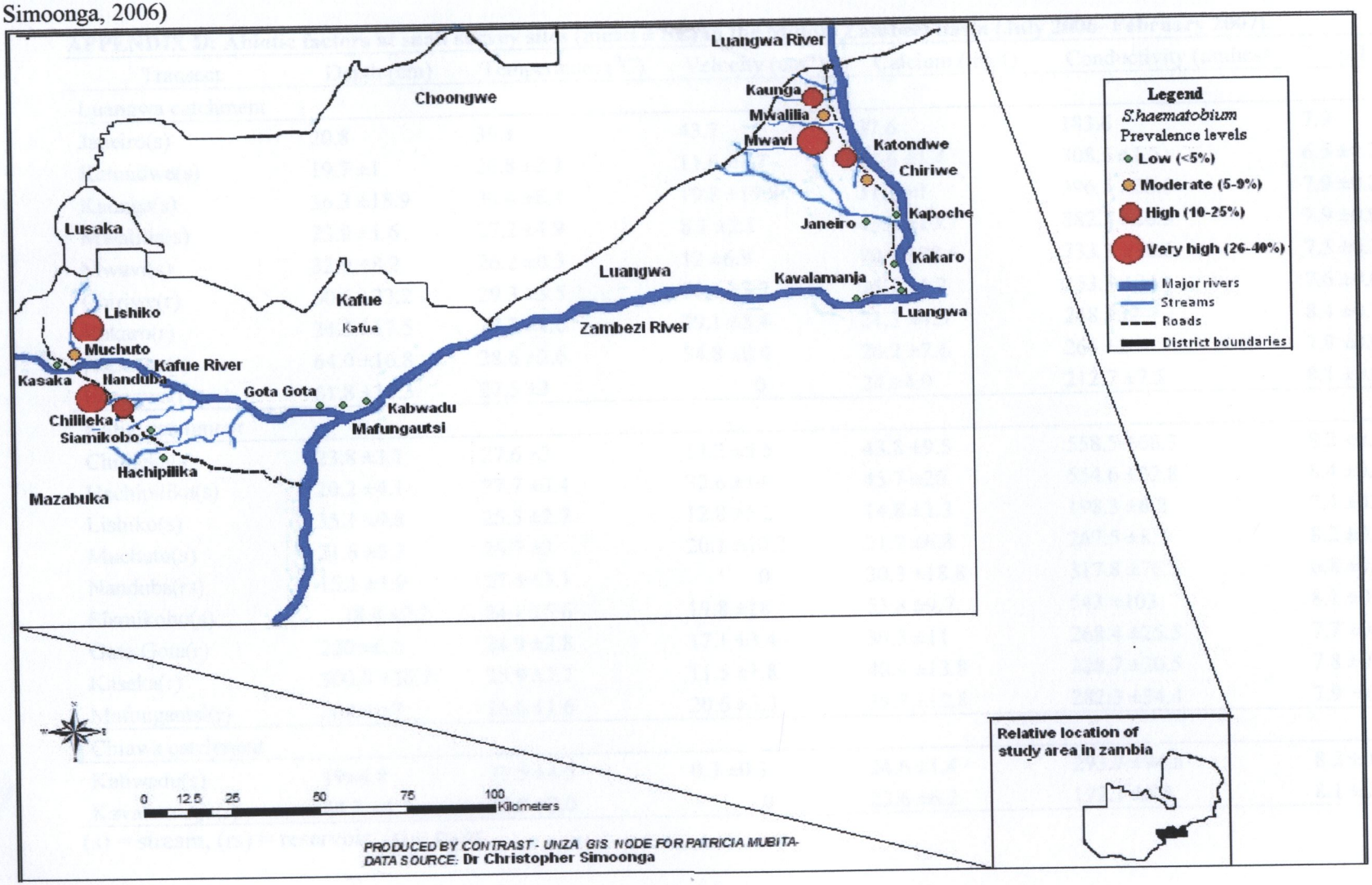
APPENDIX B: Means (± SE) of environmental factors and shan diversity in the water bodies of the Middle Lunsemfwa basin during 2006-2007.

