Impact of natural and anthropogenic factors on the trophic interactions of molluscivores and *Schistosoma* host snails

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Impact of natural and anthropogenic factors on the trophic interactions of molluscivores and *Schistosoma* host snails

Concillia Monde

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To my children and to my family

Contents

Chapter 1

General Introduction

1.1 Background

Organisms are affected by factors of the environment within which they live. These factors determine the extent to which they thrive in the environment. Snails responsible for the transmission of schistosomiasis live in water and are subject to physical, chemical and biological status of the aquatic systems they inhabit (Giovanelli et al., 2005; Ndifon and Ukoli, 1989). Human related factors also play an important role in regulating assemblages of these gastropods and supporting the transmission cycle (Adenowo et al., 2015). While there exists a large amount of data on the effects of environmental factors on aquatic organisms, little is known about their effects on trophic interactions among these organisms.

Infectious diseases largely depend on host-parasite-environment interactions for their transmission thus making them subject to environmental perturbations (Van Bocxlaer et al., 2014). Schistosomiasis is one such disease and is among 24% of global environmentally determined diseases caused by trematodes of the *Schistosoma* species (Prüss-Üstün and Corvalán, 2006). The life cycle of these *Schistosoma* depends on two independent hosts, snail intermediate hosts and mammalian definitive hosts. The snail intermediate hosts of the human *Schistosoma* belong to three genera- *Biomphalaria, Bulinus* and *Oncomelania*. *Biomphalaria* and *Bulinus* are aquatic while *Oncomelania* is amphibious. In sub-Saharan Africa where over 90% of global cases of schistosomiasis infection are found (Liao et al., 2011), *Bulinus* spp. are important hosts for *Schistosoma haematobium*, the parasites responsible for urinary schistosomiasis, while *Biomphalaria* spp. are hosts for *Schistosoma mansoni* that causes hepatic and intestinal schistosomiasis. *Schistosoma* adult worms are unisexual, reside and reproduce in the body of the definitive human hosts. Numerous eggs are passed from the definitive host's body through urine or faeces into freshwater. Here they quickly hatch into an actively swimming larva called miracidium. The miracidia actively seek suitable intermediate snail hosts in which they undergo further developmental stages until they reach the final larval stage, cercariae. These are released into the water by the host snails where they actively search for a human host (Massara et al., 2004). Infection takes place in contaminated freshwater. As a result, schistosomiasis is rampant in places with poor hygiene and heavy dependence on natural water for occupational, recreational and domestic use. This almost always coincides with poor rural communities in the tropical and sub-tropical countries.

1.2 Health problems associated with *Schistosoma* infection

Some direct health problems associated with infection with *Schistosoma* include chronic urogenital and gastrointestinal pathologies, cervical and bladder carcinoma, anaemia, and cognitive problems directly leading to poor school performances in children (Chipeta et al., 2009; Mutengo et al., 2009). In addition, schistosomiasis has been shown to facilitate the transmission of HIV/AIDS because of the increased blood in the urogenital system of those infected with schistosomes (Mbabazi et al., 2011). As a result of its debilitating nature, schistosomiasis impairs productivity. Just as health can drive economic growth, ill-health can push people into poverty and make it very difficult for them to escape the poverty trap. A vicious schistosomiasis/poverty cycle is established (Figure 1.1) (Gazzinelli et al., 2012).

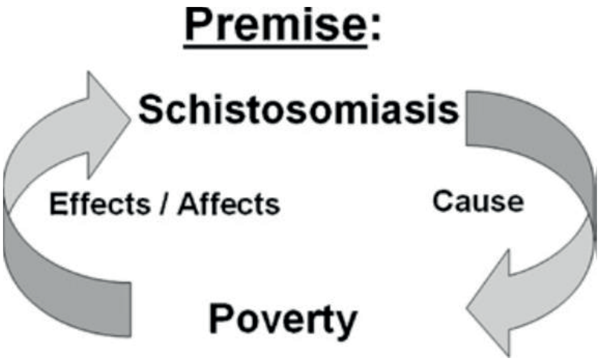

Figure 1.1 Proposed vicious cycle of schistosomiasis in the presence of poverty. Left arrow—Poverty reduces water-use options and increases risk of infection, while also influencing personal adaptation and coping for disease syndromes caused by infection. Right arrow—Increased disability, related to the impact of chronic and recurrent infection, reduces productivity and perpetuates poverty. Source: King, 2010

1.3 Control interventions

The control of morbidity in humans through chemotherapy has been the backbone of schistosomiasis control programmes implemented in many countries. Praziquantel is the drug of choice and it is widely used owing to its effectiveness against all the species of schistosomes that affect humans (Adenowo et al., 2015). Praziquantel kills the schistosomes by interfering with inorganic ion transport and glucose metabolism in the parasite as well as damaging the worm's tegument (Andrews, 1985). It has a relatively short half-life in serum of about 1 hour

(De Cock, 1984) and is tolerated well with isolated side effect cases of abdominal pain, nausea, vomiting, anorexia and diarrhoea (Fenwick et al., 2006).

The Schistosomiasis Control Initiative (SCI) helped to set up control programmes in many sub-Saharan African countries including Zambia, Burkina Faso, Mali, Niger, Tanzania and Uganda (Lai et al., 2015). Other countries also benefited from the support of the German Government and the World Bank in implementing morbidity control programmes (Fenwick et al., 2006).

While chemotherapy has the potential to reduce morbidity and mortality due to schistosomiasis, its effectiveness is limited by logistical constraints associated with health care systems and school programmes through which it is mainly implemented. School programmes are limited in that there are often very few schools and in places where schools are present, attendance is very low (43% of the world’s out of school population is in sub Saharan Africa) (UNESCO, 2011). Weak health care systems are characteristic of many developing countries (Chudi, 2010; Reerink and Sauerborn, 1996) defined by persistent inadequate availability of skilled health professionals which results in low access to health services in all communities and facilities; poor physical access to functional health centres in rural areas; inadequate funding to the health care sector and weak health information systems which need to be strengthened at all levels. Additionally, schistosomiasis is not perceived as a priority in resource poor countries because of the seemingly mild effects (Kalungwana, 2011). Coupled with the priority given by funding organisations to other diseases, such as malaria, tuberculosis, and HIV/AIDS (Gray et al., 2010), and praziquantel’s lack of activity against immature schistosomes (Fenwick et al., 2006) and the need for repeated treatment due to reinfection, makes developing countries unable to stock enough quantities of the drug to administer on all those that are infected. As a result the global burden of schistosomiasis remains unabated and somewhat increasing.

The limitations associated with the control of morbidity and mortality due to schistosomiasis necessitate for exploitation of alternative methods. Snail control is one of the methods with the potential to complement chemotherapy by eliminating the intermediate snail hosts of the *Schistosoma* parasites. Vector and intermediate host control is viewed as a means to reducing the burden of Neglected Tropical Diseases (NTD) like schistosomiasis and is expected to play a significant role in elimination of some NTDs (WHO, 2012). Three main methods are used to control populations of host snails in endemic areas. These are; chemical control by use of molluscicides both synthetic and of plant origin, mechanical control by making the environment unsuitable for snail habitation and biological control by using natural enemies of host snails. The use of chemicals to kill host snails is an effective strategy in the short term but has the disadvantage of killing non-target organisms. The need for repeated applications due to recolonization through either autogenic or allogenic recovery makes the method expensive and less available in poor endemic settings (Hamed, 2010). Mechanical control cannot be applied in large natural settings hence it is mainly suitable in man-made facilities where appropriate engineering design is incorporated during the construction. Biological control using natural enemies of host snails can keep the populations of host snails under control thereby limiting the risk of transmission (Pointier and Jourdane, 2000). However, the efficiency of these interactions depend on environmental factors acting on the parasites, target snails and their enemies. While many studies have documented the role of parasites, competitors and predators in regulating populations of host snails (Pointier and Jourdane, 2000 and citations there in) no studies have been conducted to quantify the effect of environmental factors on these biological interactions. The success and sustainability of host snail control through biological means requires greater understanding of how environmental stressors influence these trophic interactions.

The aim of this thesis is to contribute to the body of knowledge on the transmission control of schistosomiasis and the role of the predator-prey interactions of *Schistosoma* host snails and their predators, and the factors affecting these interactions.

The role of predator-prey interactions in controlling vector-borne diseases such as malaria is well appreciated (Kamareddine, 2012). Natural enemies of either the vector or the causative agent are used to suppress their populations and hence the transmission of the diseases they cause. Positive outcomes in the control of the *Anopheles* mosquitos i.e. vectors of the malaria parasites by predatory fish including *Cyprinus capio*, *Ctenopharyngodon idella*, *Catla catla*, *Labeo rohita*, and *Cirrhinus mrigala* are evident (WHO, 1982; Victor et al., 1994). Menon and Rajagopalan (1978) observed a 98% reduction in the larval density of *Anopheles stephensi* exposed to predation by *Gambusia affinis.* In China, the presence of carp fish in certain rice fields, reduced the number of cases of malaria (WHO, 1982). Given the success of this method in reducing the malaria vectors and consequently disease prevalence we designed this study whose objectives are:

1. to review *Schistosoma* transmission control methods based on the host-environment link, highlight their limitations and conditions in which they may be used in small-scale control programmes in sub-Saharan Africa

2. to assess the influence of environmental and socioeconomic factors on the population dynamics of the host snails vis-à-vis schistosomiasis in rural Zambia.
3. to evaluate the potential of crayfish and catfish as predators for *Schistosoma* host snails.
4. to investigate the effect of pesticides (endosulfan) on predator-prey interactions of host snails and catfish.

1.4 Organisation of the thesis

We will address the questions raised in this thesis in five chapters. In **Chapter 2**, we set the stage by reviewing the interventions aimed at disrupting the transmission of schistosomiasis by targeting the snail intermediate hosts of the *Schistosoma* parasite. Here we outline the factors that characterise the host-environment link and explain how they may influence this link. In this chapter, we also bring to the fore the various methods that are reported in the literature and we examine in what context they may be applied in the fight against the transmission of schistosomiasis. In order to appreciate the specific factors affecting the dynamics of schistosomiasis transmission in endemic rural settings, monitoring of both the environmental and socioeconomic factors of affected communities was inevitable. A monitoring case study was conducted in Zambia, a country in sub-Saharan Africa and the findings are outlined in **Chapter 3.** This chapter sheds light on what might be the important factors influencing the transmission of the disease. The findings of chapter 3 are important in designing intervention programmes that may be responsive to the specific requirements of the affected communities. Chapters 4 and 5 are reports of laboratory experiments whose aim was to further understand the functioning of the trophic interactions involving host snails. In **Chapter 4** the findings of laboratory experiments in which we examined the efficiency of two potential snail predators, crayfish *Cherax quadricarinatus* and hybrid catfish *Clarias gariepinus* x *Clarias ngamensis,* in reducing populations of host snails are outlined. **Chapter 5** reports on the effects of pesticides on the trophic interactions of the catfish and the host snails. In this study the aim was to understand how anthropogenic pollution of the aquatic system may impact natural processes thereby affecting the natural equilibrium which may then lead to reduced ecosystem health. The final **Chapter 6** is central to this thesis and in it I pool the findings of the preceding chapters and draw conclusions on how natural and human induced factors may enhance or hinder the transmission of schistosomiasis. I also outline the importance of understanding socioeconomic settings of the affected communities and ecological parameters affecting the disease hosts.

Chapter 2

Exploring the potential of host-environment relationships in the control of schistosomiasis in Africa – a review

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Abstract

Schistosomiasis is one of several human diseases determined by host-parasite-environment interactions. *Schistosoma* parasites are transmitted between the snail intermediate hosts and mammalian definitive hosts in an aquatic environment. This host-environment link determines the parasite transmission dynamics and is a route through which control of transmission can be achieved. Transmission control methods based on manipulating the host-environment link were reviewed, the limitations of each method were highlighted and conditions in which they may be used in small scale control programmes in sub-Saharan Africa were suggested. Chemical control may be ideal in poor rural communities, where health education strategies have little impact and where fishing is not an important livelihood strategy, because human contact with contaminated water is necessary for survival. In aquaculture and other water development project areas, biocontrol may yield positive results due to reduced predation on snail predators and competitors as a result of restricted access. Environmental modification may be ideal in man-made systems, where the planning phase includes appropriate engineering works. Control strategies must be based as much on the ecology of host snails as on social aspects of the affected community, and be implemented on a case-by-case basis.

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2.1 Introduction

Schistosomiasis is one of several human diseases determined by host-parasite-environment interactions. Managing such diseases remains a challenge to health practitioners and governments in developing countries. Management actions to control these diseases are usually directed at the parasite, the host population, or a key component of the environment, with the goal of reducing disease exposure and transmission (Wobeser, 2006). Control methods directed at the host population, such as vaccination and population reduction, remain limited in approach and suffer financial, logistical, and political constraints (Wobeser, 2006). One such disease is schistosomiasis. The global burden of schistosomiasis remains high, with over 240 million people infected (Bockarie et al., 2013) and close to 800 million at risk of getting infected (Steinmann et al., 2006). Over 90% of these infections occur in sub-Saharan Africa (Hotez and Fenwick, 2009). Schistosomiasis is caused by *Schistosoma* blood flukes which depend on freshwater snail hosts and mammalian hosts to complete their life cycle. In Africa, planorbids, *Bulinus* and *Biomphalaria* spp. are the main hosts for *Schistosoma*. Infection occurs when there is contact with cercariae-infested water through occupational (fishing and agriculture), domestic (collection of water for household use) and recreational (swimming, bathing and playing) activities (Fenwick et al., 2006; King, 2008; King and Dangerfield-Cha, 2008). Control strategies for schistosomiasis have evolved over time from those focussing on transmission control by eliminating host snails, and promoting good sanitation and health education (Bruun et al., 2008; Fenwick et al., 2003; WHO, 1985) to morbidity control by killing the worms in humans through chemotherapy (WHO, 1985, 1993). Although considerable progress has been made in reducing morbidity in humans, schistosomiasis remains a major public health problem (Bockarie et al., 2013; Lardans and Dissous, 1998) especially in sub-Saharan Africa. Counter-productive factors in the fight against schistosomiasis in this region include rapid population increase (Savioli et al., 2004), insufficient praziquantel drug availability, inadequate logistical and institutional capacity to implement control programmes (WHO, 2011), ineffectiveness of praziquantel against immature schistosomes (Fenwick et al., 2006; MacConnachie, 2012; Wang et al., 2012) and the need for repeated treatment due to reinfection (Hotez et al., 2007). These limitations prompted the World Health Organization to rethink schistosomiasis control strategies by including transmission control (WHO, 2012). The dependence of *Schistosoma* on two independent host organisms makes them vulnerable to environmental influences (Michael et al., 2010). Manipulating this host-environment link could offer greater opportunities in reducing the prevalence of schistosomiasis in poor communities in Africa. However, such interventions require an understanding of the ecology and behaviour of the host snails and how these affect the dynamics of schistosomiasis; a concept dubbed “Know your enemy” (Sturrock, 1995). The present study aimed to review transmission control methods based on manipulating the host-environment link, to highlight the limitations of each method, and to suggest conditions in which they may be used in small-scale control programmes in sub-Saharan Africa.

2.2 Interacting factors in host snail population dynamics.

Schistosomiasis disease dynamics are subject to natural and man-made environmental influences which act to restrain or synergise the transmission of schistosomiasis in endemic countries (Figure 2.1).

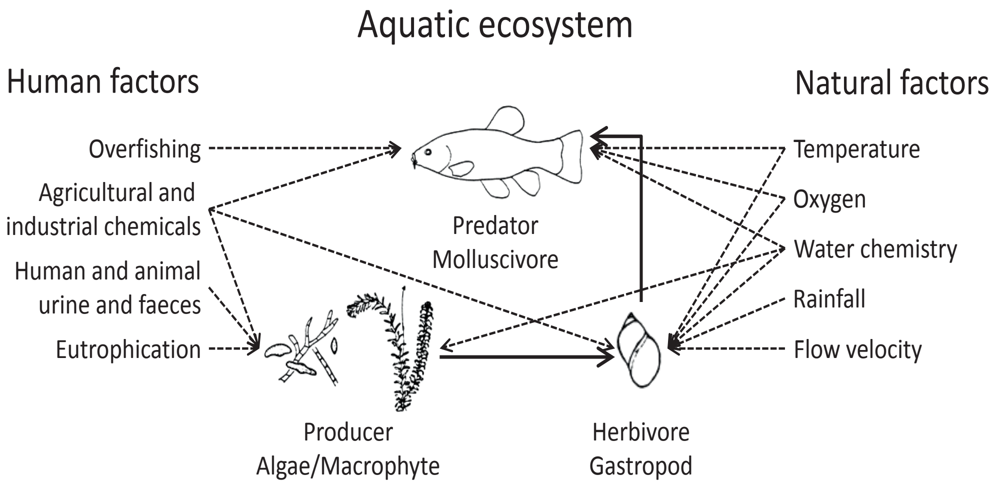

Figure 2.1: Examples of environmental factors impacting on trophic interactions of host snails

2.2.1 Natural factors

2.2.1.1 Water temperature

The effect of temperature on vector-borne diseases such as schistosomiasis is well-known (Chitsulo et al., 2000; Martens et al., 1995; Patz et al., 2005; Patz et al., 2000) because of its impact on the wellbeing of the parasite and its host. Survival and reproductive potential of snail hosts is temperature-dependent and varies among different species. Temperatures >27°C affected the fecundity of many snail species in south-eastern Africa (Appleton, 1977; Thomas and Tait, 1984). Similarly, in southern Africa, reduced fecundity in *Bulinus* and *Biomphalaria* spp. was observed at temperature ranges of 25 - 27°C and 20 to 25°C, respectively (Appleton and Madsen, 2012). Viability of the parasite is also affected by temperature, as in the case of the low infectivity of cercariae developing from sporocysts in snails maintained at temperatures of 23-25°C (DeWitt, 1955; Stirewalt, 1954). The pre-patent period also tends to be lengthened as the snail maintenance temperatures are reduced (Stirewalt, 1954).

2.2.1.2 Oxygen

Freshwater snail distribution is oxygen-dependant (Camara et al., 2012; Hussein et al., 2011; Malek, 1958; Ofoezie, 1999). Prosobranchs, such as *Melanoides*, are sensitive to low oxygen levels characteristic of polluted ecosystems, whilst pulmonates, such as *Bulinus* and *Biomphalaria,* can take in oxygen from both air and water and are generally tolerant of water with low dissolved oxygen. However, the euryok nature of aquatic snails, which can adapt to many environmental conditions (Agi and Okwuosa, 2001), enables them to inhabit places with varied oxygen levels.

2.2.1.3 Rainfall and flow velocity

Dry spells, common in tropical environments, destroy habitats for planorbid snails, although some snails survive by means of aestivation and repopulate these habitats as soon as it rains (Barbosa and Barbosa, 1994; Kariuki et al., 2004). Rainfall and subsequent run-off deposit dust, gases, toxic chemicals, nutrients such as phosphorus and nitrogen, sediments, animal wastes with faecal coliforms and pathogens, pesticides, petroleum products etc. into water bodies (Lawson, 2011) and may affect the water quality. *Schistosoma* host snails are known to inhabit water with low flow velocity (De Kock et al., 2004). Increased water velocity due to flash flooding impairs snail movement, may dislodge and wash snails downstream, and dissipates snail food (Boelee and Laamrani, 2004). Host snails have been found to tolerate a narrow range of flow velocity of up to 0.3 ms^{-1} (Appleton, 1978).

2.2.1.4 Macrophytes

Aquatic macrophytes are closely associated with the distribution of *Schistosoma* host snails (Ndifon and Ukoli, 1989; Obeng, 1966; Odei, 1973; Ofulla et al., 2013; Woolhouse and Chandiwana, 1989). In Lake Victoria the presence of water hyacinth *Eichhornia crassipes* positively influenced populations of *Biomphalaria sudanica* (Plummer, 2005). The increase may be attributed to the altered environment such as increased available food, oxygen, or shading and a decrease in current velocity or wave action due to presence of water hyacinth.

2.2.2 Human factors

Human-induced factors, such as environmental modification, poor living conditions, inadequate sanitation and water supplies, and poor personal and environmental hygiene leading to environmental pollution are some of the important factors contributing to increased transmission of schistosomiasis (WHO, 1998).

2.2.2.1 Environmental modification

Environmental modification, mainly through water resources development projects such as dams for hydro-power generation, irrigation or ponds for fish culture, has been associated with increased incidence and transmission of schistosomiasis (King and Dangerfield-Cha, 2008). This normally occurs through the creation of new habitats for snails (Berry-Cabán, 2007; Lambert et al., 1985) and the introduction of new sources of disease, or even of new diseases, by immigrants (Chitsulo et al., 2000; WHO, 1985). The impact of water development projects on schistosomiasis disease dynamics are evident in many parts of Africa (Table 2.1) and vary depending on the causative agent involved. Changes in the epidemiological importance of *Schistosoma mansoni* relative to *Schistosoma haematobium,* "the Nile shift" (Jobin, 1999), due to altered environment have been reported (Chitsulo et al., 2000; Steinmann et al., 2006; WHO, 1985). Examples from outside Africa include those in South America (Lardans and Dissous, 1998) and in China following the construction of the Three Gorges Dam (Berry-Cabán, 2007). Global estimates indicate that about 106 million people (13.6% of the total at-risk population) at risk of schistosomiasis live in proximity to large impoundments and irrigation schemes (Steinmann et al., 2006).

Table 2.1: Schistosomiasis prevalence rates in local communities before and after environmental modification. * denotes species not given

Country/Project	Prevalence		Schistosoma Species	Source
	Before	After		
Egypt (Aswan Dam)	2 -11%	44 – 75%	*S. mansoni*	(WHO, 1985)
Sudan (Sennar Dam)	< 1%	21 -45%	*S. haematobium*	(Boelee and Madsen., 2006)
	5%	77 – 86%	*S. mansoni*	
Ghana (Akosombo Dam)	5 – 10%	> 90%	*	(WHO, 1985)
Senegal	0 – 27%	1.9 – 52%	*S. haematobium*	(Steinmann et al., 2006)
	0	4.4 – 72%	*S. mansoni*	
Cote d'Ivoire	0	73%	*	(Steinmann et al., 2006)
Ethiopia	< 20%	82%	*S. haematobium*	(Steinmann et al., 2006)
Zambia (Kariba Dam)	0	34 – 60	*S. mansoni*	(Steinmann et al., 2006)
	Low	16 – 34%	*S. haematobium*	
Puerto Rico	0	40%	*	(Steinmann et al., 2006)
Burkina Faso	5 – 10%	60 – 80%	*	(Boelee and Madsen., 2006)
Proximity of local community to water impoundment				
	Close to dam	**Further away from dam**		
Cameroon	21%	7.8%		(Steinmann et al., 2006)
Madagascar	74%	7.1%		(Steinmann et al., 2006)

2.2.2.2 Agricultural, domestic and industrial waste

Leaching of nutrients and other chemical products from agricultural, domestic and industrial activities pollutes water systems and may cause eutrophication. Eutrophication leads to imbalanced functioning of the aquatic system, causing intense algal growth such as excess filamentous algae and phytoplankton blooms, the production of excess organic matter, increased oxygen consumption and consequent oxygen depletion, and ultimately the death of benthic organisms in anoxic conditions. Increased primary productivity results in improved food, nesting and refugia for host snails, especially for pulmonates which transmit schistosomiasis in the Americas and Africa. McKenzie and Townsend (2007) observed increased abundance of bird schistosomes, causing swimmer's itch, in eutrophic water. Johnson et al. (2007) also give an account of how water eutrophication increased pathogenic infection in amphibians by *Ribeiroia ondatrae*, a water-borne pathogen whose intermediate hosts are planorbid water snails including *Helisoma, Planorbella* and *Biomphalaria* spp. (Pinto et al., 2013). Industrial effluents introduce and/or increase metal and suspended sediment concentration, temperature and lower dissolved oxygen levels in the water. Heavy metals at elevated concentrations are toxic to aquatic biodiversity (Lawson, 2011). Allah et al. (1997) observed decreased survival in both *Schistosoma*-infected and uninfected *Biomphalaria glabrata* exposed to metal pollution.

