

JIMMA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF ENVIRONMENTAL HEALTH SCIENCE AND TECHNOLOGY

HABITAT SUITABILTY MODELLING OF FRESH WATER SNAILS IN OMO-GIBE RIVER BASIN, SOUTHWEST ETHIOPIA

BY: Getachew Yigezu

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ASSURANCE OF PRINCIPAL INVESTIGATOR

I, the undersigned declare that this thesis is my original work, has not been presented for a degree in this or other Universities and that all sources of materials used for this have been fully acknowledged.

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Abstract

Schistosomiasis is the most widespread water-based disease in sub-Saharan Africa. Transmission is governed by the spatial distribution of freshwater snails that act as inter- mediate hosts and human water contact patterns. The aim of this study was to identify factors that suites freshwater snails which are an intermediate hosts of schistosomiasis in Omo-Gibe basin, southwest Ethiopia. A cross-sectional study design was employed with one hundred-thirty (130) sampling sites in the study areas were selected. Ordination analysis by applying CANOCO for windows version 4.5 software was employed and since the gradient of environmental variable was greater than three (> 3SD), canonical correspondence analysis (CCA) was used for ordination plot. Classification and regression tree models (CART) was applied to develop the models. The classification tree models were built using the J48 algorithm a java reimplementation of the C4.5 algorithm in WEKA. Regression tree models were built using the M5 algorithm in WEKA and in order to relate the abundance of snail intermediate host to habitat and water quality variables. The ordination analysis indicated that pool habitat condition favors the abundance of most of the snail species such as Lymnaea natalensis, Biomphalaria sudanica, Bulinus globosus, and Biomphalaria pfeifferi. The classification tree also demonstrated that presence absence of predators was the most prominent biotic variable influencing the presence or absence of freshwater snails. This variable was selected in 75% of the classification tree models. Canopy cover, habitat type and pH were the most important variables determining the presence or absence of freshwater snail species. In addition the conditional analyses clearly indicated that the abundance of most of the snail species was decreased when the abundance of competitors' macroinvertebrates namely Heptageniidae and Helodidae abundance was greater than 40 individuals.

Key words: - CART, Freshwater Snail species, Omo-Gibe, Habitat suitability, modeling, Schistosomiasis, Ethiopia.

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Acronyms:

- APHA American Public Health Association
- ASTER Advanced Space borne Thermal Emission and Reflection
- BOD Biological Oxygen Demand
- CA Canonical Analysis
- CART Classification and Regression Trees
- COD Chemical Oxygen Demand
- DCA Detrended Correspondence Analysis
- DEM Digital Elevation Model
- DO Dissolved Oxygen
- EC Electric Conductivity
- EPA Environmental Protection Agency
- FFG Functional Feeding Group
- GF-Glass Fibre
- GPS Global Positioning System
- PCA Principal Correspondence Analysis
- SRP Soluble Reactive Phosphate
- TKN Total Kjeldahl Nitrogen
- TN Total Nitrogen
- TP Total Phosphorus
- TSS Total Suspended Solids
- UNEP United Nation Environmental Protection
- UNEPA United Nation Environmental Protection Agency
- WHO World Health Organization

CHAPTER ONE: INTRODUCTION

1.1. Background

In Africa there are several species of aquatic snails that act as intermediate hosts which are important to the transmission biology of schistosomiasis for the development of the parasite to an infective free-swimming larval stage, i.e. cercariae. Humans acquire an infection through cercarial skin penetration during water contact. There are several principal intermediate host snails belonging to two genera; *Bulinus* transmitting *Schistosoma haematobium* and *Biomphalaria* transmitting *S. mansoni*. It follows that the transmission of schistosomiasis is spatially and temporally restricted to water bodies inhabited by intermediate host snails when humans get in contact with the water infested with cercariae during occupational or recreational activities (Simoonga et al., 2009). Schistosomiasis has, therefore, been defined as an environmental disease. Hence, a deeper knowledge of ecological factors, permissive to the development of the parasite-intermediate host snail system, is important to target control interventions (Simoonga et al., 2009).