Transect	Conductivity	Depth	Simpson's diversity index	Shannon-weiner diversity index	Species richness	Green algae
Chilileka(s)	558.5 ±bc	23.8 3.1±c	0.39 ±0.25ab	1.09 ±0.6ab	1.33 ±0.88abc	1 ±0.6abc
Hachipilika(s)	554.6 ±bc	20.2 ±4.1c	0.71 ±0.13ab	1.67 ±0.39ab	2.33 ±0.33abc	2.67 ±0.3abc
Janeiro(s)	183.6 ±0e	20.8 ±0c	0b	0b	0c	0c
Kabwadu(s)	293 7 ±de	19 ±4.8c	0.49 ±0.06ab	0.86 ±0.19ab	2.67 ±0.88abc	5.33 ±0.3a
Katondwe(s)	308.5 ±1.5de	19.7 ±1c	0.76 ±0.05ab	1.86 ±0.24ab	4.33 ±0.33abc	1.33 ±0.3abc
Kaunga(s)	396.5 ±28.5cde	36.34 ±18.9c	0b	0b	0c	0c
Lishiko(s)	198.3 ±6.2e	35.1 ±9.8c	0.78 ±0.03ab	1.87 ±0.23ab	1.67 ±0.33abc	1.33 ±0.3abc
Muchuto(s)	267.5 ±8.3e	21.6 ±3.3c	0.61 ±0.07ab	1.32 ±0.24ab	2.67 ±0.33abc	3 ±1.7abc
Mwalilia(s)	882.7 ±26.5a	23.9 ±1.6c	0.74 ±0.07ab	2.23 ±0.37ab	6 ±0.58ab	2.67 ±0.9abc
Mwavi(s)	733.3 ±22.6ab	32.9 ±8.2c	0.86 ±0.03a	2.5 ±0.24a	4.33 ±0.67abc	0.67 ±0.3bc
Nanduba(rs)	317.8 ±76.5de	15 ±1.9c	0.55 ±0.29ab	1.42 ±1.18ab	0.67 ±0.33bc	4.67 ±1.5ab
Siamikobo(s)	543 ±103bcd	18.4 ±2.2c	0.17 ±0.17ab	0.23 ±0.23ab	0.67 ±0.33bc	0.3 ±0.3bc
Chiriwe(r)	253.3 24±e	50.6 ±23.2c	0b	0b	0c	0c
Gota Gota(r)	268.4 25.5±e	220 ±6.1b	0.6 ±0.19ab	1.46 ±0.51ab	2.67 ±1.67abc	3.33 ±1.5abc
Kakaro(r)	248 ±32e	34.5 ±17.5c	0.49 ±0.28ab	1.49 ±0.83ab	1 ±0.58abc	0.67 ±0.3bc
Kapoche(r)	264 ±2e	64 ±16.8c	0b	0b	0c	0.67 ±0.3bc
Kasaka(r)	328.7 ±20.5cde	299.9 ±36.7a	0.67 ±0.16ab	1.92 ±0.5ab	5 ±1.53abc	2.67 ±0.9abc
Kavalamanja(r)	192.7 ±8.8e	44.8 ±4.7c	0.43 ±0.12ab	0.84 ±0.26ab	6.33 ±2.03a	2 ±0.6 abc
Luangwa(r)	212.7 ±7.5e	61.8 ±10.3c	0.42 ±0.24ab	1 ±0.57ab	4 ±2.08abc	0.67 ±0.3bc
Mafungautsi(r)	282.3 ±34.4e	208.4 ±7b	0.31 ±0.06ab	0.6 ±0.14ab	3 ±1.53abc	2.67 ±1.7abc

(s) =streams, (r) = rivers, (rs) = reservoir

Means in the same column followed by the same letter(s) are not significantly different at 5% level using Bonferroni comparison of means test

APPENDIX C: Distribution of *S. haematobium* according to prevalence in the Middle Zambezi River Basin (Source of prevalence data, Simoonga, 2006)



APPENDIX D: Abiotic factors at snail survey sites (mean ± SE) in the Middle Zambezi basin (July 2006- February 2007).