2.2.2.3 Overfishing

Predatory and competitive mechanisms influence the functioning of aquatic interactions (Figure 2.1). Heavy predation pressure often leads to trophic cascades (Figure 2.2) where predators affect resource dynamics and energy balances in the ecosystem (Greig and McIntosh, 2006). The general decline in aquatic biodiversity due to heavy fishing pressure, as seen in Zambia (ACF/FSRP, 2009; Musumali et al., 2009) and Malawi (Stauffer and Madsen, 2012; Stauffer et al., 2006) could be examples of such trophic cascades. Decreased predation pressure on snails through overfishing, and fish kills from exposure to metals, pesticides and other chemical pollutants may reduce the top-down regulation of the trophic interactions.

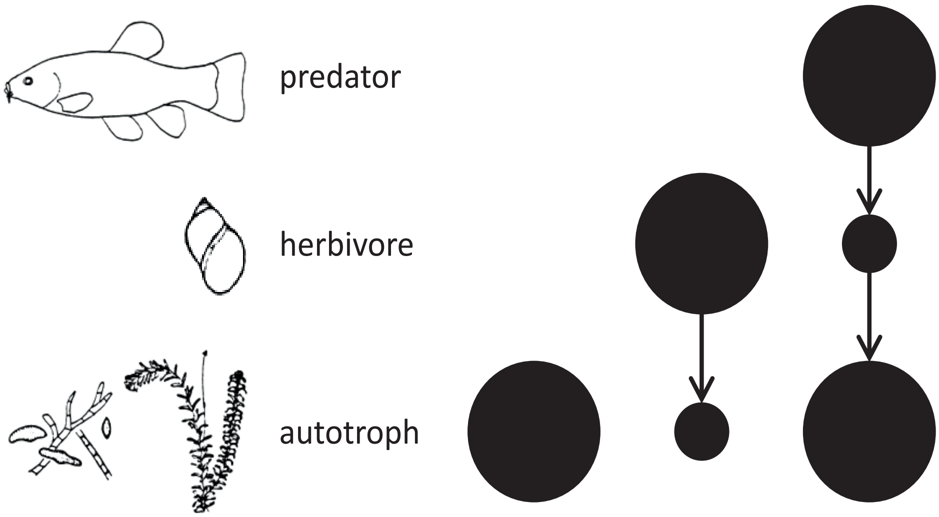

Figure 2.2: Hypothetical food chains for aquatic organisms, illustrating direct and indirect trophic interactions. Algal biomass in food chains with two trophic levels limited by grazing from a higher trophic level (snails), due to top-down regulation. Algal biomass in food chains with one and three trophic levels limited by nutrient availability in the surroundings, i.e. bottom-up regulation

2.2.2.4 Alien species

Many studies have documented the impacts of introducing alien species to new habitats. The impacts can be direct through predation and competition, or indirect through altering the food web (Baxter et al., 2004). The introduction of the Nile perch *Lates niloticus*, a voracious predator, into Lake Victoria, with the consequent disappearance of over 200 native fish species (Strayer, 2010) is perhaps the most conspicuous example. Notwithstanding the improvement of the Lake Victoria fish industry, the Nile perch affected the biodiversity of the lake by preying on the naturally-occurring haplochromines (Achieng, 1990; Ogutu-Ohwayo, 1990), altering the ecology of the lake. Similarly, in the case of molluscs, the introduction of Zebra mussels into the Hudson River, USA, resulted in a drop in phytoplankton biomass by 80% (Strayer, 2010). Snail introductions have similar results as they can invade large areas of the new suitable habitat and become dominant. These cause both negative reactions, through economic damage and disturbance to biodiversity of the area concerned, and beneficial outcomes through their competitive and predatory tendencies towards hosts of harmful parasites such as schistosomes (Pointier et al., 2005)

2.3 Control options through the host-environment interface.

The goal of the World Health Organisation is to eliminate schistosomiasis, at least in some African countries, by 2020 (WHO, 2012). Achieving this requires more than just morbidity control through chemotherapy. Transmission control strategies, including the provision of safe sanitary facilities, control of disease vectors through molluscicides, predators and competitors and environmental management as advocated by the WHO (WHO, 2012), are important. These measures can be targeted at various stages of the schistosomes' life cycle.

2.3.1 Chemical control

The use of chemicals to control intermediate host snails is one of the most important transmission control methods for schistosomiasis (Madsen, 1990; Ojewole, 2004). The synthetic chemical niclosamide is the only commercially available molluscicide applied on a large scale (WHO, 1985, 1993). In combination with other methods in Brazil, Egypt, Ghana, Madagascar, Philippines, Zimbabwe, Tanzania and Venezuela, chemical control successfully reduced snail populations in the short term (McCullough et al., 1980). In Zimbabwe, for instance, a schistosomiasis control programme carried out between 1967 and the early 1980s using molluscicides in Lake Kariba reduced the prevalence of *S. haematobium* from about 54% in 1967 to about 5% in 1985 and similar trends were observed with *S. mansoni* (68% to 8%) over the same period (Chimbari, 2012). Several years after the termination of the programme, results still indicate a lower schistosomiasis rate on the Zimbabwean side of the Kariba than on the Zambian side, where no control measures have been in effect (Chimbari, 2012; Chimbari et al., 2003). Plant-based molluscicides have also been tested in snail control activities. Research on these dates back to the 1930s (Azare et al., 2007; Lambert et al., 1985). Among those found to have molluscicidal effects were the fruits of *Sapindus saponaria*, *Swartzia madagascariensis* and *Balanites aegyptiaca*, the bark of *Entada phaseoloides*, the roots of *Derris elliptica*, the pulp of the sisal plant *Agave sisalana* and the leaves of *Schima argentea* (McCullough et al., 1980). Extracts from *Jatropha curcas* seeds showed high toxicity, with LC_{100} values of 25 ppm for *Biomphalaria glabrata* (Rug and Ruppel, 2000). *Euphorbia conspicua* (dos Santos et al., 2007), *Euphorbia splendens* var. *hislopii* (Massoud et al., 2004; Mello-Silva et al., 2006), *Allium cepa* and *Allium sativa* (Mantawy and Mahmoud, 2002), *Alternanthera sessilis* (Azare et al., 2007) and *Phytolacca dodecandra* (Gwatirisa et al., 1999; Lemma, 1970; Ndamba et al., 1989a; Ndamba et al., 1994) all proved effective under laboratory conditions. *P. dodecandra* has been the most extensively studied plant, and has been proven to be effective in reducing schistosomiasis in many parts of the tropics (Lambert et al., 1985). In Ethiopia, *P. dodecandra* applied in infested streams reduced schistosomiasis prevalence in children by 85% and concentrations of 10-20 ppm remained lethal for 24 hours, but within 48 hours the active compound had degraded and was no longer effective (Lambert et al., 1985). In Zimbabwe *P. dodecandra* applied in natural streams cleared the snails and kept the areas free from snails for seven months (Ndamba et al., 1989a). In Nigeria, *A. sessilis* caused the death of *Bulinus globosus* and snails were virtually absent in ponds where it was part of the riparian vegetation (Azare et al., 2007).

While the use of synthetic molluscicides reduces populations of host snails, limitations on their applicability and success exist. Firstly, getting all snails exposed to the molluscicide may be impeded by the snails' biological or ecological traits, such as the tendency for the planorbids *Biomphalaria* and *Bulinus* to be found in sheltered pools and their tendency to leave the water in response to molluscicide exposure. Secondly, niclosamide and all the tested plant-based molluscicides are not specific in their action to snails. Negative environmental effects on non-target flora and fauna, including fish, have been reported (Azare et al., 2007; Madsen, 1990; Mello-Silva et al., 2006; Okere and Odaibo, 2005). In the case of plant-based molluscicides, little has been done beyond their initial trials. Not much testing has been done in real field conditions to ascertain their long-term toxicological effects (McCullough et al., 1980; WHO, 1985), and producing them in sufficient quantities is a challenge to their usage (Sturrock, 1995). Notwithstanding these constraints, synthetic chemical control is a viable option with real potential to reduce snail populations if applied correctly in the right environment, as happened in Japan, Latin America, Caribbean, Saudi Arabia, Morocco and Tunisia (Tanaka and Tsuji, 1997; WHO, 2009). It is an appropriate strategy in most poor rural communities where health educational strategies may have little impact because contact with contaminated water is necessary for survival. Schistosomiasis is a disease of poverty and about 30 - 40% of the estimated 1.4 billion people living in chronic poverty live in sub-Saharan Africa (Gazzinelli et al., 2012). The burden of disease is also highest in sub-Saharan African communities (Gryseels et al., 2006; Steinmann et al., 2006; WHO, 2002). In these communities the prospects of improved sanitation and safe water supply are not feasible in the short term. Chemical control can also be useful in arid zones where water levels recede markedly during certain times of the year and is found only in seasonal pools. Chemical control is specifically ideal for places where fishing is of negligible significance, due to niclosamide's low toxicity to mammals, macrophytes, zooplankton and phytoplankton (Andrews et al., 1982; Evans, 1983; Goll et al., 1984) and its non-persistence in the aquatic environment (WHO, 2008). Provided control agents ensure minimum levels of technical expertise in identifying the appropriate time for the application of the right dose and application method, mollusciciding can complement chemotherapy and health education in poor communities.

2.3.2 Biological control

The focus of the biological control of host snails has been on the role of their natural enemies including parasites, competitors and predators.

2.3.2.1 Competitor snails

Many snail species have been used to control *Schistosoma* host snails through competitive exclusion. *Marisa cornuarietis*, a prosobranch freshwater snail, competed successfully with *B. glabrata*, a pulmonate snail host in Venenzuela (Pointier and David, 2004). Successive snail surveys showed declining populations of *B. glabrata* in the presence of *M. cornuarietis*, which was attributed to *M. cornuarietis* eating out the macrophyte species which are the preferred habitat for the host snails (Pointier and David, 2004). However, the presence of *M. cornuarietis* did not affect other molluscs such as *Biomphalaria straminea* and *Biomphalaria schrammi* present in the experimental ponds (Pointier and David, 2004). Similar results were obtained in field trials in Tanzania and Sudan (Madsen, 1990). In another study, the presence of *Indoplanorbis exustus* reduced growth rate and fecundity in *Biomphalaria pfeifferi* under laboratory conditions (Okere and Odaibo, 2005). This was attributed to *I. exustus'* superior feeding efficiency over *B. pfeifferi* and the probable removal of essential ions for the growth of *B. pfeifferi*, or the secretion of growth-inhibiting substances (Okere and Odaibo, 2005). The ability of competitors to remove essential growth ions was also seen in experiments involving *Helisoma duryi* with *Bulinus truncatus*, *Biomphalaria alexandrina* (Abdallah and Nasr, 1973) and *Biomphalaria camerunensis* (Frandsen and Madsen, 1979; Madsen, 1979; Madsen, 1983). Also Ayad et al. (1970) observed a 100% mortality rate of *B. truncatus* arising from the competition for food with *H. duryi.* Similar observations were also made in the study of the effect of food competition between *Melanoides tuberculata* and two host snails; *B. straminea* and *B. glabrata* (Pointier, 2001). Other examples include the competition between *Bulinus tropicus* (non-vector) and *B. globosus* (vector) in Zimbabwe, in which *B. tropicus* induced depressed productivity in *B. globosus* and preyed on its eggs (Ndlela and Madsen, 2001). In the Caribbean the use of the competitor snails *Thiara granifera, M. tuberculata* and *M. cornuarietis* resulted in a reduction in populations of host snails (Pointier and McCullough, 1989; Pointier, 2001; Pointier and David, 2004).

2.3.2.2 Predators

Fish, arthropods and waterfowl that feed on host snails have been considered as options for keeping snail populations under control.

Studies involving Muscovy ducks *Cairina moschata* reduced snail populations in night storage ponds in Zimbabwe (Ndlela and Chimbari, 2000). However, due to Muscovy ducks being exotic to Zimbabwe their management was too costly to justify their large-scale usage. Also studied were crayfish *Procambarus clarkii* to assess their potential as both predators and competitors for *B. pfeifferi* in laboratory and field experiments in Kenya. *P. clarkii* not only preyed on *B. pfeifferi* and its eggs (Hofkin et al., 1991), but also fed on *Nymphaea caerulea*, a macrophyte used by host snails for refuge, oviposition and food (Mkoji et al., 1999; Woolhouse and Chandiwana, 1989). Studies involving fish as predators for *Schistosoma* host snails can be traced to the early 1900s. (Slootweg et al., 1994) highlighted the use of *Mylopharyngodon piceus* in Israel, *Lepomis microlophus* in Puerto Rico and several Lake Victoria haplochromine cichlids. In these trials, both *M. piceus* and *L. microlophus* showed a preference for snails over other food items. Among the Lake Victoria haplochromines, *Astatoreochromis alluaudi* is one of the well-studied species and was used in trials in a few African countries, including Cameroon and Kenya. While positive results were obtained in Yaoundé, Cameroon and Nyanza Province, Kenya, *A. alluaudi* did not suppress snail populations in North Cameroon, owing to its low reproductive capacity and its preference for other food items when food was abundant (Slootweg et al., 1994). Similarly, in Malawi, the molluscivorous *Trematocranus placodon* (Evers et al., 2006) and *Metriaclima lanisticola* (Lundeba et al., 2007) proved to be voracious predators of host snails both in the laboratory and the field. In Zimbabwe, Makoni et al (2005) tested the potential of an upper Zambezi cichlid, *Sargochromis codringtonii* and found that it fed on host snails. However, Moyo (2004) argued that adult *S. codringtonii* are not good control candidates because they prey more on prosobranch (non-host) snails than on pulmonate (host) snails. However, Moyo’s view is explained by the fact that *S. codringtonii* prefers deep waters, where prosobranchs are common, while pulmonates prefer shallow shoreline water (ZBCP, 2009). Trials with *Serranochromis mellandi* resulted in reduced snail populations within one month of introduction (Slootweg et al., 1994).

Knowledge of the biology and ecology of the predators and competitors is necessary for success, and also to avoid the negative effects on other ecosystem components. Environmental concerns often arise when introducing species to new areas. For instance, crayfish may disturb spawning sites and prey on the eggs of tilapia, a commercially important species (Loker et al., 1991). They also are not only known to be pests in rice fields for consuming rice seedlings but also can serve as second intermediate hosts for lung flukes of the genus *Paragonimus*, including species known to infect humans (Loker et al., 1991). Predators, including fish, are suitable when the aim is to keep populations in equilibrium. Predatory organisms can seldom eradicate snails because they switch to other food items at low snail abundances (Slootweg et al., 1994). Competitor snails introduced from other habitats, though effective in displacing host snails bring a risk of displacing non-target important species and of introducing new diseases or parasites (Pointier and Giboda, 1999). The danger of competitors becoming hosts themselves is a possibility as they may get infected by the schistosomes (Ben-Ami and Heller, 2001; Lardans and Dissous, 1998). Understanding the ecological requirements of the competitor organisms assists in selection of the best control candidates. In the Caribbean, where host snail control was achieved using competitor snails, it was found that, for optimum results, permanent stable habitats which are shallow and well oxygenated were preferred by thiarid snails (Pointier and McCullough, 1989). The cichlid fishes that have been proposed for use as snail predators in Africa are also important food items for man. Their use in unprotected environments, such as open access water systems that occur in many African rural communities, may not yield positive results because people may catch them for food. However, they may be useful in aquaculture and other water development projects.

2.3.2.3 Parasites

Parasites including schistosomes that affect the fecundity of snails have been tried with little success (Frandsen 1987). Recently, Duval (2015) discovered a pathogen *Paenibacillus glabratella* which causes high mortality in snails and hatching success in snail eggs. However, it is not yet clear whether the pathogen only attacks *Schistosoma* host snails.

2.3.3 Environmental management

Environmental modification by creating barrages often leads to increased incidence and transmission of schistosomiasis. Environmental modification that makes the environment less conducive for host snail proliferation is important in the control of schistosomiasis (Boelee and Madsen, 2006). Snails are often associated with aquatic and sub-aquatic macrophytes which are both their sources of food and shelter (Hamed, 2011). Controlling vegetation growth by lining irrigation canals with concrete or plastic helps to reduce snail habitat. Alternate flooding and drying of water channels, and the filling in of water ditches, reduces potential snail habitat (Boelee and Madsen, 2006 ; Yi et al., 2005). Environmental modification, including the lining of irrigation canals, alternate flooding and drying, drop structures with stilling basins, special off-takes, and duck-bill weirs for flushing and trapping snails, at the Hippo Valley Sugar Estates and Mushandike schistosomiasis control programmes, resulted in lowering the prevalence and intensity of schistosomiasis in Zimbabwe (Chimbari et al., 1992; Chimbari, 2012; Chimbari and Ndlela, 2001). In Egypt, the prevalence of schistosomiasis fell from 60% to below 5% in 2002 due to a combination of chemotherapy and environmental modification (Fenwick et al., 2006). This reduction in prevalence was partly due to changes in irrigation patterns (perennial vs basin irrigation) in the Nile delta, which led to reduction and change in habitat for *S. haematobium (*Barakat*,* 2013). Outside Africa, China is one of the few countries that have managed to reduce the incidence and prevalence of schistosomiasis through a combination of environmental modification and other control interventions (Changsong et al., 2002). This method however is only feasible in manmade systems where proper drainage and environmental engineering are incorporated during construction.

2.4 Conclusion

It is clear from the literature that no single strategy can halt transmission of schistosomiasis by itself. Combating schistosomiasis requires a combination of strategies selected and implemented on a case-by-case basis. Valuable lessons can be drawn from Japan, which managed to eradicate schistosomiasis by affected communities working together with the Government and employing various strategies to combat the disease (Tanaka and Tsuji, 1997), and from Tunisia where schistosomiasis was eliminated through cooperation between locals and Government (WHO 2009). The adoption by the World Health Organization of strategies including both reducing morbidity due to schistosomiasis and transmission control (WHO, 2012a) is a positive step. However, poverty remains the overarching problem in the control of schistosomiasis. Unless serious efforts are made to overcome poverty in sub-Saharan Africa, the burden of schistosomiasis will remain high.

Chapter 3

Natural and human induced factors influencing the abundance of *Schistosoma* host snails in Zambia

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ABSTRACT

Schistosomiasis remains a global public health problem affecting about 240 million people. In Zambia, 2 million are infected while 3 million live with the risk of getting infected. Research and interventions relating to schistosomiasis are mainly linked to disease epidemiology. Malacological and ecological aspects of the disease are superficially understood. Developing effective control measures requires an understanding of interacting environmental and socioeconomic factors of host snails *vis-a-vis* schistosomiasis. Therefore, we examined the influence of environmental and socioeconomic factors on the population dynamics of the host snails *vis-a-vis* schistosomiasis in a large field study. Social data collected through questionnaires included demographic, educational and knowledge of schistosomiasis disease dynamics. Environmental data collected included physicochemical factors, aquatic plants and water snails. Gender ($p < 0.001$) significantly influence livelihood strategies while age ($p = 0.069$) and level of education ($p = 0.086$) have a moderate influence in Zone I (Sinazongwe and Siavonga). In Zone III (Solwezi, Mufumbwe and Zambezi) none of these factors (age, $p = 0.378$; gender, $p = 0.311$; education, $p = 0.553$) play a significant role. Environmental parameters explained 43 and 41% variation in species composition for zones I and III respectively. Most respondents' (52%, 87%) perception is that there are more cases of bilharzia in hot season than other seasons (rainy season 23%, 7%; cold season 8%, 0% and year round 17%, 6%) for Zone I and Zone III, respectively.

Manuscript submitted

3.1 Introduction

Schistosomiasis remains a global health problem in the 21st century with an estimated 240 million people in 74 countries infected, of whom 90% are living in sub-Saharan Africa (Sady et al., 2013). The parasites responsible for human schistosomiasis belong to the genus *Schistosoma* and are dependent on susceptible water snails for their asexual life stages (Boelee and Madsen, 2006). Humans are the primary hosts and transmission between the two hosts takes place in contaminated water (Ojewole, 2004). Schistosomiasis is subject to environmental perturbations such as climatic, ecological, hydrological and social-economic factors (Michael et al., 2010). As such, the management of schistosomiasis transmission requires an understanding of these interacting factors (Monde et al., 2015). Currently, advanced methodologies for predicting the prevalence of schistosomiasis using GIS and remote sensing are being used in different parts of the world including China (Zhang et al., 2013; Zhou et al., 2001) and some African countries (Simoonga et al., 2009). These techniques model disease prevalence based on temperature and vegetation data of the target area (Kristensen et al., 2001). While they provide a convenient way to map large scale (up to 50km radius; Brooker, 2007) environmental preferences of host snails, they do not model small scale local environmental factors such as demographic, educational and socio-economic aspects of affected communities which are significant in transmission dynamics of schistosomiasis (Simoonga et al., 2009). Several survey based studies indicate strong correlation between socio-economic factors and prevalence of schistosomiasis in the Americas, Asia, (Gazzinelli et al., 2006a; Ximenes et al., 2003; Yi-Xin and Manderson, 2005) and Africa (Chandiwana and Woolhouse, 1991; Kapito-Tembo et al., 2009). Similarly, many studies link environmental (physical, chemical and biological) characteristics of the aquatic body to presence of *Schistosoma* host snails (Giovanelli et al., 2005a; Ndifon and Ukoli, 1989). Environmental factors affect the *Schistosoma* host snails' ability to utilize the habitat and herewith their survival and reproductive potential (Domenici et al., 2007). This link to ecosystem conditions for host-parasite interactions (Patz et al., 2000) is what results in heterogeneities in abundance at local environmental level. Changes in these conditions, whether natural or human mediated, influence the population dynamics of parasites and their hosts and hence epidemiological aspects of the diseases they cause (Patz et al., 2000; Vora, 2008). The heterogeneous and complex nature of these interacting factors (Ojewole, 2004; WHO, 1998), partly explain why schistosomiasis is still a global public health problem especially in sub-Saharan African countries like Zambia (Chitsulo et al., 2000).

Like many tropical countries, Zambia has struggled with schistosomiasis for several decades. Out of a population of about 13 million, 2 million are said to be infected with schistosomiasis while 3 million are living in constant threat of infection (ZBCP, 2009). In some rural communities infection rate is as high as 90% of the population (ZBCP, 2009). Knowledge on the prevalence of schistosomiasis in Zambia dates back to 1855 (19^{th} century) during David Livingstone's excursions (Michelson, 1989), but the problem remains unabated in the 21^{st} century. Among other challenges, the lack of research on the ecological aspects of schistosomiasis exacerbates the problem (Monde et al., 2015). Historic and current research and interventions relating to schistosomiasis are mainly related to the epidemiology of the disease (Agnew-Blais et al., 2010; Michelson, 1989; Strahan et al., 2012). Malacological and ecological aspects of schistosomiasis are superficially understood and no snail control interventions have been attempted (Siziya and Mushanga, 1996). This may be attributed to lack of information on the influence of environmental and socioeconomic factors on the population dynamics of *B. globosus* and *B. pfeifferi* vis-a-vie schistosomiasis. Developing an understanding of the influence of environmental and socioeconomic factors on the population dynamics of these host snails *vis-a-vie* schistosomiasis would greatly contribute towards its control. Therefore, we examined the influence of environmental and socioeconomic factors on the population dynamics of the host snails *vis-a-vie* schistosomiasis. Our hypotheses were that:

i. livelihood strategies of adjacent human communities are associated with *Schistosoma* infections,
ii. habitat factors affect populations of host snails,
iii. seasonal variations influences host snail abundance.