Schistosomiasis is the most widespread water-based disease in Sub-Saharan Africa. Transmission is governed by the spatial distribution of specific freshwater snails that act as intermediate hosts and human water contact patterns (Walz *et al.*, 2015).

Schistosomiasis is a parasitic disease that affects over 200 million humans across the globe, largely in sub-Saharan Africa. It is transmitted between humans and various species of freshwater host snails. One possible technique for reducing disease transmission is biological control: introducing competitors or predators of host snails to suppress their population and reduce transmission (Swartz, 2014).

Schistosomiasis, also known as bilharziasis, is a parasitic disease that leads to chronic ill health. People infected with schistosomes expel the parasite's eggs in their faeces or urine. In villages or communities without proper latrines or sanitation, freshwater resources around them may easily become contaminated with faeces or urine containing the eggs (Ogden *et al.*, 2013). When they come in contact with water, the eggs hatch and release larvae called miracidia. If the miracidia find the right type of snail, they use it to multiply in several cycles, eventually producing thousands of new parasites, called cercariae, which are then released from the snail into the surrounding water. Humans become infected when they come into contact with skin-penetrating

cercariae in water. A child who has suffered persistent and heavy infections is likely to have chronic, irreversible disease later in life, such as liver fibrosis, cancer of the bladder or kidney failure (WHO, 2010).

Schistosomiasis is characterized as either intestinal or urogenital, depending on where the adult flukes are located. Four species of schistosomes cause the intestinal form (*Schistosoma intercalatum*, S. *japonicum*, S. *mansoni*, S. *mekongi*); in this form the adult worms occupy mesenteric veins, and their eggs pass into the lumen of the intestine and reach the faeces. Adult S. *haematobium*, which cause urogenital schistosomiasis, reside in veins draining the urinary tract, and their eggs normally pass out of the body in the urine. Adult schistosomes are sometimes found in sites other than the intestines or urogenital tract (WHO, 2010). And estimated 207 million people may have schistosomiasis. On the other hand, population growth and the increasing demand for water leads to developments that result in increased transmission and a changing epidemiology of the infection and disease (WHO, 2010).

Hence, among factors governing the distribution of these species, type of water-body, total dissolved chemical composition, or salinity, of the water, current velocity, stream geology and temperature, amongst others, are important (Kock *et al*, 2004).

1.2. Statement of the Problem

Schistosomiasis is endemic in 76 countries, but transmission has been significantly decreased or interrupted in a number of countries, including the Islamic Republic of Iran, Japan and Tunisia, through the implementation of control interventions. A process for certifying elimination of the disease or interruption of transmission has not yet been put in place (WHO, 2010).

High schistosomiasis transmission areas are located in sub-Saharan Africa (mainly *S. haematobium* and *S. mansoni*), Brazil (*S. mansoni*) and the Philippines (*S. japonicum*). A low risk of acquiring Schistosoma infections occurs in the Middle East (*S. haematobium* and *S. mansoni*), Surinam, Venezuela and some of the Caribbean islands (*S. mansoni*), Indonesia and the People's Republic of China (*S. japonicum*), Laos People's Democratic Republic and Cambodia (*S. mekongi*) (Knopp *et al.*, 2015).

Schistosomiasis is most prevalent in Sub-Saharan Africa, where more than 90% of those who are infected people are living. About 62% of all cases live in 10 African countries. In the Caribbean

islands and Suriname, endemicity has been reduced by development and ecological changes. In the Bolivarian, Republic of Venezuela and in Brazil, transmission continues despite achievements made in controlling the disease. In WHO's Eastern Mediterranean Region, control has been successful; and transmission is expected to be interrupted in Egypt, the Libyan Arab Jamahiriya, Morocco, Oman and Saudi Arabia. A control programme has been launched in Yemen; Somalia and Sudan remain the most endemic countries in the region (WHO, 2010).