Transect	Depth (cm)	Temperature (^{0}C)	Velocity (cm^{-s})	Calcium (mg/l)	Conductivity (umhos)	pH
Luangwa catchment						
Janeiro(s)	20.8	36.1	43.7	37.6	183.6	7.9
Katondwe(s)	19.7 ±1	26.8 ±2.3	11.6 ±1.7	24.6 ±1.4	308.5 ±1.5	6.5 ±0.2
Kaunga(s)	36.3 ±18.9	30.6 ±8.1	19.8 ±19.8	31.1 ±1	396.5 ±28.5	7.9 ±0.2
Mwalilia(s)	23.9 ±1.6	27.2 ±4.9	8.1 ±2.1	43.6 ±10.7	882.7 ±26.5	7.9 ±0.06
Mwavi(s)	32.9 ±8.2	26.2 ±0.3	12 ±6.9	70.7 ±28.6	733.3 ±22.6	7.5 ±0.2
Chiriwe(r)	50.6 ±23.2	29.3 ±3.5	20.1 ±3.7	25.1 ±1.3	253.3 ±24	7.6 ±0.09
Kakaro(r)	34.5 ±17.5	27.7 ±1.6	29.1 ±5.4	21.3 ±4.5	248 ±32	8.4 ±0.25
Kapoche(r)	64.0 ±16.8	28.6 ±2.6	34.8 ±8.4	26.2 ±7.6	264 ±2	7.9 ±0.06
Luangwa(r)	61.8 ±10.3	27.5 ±3	0	24 ±4.9	212.7 ±7.5	8.1 ±0.09
Kafue catchment						
Chilileka(s)	23.8 ±3.1	27.6 ±3	14.2 ±5.5	43.8 ±9.5	558.5 ±68.3	8.2 ±0.3
Hachipilika(s)	20.2 ±4.1	27.7 ±3.4	32.6 ±14	45.7 ±20	554.6 ±32.8	8.4 ±0.2
Lishiko(s)	35.1 ±9.8	25.5 ±2.7	12.8 ±5.2	14.8 ±1.3	198.3 ±6.2	7.4 ±0.2
Muchuto(s)	21.6 ±3.3	26.7 ±3	20.1 ±10.2	21.7 ±6.8	267.5 ±8.3	8.2 ±0.4
Nanduba(rs)	15.1 ±1.9	27.4 ±3.1	0	30.3 ±18.8	317.8 ±76.5	6.8 ±0.3
Siamikobo(s)	18.4 ±2.2	24.1 ±5.6	19.8 ±18	53.8 ±9.7	543 ±103	8.1 ±0.05
Gota Gota(r)	220 ±6.1	24.9 ±2.8	17.1 ±3.4	39.3 ±11	268.4 ±25.5	7.7 ±0.1
Kasaka(r)	300.0 ±36.7	25.9 ±3.2	11.5 ±1.8	40.4 ±13.8	328.7 ±20.5	7.8 ±0
Mafungautsi(r)	208.4 ±7	26.6 ±1.6	20.6 ±3.3	39.7 ±12.8	282.3 ±34.4	7.9 ±0.18
Chiawa catchment						
Kabwadu(s)	19±4.8	32.5 ±4.5	0.3 ±0.3	24.6 ±1.4	293.7 ±94.5	8.2 ±0.2
Kavalamanja(r)	44.7 ±4.7	28.8 ±2.0	0	23.6 ±6.2	192.7 ±8.8	8.1 ±0.03

(s) = stream, (rs) = reservoir, (r) = river

	Chlamydomonas	*Ulothrix*	*Ankistrodesmus*	*Pediastrum*	*Chlorela*	*Closterium*	*Spirogyra*	*Cosmarium*	*Chaetophora*	*Clorococum*	*Mougeotia*	*Cladophora*	*Oedogonium*	*Anacystis*
Luangwa catchment														
Mwalilia(s)	-	+	+	-	-	-	+	-	-	-	-	-	-	-
Katondwe(s)	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Mwavi(s)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Janeiro(s)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Kaunga(s)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Kakaro(r)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Chiriwe(r)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Kapoche(r)	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Luangwa(r)	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Kafue catchment														
Hachipilika(s)	-	-	-	-	+	+	+	+	+	-	-	-	-	-
Chilileka(s)	+	+	-	-	-	-	-	-	-	-	-	-	-	-
Muchuto(s)	+	+		+	+				+	+		+		
Lishiko(s)	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Nanduba(rs)	+	+	+	+	+	+	-	-	-	-	-	-	-	-
Siamikobo(s)	-	-	-	-	+	-	-	-	-	-	-	-	-	-
Kasaka(r)	+	+	-	-	+	-	-	-	-	+	-	-	-	-
Mafungausti(r)	-	+	-	+	-	+	+	-	-	-	+	-	+	-
Gota Gota(r)	-	+	+	+	+	+	+	-	-	-	-	-	-	-
Chiawa catchment		-	-	-	-	-	-	-	-	-	-	-	-	-
Kabwadu(s)	-	+	+	+	+	+	+	-	-	-	-	-	-	-
Kavalamanja(r)			+			-	-	+	-					