3.2 Materials and Methods

3.2.1 Description of the study sites

The study was conducted in two ecologically distinct zones in Zambia; Zone I (Sinazongwe and Siavonga) and Zone III (Solwezi, Mufumbwe and Zambezi). The two zones receive different amounts of rainfall; Zone I receives less than 700 mm while Zone III receives between 1000 – 1500 mm annually (MAFF, 2001). Zone I is prone to drought, and temperature ranges between 10°C – 37°C. Snail habitat is mainly concentrated in seasonal pools and tributaries of large water systems like Kafue, Zambezi and Kariba system. Zone III on the other hand is relatively cooler with temperatures ranging between 6°C – 32°C. It is a high rainfall area and has many perennial water systems. Subsistence agriculture (Ndambo, 2005) and fishing (Mudenda et al., 2005) are the major livelihood strategies in the study areas. In Zone I, small-scale farming (maize) and animal husbandry predominate while small scale-farming (cassava) and fishing are the dominant activities in Zone III. Vegetable gardening is an off season activity common to both zones.

3.2.2 Experimental design

Data collection was divided into two parts namely; questionnaire survey and environmental data collection. Each of these surveys was designed differently.

3.2.2.1 Questionnaire survey

The questionnaire survey was designed to collect social data that have an impact on schistosomiasis dynamics in the study areas. In our survey, all the members of the community who were aged 10 years and above were eligible to participate. The 10 years cut-off point was thought to be adequate to ensure coherent responses. The questionnaire was pre-tested by administering it to ten randomly selected community members and adjustments made accordingly. The questionnaire was designed to collect information ranging from demographic (gender, age, livelihood strategy), educational to knowledge levels of schistosomiasis disease dynamics among the respondents. The questionnaire was administered by the researcher and an assistant who served as translator in some cases. A total of 178 (Zone I, n = 92; Zone III, n = 76) people participated in this survey. Members of households within 1km radius from the water body were eligible to participate.

3.2.2.2 Environmental data

Clinical records obtained from District Health Officials indicating the prevalence of schistosomiasis and communities affected informed the selection of sampling sites. Sampling was done in rivers, streams and/or dams selected by the researcher based on information from members of communities affected by schistosomiasis and own observations by the researcher. Sample plots of 10m x 10m were established in selected portions of rivers, streams, ox-bow lakes and reservoirs. Where the size of the water body did not allow for establishment of 10m x 10m plots, the length of the plot was maintained at 10m while the width was up to the midpoint of the water body. Collection of water samples for determination of physico-chemical parameters, estimation for macrophyte cover and snail surveys were done in these plots.

Physico-chemical Properties

Dissolved oxygen, pH, conductivity, turbidity, temperature, nitrate, phosphate and calcium were measured bi-monthly in-situ from March, 2013 to December, 2013 using an AM-200 Aquaread GPS Aquameter. Water and sediment samples for ex-situ metal analysis were collected into rinsed 500ml polyethylene bottles with polyethylene caps. Each sample was preserved by adding concentrated nitric acid (HNO_3) to bring the pH to > 2 (1.5 ml HNO_3 to 500 ml of sample water) and stored at room temperature. A weak acid extraction method (Nitric Acid-Hydrochloric Acid Digestion) was used to analyse for metals in water and sediment samples (APHA, 1995; Giddings et al., 2001). The flow velocity of water was measured by placing a floating object in the middle of the water body and recording the time it took to drift over a 10m distance. The substratum was visually categorized as gravel, sandy or muddy at all sites.

Aquatic plants

All aquatic plants (macrophytes) were identified based on (Cook, 2004; Gerber et al., 2004) while observation based on the established Likert scale of 0 to 5 was used to estimate percent cover of macrophytes over the area. For this scale, 0 indicated no macrophytes while 5 indicated 100% macrophyte cover. In order to ensure consistency, estimates of macrophyte cover were made by the same person throughout the study.

Snails

Two methods were used to collect live snails: by a gloved hand in shallow water and by using a scoop net where water depth could not permit collection by hand. The net was made of 2 x 2 mm mesh size kitchen sieve that was supported by a metal frame mounted on a 1.5m wooden handle. Search for snails by either way was conducted for 20 minutes at each site. The search team comprised of the same persons all the time. All materials such as boulders, gravel and floating, submerged and emerging vegetation, were searched for snails. Contents of the scoop net were emptied into a tray where snails were identified based on their morphological features (Mandahl-Barth, 1962) and counted.

3.2.3 Data analysis

Two sets of data were analysed namely; social survey, and environmental and biological data in each zone (Zones I & III).

3.2.3.1 Social survey data

Questionnaire responses were coded and entered into SPSS (IBM SPSS Statistics for Windows, Version 22.0) software. Both descriptive and inferential statistics were performed. Descriptive statistics gave an overview of trends in livelihood strategies and water use patterns and were presented as frequencies and percentages. Inferential statistics were performed by multinomial logistic regression with gender, age and educational level as factors. Response variables were livelihood strategies, water use patterns and knowledge levels of disease dynamics.

3.2.3.2 Environmental and biological data

To identify environmental factors (Table 3.1) that act as habitat filters which influence distribution and abundance of aquatic snails (including *Schistosoma* host snails) and to explore how these effects can be quantified, biological and physico-chemical parameters were linked to observed densities of aquatic snails.

Table 3.1: Environmental variables monitored in the field for determination of possible impact on abundance and composition of freshwater snails in Zambia.

Explanatory variables		**Response variables**
System attribute	**Habitat filter**	**Molluscs**
Chemical properties	pH, nutrients (nitrates, phosphates), heavy metals (Cu, Co, Cr, Cd, Pb, Ni) DO, ORP, TDS, EC.	Species of freshwater molluscs (composition & abundance)
Physical properties	Turbidity, colour, flow.	
Substrate	Gravel, sandy, muddy, detritus.	
Thermal Regime	Temperature fluctuations.	
Biological Regime	Competitors, predators, macrophytes.	

Identification of single variables and combinations of variables with high explanatory potential, was achieved by use of a linear regression selection method using RSEARCH procedure in GenStat release 12.1 with sampling date as covariate (Payne, 2007). For all variables, simple single linear regressions were performed with composition and log-transformed (Ln(x+1) abundance of snails as endpoints separately. Each variable was paired with each species (excluding species with very low abundances) and the variables with significant correlation coefficients were selected for forward multiple regression. Two levels of significance were adopted: i) the 5% probability for Type I error ($p \leq 0.05$) and ii) the 10% accepted error probability ($0.05 < p \leq 0.1$) due to possible high levels of variation in sampling sites.

Partial RDA

Partial Redundancy Analysis (RDA) was performed in order to identify factors with the most significant impact on the distribution of snail fauna and their correlation. Physical factors including turbidity, colour of water, flow velocity, type of substrate and chemical factors such as pH, nutrients (nitrates, phosphates), heavy metals (copper, cobalt, chromium, cadmium, lead, nickel) dissolved oxygen, oxygen reduction potential, total dissolved solids and electric conductivity were used as predictor variables for snail abundance and distribution. The significance of these factors in explaining the distribution of the snails was determined using permutation tests under the RDA option, using the sampling months as covariables. A partial RDA was performed which only included the significant explanatory variables ($p < 0.10$) and the sampling months as covariables. Analyses were performed separately for the samples taken from Zone I and III.

3.3 Results

3.3.1 Water contact patterns

Livelihood strategies are significantly ($p < 0.001$) influenced by gender in Zone I but not in Zone III (Figure 3.1, Table 3.2). While more women tend to be small scale farmers, men combine farming and fishing to sustain their families in Zone I. Age ($p = 0.069$) and level of education ($p = 0.086$) have a moderate influence on the livelihood strategies in Zone I. More people with primary education of ages between 20 and 30 years depend on farming for their livelihood instead of fishing. In Zone III, however, none of these factors (age, $p = 0.378$; gender, $p = 0.311$; education, $p = 0.553$) play a significant role.

Table 3.2: Results of multinomial logistic regression analysis. Significant *P* values are indicated by an asterisk (*), moderately significant ones by two asterisks (**).

Response variable	**Predictor variables**	**P-value @95% CI**	
		Zone I	**Zone III**
Livelihood strategy	Age-group	0.069**	0.378
	Gender	<0.001*	0.311
	Level of education	0.086**	0.553
Pattern of river/stream/dam water use	Age-group	0.219	<0.001*
	Gender	0.001*	0.013*
	Level of education	0.079**	0.538
Knowledge of problems due to river/stream/dam water use.	Age-group	0.442	0.185
	Gender	0.632	0.075**
	Level of education	0.040*	0.357

Overall, subsistence farming is the main livelihood strategy in Zones I and III. Fishing which is often the next option for rural communities is dominated by men relative to women in Zone I and Zone III (Figure 3.1).

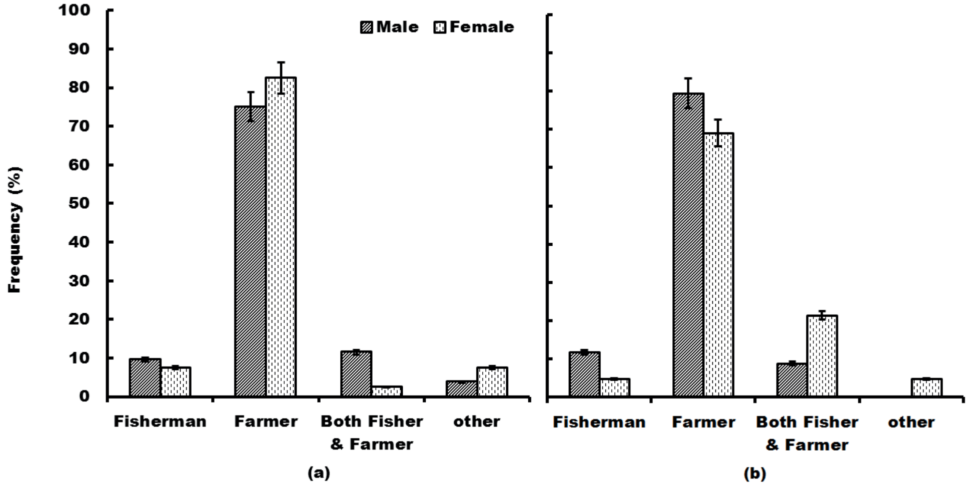

Figure 3.1: Livelihood strategies across gender in Zone I (a) and Zone III (b).

Bathing/swimming, gardening, washing and cassava soaking are the main avenues through which members of the study communities get in contact with possibly *Schistosoma* contaminated water (Figure 3.2). These patterns are gender-dependant in both zones ($p \leq$

0.001, Zone I; p = 0.013, Zone III) (Table 3.2). More women than men access water contact points during domestic chores like washing plates, clothes and soaking cassava for processing into cassava meal. On the other hand, more men than women are exposed through recreational activities such as bathing and swimming and occupational activities (Figure 3.2). Level of education moderately influence stream water use patterns in Zone I with less educated people (primary level) having more contact with stream water. In Zone III, age significantly affects how people get exposed to possibly *Schistosoma* contaminated water (Table 3.2). Younger people (16 – 20) are more likely to get exposed through recreational activities while older people (26 -30) through domestic chores.

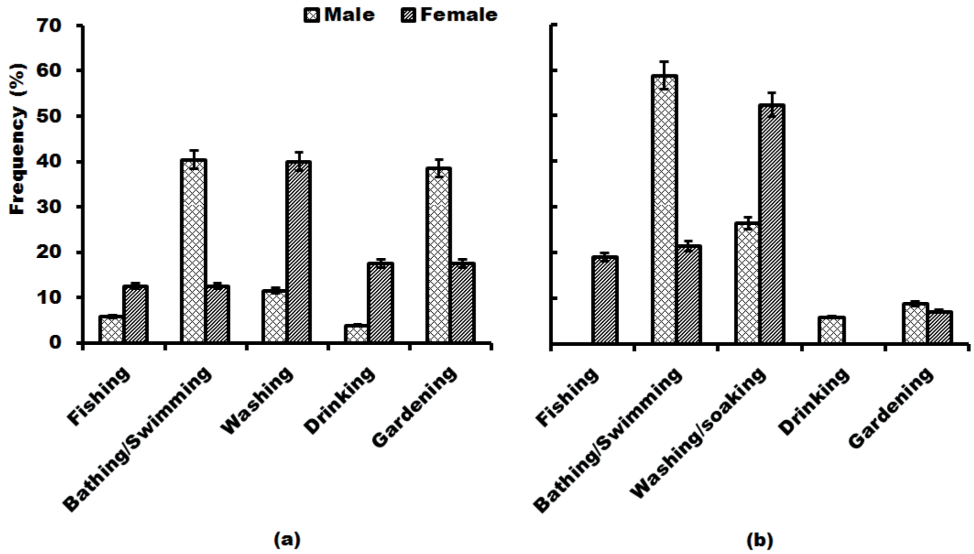

Figure 3.2: Stream water use across gender in Zone I (a) and Zone III (b).

Level of education significantly influenced the knowledge about the *Schistosoma* problem resulting from use of water obtained from streams and dams in Zone I (Table 3.2). Most respondents with some level of education (primary to tertiary) knew that contact with stream/dam water resulted in many health related problems including schistosomiasis. In Zone III on the other hand, gender has a moderate influence on knowledge levels regarding schistosomiasis (Table 3.2). Females are comparatively more aware of waterborne diseases than are males. Overall in Zone I, most of the respondents associate bilharzia to contact with stream or dam water followed by diarrhoea, rush and malaria. Similarly, in Zone III contact with stream/dam water is associated by most people with bilharzia, followed by rush, diarrhoea, worms and malaria (Figure 3.3). Season is perceived to influence the prevalence of schistosomiasis among the community members. Most of the respondents' (52%, 87%) perception is that the hot season has more cases of bilharzia in the communities than in all other seasons (rainy season 23%, 7%; cold season 8%, 0% and year round 17%, 6%) for Zone I and Zone III, respectively.

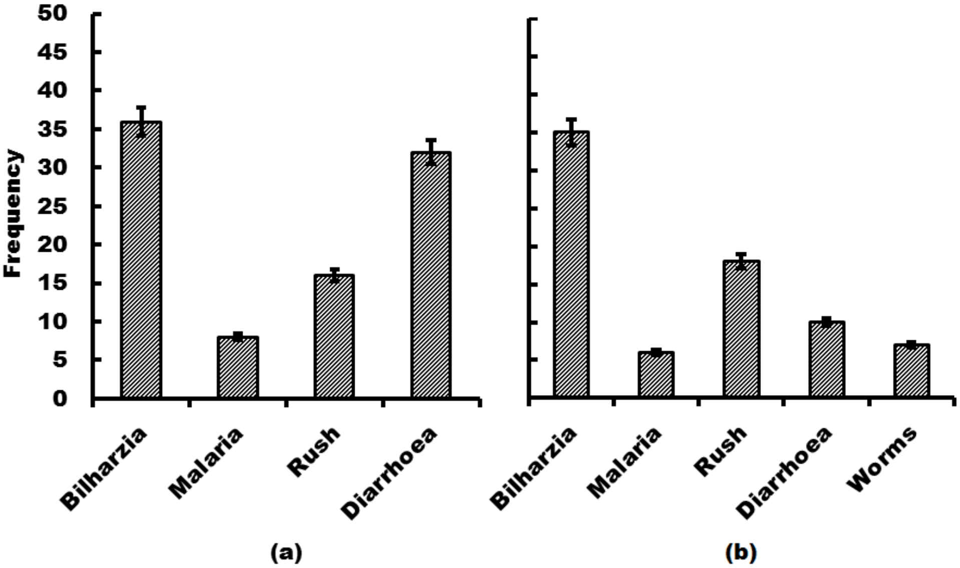

Figure 3.3: Participants' perceived problems associated with contact with contaminated water in Zone I (a) and Zone III (b).

3.3.2 Environmental parameters and snail abundance

Five snail species were collected during four repetitive sampling events in both Zone I and III; four from class pulmonata (*Lymneae natalensis, Bulinus globosus, Biomphalaria pfeifferi, Physa acuta)* and one from class prosobranchia (*Melanoides tuberculata)*. Three of the four i.e. *Lymneae natalensis, Bulinus globosus, Biomphalaria pfeifferi* snail species collected in class pulmonata are of medical and veterinary importance. In both zones, *M. tuberculata* showed the highest density, 93% in Zone I and 42% in Zone III over the whole period, though with a somewhat erratic distribution over the samples especially in Zone I (Figure 3.4). In Zone I, *L. natalensis* accounted for 4% with the highest numbers recorded at the onset of the hot dry season and declining markedly towards the end of the season. *B. globosus* accounted for 2% while *B. pfeifferi* accounted for 1% (Figure 3.4). *P. acuta* was the least abundant with n = 1. In

Zone III, M. *tuberculata* was followed by *B. globosus* with 37%. *P. acuta* was the least abundant with n = 1, while *B. pfeifferi* and *L. natalensis* accounted for 3% and 18%, respectively. In Zone I, pulmonates *B. globosus* and *L. natalensis* generally showed a similar trend with peaks during the hot dry season (August to November). *B. pfeifferi* was abundant during the hot months of the year. In Zone III pulmonates *B. globosus* and *B. pfeifferi* showed a similar trend with peaks during the hot dry season (August to November). Prosobranch *M. tuberculata* on the other hand, was present in sufficient numbers during four sampling events but peaked in the cool dry season (April to August) in both zones (Figure 3.4).

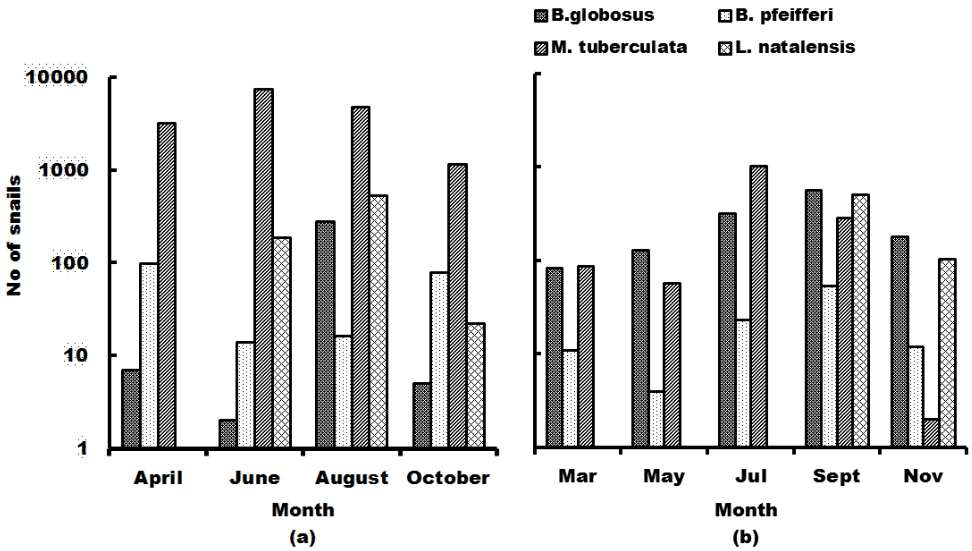

Figure 3.4: Total number of snails collected over time in Zone I (a) and Zone III (b).

NB: *P. acuta* not included in the diagram as it was only found once.

3.3.2.1 Redundancy analysis

The results presented in the RDA biplots (Figure 3.5) show all pulmonate snails recorded are positively correlated with gravel as a substrate and flow velocity in Zone I. Prosobranch *M. tuberculata* is positively correlated with clear water and negatively correlated with cloudy water, EC, TDS and nitrates. *P. acuta* was only found once in both Zones. While in Zone III, the results show marked variability in habitat selection by the pulmonate snails recorded. *B. globosus* is positively correlated with sandy substrate, ORP and turbid water while *B. pfeifferi* is positively correlated with flow velocity, macrophytes and cobalt. M. *tuberculata* is positively correlated with EC, TDS and to a lesser extent calcium.

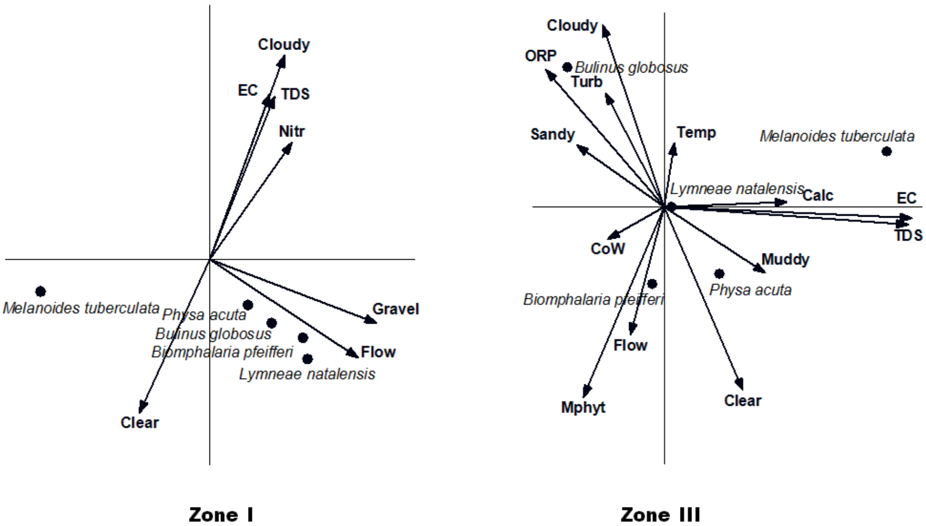

Figure 3.5. Partial RDA biplot showing snail species found in the study sites and their correlation with environmental parameters of Zone I and Zone III. For Zone I, the explanatory variables explained 43% of the variation in species composition, while the first and second axis displays 82 and 14 % of this variation. For Zone III, the explanatory variables explained 41% of the variation in species composition, while the first and second axis displays 52 and 21 % of this variation. Key: Nitr = Nitrates, EC = Electric Conductivity, TDS = Total Dissolved Solids. CoW = Cobalt in water, Calc = Calcium, EC = Electric Conductivity, TDS = Total Dissolved Solids, ORP = Oxygen Reduction Potential, Temp = temperature, Turb = turbidity, MPhyt = macrophytes.

3.3.2.2 Linear regression

Table 3.3 gives results of the single linear regressions between environmental parameters and snail populations in both zones. Parameters with significant ($p \leq 0.05$) correlations were selected for multiple regression analysis in order to come up with factor combinations with the best explanatory power for the distribution and abundance of snails. The positive (+) and negative (-) signs beside the Adj R^2 values represent the direction of relationship.

Table 3.3. Results of single linear regression where each habitat factor is regressed against each snail species showing P-values and adjusted R^2 for Zones I and III.

Zone I

B. globosus			**Bi. pfeifferi**			**M. tuberculata**			**L. natalensis**		
Predictor	**P-value**	**Adj. R^2**	**Predictor**	**P-value**	**Adj. R^2**	**Predictor**	**P-value**	**Adj. R^2**	**Predictor**	**P-value**	**Adj. R^2**
Cd-W	0.001	+ 29.0	Flow	0.001	+ 18.9	Gravel	0.001	- 17.1	Cd-W	0.002	+ 18.1
Flow	0.019	+ 21.3	Pb-W	0.003	+ 14.9	Flow	0.006	- 12.1	Gravel	0.004	+ 15.8
Cd-S	0.024	+ 20.6	Ni-W	0.011	+ 10.5	Nitr	0.017	- 8.6	Flow	0.006	+ 14.7
Phos-S	0.027	-20.3	Gravel	0.016	+ 9.5	Cloudy	0.018	- 8.2	Cd-S	0.027	+ 10.1
Chloro	0.056	+ 18.2	DO	0.037	+ 6.6	TDS	0.033	- 6.3	Chloro	0.075	+ 6.8
Gravel	0.082	+ 17.1	Muddy	0.057	- 5.2	EC	0.041	- 5.6	Cloudy	0.097	-6.0
			Co-S	0.064	-4.8	Clear	0.042	+ 5.5			
			Calc	0.094	+ 3.6						

Zone III

ORP	0.006	+ 17.3	Flow	0	+ 19.4	EC	0	+ 32.7	Co-W	0.002	- 47.6
Cloudy	0.006	+ 17.1	Clear	0.003	+ 8.4	TDS	0	+ 31.2	Turb	0.042	- 42.2
Clear	0.008	- 16.6	Cloudy	0.006	-6.7	Calc	0.002	+ 9.0	Temp	0.054	+ 41.9
TDS	0.037	- 12.8	Muddy	0.025	+ 2.8	Mphyt	0.019	- 4.2	DO	0.069	+ 41.5
EC	0.038	- 12.7	Co-W	0.04	+ 1.7	Muddy	0.08	+ 0			
Turb	0.06	+ 11.6	Turb	0.054	- 0.9	ORP	0.09	- 0			
Gravel	0.06	+ 11.6	Sandy	0.06	- 0.6						
Mphyt	0.08	- 10.9									

Key: Cloudy, clear, turb = condition of water; Gravel, muddy, sandy = substrate type; Mphyt = Macrophytes; Co-W, Pb-W, Ni-W = Metals in water; Cd-S, Co-S = metals in sediment; Flow = Flow velocity; Nitr = nitrates; Phos = phosphates; Chloro = clorophyll-a.

3.3.2.3 Multiple regressions (factor combinations)

For Zone I, seven significant combinations of two parameters were found for *B. globosus*. The combination with the highest explanatory potential was that of metal content (Cd-W) and flow velocity explaining 38% (Adj R^2) of the variance in the population dynamics of *B. globosus.* Followed by another two-way combination of flow velocity and chlorophyll content explaining 32% (Adj R^2) of the variance. For *B. pfeifferi* seventeen combinations were found; ten two-way and seven three-way. The highest variance (Adj R^2 = 41%) was explained by a three-way combination of flow velocity, metal content (Co-S and Pb-W). Metal content in combination with other factors including type of substrate, and levels of dissolved oxygen explain variance ranging from 32% to 39%. For *M. tuberculata* twelve factor combinations were found; eight two-way and four three-way. The three-way factor combination of condition of water (cloudy) flow velocity and type of substrate (gravel) explain 39% (Adj R^2) of the variance. While a two-way combination of flow velocity and condition of water (cloudy) explain 33% (Adj R^2) of the variance. In case of *L. natalensis* nine combinations were possible; seven two-way and two three-way combinations. The highest variance 32% (Adj R^2) was obtained from a two-way factor combination of metal content (Cd-W) and flow velocity. The highest three-way combination (Adj R^2 = 30%) was that of chlorophyll-a, flow velocity and type of substrate (Gravel).

For Zone III, nine factor combinations were found for *B. globosus*. Of these seven were two-way and two were three-way combinations. The highest explanatory potential was from a three-way combination of condition of water (cloudy), Oxygen Reduction Potential (ORP), and type of substrate (gravel) explaining 28% (Adj R^2) of the variance in the population dynamics of *B. globosus.* The second best combination with explanatory potential of 25% (Adj R2) of the variance was a two-way combination of condition of water (cloudy), Oxygen Reduction Potential (ORP). For *B. pfeifferi* eight combinations were found; six two-way and two three-way. Similarly, the highest variance Adj $R^{2\,=}$ 28.4 was explained by a three-way combination of flow velocity, metal content (Co) and type of substrate (muddy). For *M. tuberculata* and *L. natalensis* only two-way combinations were observed to have significant explanatory potential. Electric conductivity and macrophytes explain 43% (Adj R^2) while metal (Co) content and turbidity explain 50% (Adj R^2) of the variance in *M. tuberculata* and *L. natalensis* respectively.

3.4 Discussion

3.4.1 Water contact patterns.

The exposure patterns to *Schistosoma* infection in the study sites include those through domestic, recreational and occupational activities (Figures 3.1 & 3.2). Such exposure patterns were also observed by various researchers in other parts of the world including sub-Saharan Africa (Fenwick et al., 2006; King and Dangerfield-Cha, 2008). However, differences were seen to occur at smaller units such as village groupings. These have important implications to the transmission dynamics of schistosomiasis. In Kenya (Satayathum et al., 2006) and China (Liang et al., 2007) differences in infection risks and rates were observed within climatologically homogeneous region. This heterogeneity was attributed to differences in land use, age and gender structure, and kinship at the village level (Satayathum et al., 2006). Our study also confirms the existence of differences in potential transmission pathways based on gender, age and level of education (Table 3.2). The influence of gender on water use patterns was found to be significant in both zone I and zone III, with the differences being the same for the bathing/swimming and washing activities, but not for the others (Figure 3.2). In both zones recreation activities of bathing and swimming are important exposure pathways for males. While for females, domestic chores including washing of clothes and utensils and soaking of cassava expose them to contaminated water. Similarly, the role of gender in exposure patterns was also reported in Malawi with boys having a higher infection rate than girls due to exposure through swimming and bathing (Kapito-Tembo et al., 2009), in Senegal where females spent more frequent and longer time exposed to contaminated water than males through domestic chores (Sow et al., 2011) and in Zimbabwe with males having higher exposure through recreation activities than women (Chandiwana and Woolhouse, 1991). Age is another important factor influencing exposure patterns. In this study, the effect of age on exposure was significant ($p < 0.001$) in Zone III but not in Zone I. Adolescents and young adults (16 – 25 years) are more likely to swim and/or bath in streams and other open water areas while older people get exposed through domestic and occupational activities. Similarly in Brazil, Gazzinelli et al. (2006) found a positive correlation between age and prevalence of schistosomiasis and adolescents and young adults (15 – 29 years) were most affected. Education is an important tool which governs individual behaviour. In this study, education had a significant ($p = 0.040$) positive correlation with knowledge levels of schistosomiasis in Zone I but not in Zone III. Educated people are more likely to avoid behaviours that put them at risk of infection. Yang et al*.* (2015) concluded that at the individual level, information, education and communication are an essential measure to decrease human exposure to water potentially containing cercariae. The significant impact of education in Zone I can be attributed to the work of the Zambia Bilharzia Control Programme (ZBCP) which has prioritized two Provinces (Southern and Eastern Provinces). Although, the ZBCP's main focus is on acquiring and correctly administering praziquantel, health education using information, education and communication materials is an important element of the programme (Kabatereine et al., 2006). These differences in factors influencing exposure patterns in this study confirm the heterogeneous nature of disease patterns which may also reflect the cultural differences among communities (Huang and Manderson, 1992). In Zone I, for instance, where cattle is a major part of the culture, cattle herding is a risk factor as it involves wading in potentially infested water and is a male activity. This claim is in agreement with Yi-Xin and Manderson (2005) who observed that livestock breeding increases human-water contact activities when they allow their animals to graze near water. In Zone III, on the other hand, soaking of cassava for processing into cassava powder a staple food involves exposure to potential transmission sites over considerable time for females. These cultural differences could model the risk of disease in a community between gender.

3.4.2 Environmental parameters and snail abundance

From the analysis of habitat parameters in both zones, one apparent conclusion is that aquatic snails including hosts of important parasites can tolerate wide variations in their habitat. Therefore, based on these results, it has not been possible to identify a single environmental factor that is a major determinant of host snail population dynamics. Single parameters explained on average a maximum amount of 25% (min - max: 17 - 48%) of the difference in occurrence of the snail species (Table 3.3). Analysis of various factor combinations yielding an explanatory potential of maximally 50%, indicating that many other factors than investigated in this study interact to condition the habitat for host snails. Utzinger et al. (1997) and literature cited therein allude to the fact that it is difficult to isolate a single environmental factor as a major determinant for the distribution of host snails. However, in the present study, pulmonate snails in Zone I seem to have similar habitat requirements with substrate type and flow velocity being important habitat conditioning parameters (Figure 3.5). To the contrary, pulmonates *B. globosus* and *B. pfeifferi* are not influenced by the same factors in Zone III (Figure 3.5). Differences in ecological and climatic conditions between the zones may be responsible for this result. Zone I is a low rainfall area with mostly disconnected water bodies for most of the year. Water movement in these systems is through wave action and was found to be on average $0.01ms^{-1}$. In Zone III, a high rainfall area, the water systems are connected for most of the year with an average flow velocity of 0.03 ms^{-1}. Flow velocity impairs snail movement and dissipate snail food (Boelee and Laamrani, 2004). Appleton (1978) suggested an upper limit of $0.3ms^{-1}$ which is above the flow regime for the study sites. Heavy metal Co is positively correlated with *B. pfeifferi* snail in Zone III. Although many metals are known to be nutrients at low concentration, Pb and Cd have no known biological function and are generally toxic to most organisms (Allah et al., 1997). The positive correlation observed in this study could be as a result of the indirect effects of metals on snails through negatively impacting on the snail parasites and predators. Parasites, like predators may have a regulatory effect on the snails (Brown et al., 1988; Loker et al., 1981). Lefcort et al. (2002) in their study of populations of two closely related pulmonate snails *Physella columbiana* and *Lymnaea palustris* found that heavy metal pollution had a direct negative effect on parasites that resulted in an indirect positive effect on snails. While metals can result in reduced growth rate, reproduction and survival (Allah *et al*., 1997; Factor and de Chavez, 2012) in snail hosts, it is hypothesized in the present study that metals could have had a more depressing effect on snail parasites and/or predators.

3.4.3 Seasonal variations

Although population numbers were not rigorously estimated it was observed that all of the species of medical and veterinary importance exhibited a seasonal trend (Figures 3.4). Snail distribution follow a rhythm in response to seasonal climatic variations (Phiri et al., 2007). Temperature and rainfall are important climatic determinants of snail populations (Appleton, 1978). In the present study, both *Schistosoma* host snails tended to peak around the hot dry season (August to November). Similar results were observed in Zimbabwe (Woolhouse, 1992) for *B. pfeifferi*. A lot of *B. globosus* juveniles were observed during this period unlike for *B. pfeifferi* where mostly juveniles were recorded throughout the study period. Other studies indicate the role of temperature on growth rate, reproductive success (El-Emam and Madsen, 1982; Woolhouse and Chandiwana, 1989) and survival (McCreesh and Booth, 2014b). Water flow velocity is a seasonal factor regulated by rainfall in Zambia. Impact of rain on stream hydrology and consequent flow velocity and snail prevalence have been observed in Kenya (Teesdale, 1962), Tanzania (Marti and Tanner, 1988) and Zimbabwe (Woolhouse and Chandiwana, 1990; Woolhouse, 1992).

3.5 Conclusions

The aim of this study was to provide baseline data on human and environmental factors that influence the prevalence and population dynamics of *Schistosoma* host snails vis-a-vis schistosomiasis in two ecologically distinct zones in Zambia. The focus was twofold covering the social and the physicochemical aspects of host snail population dynamics. We have established from this study firstly that, like in many other studies, rural livelihoods have an impact on the patterning of exposure to schistosomes. While adhering to the general dynamics of schistosomiasis transmission through domestic, occupational and recreational activities, the study has shown local level heterogeneities influenced by culture. Cassava processing and cattle herding are culturally determined exposure patterns that vary between the two zones. Secondly, that although physicochemical parameters including heavy metals, water flow velocity, type of substrate and condition of water seem to have a significant influence, no single environmental parameter is a major determinant for the distribution of host snails. Thirdly, that season has a profound influence on the prevalence of host snails. Therefore, to address the problem of schistosomiasis requires a delicate balance between disease epidemiology and malacology of the disease vectors. The work being reported in this paper forms a baseline in generating social and malacological information which is paramount in addressing schistosomiasis in Zambia. Such information is critical in designing and focussing sustainable control programmes. It highlights habitat conditioning factors including human-water contact patterns that may influence population dynamics of host snails hence schistosomiasis incidence and prevalence. However, the down side of this study is that combining social and environmental aspects did not allow for in-depth examination of anyone of the aspects. But it being baseline it has pointed out areas that need more investigation like:

- The relationship between pulmonate species and prosobranch *M. tuberculata* because *M. tuberculata* was always found not associated with pulmonate species. This would be important because there are conflicting results from other studies (Giovanelli et al., 2005b; Mkoji et al., 1992; Ndifon and Ukoli, 1989; Pointier et al., 1991) regarding the predatory and competitive nature of *M. tuberculata*.
- The breeding patterns of the host snails in these areas. This information can help in planning intervention measures.
- The effect of rainfall on the snail populations. This was not accounted for in this study due to logistical constraints.

Chapter 4

The potential of using the red claw crayfish (*Cherax quadricarinatus)* and African catfish (*Clarias gariepinus/Clarias ngamensis* hybrid) as biological control agents for *Schistosoma* host snails

Concillia Monde, Stephen Syampungani, Paul J. van den Brink.

Abstract

We evaluated the potential of the red claw crayfish and African catfish hybrid as predators for *Schistosoma* host snails. This was done by monitoring the consumption of snails by crayfish and catfish in experimental tanks over a period of time under laboratory conditions. After 15 days, both crayfish and catfish significantly reduced the population of B*. globosus.* Crayfish was a slightly more efficient predator with a feeding rate of 6.9 snails per day than catfish with a rate of 5.9 snails per day. However, when supplied with an alternative prey (*M. tuberculata*) organism, crayfish had a clear preference for *M. tuberculata* (100% consumed over 7 days) over *B. globosus* (54% consumed over same period). Catfish on the other hand did not have a clear preference for any prey (consuming 77 and 88% of *M. tuberculata* and *B. globosus* respectively). In the case of catfish we found that small catfish are more efficient predators than the larger ones. This is due to the ontogenetic shifts in diet of the catfish as they mature becoming more filter feeders feeding on zooplankton than on larger prey. This study has shown that hybrid catfish retains the molluscivorous characteristics of the parent stock and that like *P. clarkii*; *C. quadricarinatus* also preys on *Schistosoma* host snails. However, effectiveness of both predators is affected by presence of an alternative prey. Therefore, these species can be considered for biological control of schistosomiasis transmission under suitable conditions.

Manuscript submitted

4.1 Introduction

Vector and intermediate host control is among the strategies advocated by the World Health Organisation (WHO) in the fight against Neglected Tropical Diseases like schistosomiasis (WHO, 2012). Chemical, environmental and biological methods are used to control populations of host snails in schistosomiasis control programmes. However, chemicals tend to be expensive and pose environmental concerns including killing non-target organisms (Okere and Odaibo, 2005). Environmental modifications that render the habitat unsuitable for colonization by host snails are important but their applicability is restricted to manmade facilities where proper drainage and environmental engineering are incorporated during construction (Monde et al., 2015).

Biological control using natural enemies of host snails such as predators, parasites and competitors has been proposed for possible regulation of the populations of host snails. For instance, using fish, arthropods and waterfowls as control agents for schistosomes has long been ascertained as in the case of the cichlids *Trematocranus placodon* and *Metriaclima lanisticola* in Malawi (Evers et al., 2006; Lundeba et al., 2007; Madsen et al., 2004; Stauffer et al., 1997) and several cichlids of Lake Victoria in East Africa (Slootweg et al., 1994). A study on the potential of the Green Bream *Sargochromis codringtonii*) as a biological control organism performed in Zimbabwe (Moyo, 2004) further elucidates this point. However Moyo (2004) observed that the diet of this species was dominated by prosobranch (non-host) snails than by pulmonate (host) snails because the fish's feeding habitat coincided with the habitat for prosobranch snails rather than that of pulmonate snails. These findings are consistent with Makoni et al. (2005) who concluded that the use of *S. codringtonii* for controlling snails that transmit schistosomiasis should, therefore, be recommended for small irrigation ponds (night storage ponds) where the fish's habitat coincides with that of the snails. Other than fish, crayfish *Procambarus clarkii* has also been tested to assess its potential both as predator and as competitor for *Biomphalaria pfeifferi* in laboratory and field experiments in Kenya (Hofkin et al., 1991). Results from these experiments show that *P. clarkii* did not only prey on *B. pfeifferi* and its eggs but also fed on *Nymphaea caerulea*, a macrophyte used by snails for refuge, oviposition and food (Hofkin et al., 1991). However, although the use of *P. clarkii* shows promising results in the control of snail hosts, it is destructive to the environment due to its burrowing tendencies (FAO, 2011) causing erosion and turbidity of the water. This characteristic makes it undesirable for use in water control and aquaculture structures. Table 4.1 gives a summary of studies on predator-prey interactions of host snails and results obtained mainly in Africa. From these studies all the predatory organisms used reduced the populations of host snails under specific conditions.

Table 4.1: Summary of studies on snail predation.

Predator	Prey	Type of test	Source	Results
Birds:				
Cairina moschata	*Biomphalaria pfeifferi, Bulinus globosus*	Pond	(Ndlela and Chimbari, 2000)	Populations of both host snails reduced in ponds where muscovy ducks were introduced
Crustaceans:				
Macrobrachium rosenbergii	*Biomphalaria glabrata, Biomphalaria tenagophila, Biomphalaria glabrata*	Lab	(Lee et al., 1982; Roberts and Kuris, 1990)	Reduced populations of both snails at a feeding rate 2.65-3.5% of body weight per day.
Macrobrachium vollenhovenii, *Macrobrachium rosenbergii*	*Biomphalaria glabrata*	Lab	(Sokolow et al., 2014)	Eat snails, hatchlings and eggs at a feeding rate of 12% of body weight per day.
Procambarus clarkii	*Biomphalaria alexandrina, Bulinus truncatus, Limnaea natalensis, Physa acuta, Bellamya unicolor, Cleopatra bulimoides, Melanoides tuberculata, Lanistes carinatus*	Lab	(Khalil and Sleem, 2011)	Had a preference of *B. alexandrina, P. acuta, L. natalensis* and *B. truncatus* over other snails and other food items.
	Bulinus Africanus	Field observ.	(Mkoji et al., 1999)	Populations of *B. africanus* reduced in places where *P. clarkii* was introduced.
	Biomphalaria pfeifferi, Biomphalaria glabrata	Lab	(Hofkin et al., 1992)	Consumed both snails and their eggs.
	Biomphalaria pfeifferi	Field exp.	(Hofkin et al., 1991)	Field enclosure experiments indicate that crayfish exert a significant negative impact on the abundance of *B. pfeifferi*.
Fish:				
Trematocranus placodon	*Bulinus nyassanus, Melanoides spp, Bellamya spp.*	Field observ.	(Evers et al., 2006)	There were more *B. nyassanus* in the stomachs of *T. placodon* than *Melanoides* spp. which are more abundant in the field.
			(Madsen and Stauffer, 2011)	Madsen and Stauffer observed a negative effect of *T. placodon* on densities of *B. nyassanus* and *Melanoides* spp.
Metriaclima lanisticola	*Bulinus nyassanus, Bulinus globosus, Bulinus africanus*	Lab	(Lundeba et al., 2007)	Eat all the three species of snails offered by orally shelling them from the shells.
Sargochromis codringtonii	Various snails	Field observ.	(Moyo, 2004)	More prosobranch snails were found in the stomachs of sampled *S. codringtonii*.
	Bulinus globosus, Bulinus tropicus, Biomphalaria pfeifferi, Lymnaea natalensis, Melanoides tuberculata.	Field exp	(Makoni et al., 2005)	At the end of the experiment the density of *B. globosus* was 150.5snails m^2 in the exclosures and 4.7snails m^2 in the control areas. The other snail species showed the same trend, but the differences were less pronounced.
			(Chimbari et al., 2007)	Had minimal impact on snails which could be attributed to low fish population sizes and the relatively short period of observation.
Clarias gariepinus	*Biomphalaria pfeifferi*	Lab	(Gashaw et al., 2008)	Fish fed on the snails and reduced its egg-laying potential.

N.B: Only studies of the past 20 years in Africa involving fish are reported. For earlier studies is referred to Slootweg, 1994.

In this paper we studied the potential of another species of crayfish, the invasive Australian red claw crayfish *Cherax quadricarinatus* as predators for *Schistosoma* host snails. The red claw crayfish is a non-burrowing species (Wingfield 2002) but no studies have been conducted to ascertain its molluscivorous tendencies towards *Schistosoma* host snails. It is worth noting also that *C. quadricarinatus* has invaded most aquatic systems in many African countries (Harlioğlu and Harlioğlu, 2006) and owing to their invasive nature and tolerance to wide environmental conditions (FAO, 2011) any hope of eradicating them is not feasible. Additionally, crayfish are not considered important food items for humans by some African countries' standards (Lodge et al., 2012; Loker et al., 1991). We also studied the potential of hybrid African catfish *Clarias gariepinus* X *Clarias ngamensis* in host snail control because, to date, studies on the biological control of *Schistosoma* host snails in most African countries using fish have focused more on the use of cichlids. Fish, especially cichlids are important food items in the diets of most people in sub-Saharan Africa (Béné and Heck, 2005). It is the most readily available and affordable source of animal protein for rural and poor urban and peri-urban dwellers (Béné and Heck, 2005). Therefore, the use of these fishes as biological control agents is compromised by their demand for human consumption. In Malawi, Stauffer et al. (2006) observed a negative correlation between populations of host snails and abundance of molluscivorous cichlids. The reduced density of *T. placodon* due to overexploitation for food led to the increase in host snail abundance. Hybrid catfish were chosen firstly, because catfish are less demanded for relative to cichlids in sub-Saharan African (El-Sayed, 2013) and secondly, to ascertain whether hybrids retain the molluscivorous characteristics of the parent stock because more and more hybrids are being released into fish ponds and adjacent streams (Bbole, 2015; pers. Com). Additionally, no studies have investigated the effect of the size of catfish on its feeding efficiency. The objectives of this study therefore, were to:

1. assess whether hybrid catfish retain the molluscivorous characteristics of the parent stock.
2. assess whether the Australian red claw crayfish feed on *Schistosoma* host snails.
3. evaluate the effect of alternative prey on predation of host snails.
4. assess whether the size of the predator has an effect on the rate of predation.

4.2 Materials and methods

Indoor tank experiments were performed at the National Aquaculture Development and Research Centre (NADRC) in Mwekera, Zambia. Mwekera is located on the Copperbelt Province about 26 kilometres east of the Kitwe City centre.

4.2.1 Biological material

Catfish (*Clarias gariepinus/Clarias ngamensis* hybrid) were collected from outdoor ponds at the NADRC. African catfish are native to water bodies in many African countries including Zambia. The hybrids used in this experiment are a result of the hybridization programme being spearheaded at the National Aquaculture and Development Centre of the Fisheries Department. The aim of this hybridization is to increase the disease resistance and increase the size of the fish (Bbole 2015; Pers.com). These hybrids are supplied to fish farmers who raise them in small fish farms and aquaculture facilities which also often serve as breeding sites for host snails in countries where schistosomiasis is endemic (Chiotha, 1994). Fish of 250 to 600g in weight were collected by draining the ponds and scooping with a scoop net made of 2 x 2 mm mesh size sieve that was supported by a metal frame mounted on a 1.5m wooden handle. These were collected into plastic buckets and immediately transferred into 100L storage tanks at a stocking density of one fish per tank. Prior to experimentation, the fish were fed on commercial fish meal (pellets of 30 % crude proteins, 12 % crude fat) twice a day at 4% body weight and left to acclimatize to laboratory conditions for 10 days.

Crayfish- Freshwater crayfish are not native to Africa (Mikkola, 1996) but were introduced to African countries mainly for economic purposes (Harlioğlu and Harlioğlu, 2006). *P. clarkii* was introduced into east African countries, Uganda and Kenya between 1966 and 1970 with the aim of broadening the range of commercial fisheries (Foster and Harper, 2007). This species has spread into many Kenyan water bodies and is believed to have been translocated into Zambia and Sudan (Mikkola, 1996) and possibly other neighbouring countries like Rwanda and Burundi (Foster and Harper, 2007). Apart from *P. clarkii*, three Australian crayfish species from the genus *Cherax* have also been introduced to South Africa and consequently spread to other Southern African countries including Swaziland (Du Preez and Smit, 2013). *C. quadricarinatus* one of the three crayfish native to Northern Australia and Papua New Guinea (Horwitz, 1990), was first introduced into South Africa in the Orange Free State for Aquaculture purposes (Mikkola, 1996). This species together with the other two *Cherax albidus* and *Cherax tenuimanus* were then introduced into Zambia from South Africa through a farmer known as

Grubb near Livingstone in 1992 (Mikkola, 1996; Thys van den Audenaerde, 1994). It is believed to have escaped from a farm in Kafue into the Kafue river where it has established feral populations (Tyser and Douthwaite, 2014). Marufu et al. (2014) and Nakayama et al. (2010) show evidence of the presence of *C. quadricarinatus* also in Lake Kariba on the Zambezi River system.