(s) = Stream, (rs) = reservoir, (r) = river

APPENDIX F: Diatom genera at the twenty snail survey sites in the Middle Zambezi basin (July 2006- February 2007).

	Amphora	*Cymbella*	*Cymatopleura*	*Fragillaria*	*Gomphonema*	*Gyrosigma*	*Melosira*	*Navicula*	*Pinnularia*	*Pleurosigma*	*Stauroneis*	*Synedra*	*Tabellaria*
Luangwa catchment													
Mwalilia(s)	-	-	-	-	-	+	-	-	-	-	+	-	-
Katondwe(s)	-	-	-	-	-	-	-	-	-	-	-	-	-
Mwavi(s)	-	-	-	+	-	-	-	-	-	-	-	-	-
Janeiro(s)	-	-	-	-	-	-	-	-	-	-	-	-	-
Kaunga(s)	-	+	-	-	-	-	+	-	+	-	+	-	-
Kakaro(r)	-	-	-	-	-	-	-	-	-	-	-	-	-
Chiriwe(r)	-	-	-	-	-	-	-	-	-	-	-	-	-
Kapoche(r)	-	-	-	-	-	-	-	-	-	-	-	+	-
Luangwa(r)	-	+	-	-	-	-	-	+	+	-	-	-	+
Kafue catchment													
Hachipilika(s)	-	+	-	-	-	-	-	+	+	-	+	-	+
Chilileka(s)	+	+	+	-	-	-	-	-	+	-	+	+	+
Muchuto(s)	+	+	+		+	+		+	+			+	+
Lishiko(s)	-	-	-	-	-		-	-	-	-	-	-	-
Nanduba(rs)	-	-	-	-	+	-	-	+	+	-	-	+	-
Siamikobo(s)	-	+	-	-	-	-	-	-	-	-	-	+	-
Kasaka(r)	-	-	-	-	-	-	-	-	+	-	-	+	+
Mafungausti(r)	-	-	-	-	-	-	-	+	-	-	-	+	-
Gota Gota(r)	-	-	-	-	-	-	-	-	-	-	-	+	-
Chiawa catchment													
Kabwadu(s)	-	+	-	-	-	+	-	+	+	+	+	+	+
Kavalamanja (r)	+	+	-	-	-	-	-	+	+	-	-	+	-

(s) = Stream, (rs) = reservoir, (r) = river

PENDIX G: Snail data sheet

HOOL.....WATER BODY.....

TE..... TIME.....

S Start: Lat.....Long.....

End: Lat.....Long.....

coop nt dist)	Total Snail sp	1 beca	2 bmpf	3 bucn	4 bufs	5 bugb	6 cpns	7 frbn	8 gabi	9 grcs	10 lnov	11 lmnt	16 other sp
(0m)													
2(4m)													
3(8m)													
4(12m)													
5(16m)													
6(20m)													
7(24m)													
8(28m)													
9(32m)													
10(36m)													
11(40m)													
12(44m)													
13(48m)													
14(52m)													
15(56m)													
16(60m)													
17(64m)													
18(68m)													
19(72m)													
20(76m)													
21(80m)													
22(84m)													
23(88m)													
24(92m)													
25(96m)													
26(100m)													
27(104m)													
28(108m)													

APPENDIX H: Environmental data sampling form

SCHOOL.....WATER BODY.....

DATE.....TIME.....

GPS Start: Lat.....Long.....

End: Lat.....Long.....

Quadrant Geocoordinates	Depth in cm	Water velocity cm^{-1}	Temperature (^0C)	Conductivity	Calcium	DO	pH
1							
2							
3							
4							
5							

Quadrant Waypoint	Vegetation		Phytoplankton
	Type	Genera	Genera
1			
2			

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