Crayfish (*C. quadricarinatus*) were collected along the Kafue river in Chikankata District of Southern Province, Zambia. Traps made of wire and nylon mesh were baited with nsima (thick starchy porridge made from corn) and set up in strategic points overnight and checked in the morning for trapped crayfish. The collected crustaceans were transported to NARDC in moist hessian sacks. At NARDC the crayfish were maintained in transparent plastic tanks of 1000 L capacity with continuously aerated borehole water. The crayfish were fed on fingerlings and left to acclimatize to laboratory conditions for 10 days.

Snails (*Bulinus globosus* and *Melanoides tuberculata* were collected from ponds at NADRC. These were picked by gloved hands and kept in plastic tanks. Raw lettuce was provided as feed for snails *ad libitum*.

4.2.2 Experimental set up.

Indoor tank experiments were performed at the National Aquaculture Development and Research Centre (NADRC) in Mwekera, Zambia. Mwekera is located on the Copperbelt Province about 26 kilometres east of the Kitwe City centre.

4.2.2.1 Predation experiment on B. globosus

All the experiments performed followed a completely randomised design. For the experiment to assess the feeding rates of the catfish and the crayfish on *B. globosus*, fifteen transparent plastic tanks of 50cm x 70cm x 50cm were filled with 30L of water and 2cm of clean fine sand as sediment. Water was exchanged among all the tanks to ensure uniformity of conditions prior to the introduction of snails. One hundred snails of sizes ranging between 9 – 15mm were placed in each of the tanks and left to acclimatize for 48 hours. Three treatments; T0 = control, T1 = Crayfish, T2 = Catfish were assigned to the experimental tanks containing snails at random using the lottery method resulting in 5 replicates for each treatment. One specimen (300 ± 5g weight for catfish and 4 ± 1cm length for crayfish) was placed in each tank for T1 and T2 while no predator was added in T0 as it served as a control. Observations (snail counts) were made every 24 hours for fifteen days. Water in the tanks was changed every other day by syphoning 50% and filling with fresh borehole water whilst ensuring continuous aeration.

4.2.2.2 Prey selection experiment on host snails

Two snail species *B. globosus* and *M. tuberculata* were used to assess prey preference by the catfish and the crayfish. Each tank was stocked with 100 *B. globosus* and 100 *M. tuberculata.* Three treatments; T0 = control, T1 = Crayfish, T2 = Catfish were assigned to the experimental tanks containing snails at random using the lottery method resulting in 5 replicates for each treatment. One specimen (300 ± 5g weight for catfish and 4 ± 1cm length for crayfish) was placed in each tank for T1 and T2 while no predator was added in T0 as it served as a control. Observations (snail counts) were made every 24 hours for eight days. Water in the tanks was changed every other day by syphoning 50% and filling with fresh borehole water whilst ensuring continuous aeration.

4.2.2.3 Experiment on the effects of the size of the predator

To assess the effect of predator size on the predation rate, fifteen transparent plastic tanks of 50cm x 70cm x 50cm were filled with 30L of water. Each tank was stocked with 100 *B. globosus* snails with an average size of 10.2mm. Each tank was supplied with 2g of raw lettuce as food for snails. Five catfish of average weight 250g were placed in five tanks and another five catfish of average weight 600g in another five tanks. Five tanks were left as control containing only snails and no predator. The catfish were starved for 48 hours prior to the experiment. Observations (snail counts) were made every 24 hours for 96 hours. Water in the tanks was changed after 48 hours by syphoning 50% and filling with fresh borehole water whilst ensuring continuous aeration.

4.2.3 Statistical Analysis

The predation rate for *B. globosus* by crayfish and catfish and the effect of an alternative prey organism (*M. tuberculata*) was determined by Generalized Linear Models (GLMs) using GenStat 11th (VSN International Ltd., Oxford, UK). GLM was selected because the response variable involved discrete count data of residual snails. The GLM analysis was performed on residual snail abundance at each sampling day after adapting the model to the data distribution type for both the predation and prey selection experiments. The binomial distribution with a logit link function was adopted. For the prey selection experiment, after establishing the effect of crayfish, catfish and alternative prey using the GLM, we further performed 2- sample t-tests. The t-tests assessed whether each of the predators had a significant preference between the two preys. To assess the effect of the size of the predator (catfish) on predation of *B. globosus* we compared the means of the three treatments using ANOVA. The response variables for both the prey selection and the effect of size experiments were arcsine transformed prior to analysis. Tukey's honestly significant difference (HSD) was used to separate the means of the three treatments. All the tests were considered statistically significant when the resultant *p*-values were < 0.05.

4.3 Results

4.3.1 Predation experiment on B. globosus

At the end of the experiment (day 15), there were on average more snails consumed in both the crayfish and catfish tanks relative to the control (Figure. 4.1; Table S4.1). Overall, crayfish had a slightly higher rate of predation (*B. globosus* = 6.9/day, R^2 = 99.3%) than catfish (*B. globosus* = 5.9/day, R^2 = 96.8% while the rate of (natural) mortality in the control tanks (*B. globosus* = 1.91/days, R^2 = 95.8%) was significantly lower than those observed in both catfish and crayfish tanks.

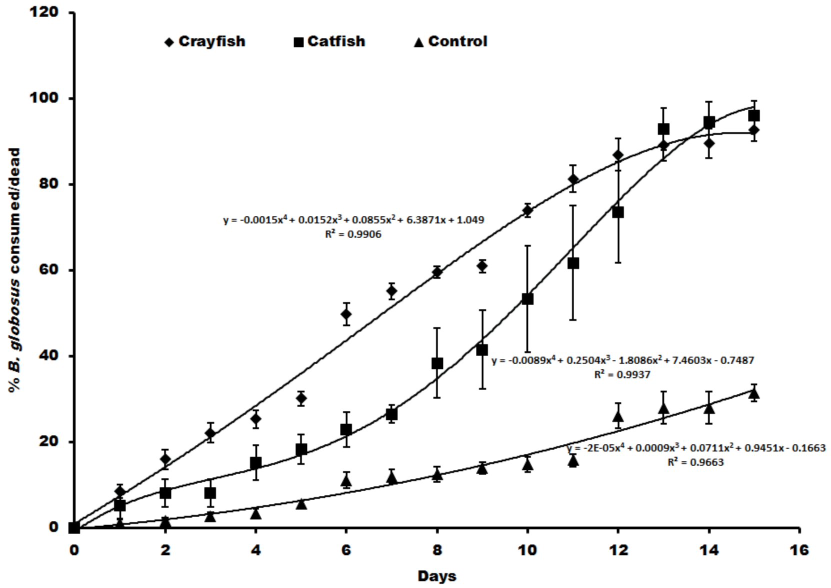

Figure 4.1: Mean daily consumed snails for the three treatments with standard error.

The daily differences in snail predation between experimental groups (crayfish, catfish and control) were tested using a Generalized Linear Model. There were significant differences in residual snails between crayfish tanks and the controls on all days. For catfish, there was no significant difference for days 1 and 3 while the rest of the days showed significant difference from the control group (Table 4.2).

Table 4.2: Results of GLM analysis showing the daily coefficients of estimate and their associated *p*-values for each predator against the control group. Coefficients in brackets show the daily rate of predation by the two predators. Non-significant *p*-values are in bold.

Predator	Coefficient/*p*-value							
	d1	d3	d5	d7	d9	d11	d13	d15
Crayfish	(-2.047) < 0.032	(-2.293) < 0.001	(-1.987) <0.001	(-2.201) <0.001	(-2.263) <0.001	(-3.149) <0.001	(-3.056) <0.001	(-3.329) 0.001
Catfish	(-1.508) **0.112**	(-1.132) **0.068**	(-1.335) <0.001	(-0.977) <0.001	(-1.476) 0.003	(-2.154) 0.004	(-3.531) <0.001	(-3.950) <0.001

4.3.2 Prey selection experiment on host snails

For the alternative prey experiment, daily *p*-values for effect of predator, alternative prey and their interaction on predation of *B. globosus* are given in Table 4.3. The effect of both the species of the predator (crayfish and catfish) and alternative food (*M. tuberculata*) was significant on all days. However, their interaction was non-significant on the first two days. At the end of a period of seven days of observation the crayfish had consumed 54% of *B. globosus* and 100% of *M. tuberculata*. The catfish on the other had consumed 88% of *B. globosus* and 77% of *M. tuberculata* (Figure 4.2).

Table 4.3: Daily *p*-values showing the effect of predators, alternative prey and their interaction on predation of *B. globosus*. Non-significant *p*-values are in bold

FACTOR	DAYS/ *p*-value						
	1	2	3	4	5	6	7
Predator (Catfish/Crayfish)	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001	< 0.001
Alternative prey *(M. tuberculata)*	0.002	0.008	< 0.001	< 0.001	< 0.001	0.003	0.002
Interaction (Pred x Alt. prey)	**0.140**	**0.195**	0.008	0.002	0.001	< 0.001	< 0.001

Two sample t-test gave significant differences in prey selection for crayfish but not for catfish. Crayfish showed a significantly higher (T = 6.04, DF = 57, $P < 0.001$) affinity for *M. tuberculata*. On the other hand although statistically not significant (T = -1.58, DF = 76, $P = 0.119$) catfish consumed slightly more *B. globosus* than *M. tuberculata* (Figure 4.2; Table S4.2).

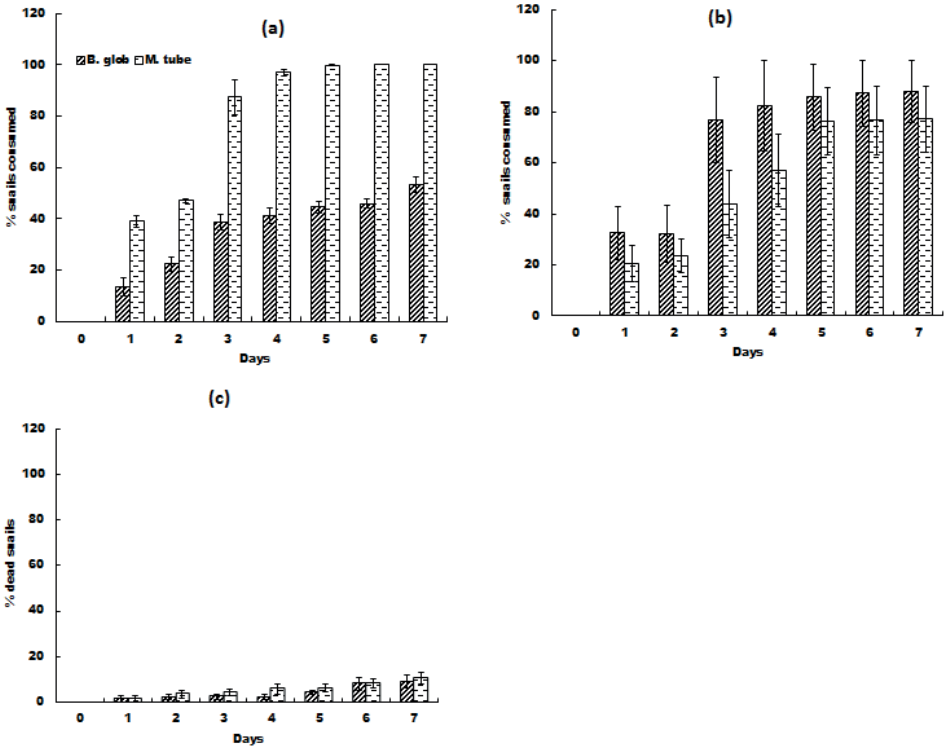

Figure 4.2: Prey selection by two snail predators; (a) – crayfish, (b) – catfish and (c) – no predator control.

4.3.3 Experiment on the effects of the size of the predator

We observed differences in feeding rates between different sized catfish. Smaller catfish (250g) were more active predators than their larger (600g) counterparts and consumed all the snails (n = 100) available while larger catfish still had snails remaining over a 96 hours period. For small catfish active feeding occurred within the first 24 hours with about 90.2% of the prey eaten up while larger catfish only ate 32% within the same period (Figure 4.3). However, there was a marked increase in feeding rate by the larger catfish beyond the 72 hours threshold.

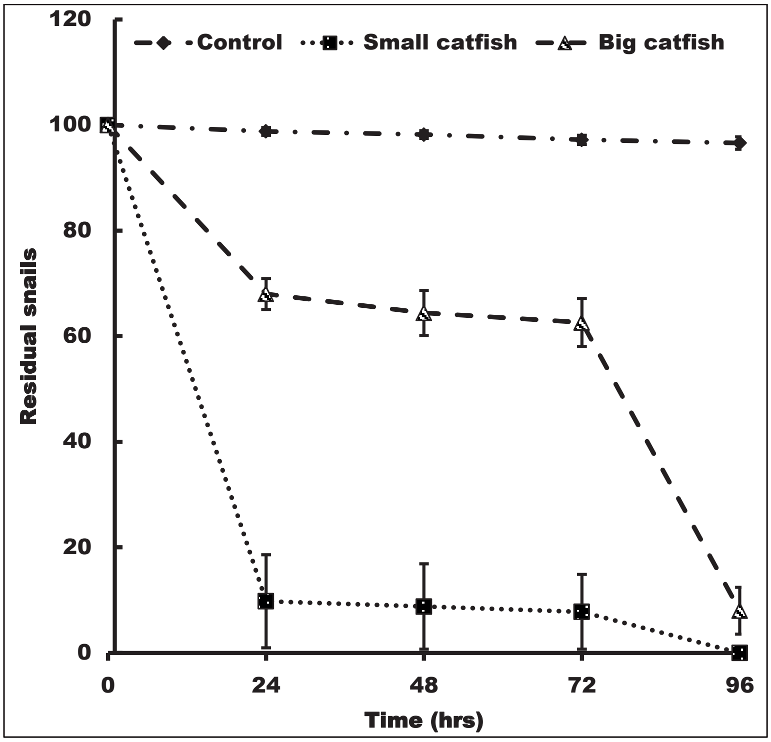

Figure 4.3: Effect of predator size on snail predation by catfish.

ANOVA showed that the difference in residual snails between the control group (*n* = 100, $\bar{x}$ = 98.6), the small catfish group (*n* = 100, $\bar{x}$ = 11.8) and the large catfish group (*n* = 100, $\bar{x}$ = 54.1) were statistically significant, $F_{2, 12} = 33.5$, $p < 0.001$. Tukey's HSD tests showed that both experimental groups scored statistically significantly higher than the control group and the two experimental groups also differed with each other significantly. The 95% CI for these treatments were (97.3, 99.0), (8.6, 41.9) and (47.7, 73.4) for the control, small catfish and large catfish respectively.

4.4 Discussion

4.4.1 Crayfish/catfish predation on *B. globosus*

We evaluated the potential of using the hybrid catfish and the red claw crayfish in the biological control of *B. globosus* a snail host of *Schistosoma haematobium* (Bilharz, 1852). In this study, we found that the red claw crayfish *C. quadricarinatus* readily fed on *B. globosus*

(Figure 4.1 and 4.2) at a daily average feeding rate of 6.9 snails. Studies on the predatory tendencies of crayfish towards aquatic snails including vectors of important parasites affecting humans like *Schistosoma* have been done in some parts of Africa (Table 4.1). For example, in Kenya the North American red swamp crayfish *P. clarkii* was used to control *B. pfeifferi* an intermediate host for *Schistosoma mansonii* (Hofkin et al., 1991) and it induced a significant negative impact on populations of *B. pfeifferi* in both laboratory experiments and field observations. The crayfish was found to feed on both the snails and their eggs. In another field study, still in Kenya, there was a substantial reduction in *Bulinus africanus* (Krauss, 1848) snail populations (a host for *S. haematobium*) in water bodies where crayfish *P. clarkii* established compared to places where either it failed to establish or it was not introduced (Table 4.1) (Mkoji et al., 1999). Similarly, in a study conducted in Egypt there was a complete wipe out of or a significant reduction in *Schistosoma* and *Fasciola* host snails in canals in which red swamp crayfish were present, compared to those where crayfish were not present. Laboratory experiments also indicated a significant negative correlation between presence of crayfish and abundance of host snails (Khalil and Sleem, 2011). Kreps et al. (2012) found evidence of reduced snail abundance in the presence of rusty crayfish *Orconectes rusticus* in a large scale study over time (Table 4.1). *C. quadricarinatus* are widely known as detritivores (Jones, 1989; Loya-Javellana et al., 1993) but our finding confirms the argument that *C. quadricarinatus* are polytrophic feeders that can occupy different levels in the aquatic food chain (Saoud et al., 2012). We have also demonstrated that, like *P. clarkii*, *C. quadricarinatus* preys on *B. globosus* a *Schistosoma* host snail under laboratory conditions. However, unlike *P. clarkii, C. quadricarinatus* is not known to engage in destructive burrowing (FAO, 2011) a tendency which can result in damage to levees, dams, drainage systems and water control structures (De Moor, 2002). This gives it an advantage over *P. clarkii* for use as a control agent in aquaculture and water development facilities. Additionally the non-aggressiveness and non-territorial tendencies of *C. quadricarinatus* (Jones, 1989) makes it a more suitable species in aquaculture than the aggressive and territorial *P. clarkii* (FAO, 2007-2015).

Like in the case of red claw crayfish, hybrid catfish were able to significantly reduce the population of *B. globosus* at a daily average feeding rate of 5.9 snails relative to the control groups (Figure 4.1, 4.2 and 4.3). This implies that the hybrid catfish retains the molluscivorous characteristics of the parent stock. Catfish *C. gariepinus* are known to reduce snail populations by either directly feeding on the snails or negatively impacting their fecundity by eating out the eggs or disturbing the snails' sponying areas (Gashaw et al., 2008). In the present experiments, hybrid catfish consumed on average 96 *B. globosus* in 15 days (Table S4.1) compared to 15 *B.*

pfeifferi in 16 days by *C. gariepinus* under laboratory conditions in Ethiopia (Gashaw et al., 2008). These differences can be attributed to the differences in the experimental design, where as in the present study no supplementary feed was provided for the catfish unlike in the Ethiopian study, the size of catfish- 300 ± 5g this study against 163 ± 55g Ethiopian study, the species of prey- *B. pfeifferi* against *B. globosus* for this study and the type of catfish- pure breed against hybrid catfish in this study. Similar observations were made by Su Sin (2006) where catfish successfully fed on apple snails in laboratory experiments. In this experiment, the hybrid catfish exhibited a feeding mechanism whereby the fleshy parts of the snails were sucked out of the shell. This was evident from whole or slightly damaged shells that were seen on the floor of the aquaria.

4.4.2 Prey selection by crayfish and catfish

In our prey selection experiment we saw that crayfish consumed significantly more prosobranch *M. tuberculata* than pulmonate *B. globosus* (Figure 4.2). This could be due to the fact that during our experiments we observed that *M. tuberculata* was a bottom dweller occupying the floor of the tank while *B. globosus* escaped by climbing up above the water column. *B. globosus'* escape behaviour was also observed with *Physa acuta* which left the water in the presence of crayfish thereby avoiding predation (Hofkin et al., 1992). This is consistent with Lodge et al. (2005) who states that, "the reduced abundance of slow-moving, benthic invertebrates in the presence of crayfish is expected because crayfish are tactile feeders, consuming what they encounter. Faster-moving benthic invertebrates and pelagic organisms presumably can avoid crayfish." However, contrary to our result Khalil and Sleem (2011) observed that *P. clarkii* preferred pulmonates over prosobranchs. This preference was based on the fact that pulmonates have thinner and easier to break shells than the prosobranchs. On the contrary, catfish did not show strong affinity towards any of the prey organisms implying that it fed on whichever prey it came across (Figure 4.2). Catfish are opportunistic feeders which can feed on a range of food from detritus, macrophytes to fish. In a study on food and feeding habits of *C. gariepinus* in Ethiopia, Dadebo et al. (2014) reported a diet dominated by detritus, fish, zooplankton, macrophytes and insects while the contributions of phytoplankton and gastropods were low. Similarly, Makoni et al. (2005) did not find snails in the stomachs of *C. gariepinus* in an experiment involving mixed fish species and a number of snail species in Zimbabwe. These findings signal a need for more semi-field experiments to investigate the behaviour of hybrid catfish towards *Schistosoma* host snails.

4.4.3 Effect of predator size on predation

In the study on the effect of predator size on predation of *B. globosus*, we found a significant difference between the two size classes. Smaller catfish were more active predators than their larger counterparts regardless of size of prey provided at least for the first 72 hours (Figure 4.3). This may be a result of the ontogenetic shift in diet which occurs as the fish get bigger. As the catfish develop long, numerous and compact gill rakers with age, they become more of filter-feeders feeding mainly on zooplankton. Juveniles on the other hand feed more on larger prey including insects, fish and molluscs (Dadebo et al., 2014; Munro, 1967). The observed increased feeding rate of bigger catfish after 72 hours could have been a response to hunger due to lack of preferred food in the experimental tanks.

4.5 Conclusion

The interruption of the *Schistosoma* life cycle through intermediate host control will continue to be an important complementary strategy to chemotherapy in the fight against schistosomiasis (King and Bertsch, 2015). While there are many snail control options, effective strategies will need to be decided on a case by case basis (Monde et al, subm.). This study has shown that both *C. quadricarinatus* and a cross breed of *C. gariepinus* and *C. ngamensis* feed on *B. globosus* a *Schistosoma* host snail. However, presence of an alternative gastropod affects the predation efficiency of these predators. The preference by *C. quadricarinatus* of *M. tuberculata* (non-host) over *B. globosus* (host) may reduce its effectiveness as a biocontrol organism in places where the two species occur together. However, in a study on habitat preferences of freshwater snails in Nigeria Ndifon and Ukoli (1989) observed that most snails including *M. tuberculata* were most frequently found alone an observation similar to that reported by Monde et al. subm. where *M. tuberculata* were never found occupying the same space with either *B. globosus* or *B. pfeifferi*. The non-selectiveness observed in this study and the opportunistic feeding behaviour reported by Dadebo et al. (2014) and Munro (1967) in catfish may also reduce its efficiency in reducing populations of host snails. However, the fact that both organisms feed on host snails as demonstrated in this study, there is potential that they can control the populations of host snails. Young catfish may be preferred over older ones because of the ontogenetic changes that result in dietary shifts as the catfish gets older.

It is worth noting that the invasive *C. quadricarinatus* may be valuable in small manmade dams meant to supply water for domestic and livestock needs which are numerous in drier regions. Their isolation from other water bodies reduces the chances of the crayfish escaping although there are already substantial populations in the major water bodies in Africa. These impoundments are often active *Schistosoma* transmission points.

Acknowledgement

This study was made possible by funding from the NICHE project a partnership between the NUFFIC and Copperbelt University. We are grateful to both institutions and to the National Aquaculture and Development Centre for allowing us to use their wet lab to conduct these experiments. We would also like to thank the staff and workers of NARDC notably Mr. Ian Bbole, Mr. Mwakawaya and Lameck for ensuring that all the necessary materials and equipment needed were available. Other thanks go to Mr. Mushanga the driver for Copperbelt University for his services including help with collection of specimens in the field.

Supplementary Information

Table S4.1: Daily means and standard deviations for snails eaten by crayfish, catfish and average daily mortality for the control.

Day	Crayfish	Catfish	Control
	Mean (± S.D)	Mean (± S.D)	Mean (± S.D)
1	8.6 ± 3.6	5.5 ± 6.6	1.2 ± 1.6
2	16.0 ± 5.1	8.2 ± 7.3	1.8 ± 1.6
3	22.2 ± 5.2	8.2 ± 7.3	2.8 ± 1.9
4	25.4 ± 4.5	15.2 ± 9.1	3.4 ± 2.6
5	30.7 ± 3.7	18.4 ± 7.9	5.6 ± 1.8
6	47.8 ± 5.8	23.0 ± 8.9	11.2 ± 4.1
7	55.2 ± 4.1	26.6 ± 4.5	12.0 ± 3.7
8	59.6 ± 3.1	38.4 ± 18.1	12.6 ± 4.0
9	61.0 ± 3.1	41.6 ± 20.6	14.0 ± 3.0
10	74.0 ± 3.3	53.4 ± 27.8	14.8 ± 3.9
11	81.4 ± 6.8	61.8 ± 29.6	15.8 ± 3.3
12	87.0 ± 8.5	73.6 ± 26.5	26.5 ± 6.3
13	89.2 ± 8.3	93.0 ± 10.9	28.0 ± 8.3
14	89.6 ± 7.8	94.6 ± 10.5	28.0 ± 8.3
15	92.8 ± 5.9	96.0 ± 7.9	36.6 ± 4.4

Table S4.2: Means, standard deviations and confidence intervals for consumed snails for the treatments.

Day	B. globosus			M. tuberculata		
	Crayfish	Catfish	Control	Crayfish	Catfish	Control
	Mean (± S.D)	Mean (± S.D)	Mean (± S.D)	Mean (± S.D)	Mean (± S.D)	Mean (± S.D)
1	13.4 ± 7.7	32.4 ± 23.6	1.4 ± 2.6	38.8 ± 4.9	20.4 ± 16.1	1.6 ± 2.1
2	22.2 ± 6.5	32.0 ± 24.8	2.0 ± 2.5	47.0 ± 2.1	23.4 ± 14.9	3.4 ± 3.6
3	38.6 ± 6.5	76.6 ± 37.3	2.4 ± 2.5	87.2 ± 15.3	43.8 ± 29.6	4.2 ± 3.8
4	41.2 ± 7.1	82.2 ± 39.8	2.2 ± 2.7	97.0 ± 2.6	57.0 ± 31.9	5.8 ± 5.2
5	44.4 ± 4.8	85.6 ± 29.0	4.4 ± 2.1	99.8 ± 0.4	76.2 ± 29.5	6.2 ± 3.8
6	45.8 ± 4.1	87.2 ± 28.6	8.0 ± 6.8	100 ± 0	76.6 ± 29.8	8.2 ± 4.3
7	53.2 ± 7.1	88.0 ± 26.8	9.0 ± 6.9	100 ± 0	77.0 ± 29.3	10.4 ± 5.5

Chapter 5

Effect of endosulfan on predator-prey interactions between *Clarias gariepinus/Clarias ngamensis* hybrid and *B. globosus*

Concillia Monde, Stephen Syampungani, Paul J. van den Brink.

Abstract

The effect of the pesticide endosulfan on the predator-prey interactions between catfish and *Schistosoma* host snails was assessed in static tank experiments. Hybrid catfish (*Clarias gariepinus* x *Clarias ngamensis*) and *Bulinus globosus* were subjected to various endosulfan concentrations, including an untreated control. The 48h and 96h LC50 for catfish were 1.0 and < 0.5 µg/L, respectively, while the 96h LC50 for snails was 810 µg/L. To assess the sub-lethal effects on the feeding of the catfish on *B*. *globosus*, endosulfan concentrations between 0.03 and 1.0 µg/L were used. Predation was significantly higher ($p < 0.001$) in control tanks than in all other treatments. There was a progressively decreasing predation with increasing toxicant concentration. Biological control of *Schistosoma* host snails using fish may be affected in endosulfan polluted aquatic systems of Southern Africa as it has been found present at concentrations that are indicated to cause lethal effects on the evaluated hybrid catfish and to inhibit the predation of snails by this hybrid catfish.

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5.1 Introduction

As the global human population continues to increase, the pressure on natural resources to provide goods and services also increases (Millennium Ecosystem Assessment 2005). This entails an increase in food production through agriculture. The use of chemicals is viewed as a panacea to improve the productivity of agriculture. As a result, application of chemical fertilizers and pesticides to improve crop health and yield has increased worldwide. Global estimates of pesticide use in 2006 and 2007 are at about 2.4 million tonnes of active ingredients per year (EPA 2011). However, not all the pesticides applied will reach the targeted organisms. Estimates show that only about 0.3% of pesticides applied reach the target organisms while 99.7% contaminates the surrounding environment including air, soil and water through spray-drift, leaching and run-off (van der Werf 1996). Pesticides may have direct and indirect effects on non-target organisms in both terrestrial and aquatic ecosystems. Fish kills and alteration in the structure of invertebrate communities have been reported as well as effects on higher predators such as birds (Schäfer et al. 2011).

The majority of studies investigating the effects of pesticides on non-target organisms have focused on their effect on individuals or single species. Several studies on how pollutants affect fish behaviour had been previously reviewed (Weis & Candelmo 2012). However, to understand the effects of these pollutants on interspecific interactions such as predation, predators and their prey should be studied simultaneously in order to mimic natural conditions (Junges et al. 2010). Few studies focusing on the effect of pesticides on predator-prey interactions have been reported. Results from these studies have shown that different pesticides can disrupt intra- and interspecific interactions between organisms and, therefore, alter the ecological functioning of ecosystems (Bridges 1999, Relyea & Hoverman 2006, Junges et al. 2010). This could be by causing mortality of either the predator or prey or by interfering with their physiology. However, there is no evidence of studies looking specifically at effect of pesticides on snail predation by fish in the literature. Organophosphate, carbamate and organochlorine pesticides affect the nervous system of vertebrates by inhibiting the enzyme cholinesterase which regulates acetylcholine a neurotransmitter which is important for nerve function (DeLorenzo et al. 2001). Boone & Semlitsch (2003) observed increased survival of bullfrogs *Rana catesbeiana* after the extermination of predatory crayfish *Orconectes* sp. and bluegill sunfish *Lepomis macrochirus* in a pond experiment. The death of the predators was due to toxicity of the carbamate insecticide carbaryl. *Micropterus salmoides* exposed to organochlorine pesticide pentachlorophenol (67-88µg PCP/L) showed a reduced feeding capacity, made more mistakes by aiming at non-prey items and failed to capture its targets more often than the unexposed fish (Mathers et al. 1985, Brown et al. 1987). Cessation of all locomotion, inability to maintain position and reduced feeding in *Oncorhynchus kisutch* when exposed to organophosphate insecticide fenitrothion was reported by Bull & McInerney (1974). Increased prey survival was the outcome of the predator-prey interactions of *Synbranchus marmoratus* and *Hypsiboas pulchellu*s when exposed to 2500 µg/L of fenitrothion. Fenitrothion toxicity appeared to have modified prey behaviour by making them less mobile hence less visible to the predator (Junges et al. 2010).

Agriculture makes an important contribution to livelihoods and economies of southern African countries. It contributes about 8% to the gross domestic product (GDP) of southern Africa (Chilonda & Minde 2007) and over 80% of the populations of Malawi, Zambia and Mozambique depend on agriculture for subsistence (Mucavele 2013). As a coping strategy for food production and income generation, wetlands and uplands are used in an integrated manner by the rural people to achieve sustained livelihoods. Agricultural fertilizers and pesticides are used in these farming systems to boost crop yields. Unfortunately, like in many developing countries, banned, non-patented, obsolete and environmentally persistent pesticides are widely used in southern African countries including Zambia, Zimbabwe and South Africa (Stockholm Convention on Persistent Organic Pollutants Review Committee 2010). However, in many of these countries not much has been done to quantify the effects of these pollutants on the environment. One such pesticide with persistent effects to the environment still in use in southern African is endosulfan (Deedat et al. 1997, Stockholm Convention on Persistent Organic Pollutants Review Committee 2010).

Endosulfan is an organochlorine insecticide which is hazardous to the environment. It is composed of two isomers, a- and b-endosulfan, which degrade into endosulfan sulphate and is persistent, non-biodegradable and capable of bio magnification as it moves up the food chain (Agbohessi et al. 2014). Endosulfan may enter the aquatic ecosystem through run off, direct spray, leaching through the soil and volatilization into the atmosphere and later as precipitation. While monitoring data for pesticides is very difficult to find for developing countries (Van Dyk & Pletschke 2011), endosulfan has been found in water and sediment of many water bodies in Africa (Nyangababo et al. 2005, Syakalima et al. 2006, Ezemonye et al. 2010, El Bouraie et al. 2011, Ansara-Ross et al. 2012, Ibigbami et al. 2015) (Table 5.1).

Table 5.1: Endosulfan concentration in water and sediment recorded for water bodies in some African countries.

Location	Aquatic compartment		Source
	Water (µg/L)	Sediment (µg/Kg)	
Western Cape (Elgin farm dams)	626	-	(Ansara-Ross et al., 2012)
KwaZulu-Natal (Ubombo and Ingwavuma districts)	-	0.09 – 2.36	(Ansara-Ross et al., 2012)
Western Cape (Hex River valley)	1.79	-	(Ansara-Ross et al., 2012)
Western Cape (Lourens River)	0.03 – 0.16	3.9 – 245	(Ansara-Ross et al., 2012)
Western Cape (Hex River valley, Grabouw and Piketberg)	<0.10 – 26.3	-	(Ansara-Ross et al., 2012)
Eastern Cape (East London and Umtata)	<0.02 – 0.10	<bdl – 92.0	(Ansara-Ross et al., 2012)
Gauteng (Jukskei River)	7.28 –364	44.7 – 3 705	(Ansara-Ross et al., 2012)
Western Cape (Berg and Franschhoek rivers)	0.01	4.60 – 156	(Ansara-Ross et al., 2012)
Warri River (Nigeria)	0.01 - 9.23	0.06 – 11.98	(Ezemonye et al., 2010)
Ogbese River (Nigeria)	bdl[a] <bdl – 6.17[b] 3.01 – 10.9[c]	25.6 – 199 8.35 – 69.4 4.69 – 63.6	(Ibigbami et al., 2015)
El Rahaway drain (Egypt)	0.021 – 0.823		(El Bouraie et al., 2011)
Lake Volta (Ghana)	0.039	1.3	(Gbeddy et al., 2015)
Ghana	0.062[a] 0.031[b] 0.031[c]	0.19 0.13 0.23	(Ntow, 2001)
Lake Victoria basin (East Africa)	0.003 - 0.034[a] 0.012 – 0.031[b] 0.017 – 0.038[c]	0.12 – 0.43[a] 0.39 – 0.45[b] 0.27 – 0.42[c]	(Nyangababo et al., 2005)
Kafue River (Zambia)	-	3.00	(Syakalima et al., 2006)

NB: bdl = below detection limit, a = alpha-endosulfan, b = beta endosulfan, c = endosulfan sulfate

Many studies have documented the toxicity of endosulfan to aquatic organisms including tadpoles, snails and fish (Ellis-Tabanor & Hyslop 2005, Jones et al. 2009, Agbohessi et al. 2014). These effects can be direct on the organism's health by affecting the animal's physiological function or indirect by impacting the trophic interactions such as competition and predation (Schäfer et al. 2011). Although, it has been found not to be as persistent in tropical climates as in temperate climates (Mwangala et al. 1997), endosulfan toxicity to aquatic organisms may have a bearing on the functioning of aquatic interactions in the tropics as it is found at concentrations that are likely to affect arthropods, invertebrates and fish (Table 5.1; Hose and Van den Brink, 2004).

To date, despite the fact that many studies having documented the effects of pesticides on aquatic invertebrate and vertebrate fauna, none of these studies have assessed the effect of pesticides on the predator-prey interaction of *Schistosoma* host snails and their fish predators. However, the studies specifically looking at the effects of pesticides on host snails mainly focussed on organophosphate pesticides include the insecticides profenophos and chlorpyrifos as studied by Hasheesh & Mohamed (2011) and Mohamed (2011) which were found to have molluscicidal effects. Ibrahim et al. (1992) observed reduced egg production and egg hatchability at sub lethal concentrations and mortality at higher concentrations of chlorpyrifos by *Biomphalaria alexandrina*. The effect of organochlorine pesticides on host snails are largely unknown. Similarly, several studies have documented the effect of pesticides on fish. These include Bacchetta et al. (2014) who observed deleterious effects of endosulfan and lambda-cyhalothrin on *Piaractus mesopotamicus.* Refer to Bacchetta et al. (2011) and Napit (2013) for more studies on effects of various pesticides on different species of fish. Hence the present study investigated the effect of an organochlorine pesticide endosulfan on the predator-prey interactions of hybrid catfish and *Bulinus globosus* snails using environmentally relevant concentrations (Table 5.1). In doing so we were specifically seeking to address two objectives i.e. 1) to determine what pesticide concentration would impact both the host snails and the hybrid catfish and 2) to investigate whether sub-lethal pesticide concentrations have an effect on the predation efficiency of catfish (*Clarias gariepinus/Clarias ngamensis* hybrid).

5.2 Materials and Methods

5.2.1 Test animals

Hybrid African catfish were collected from outdoor ponds at the Aquaculture Development and Research Centre (NARDC) in Mwekera, Zambia. The ponds were drained and a scoop net made of 2 x 2 mm mesh size sieve that was supported by a metal frame mounted on a 1.5 m wooden handle was used. One hundred and twenty four fish of weight ranging from 180 to 310 g were kept in plastic tanks (100 L) at a stocking density of two fish per tank due to their aggressive behaviour. The fish were left to acclimatize for 5 days during which they were fed on commercial fish meal (pellets of 30 % crude proteins, 12 % crude fat) twice a day at 4% body weight. To reduce on accumulation of excess food and fish faeces, water in the tanks was changed every other day by siphoning 50% of the spent water and filling with fresh borehole water.

One thousand snails (*Bulinus globosus*) were collected from ponds at NADRC. These were picked by gloved hands and kept in plastic tanks. Raw lettuce was provided as feed for snails *ad libitum*.

5.2.2 Test water parameters

We monitored three water quality parameters, temperature, pH and dissolved oxygen for the predation experiment using an AM-200 Aquaread GPS Aquameter. All tests were done in static conditions meaning that the test water was not changed for the duration of the experiment. This was important in order to capture the short-term peak concentration effects rather than long-term, chronic exposure effects of endosulfan as it is known not to be persistent in tropical environments and since application of pesticides are usually many months apart.

5.2.3 Endosulfan preparation and analysis

The insecticide endosulfan was chosen because it is one of the commonly used pesticides for both seasonal and off season farming in Zambia. Vegetable gardening is an off season activity in most parts of the country and is done on the banks of rivers, streams and other water impoundments. Sionex 35 EC was purchased from VINCO agrochemicals in Zambia. The concentration of the active ingredient (endosulfan) in the Sionex 35 EC was 350 g/L on which all dilutions to make the test solutions were based. A stock solution of 10 mg/L was made from the original concentration. Desired test solutions were obtained by diluting various amounts of the stock solution in 500 L of borehole water (Table 1). All test solutions were freshly prepared prior to the commencement of the tests.

Analytical verification of pesticide concentration in the test water was done through HPLC-UV procedure prior to and after experimentation. For the extraction of the pesticide residues from water, liquid-liquid extraction method was adopted. We collected water samples at the beginning and at the end of each experiment from the test water into 500 ml amber-glass bottles. Ethyl acetate was used to extract endosulfan from the water in the ratio 2:1 sample to solvent ratio. The sample-solvent mixture was then shaken with a manual shaker for 30 minutes to extract the endosulfan to the organic solvent. The mixture was transferred into separatory funnels and allowed to stand for 20 minutes to allow separation of the organic layer from the water layer. The organic layer was collected into 100 ml amber-glass bottles and sealed with glass tops. Prior to analysis by HPLC-UV, the samples were further dried using anhydrous sodium sulphate. HPLC operating conditions for the analysis were as follows; mobile phase; acetonitrile: water (70:30) using an isocratic elution. Injection volume of the sample was 10 µL with a flow rate of 1.0 mL/min. Oven temperature was at 40 degrees centigrade, while the run time was 20 minutes. The column used was a Shim-pack VP-ODS;250 x 4.6 mm I.D while the UV detector used ran at 254 nm. The used standard was endosulfan (mixed isomers) CAS# 115-29-7 with a purity of 100%. Actual concentration of the standard was 1002 µg/mL or 0.1% in methanol solvent. This standard was purchased from Accu Standard, Inc.

5.2.4 Experimental Design

Two sets of tank experiments were set up to: 1) determine effect of endosulfan exposure on snail and catfish survival, 2) determine effect of sub-lethal endosulfan exposure on host snail predation by catfish.

5.2.5 Observable behavioural Responses

Changes in fish behaviour were qualitatively quantified either as normal (-), low (+), moderate (++) or severe (+++). The observations were terminated after 72 hours because no catfish survived beyond 72 hours at all concentrations except in the control.

5.2.6 Effect on catfish and snail survival

In the toxicity experiment with hybrid catfish, 20 tanks of 200 L were filled with 50 L of endosulfan treated water. Five concentrations (0, 0.5, 1.0, 1.5 and 2.0 µg/L) were tested in the range-finding test which lasted for 24 hours. Since mortality was observed in a few hours in the two highest test concentrations we decided to use five concentrations of 0, 0.5, 1.0, 1.1 and 1.2 µg/L to test for catfish survival to technical endosulfan toxicity in the final test. These concentrations were selected based on the results of a range-finding test which was itself based on the Species Sensitivity Distribution (SSD) and the resulting Hazardous Concentration 5% (HC5) value for fish of 0.31 µg/L as reported by Hose & Van den Brink (2004). Juvenile catfish of weight 190 to 250 g were stocked, five in each tank. All concentrations were replicated four times. Observations started immediately after the fish were exposed to endosulfan and continued every 12 hours. In the experiment for endosulfan toxicity to snails, 28 tanks of 50 L were used. Seven pesticide concentrations of 0, 100, 200, 400, 500, 1000 and 1200 µg/L were prepared based on the LC_{50} values provided by Yasser et al. (2008) for other gastropods and a range-finding test involving ten (0, 200, 400, 600, 800, 1000, 1200, 1400, 1600, 1800 µg/L) concentrations. In the range finding test, we observed more than 50% mortality in the 1400 to 1800 µg/L concentrations during the 24 h observation time. All definitive test concentrations were evaluated in four replicates. Twenty eight groups of twenty snails (9 – 15 mm size range) were each randomly stocked in the prepared experimental units. Observations started immediately after the snails were exposed to the endosulfan treated water and continued every 24 hours for 96 hours. All tanks were covered with netting material to prevent test organisms from escaping. A completely randomized design was used to perform these experiments. The endpoints for both hybrid catfish and *B. globosus* was death of the exposed individuals recorded on a daily basis. Fish were considered dead when they were found either floating on the surface of the water upside down or lying motionless at the bottom even when provoked. Snails were considered dead when they did not respond to pricking on their soft parts by a sharp object. All dead specimens were removed from the experimental units.

5.2.7 Effect on snail predation by catfish

In order to evaluate the sub-lethal effect of endosulfan, predation of snails by hybrid catfish was evaluated in twenty tanks of 50 L by stocking each tank with 50 *B. globosus* of sizes ranging between 8-15 mm shell height. The tanks were filled with 20 L of water with one of the endosulfan test concentrations. The control tanks received water with no endosulfan while the other tanks received water with 0.03, 0.1, 0.3 or 1.0 µg/L concentrations, each replicated 4 times. These concentrations were selected based on the results of the lethality test which yielded 48 h LC_{10} of approximately 1 µg/L. The LC_{10} was preferred over the LC_{50} in order to reduce the chances of mortality in the sub lethal tests. A single catfish was introduced in every tank and observations commenced 24 hours later for 96 hours. All the catfish used fell within a 300 ± 15 g size range and were starved for 24 hours prior to the start of the experiment. Snails continued receiving 2 g of raw lettuce per tank *ad libitum* and the medium was not renewed for the whole duration of the experiment. The effect of endosulfan on the snail predation efficiency of hybrid catfish was measured through the number of snails eaten per day against the control. A snail was considered eaten only when it was missing from the tank.

5.2.8 Statistical analysis

The $LC_{10,\ 50}$ and $_{90}$ values of the toxicity experiments were calculated by means of log-logistic regression using the software GenStat 11th (VSN International Ltd., Oxford, UK) according to Rubach et al. (2011). Because we used a static bioassay, $LC_{10,\ 50}$ and $_{90}$ values were calculated using the geometric means of the start and end concentrations for Catfish and *B. globosus* at each sampling period. The effect of endosulfan toxicity on predator-prey interactions of catfish and snails was assessed by comparing the number of eaten snails at different exposure concentration over time through regression analysis. Generalized Linear Models (GLM) were used to assess the significance of the differences among the treatments for each sampling period. The model used for the GLM analysis was adapted to the data distribution of the measured endpoints. Mortality was assessed using a binomial distribution and logit as the link function. The effects of the pesticide concentration on the evaluated endpoints were considered to be significant when the calculated *p*-values were < 0.05.

5.3 Results

5.3.1 Test water parameters

There were no large differences between the nominal and the measured concentrations (Table 5.2). The concentrations were on average 14% (± 20%) higher than the nominal concentrations. Endosulfan did not show a clear dissipation rate for the three experiments. An average dissipation of 8.1% (± 4.8%) was found in the two days and of 6.6% (± 7.4%) in four days for the lethal effects experiment on catfish and snails respectively while a dissipation of 20% (± 16%) was found in the four days experiment evaluating the sub-lethal effects of endosulfan on predator-prey interactions (Table 5.2).

Table 5.2: Nominal test concentration and the subsequently HPLC measured concentrations at the start and end of experimentation.

Experiment	Nominal concentration (µg/L)	Measured concentration at start (µg/L)	Measured concentration at end (µg/L)
Effect on feeding	0.03	0.033	0.028
	0.1	0.17	0.096
	0.3	0.33	0.31
	1.0	1.3	1.1
Effect on catfish	0.5	0.46	0.39
	1.0	1.2	1.2
	1.1	1.3	1.2
	1.2	1.5	1.4
Effect on snails	100	101	100
	200	267	212
	400	399	365
	500	451	427
	1000	997	982
	1200	1210	1180

The levels of the three water quality parameters (temperature, dissolved oxygen and pH) monitored in the experimental units over time during the predation experiment are shown in Fig. 5.1 of the supplementary information. There were fluctuations in these parameters over the duration of the experiments. Temperature ranged between 23.0 and 22.7^{o}C, pH between 4.15 and 5.70 and DO between 3.5 and 6.7 mg/L. There was no significant difference (p = 0.892) in the temperature between the treatments and the control. However significant differences were observed in pH (p = 0.002) and DO (p < 0.001). The control units (0.0 µg/L) had the highest DO while the highest endosulfan concentration (1.0 µg/L) units had the lowest. The pH reached its lowest (4.15) in the highest endosulfan concentration at 96 h (Fig. 5.1).

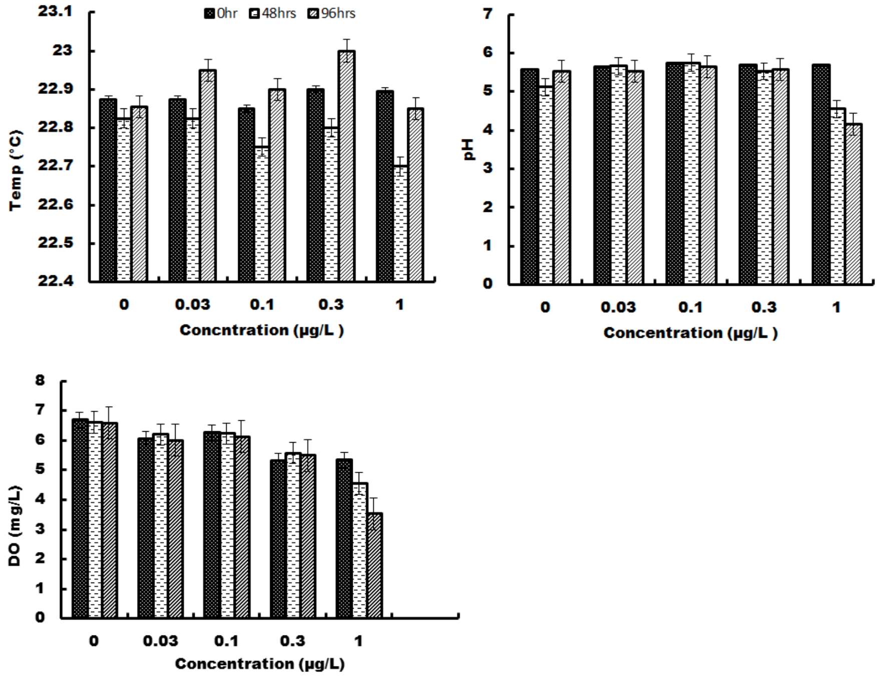

Fig 5.1: Temperature, pH and DO values at the beginning, halfway and at the end of the predation experiment at different concentrations of endosulfan**.**

5.3.2 Observable behavioural Responses

Five responses were observed in fish exposed to endosulfan. These include increased or erratic swimming, attempts to jump out of the tanks, gasping for air, disorientation and secretion of thick layer of mucus on the body. The intensity of these responses were dependent on endosulfan concentration. Fish in the 0.5 and 1.0 µg/L concentrations exhibited severe jumping and swimming upon exposure to endosulfan while these traits were low for fish in higher concentrations. Fish in higher concentrations showed more signs of exhaustion, gasping for air and disorientation. All fish exposed to endosulfan secreted thick masses of mucus on their bodies (Table 5.3).

Table 5.3: Observed behavioural responses for catfish exposed to various concentrations of endosulfan.

Response	Concentration																			
	0.0µg/L				0.5µg/L				1.0µg/L				1.1µg/L				1.2µg/L			
	R 1	R2	R 3	R 4	R 1	R 2	R 3	R 4	R 1	R 2	R 3	R 4	R 1	R2	R 3	R 4	R 1	R 2	R 3	R4
Swimming	-	-	-	-	+++	+++	+++	+++	+++	+++	+++	+++	+	+	+	+	+	+	+	+
Jumping	-	-	-	-	+++	+++	+++	+++	++	++	++	++	+	+	+	+	+	+	+	+
Gasping	-	-	-	-	++	++	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Disorientation	-	-	-	-	++	++	++	++	++	++	++	++	+++	+++	+++	+++	+++	+++	+++	+++
Mucus	-	-	-	-	++	++	++	++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++	+++
Resting	-	-	-	-	+	+	+	+	+	+	+	+	+++	+++	+++	+++	+++	+++	+++	+++

5.3.3 Effect on catfish and snail survival

For catfish, survival in the control remained at 95% at both 24 and 48 h (Table 5.4). In the lowest endosulfan concentration survival was equally high 95 and 90% for 24 and 48 h respectively. However marked reduction in survival rates were recorded for the concentrations ranging between 1.0 and 1.2 µg/L for both days. Only 55 and 45% survival was found in the 1.0 µg/L concentration, 5 and 0% in the 1.1 µg/L concentration and no survival for the highest concentration (1.2 µg/L) at both 24 and 48 h respectively (Table 5.4). At 72 h all the catfish exposed to endosulfan toxicity were dead leaving only those in the control tanks. Table 5.6 shows the associated LC_{10}, $_{50}$ and $_{90}$ values for the catfish, which are all close to 1 µg/L.

Table 5.4: Catfish survival at each concentration over time in the toxicity experiment with endosulfan. In all replicates 5 individuals were included at the start of the experiment. At 72 hours all fish were dead at all concentrations except the control.

Conc.	24 h					48 h				
(µg/L)	R 1	R 2	R 3	R 4	% surv	R 1	R 2	R 3	R 4	% surv
0	5	5	5	4	95	5	5	5	4	95
0.5	5	5	4	5	95	4	5	4	5	90
1.0	3	3	2	3	55	2	3	2	2	45
1.1	0	1	0	0	5	0	0	0	0	0
1.2	0	0	0	0	0	0	0	0	0	0

N.B: R = Replicate

During the first 24 h, *B. globosus* survival ranged between 80 and 100% for all treatments lower than 1000 µg/L and between 67 and 76% for the treatments higher or equal to 1000 µg/L (Table 5.5). However, at 48 h the survival in the control and the concentrations lower than1000 µg/L dropped to between 67 and 82% while for the concentrations 1000 and 1200 µg/L survival declined to 59 and 39% respectively. At 96 h only 20% and 11% *B. globosus* were still surviving at the two highest concentrations respectively, while survival remained above 58% for the lower concentrations over the same period (Table5.5). Table 5.6 shows the associated LC_{10}, $_{50}$ and $_{90}$ values for *B. globosus*, which are much higher than those found for the catfish.

Table 5.5: *B. globosus* survival at each concentration over time in the toxicity test with endosulfan. In all replicates 20 individuals were included at the start of the experiment.

Conc.	24 h					48 h					72 h					96 h				
(µg/L)	R1	R2	R3	R4	% surv	R1	R2	R3	R4	% surv	R1	R2	R3	R 4	% surv	R 1	R 2	R 3	R 4	% surv
0	19	20	20	20	98.7	19	20	17	20	81	17	20	17	20	79	15	15	13	15	61.2
100	20	20	20	20	100	20	18	19	20	82	17	17	18	20	77	15	17	17	14	70.2
200	19	17	19	20	80	19	14	14	16	67	18	14	14	16	66	10	14	14	16	58
400	20	19	20	20	89	20	19	17	19	79.7	18	12	17	19	70.5	15	10	9	13	58.7
500	18	18	19	20	80	16	17	16	18	71.5	14	13	15	16	62	14	11	15	16	60
1000	18	12	17	16	67	10	10	14	13	58.7	6	7	9	9	38.7	2	5	5	4	20
1200	17	18	17	19	75.7	7	9	10	5	38.7	5	7	7	4	28.7	0	3	6	0	11.2

N.B: R = Replicate

Table 5.6: LC values (µg/L) and their confidence intervals at the various time points.

Experiment	Endpoint	Time			
		24hrs	**48hrs**	**72hrs**	**96hrs**
Catfish	LC10	0.948 (0.896 -1.003)	0.988 (0.978-0.998)	< 0.5 *	< 0.5 *
	LC50	1.009 (0.983-1.036)	1.000 (0.995-1.005)	< 0.5 *	< 0.5 *
	LC90	1.075 (1.023-1.13)	1.011 (1.003-1.02)	< 0.5 *	< 0.5 *
B. globosus	LC10	807 (551-1181)	682 (485 - 959)	372 (257- 540)	485 (336 -701)
	LC50	4160 (1336 -12956)	1137 (1041-1245)	907 (787-1044)	810 (690-950)
	LC90	21457 (2056 - 223941)	1902 (1306 - 2769)	2209 (1585-3077)	1351 (1139-1602)

*: no catfish survived beyond 48 hrs except in the controls.

5.3.3 Effect on snail predation by catfish

Exposure to sub-lethal concentrations of endosulfan resulted in significant differences in catfish rate of predation. At the end of the four days observation period there was over seven times more snails eaten in the controls than in the high endosulfan concentration (1.30 µg/L) (Fig 5.2a) . There were deaths of catfish in the 0.3 and 1.2 µg/L treatments and replacements were made immediately (Table 5.7).

Table 5.7: Daily residual snails for all treatments of the predation experiment. Replicates (R) in which the predator died and was replaced by a new individual are indicated in bold.

Time	Concentration																			
	0.0µg/L				0.03µg/L				0.1µg/L				0.3µg/L				1.0µg/L			
	R 1	R2	R 3	R 4	R 1	R 2	R 3	R 4	R 1	R 2	R 3	R 4	R 1	R2	R 3	R 4	R 1	R 2	R 3	R4
0 hrs	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50	50
24 hrs	35	33	32	36	39	36	40	40	43	44	44	43	46	45	45	44	49	**50**	50	**50**
48 hrs	14	11	15	18	32	31	35	34	36	34	36	33	34	**38**	36	37	**49**	46	**44**	47
72 hrs	5	9	11	10	21	26	24	28	27	29	29	33	30	30	33	34	47	**46**	**43**	47
96 hrs	1	4	4	2	16	23	19	24	22	25	27	29	27	28	31	27	44	44	43	45

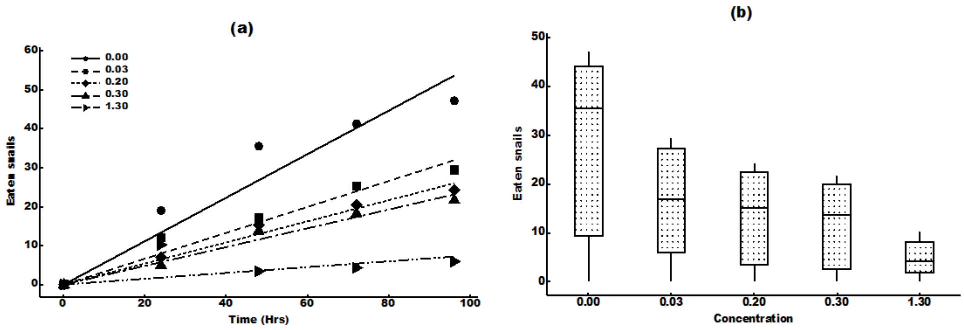

Figure 5.2 Average of the cumulative number of snails eaten by the catfish per treatment level over time and the associated GLM fits (a) and variation in the cumulative number of snails eaten at the different endosulfan concentrations at the end of the experiment (b).

GLM tests yielded significant differences in snail predation ($p < 0.001$ for all sampling dates). Number of snails consumed per day was dependent on pesticide exposure regimes with predation being significantly higher in the controls than in all other treatments (Figure 5.2b).

5.4 Discussion

5.4.1 Test water parameters

The aim of this study was to assess the toxic effect of endosulfan on both the predator hybrid catfish and the prey *B. globosus*, and on the predation efficiency of the hybrid catfish. Endosulfan toxicity to aquatic organisms as reported in many studies has also been demonstrated in the present study. The decrease in the levels of pH and DO observed in this study in the highest treatment level of the predation experiment (Fig. 5.1) reveal the way the fish is affected by the toxicant. Endosulfan inhibits the action of the neurotransmitter gamma-aminobutyric acid (GABA), which leads to a state of uncontrolled neuronal excitation (Rozman & Klaassen 2007). Interference in activity of Ca^{2+}-ATPase hence calcium transportation as well as phosphokinase activities may also be induced by exposure to endosulfan (WHO 2000). Matthiessen & Roberts (1982) reported hyperactivity in *Tilapia rendalli* exposed to endosulfan. This behavioural response may have caused an increase in the rate of respiration hence a reduction in levels of dissolved oxygen and an increase in dissolved carbon dioxide in the water in the present study. Muthukumar et al. (2009) observed a rapid increase in opercular movements of *Tilapia mossambicus* exposed to sub lethal concentrations of endosulfan as a way of increasing the oxygen uptake to mitigate the stress caused by the toxicant. Dissolved carbon dioxide reacts with water to form carbonic acid which in turn reduces the pH (Wurts & Durborow 1992). In the present study although there was a significant decrease in dissolved oxygen in the highest endosulfan concentration it remained within the tolerable range for catfish (Mallya 2007). The pH observed in this experiment (between 4.15 and 5.70) was in some cases not optimal for fish which according to Alabaster & Lloyd (2013) falls between 5 and 9. Although fish may survive at lower pH ranges as the case was in our study acidic conditions may affect the fish's endocrine system, breakdown the gill structure or even suffocate the fish due to excessive accumulation of mucus (Kwong et al. 2014).

5.4.2 Behavioural responses and effect on catfish survival

In the toxicity test, the hybrid catfish exhibited a number of behavioural responses upon exposure to endosulfan at all concentrations. These included increased swimming activities, attempts to jump out of the tanks, violent and erratic swimming, gasping for air, disorientation and secretion of thick masses of mucus from their bodies. Capkin et al. (2006) reported similar behavioural changes for rainbow trout (*Oncorhynchus mykiss)* exposed to endosulfan. These behavioural changes may be an indication of the effect of endosulfan on the nervous system of the fish. Endosulfan is highly toxic to fish and other aquatic organisms but its toxicity varies among different species. The 48 h, LC_{50} value for hybrid catfish found in this study is 1.0 µg/L. The LC_{10} and LC_{90} values are also quite close to 1.0 µg/L (Table 5.6) implying that this species has a low intraspecific variation in sensitivity to endosulfan toxicity. However, the death of catfish occurred within one hour of exposure in the 1.2 µg/L experimental units and all except those in the control died between 48 and 72 h. Catfish are known to rapidly accumulate high levels of endosulfan in their body tissues. Zeid et al. (2005) reported elevated levels of endosulfan in juvenile African catfish after 6 h of exposure. Jenyo-Oni et al. (2011) recorded a 48 h, LC_{50} value of 4.68 µg/L while Yekeen & Fawole (2011) reported a 96 h LC_{50} of 52 µg/L for juvenile *C. gariepinus.* Our finding are similar to those for several other species of fish of the Okavango Delta in Botswana ranging from 1.2 to 7.4 µg/L (Fox & Matthiessen 1982). The differences in LC_{50} values observed in many studies could be attributed to differences in the length of observation, age and species of fish used and in the case of this experiment the use of a hybrid fish in contrast to pure breeds used in other experiments. From the results of this toxicity experiment we only expected effects of endosulfan on fish survival in the highest concentration of the predation experiment.

5.4.3 Effect on snail survival

For *B. globosus* we found a 96 h, LC_{50} value of 810 µg/L, so we did not expect any direct effect of endosulfan on snail survival in the predation experiment. The control mortality of the experiment was, however, rather high (Table 5.5). This could have been a result of stress due to the long period the animals were kept in artificial conditions in the laboratory prior to experimentation. Our finding is within the range of values obtained in other studies involving the acute toxicity of endosulfan to freshwater snails. Yasser et al. (2008) performed a toxicity test with *Lymnaea radix* and found 96 h LC_{50} values of 380 and 910 µg/L for juveniles and adults, respectively. Jonnalagadda & Rao (1996) performed a test with *Bellamya dissimilis* and recorded a 96 h LC_{50} of 1800 µg/L, while Oliveira-Filho et al. (2005) found 96 h LC_{50} values of 120 and 890 µg/L for *Biomphalaria tenagophila* juveniles and adults, respectively. Higher values were reported, however, for three freshwater snails by Ellis-Tabanor & Hyslop (2007) who obtained 96 h LC_{50} values of 2300, 1740 and 1350 µg/L for *Melanoides tuberculata*, *Thiara granifera and Planorbella duryi*, respectively, while Otludil et al. (2004) reported one of 3230 µg/L for *Planorbarius corneus*.

5.4.4 Effect on snail predation by catfish

The sub-lethal effects were assessed by monitoring the changes in the catfish rate of predation of *B. globosus*. The effect of endosulfan on predation was dose and time dependent (Fig 5.2). However, mortality of some predators was observed in the highest concentration (Table 5.7). Replacement of the dead individuals with new previously unexposed individuals may have resulted in underestimation of the inhibition on predation in the high concentration treatments. Due to its effect on the nervous system (Ellis-Tabanor & Hyslop 2005) and enzyme activity (Tripathi & Verma 2004), endosulfan affects physiological and metabolic functions in organisms. One such effect is the inhibition of the activity of phagocytic cells and renders the organism less able to defend itself against disease. Girón-Pérez et al. (2008) in a study to determine the effect of endosulfan on the phagocytic activity in *Oreochromis niloticus* focused on two parameters, the phagocytic index and percentage of active cells and found that endosulfan had a significantly negative effect on both parameters. Blood is a reflector of the pathological and physiological status of the body of an organism and hence it shows the structural and functional wellbeing of the organism. The reduction in feeding observed in this study may be a reflection of the adverse effect that endosulfan may have exerted on the haematological parameters of the catfish. Several studies have reported the effects of endosulfan on haematological parameters of aquatic organisms. For instance Jenkins et al. (2003) observed a decrease in haematological parameters such as erythrocyte counts, haemoglobin percentage and haematocrit values in *Cyprinus carpio* exposed to endosulfan and the effects were dose dependent. Ndimele et al. (2015) reported microcytic hypochromic anaemia in fingerlings of catfish (*C. gariepinus*) after 24 h of exposure to endosulfan. A reduction in the haemoglobin has a direct effect on the amount of oxygen available to an organism. In our experiment the reduced feeding may have been a coping strategy to depressed oxygen supply to the various organs of the catfish as the fish's affinity for oxygen reduced due to endosulfan toxicity.

The inhibitory effect of endosulfan on fish's acetylcholinesterase (AChE) may also have affected interspecific relations between the catfish and the snails. AChE is an important enzyme catalyzing activities of acetylcholine a neurotransmitter responsible for psychomotor activities of fish. This inhibitory effect was observed in *Lepomis macrochirus, Labeo rohita, Danio rerio, Jenynsia multidentata* exposed to endosulfan (Dutta & Arends 2003, Ballesteros et al. 2009, Kumar et al. 2012, Pereira et al. 2012). These alterations in fish behaviour have an effect on their ecological functions such as feeding, predator avoidance, foraging and reproduction hence their survival (Banaee 2012).

5.5 Consequences for the transmission of schistosomiasis*.*

From this study, it is clear that endosulfan pollution has a negative impact on the predator-prey interactions of *Schistosoma* host snails and their fish predators. This effect appear to favour the survival of the host snails over their predators. This finding has further augmented the claim that endosulfan is generally less toxic to non-arthropod invertebrate taxa than it is to fish (Hose & Van den Brink 2004). The reduced predation pressure of the catfish towards their prey due to pesticide pollution has the potential to foster rapid population growth in the prey population. This increase may make it easier for the *Schistosoma* to encounter and infect the snails. Endosulfan is present in water and sediment samples taken in Africa (Table 5.1) at concentrations capable of eliciting both sub lethal and lethal effects on catfish, which may reduce the effectiveness of biocontrol using hybrid catfish. The implications of this finding to biological control of schistosomiasis is that in places where pesticides such as endosulfan are used adjacent to transmission sites fish may not be effective control agents. While fish may be affected at very low doses, snails remain unaffected by these concentrations.

Since agriculture is an important economic activity in most poor countries (Dao 2012), a shift to using pesticides which are less lethal to snail predators would be an important factor in balancing between agricultural productivity and reduced prevalence of schistosomiasis in endemic areas. Besides endosulfan, there are several insecticides that are found in aquatic ecosystems in many African countries including South Africa (Quinn et al. 2011), Mali (Dem et al. 2007), Nigeria (Ibigbami et al. 2015, Ogbeide et al. 2015) and Zambia (Syakalima et al. 2006). Evaluation of the effects of these pesticides and their combinations on predator-prey interactions of host snails and their predators should be considered.

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Chapter 6

General discussion and conclusions

6.1 Introduction

With the increasing human population leading to negative effects on the economic growth in developing countries (Dao, 2012), sub-Saharan African countries continue to face challenges in provision of good quality healthcare (Gold and John, 2013). Many African country governments fall short of allocating at least 15% of their budgets to provision of healthcare as per requirement of the Abuja declaration (OAU, 2001). Additionally, while sub-Saharan Africa has 24% of the global disease burden, the healthcare system of this region includes only 3% of global health professionals and 1% of global health expenditure (Gold and John, 2013). As a result, exploring cheaper and sustainable ways of reducing disease burden in this region will continue to be a priority if the Sustainable Development Goals (SDGs) (United Nations General Assembly, 2015) are to be achieved.

Schistosomiasis is among the most prevalent diseases in sub-Saharan Africa second only to malaria (Hotez et al., 2007). Globally, an estimated 240 million people are infected (Bockarie et al., 2013) and close to 800 million are at a risk of getting infected (Steinmann et al., 2006). The majority of these infections (over 90%) occur in sub-Saharan Africa (Hotez and Fenwick, 2009). It is a poverty related disease as it thrives mainly in poverty stricken areas without proper sanitation and water supply (King, 2010). In the fight against schistosomiasis, the World Health Organisation recommend a two-pronged approach of chemotherapy (killing the parasite in the human host) on one hand and blocking the transmission of the parasite between the intermediate snail and definitive human hosts on the other hand (WHO, 2012). Chemotherapy using praziquantel significantly reduces morbidity due to schistosomiasis (Koukounari et al., 2007) but it is limited in that it is only effective against adult worms (Ona, 2015). Therefore, eradication of the *Schistosoma* through chemotherapy requires repeated administration of the drug which may not be feasible in resource poor countries. Blocking the transmission between the human and the snail hosts can mitigate against this limitation.

Elimination of the snail intermediate hosts is one strategy for disrupting the life cycle of the *Schistosoma* parasites. Three main methods (chemical, biological, mechanical) of eliminating snails have been proposed and implemented in some cases in endemic areas. Biological control through trophic interactions of host snails is a well exploited field (Chimbari, 2012;

Duval et al., 2015; Hofkin et al., 1992; Pointier and Jourdane, 2000; Slootweg et al., 1994; Sokolow et al., 2014). However, the effects of natural and anthropogenic factors impacting on the interactions of these snails and their natural enemies are largely unknown. Therefore, in this thesis, I add to the body of knowledge on the role of some trophic interactions of *Schistosoma* host snails and the factors affecting these interactions in schistosomiasis control. To this effect, I studied the factors affecting predator-prey interactions of host snails and two predatory organisms, i.e. catfish and crayfish. The work reported in this thesis, therefore, is pioneer in assessing the potential of hybrid catfish and red claw crayfish as predators of host snails and in assessing the impact of anthropogenic pesticide pollution on predator-prey interactions of host snails and catfish. Four objectives outlined in **Chapter 1** guided my analysis.

I addressed these objectives by answering a set of questions which collectively address the overall aim of this thesis: To quantify the **impact of natural and anthropogenic factors on trophic interactions of molluscivores and *Schistosoma* host snails.**

6.2 Influence of environmental and socioeconomic factors on the population dynamics of the host snails vis-à-vie schistosomiasis.

One of the prominent features of factors conditioning the habitats of host snails is their spatial and temporal heterogeneity (**Chapter 2**). The results of a field monitoring case study in Zambia reported in **chapter 3** of this thesis augments findings of chapter 2. Two climatically distinct zones (based mainly on rainfall) endemic with schistosomiasis were studied. The aim of **Chapter 3** was to identify a combination of factors that explain prevalence of host snails and hence the risk factors for schistosomiasis transmission in specific community settings. This section analyses the implications of the findings of the field monitoring study to population dynamics of host snails and consequently the transmission of schistosomiasis.

6.2.1 Demographic factors and schistosomiasis dynamics.

The importance of demographic and socioeconomic parameters in the schistosomiasis disease dynamics has been widely acknowledged (Enk et al., 2010; Geleta et al., 2015; Houmsou et al., 2013; Yang et al., 2009). Within this thesis, health education, age and gender were found to be important factors which influence the risk of getting infected by the *Schistosoma* parasites (**Chapter 3**). Informed people are less likely to engage in risky behaviours. Provision of information through both formal and informal education is an option available to effect behaviour change in affected communities. The impact of education on schistosomiasis infection was demonstrated in this thesis (**Chapter 3**) and elsewhere (Zhou et al., 2013). However, changing behaviour especially that associated with occupational and recreational activities is not easy to achieve (Jamda et al., 2007; Wang et al., 2013). Incorporating health education into formal school curricular can help enhance knowledge and improve attitude towards schistosomiasis (Stothard et al., 2006). Gender and age determine activities that community members are involved in. Our monitoring study showed that young people come in contact with contaminated water through recreational activities while older people are exposed through domestic and occupational activities. Designing awareness and educational programmes based on the social representation theory can help in attitude change that may be required to change risk behaviours (Gazzinelli et al., 2006).

Livelihood strategies of members of adjacent human communities is an important exposure pathway to *Schistosoma* contaminated water. An understanding of these strategies helps to appreciate risk factors that may be associated with the *Schistosoma* transmission cycle thereby informing the development of sustainable control strategies. Overall, from the two case studies, there were differences in the demographic parameters which influenced exposure to the risk of getting infected. Culture seemed to play an important role in the way the human hosts get in contact with snail infested areas. Animal husbandry for instance has a significant effect by bringing the two hosts together in Zone I while cassava processing is a more important pathway in zone III (**Chapter 3**).

The relationship between exposure patterns and livelihood strategies imposes a constraint on control strategies aimed at reducing or severing contact with contaminated water. This is due to the fact that schistosomiasis is a disease of poverty (Hotez et al., 2007) mainly affecting people with limited capacity to diversify to other livelihood strategies as well as those with no access to improved sanitation and safe water. Schistosomiasis is high in communities where agriculture and fishing are dominant livelihood strategies (Adenowo et al., 2015). In this case, the increasing poverty levels in sub-Saharan Africa (Livingston et al., 2011) and the dependence on small scale farming (62% of population of SSA) (Staatz et al., 2007) and fishing perpetuate and possibly expand the incidence and prevalence of schistosomiasis in SSA. Additionally, small-scale farming, offseason gardening and fishing support snail populations by adding pollutants to the water bodies. Leaching of fertilizers from farms and gardens and organic matter (animal and human excreta) input increases the primary productivity of these systems. *Schistosoma* host snails thrive in places with abundant aquatic vegetation (Dida et al., 2014; Hilali et al., 1985) which they use for shelter, food and oviposition.

6.2.2 Habitat factors and trophic interactions of *Schistosoma* host snails.

Similarly, physicochemical and biological factors play a significant role in making the environment suitable for colonization by host snails. However, there are often differences in the importance of these factors from place to place. The monitoring studies presented in **chapter 3** show significant variations between the two zones. Figure 3.5 shows the clustering of pulmonate snails including host snails around physical properties of the water bodies in Zone I and a clear lack of clustering in Zone III. This confirms the large number of environmental factors that influence prevalence of host snails (Hussein et al., 2011; **Chapter 2**). **Chapter 4** showed the significant effect of a biological factor; an alternative food item on predation of host snails. In this study, we observed that crayfish preferred the alternative *M. tuberculata* over *B. globosus* while catfish did not show preference of any of the provided gastropods. The reviewed studies in **Chapter 2** also showed the significance of biological and physicochemical factors of the environment in which host snails are present. Among the physical factors, climatic factors such as temperature, and rainfall due to its effect on water velocity (Appleton, 1978; Opisa et al., 2011; **Chapter 2**) can have both positive and negative effects for snail survival. Members of the genera *Biomphalaria* and *Bulinus* which are responsible for transmitting schistosomiasis in Africa can tolerate a wide range of temperature, i.e. 18-27^{o}C for *B. globosus* (Parashar and Rao, 1988) and 15-31^{o}C for *B. pfeifferi* (McCreesh and Booth, 2014). However outside that range, temperature does affect host snails both directly by impacting snail fecundity, growth and survival (McCreesh et al., 2014) and indirectly by its effect on primary productivity and rate of bacterial decomposition of organic matter. Additionally, due to its effect on the survival, growth and fecundity of organisms, temperature may affect the interspecific interactions of the parasites, prey and predators. Because species respond differently to temperature fluctuations and have different tolerance ranges, mismatches in prey and predator populations can result in trophic cascades (Mas-Coma et al., 2009; **Chapter 2**) which are important in the transmission dynamics of vector-borne diseases like schistosomiasis. The differences in generation time, where smaller organisms tend to have faster generation time (Pörtner, 2002) and increased fecundity due to temperature rise, may result in population increase for parasites and host organisms thereby disturbing the equilibrium and affecting the efficiency of the predators.

Rainfall, and the resulting increase in water flow velocity is another important physical factor influencing population dynamics of *Schistosoma* host snails (Mas-Coma et al., 2009). Host snails are adapted to low water flow rates (Dida et al., 2014; **Chapters 2 and 3)**. The flash flooding events following heavy down pours allow for translocation of host snails to other areas. Additionally, rainfall also determines the length of the dry period (Martens et al., 1995) hence the survival of aestivating snails which serve as seed for repopulating the water bodies during favourable periods (Moser et al., 2014). The effect of water flow velocity on the substrate is another important factor determining the effects of rainfall on host snail population dynamics. Both *Bulinus* and *Biomphalaria* spp. live on aquatic plants and other debris that are rich in decaying organic matter on which they subsist. Fast flowing water washes away small and light substances like organic matter and deposits it down stream where flow rates reduce. Rainfall is a seasonal phenomenon in most of the sub-Saharan African region. Differences in snail abundance were observed for the different seasons. Populations of *Schistosoma* host snails showed a peak during the hot dry season (**Chapter 3**). This can be explained by the favourable temperatures and the reduced water flows during this time in the study areas.

The link between the physical factors especially temperature and water flow velocity influenced by season should be considered in planning control strategies. The population increase during the hot dry season (**Chapter 3**) coincides with reduced flow velocities and fragmented pools of water thereby decreasing the chances of translocation to other areas during this period and allowing for concentrating snail control measures in localised areas. Consequently, control interventions should be planned around these dynamics to increase their effectiveness.

In conclusion, schistosomiasis is a water-based environmental disease (Malone, 2005) due to its dependence on two independent hosts and the parasite's free living phase in water, which makes it highly sensitive to environmental fluctuations. The suitability of a habitat for host snail colonization is dependent on the interaction of biological, physical and chemical factors (**Chapter 2**). These factors have an influence on population dynamics of host snails and hence the schistosomiasis disease dynamics. However, the importance of these factors shows significant variation at both macro and micro levels (**Chapters 3**). These variations underpin the importance of local level investigations to identify important interacting factors in each area. Risk mapping using climate and vegetation data obtained through GIS and remote sensing is valuable as far as mapping potential risk areas is concerned. However, because transmission of schistosomiasis is highly focal and varies within climatologically homogenous regions, small-scale village or community level surveys will continue to be more informative than large scale aggregated risk models (Brooker et al., 2009; Chipeta et al., 2013).

Also the relationship between poverty and schistosomiasis on the one hand and the importance of snail habitat factors on the other hand are important considerations in planning sustainable control strategies. Strategies aimed at reducing the probability of getting infected by reducing the sources of contamination such as removal of host snails provide an effective complementary strategy to chemotherapy in sub-Saharan Africa. Among other methods, biological control may be a suitable option in small-scale control programmes for vulnerable communities. However, the success of biocontrol depends on identification of organisms that are both environmentally and socially acceptable, and adaptive to the site conditions. The findings of this thesis, particularly **Chapter 4**, have contributed to this need.

6.3 Crayfish and catfish as optional predators for *Schistosoma* host snails.

From the literature review in **Chapter 2** it is apparent that reducing or eliminating the host snails is one of the most commonly used *Schistosoma* transmission control mechanism. As already alluded to, the use of chemicals, environmental modification and biological control are the main strategies for this purpose. However, because schistosomiasis often affects poor people with limited capacity to access required chemicals and because environmental modification is more feasible in manmade facilities, biological control could be the most suitable option in natural water bodies. Fish, waterfowl and crayfish are the commonly used snail predators (**Chapter 2**). Limitations such as demand for human consumption associated with the commonly used species of fish i.e. cichlids and the destructive nature of the crayfish, *P. clarkii* affect their usefulness as host snail control agents (**Chapter 4**). The study carried out in this thesis (**Chapter 4**) is an attempt to address these limitations by suggesting other species. The red claw crayfish *C. quadricarinatus* and the hybrid catfish, (a cross breed of *C. gariepinus/C. ngamensis*) were the alternative predators to those reported in **Chapter 2**. Both of these predators successfully preyed on *B. globosus***.** To keep *Schistosoma* host snails away from water development projects *C. quadricarinatus* can be used in place of *P. clarkii* because it is less destructive to these facilities (Alcorlo and Baltanás, 2013; Gherardi, 2006). It can also be an ideal organism to control host snails in water impoundments which are made to supply water for animal and domestic use in arid and semi-arid regions. The lack of appeal of *C. quadricarinatus* as food for most African people is an advantage because it will not be fished out for human consumption. With regard to the hybrid catfish, we observed that its effectiveness as a snail predator is higher when they are still young at which point they are less likely to be caught for human consumption (**Chapter 4)**. This makes them ideal for snail control especially in aquaculture facilities and protected water impoundments to keep snail populations under control.

Because the main aim of this thesis was to assess the impact of interacting factors on trophic interactions of the host snails and their predators we went further to investigate the effect of a biological factor on this interaction. An alternative prey item *M. tuberculata* a non-host gastropod compromised the efficiency of both predators. *C. quadricarinatus* preference of *M. tuberculata* over *B. globosus* limits its efficiency as a biological control organism of *Schistosoma* hosts snails. This observation parallels *S. codringtonii* which preyed more on prosobranch non-host than on pulmonate host snails in Zimbabwe (Moyo, 2004). The opportunistic feeding behaviour of catfish, reported by Dadebo et al. (2014) and observed in this thesis (**Chapter 4**), limits its ability to regulate snail populations in places where food is abundant.

However, the molluscivorous nature of these organisms established for the first time in this thesis enable them to be considered as alternatives to those reported in the literature (**Chapter 2**). The efficiency of both predators under field conditions still remains to be investigated but it is hoped that their use in ideal conditions can play a complementary role to other control strategies.

6.4 Impact of anthropogenic pollution on biological control of *Schistosoma* host snails.

The reviewed literature lacks information (**Chapter 2**) on the effect of pollution on predator-prey interactions between host snails and their predators. What is rather abundant in the literature is the effect of pollutants on individual organisms or individual species. Our study on the effects of pesticides on predator-prey interactions of *B. globosus* and hybrid African catfish represent an attempt to partly cover this gap (**Chapter 5**). We carried out a study in which the predators and prey were simultaneously exposed to the insecticide endosulfan. Our interest was to find out what effect the pollutant would have on the predation efficiency of the hybrid catfish and on the survival of both the predator and the prey. An agricultural pesticide was chosen because agriculture is the backbone of most of sub-Saharan African countries' economies and a source of livelihood for a large part of the rural communities (Chatterjee, 2014). At present agricultural productivity in sub-Saharan Africa is below population growth rate (McIntyre et al., 2009) and food demand. More intensified methods to improve productivity are being introduced hence increasing use of agrochemicals. As observed in this thesis**,** the organochlorine insecticide endosulfan influenced the functioning of predator-prey interactions of the *Schistosoma* host snails. Pesticide pollution has the potential to alter assemblages of aquatic organisms. According to results of this thesis (**Chapter 5**) there was a significant reduction in the rate of predation of snails by catfish as a result of endosulfan pollution. This reduction in predation pressure can lead to increases in snail populations. Additionally, the higher susceptibility of vertebrates than non-arthropod invertebrates to pesticide pollution (Hose and Van den Brink, 2004; **Chapter 5**) can adversely affect predator-prey interactions between *Schistosoma* host snails and fish. It is observed in this thesis that catfish die at much lower endosulfan concentration than at the level where snails are affected. The concentrations at which snails responded to insecticide pollution in this study were several times higher than field relevant concentrations. Based on endosulfan concentration in water and sediment from a few selected water bodies in Africa (Ansara-Ross et al., 2012; El Bouraie et al., 2011; Ezemonye et al., 2010; Gbeddy et al., 2015; Ibigbami et al., 2015; Ntow, 2001; Nyangababo et al., 2005; Syakalima et al., 2006), both lethal and sub-lethal effects are expected on the vertebrate predators while the host snails remain largely unaffected. Evaluation of pesticide contamination of the aquatic systems should be considered in designing transmission control strategies involving biotic organisms in endemic areas. Because aquatic systems receive pollutants of various types from various sources, mixtures of different pollutants are expected. Our study focused on only a single insecticide hence there is a possibility of underestimating or overestimating the effect of pesticides on predator-prey interactions of fish and host snails due to additive, synergistic or antagonistic effects that may form between and among mixtures of agrochemicals (Relyea, 2009). It is recommended that future studies should investigate the effect of realistic mixtures of agrochemicals under semi-field conditions in order to capture both direct and indirect effects on the host snail populations and their predators.

6.5 Integrating socio-economic, environmental factors and predator-prey interactions in the control of *Schistosoma* host snails and schistosomiasis.

The problem of schistosomiasis is multifaceted and its control requires an integrated and interdisciplinary approach. The need for an integrated approach in the control of Neglected

Tropical Diseases, of which schistosomiasis is a part, has been recognised and efforts to implement such a strategy are evident (Molyneux et al., 2005). Some of the benefits of such an integrated approach are reduced costs of implementation which is an advantage for resource poor countries (Sabin Vaccine Institute, 2013). However, this approach is only promoted for the morbidity control of schistosomiasis while integration of efforts in transmission control is not evident. Therefore, it is recommended that;

Firstly, endemic country governments must be proactive in supporting research pertaining to the population dynamics of host snails due to their importance in defining the schistosomiasis disease dynamics. A thorough knowledge of all the interacting factors is fundamental to transmission control of schistosomiasis. According to the findings of this thesis, Zambia is an example of an endemic country where there is no evidence of effort exerted towards understanding the socioeconomic and ecological basis of schistosomiasis. Hence no attempts have been made to control the transmission of schistosomiasis through the *Schistosoma* host snails (Siziya and Mushanga, 1996). To this effect results of this thesis, particular **Chapter 3** are pioneer in addressing this deficiency.

Secondly, in order to raise awareness on the schistosomiasis disease burden and risk factors, endemic country governments like the Zambian Government should deliberately set up programmes to disseminate information about factors governing schistosomiasis like those reported in this thesis. Incorporating health education programmes both through formal and informal learning should be explored. At the formal level, the government through the ministry of education should incorporate into formal school curricular topics specifically looking at schistosomiasis. At an informal level dissemination of information can be achieved through drama groups and clubs especially focussing on community members who may not be enrolled in schools.

Thirdly, although the main focus of this thesis was to quantify the impact of natural and anthropogenic factors on biological control of *Schistosoma* host snails, I also explored the potential of red claw crayfish and hybrid catfish as biocontrol agents. The information generated in **Chapter 4** is valuable to health practitioners as well as to fisheries management. The Fisheries Department could incorporate the findings of this thesis through their extension services, with regard to the benefits of conserving fish species such as the catfish. People are more likely to cooperate in fish conserving measures if they see the health benefits of such a measure.

C. quadricarinatus has invaded many aquatic systems in sub-Saharan Africa (**Chapter 4**) and are generally viewed as a nuisance species especially to the fishing industry. Its use as a snail predator can therefore, be a positive contribution since eradicating it may not be feasible.

Fourthly, the Department of Agriculture extension services can use the findings of **Chapter 5** of this thesis to promote good practices in the use of agrochemicals in schistosomiasis endemic areas. Because offseason agriculture is a common livelihood strategy for rural people (**Chapter 3**) and due to the pivotal role that pesticides play in improving crop health and productivity, the use of pesticides cannot be excluded in these farming systems. However, to strike a balance between improved crop yield and reduced water pollution the Department of Agriculture can adopt and promote practices such as proposed by Nesheim and Fishel (2009) which mitigate against water pollution by agrochemicals.

Finally, statutory bodies such as the Environmental Council of Zambia whose core functions include to: draw up and enforce regulations related to water, air and noise pollution, pesticides and toxic substances, waste management and natural resources management, advise the Government on the formulation of policies related to good management of natural resources and environment and advise on all matters relating to Environment conservation, protection and pollution control, including necessary policies, research investigations and training should consider information on the effects of pesticides on *Schistosoma* host snails in the registration process of pesticides.

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Summary

With a global disease burden of 240 million infected people, of which 90% live in sub-Saharan Africa, schistosomiasis is one of the most prevalent Neglected Tropical Diseases affecting people in the tropical and sub-tropical regions of the world. *Schistosoma* parasites are the causative agents of schistosomiasis. They complete their asexual life stages in susceptible aquatic snails while the sexual stages are completed in susceptible mammals including man.

Control of this disease is targeted at killing the worms in man through chemotherapy using drug praziquantel or disrupting transmission from snails to humans through provision of improved sanitation and clean water, health education or eliminating of susceptible snails. Praziquantel is effective against adult worms but not against the *Schistosomula*. Due to this limitation and coupled with presence of contaminated water and susceptible snails transmission of schistosomiasis continues. Therefore snail control is viewed as an essential complementary strategy to chemotherapy. Use of chemicals, environmental modification and biological control methods can eliminate host snails but suitability of any of these methods depend on many considerations of the environmental and socio-economic setting of the target areas.

The biological control of schistosomiasis using predator-prey interactions is the subject of this thesis. The main premise of this focus is the fact that schistosomiasis occurs among poor people in poor countries who have limited capacity to avoid contaminated water. Chemicals and environmental management are often expensive and out of reach for affected communities. Given this background this thesis aims to contribute to the body of knowledge on the predator-prey interactions of *Schistosoma* host snails and their predators, and the factors affecting these interactions. A clear understanding of the factors affecting predator-prey interactions is fundamental in recommending suitable organisms as biocontrol agents in schistosomiasis control programmes.

Chapter 2 begins by exploring the potential of host-environment relationships in the control of schistosomiasis in Africa through a literature review. A number of human diseases including schistosomiasis are supported by host-parasite-environment interactions. S*chistosoma* parasites are transmitted between the snail intermediate hosts and mammalian definitive hosts in an aquatic environment. This host-environment link determines the parasite transmission dynamics and is a route through which control of the transmission can be achieved. Understanding interacting factors determining host snail population dynamics and

what control options through the host-environment interface are available was the focus of the review. The main result of the review was that quite a large number of factors, both environmental and socioeconomic, affect the transmission dynamics of schistosomiasis. However, temperature and water flow velocity seem to be the more important environmental factors while occupational and recreational activities are the more significant human factors. Control options through the snail intermediate hosts are done through the use of chemicals, environmental modification and by using competitor or predatory organisms. Given the fact that schistosomiasis is a disease of poverty, it became apparent that sustainable management of the disease would require low cost interventions. Biological control is, therefore, better suited as a complementary strategy to chemotherapy. However, it was apparent from the literature that, there was need to study the effect of natural and human factors on the trophic interactions associated with the biocontrol of host snails.

To appreciate the specific factors that may influence the transmission of schistosomiasis in affected communities, two monitoring case studies were conducted in Zambia (**Chapter 3**). From these case studies, we confirm the importance of site specific investigations because the transmission of schistosomiasis is highly focal and neatly linked to the socioeconomic parameters of the affected community. While, in broad terms, occupational, domestic and recreational activities predispose people to infection, the importance of these factors vary from place to place. From these case studies in **Chapter 3** the culture of the communities involved is a significant exposure pathway and influences exposure between gender. For the predominantly cassava growing zone, the processing of cassava exposes females more to contaminated water than males. For the cattle rearing zone on the other hand, pastoralism exposes the males more to contaminated water than females. We also saw that host snails are euryok in nature, being able to adapt to a lot of environmental conditions.

In **Chapter 4**, we shifted our focus from the field to the lab by studying the potential of alternative predatory organisms. Our aim was to broaden the choice base for candidate predators owing to the fact that many organisms that were reported in chapter 2 have limitations in different situations. For instance, the red swamp crayfish's burrowing tendencies does not make them suitable for use in aquaculture and water development projects. The cichlids are important and the most available sources of protein for poor people. These are often caught for food and their depletion allows the growth of the host snail populations. We therefore, studied the potential of the red claw crayfish which does not possess the destructive burrowing characteristic and the catfish which on comparative basis is less demanded for than cichlids. Both these species feed on the host snails but their efficiency is compromised by presence of alternative food. However, in suitable conditions they can reduce the populations of host snails.

Agriculture is an important activity for the rural communities of sub-Saharan Africa. Therefore pollution of the aquatic bodies by agrochemicals is a common feature. We examined in **Chapter 5** the effect of a pesticide on the predator-prey interactions of host snails. The aim was to appreciate how endosulfan may impact on biological control of host snails, hence schistosomiasis. Our findings were that fish are more sensitive to endosulfan toxicity than host snails. We also observed inhibition of predation of snails by catfish at sub-lethal concentrations of endosulfan. Our conclusion is that since the fish are more susceptible to endosulfan toxicity and that endosulfan is found in the field at concentrations known to cause both lethal and sub-lethal reactions, the level of pollution must be taken into consideration when designing control programmes.

Finally, I discuss the results of the various studies in **Chapter 6.** The main conclusion is that identification of suitable predators and understanding the functioning of the predator-prey interactions is important in the biological control of *Schistosoma* host snails and therewith schistosomiasis transmission. This thesis has contributed to this requirement by outlining i) the role of two alternative organisms as predators of host snails, ii) the effect of alternative prey on the efficiency of the predators and iii) the effect of pesticide pollution on the predator-prey interactions. From these studies, it is recommended that more efforts should be put towards identification of more suitable predators to be used as snail control agents. Additionally, due to the multifaceted nature of the schistosomiasis disease, affected country governments should adopt an interdisciplinary approach towards the control of both the disease and its transmission.

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About the author

Concillia Monde was born in 1970 in Choma, Zambia. She obtained a Diploma in Forestry in 1994 from the Zambia Forestry College. She started work at the Zambia Forestry college as an assistant Training Officer. In 1997, she obtained a Diploma in Technical Education from the University of Zambia.

After working as a training officer at Zambia Forestry College, she enrolled into the Bachelor of Science in Forestry programme at the Copperbelt University. For her special project, she focussed on root nodulation for *Sesbania sesban* growing in low P supplied soils. For this she worked with *Ipomoea batatas* as a mycorrhiza trap plant. From this, she found out that growing *S. sesban* with *I. batatas* enhanced quality and quantity of nodules in low P soils. Upon completion of the BSc programme and being the best graduating student, she was then retained as a Staff Development Fellow in the School of Natural Resources of the Copperbelt University. During this time, the School of Natural Resources introduced a BSc programme in Fisheries and Aquaculture. This prompted the school to develop human resource who would handle this programme. As a result, Concillia had to divert from Forestry to Aquatic Sciences. For her Masters, she studied Inland Fisheries in 2007-2008 academic year at the University of Hull in the United Kingdom. Upon completion, she was awarded an MSc in Fisheries Management.

In 2012, an opportunity availed itself for Concillia to pursue her PhD studies. She enrolled at Wageningen University in the Aquatic Ecology and Water Resource Management Group under the supervision of Professor Paul van den Brink. This thesis is a product of 4 years of field and laboratory work. Here she focuses on the role of ecological interactions in the transmission dynamics of schistosomiasis. Going forward, Concillia wishes to continue researching on natural processes that can enhance ecosystem stability.

Outside academics, Concillia is a committed Christian who enjoys attending church and singing. She also enjoys cooking and watching movies whenever time allows.

Netherlands Research School for the
Socio-Economic and Natural Sciences of the Environment

D I P L O M A

For specialised PhD training

The Netherlands Research School for the
Socio-Economic and Natural Sciences of the Environment
(SENSE) declares that

Concillia Monde

born on 24 August 1970 in Choma, Zambia

has successfully fulfilled all requirements of the
Educational Programme of SENSE.

Wageningen, 25 April 2016

the Chairman of the SENSE board	the SENSE Director of Education
Prof. dr. Huub Rijnaarts	Dr. Ad van Dommelen

The SENSE Research School has been accredited by the Royal Netherlands Academy of Arts and Sciences (KNAW)

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The SENSE Research School declares that **Ms Concillia Monde** has successfully fulfilled all requirements of the Educational PhD Programme of SENSE with a work load of 34.4 EC, including the following activities:

SENSE PhD Courses

- Basic Statistics (2012)
- Research in Context Activity: 'Organising committee member for the 6th SETAC Africa Conference - 21st Century Africa and Beyond–Balancing Economic Growth Opportunities With Environmental Sustainability', Zambia (2013)
- Mixed Linear Models (2014)
- Generalized Linear Models (2014)
- Environmental Research in Context (2015)

Other PhD and Advanced MSc Courses

- Scientific Publishing, Wageningen University (2012)
- Information Literacy and EndNote, Wageningen University (2012)
- Introduction to R for statistical analysis, Wageningen University (2012)
- Chemical Stress Ecology and Risk Assessment, Wageningen University (2012)
- Techniques for Writing and Presenting a Scientific Papers, Wageningen University (2012)

Management and Didactic Skills Training

- Teaching in the BSc course 'Aquatic Ecology, Fisheries Management', Copperbelt University, Zambia (2013)
- Consultant on Preliminary study on the drivers of deforestation & potential for REDD+ in Zambia (2012)

Oral Presentations

- *Natural and human induced factors influencing the abundance of Schistosoma host snails in Zambia*. 6th SETAC Africa Conference, 2-3 September 2013, Lusaka, Zambia

SENSE Coordinator PhD Education

Dr. ing. Monique Gulickx