Schistosomiasis continues to exert pressure against social and economic development, particularly in sub-Saharan Africa where more than 80% of the total number of infected individuals and the global burden of this often neglected tropical disease are concentrated. Despite considerable progress made in morbidity control of schistosomiasis in several African countries –facilitated through large-scale administration of Praziquantel to school-aged children and other high- risk groups– the disease has expanded elsewhere and the transmission has intensified in areas where water resources have been developed, such as large dams and irrigation systems. Hence, a deeper knowledge of ecological factors, permissive to the development of the parasite-intermediate host snail system, is important to target control interventions (Simoonga et al., 2009).

Two main forms of human schistosomiasis or bilharzia exist in Africa – urinary schistosomiasis caused by *Schistosoma haematobium* infection and intestinal schistosomiasis caused by *Schistosoma mansoni* infection. There are around 165 million people in sub-Saharan Africa with the disease: about 112 million with urinary schistosomiasis and about 54 million with intestinal schistosomiasis. The mainstay of the current strategy recommended by WHO against schistosomiasis is morbidity control through preventive chemotherapy with praziquantel (PZQ) (Touré *et al*, 2008).

As indicated by (Kock *et al.*, 2004), intermediate host for *Schistosoma mansoni*, *Biomphalaria pfeifferi* plays a major role in the transmission of intestinal bilharziasis in the endemic areas of South Africa. It is evident from his study that details of each habitat, as well as mean altitude and mean annual temperature and rainfall of each locality, was used to construct an integrated decision tree that makes a selection of those variables that could maximally discriminate between this snail and all the other species in the database. The results of his study indicated that

temperature and type of water-body are the major factors determining the distribution of *B*. *pfeifferi* in South Africa.

Moreover, according to (Tadesse, 2008), further studies are needed to monitor and control parasitic infections such as creating community awareness on the proper disposal of wastes and control of the snail. Climate change may drive schistosomiasis and fascioliasis towards elimination in the far future of 2090, although other factors such as land-use changes, transmission awareness and interventions may play an important role on the distribution and may in fact overrule that of climate (Pedersen et al., 2014).

Most studies done in different countries of Africa on snail intermediate hosts of schistosomiasis focuses on the geographical distribution and habitats of this species (i.e. distribution and habitats of *Biomphalaria pfeifferi*, snail intermediate host of *Schistosoma mansoni*, in South Africa (Kock *et al.*, 2004), the factors influencing patterns of freshwater snails distribution in Oyan Reservoir, a typically medium sized manmade reservoir in southwest Nigeria (Ofoezie, 2015), survey of intermediate host snails of schistosomiasis to determine their abundance and distribution in the lake and land aquatic habitats of Lake Victoria basin of Kenya (Ofulla *et al.*, 2013), an overview of the progress made in the use and application of geographical information systems (GIS) and remotely sensed (RS) satellite sensor data for the epidemiology and control of schistosomiasis in sub-Saharan Africa (Brooker, 2002), the distribution, habitats and role as intermediate host of the freshwater snail, *Bulinus forskalii*, in South Africa (Kock & Wolmarans, 2005).

In Ethiopia, different studies were conducted on the assessment of the prevalence and intensity of *S. mansoni* infection and other intestinal helminth among different water source users (Dejenie & Asmelash, 2010), an epidemiological study with the objective to assess the establishment of transmission of *Schistosomiasis mansoni* in Tikur Wuha area, southern Ethiopia (Mitiku *et al.*, 2008), epidemiological study to determine the status of schistosomiasis and soil-transmitted helminthiasis among primary school children in Adwa Town, northern Ethiopia (Legesse and Erko, 2010), an infection prevalence of human intestinal schistosomiasis (*S. mansoni*) in communities living near three rivers of Jimma town, south western Ethiopia (Mengistu *et al.*, 2011), epidemiological survey in children of four primary schools in Raya Alamata District of Ethiopia to determine the prevalence and intensity of infection, and index of potential contamination of intestinal schistosomiasis (Dejenie, Legese, Tomas, & Kiros, 2013), a review

work was undertaken to compile available literature information on the characteristics of the parasite; about its epidemiology, public health significance and economic impact of bovine Fasciolosis (Engdaw & Gebrie, 2015).

On the other hand, water quality and habitat factors are used to develop habitat suitability modeling. Snail species assemblages are dependent on physical factors such as canopy cover, substrate type, water current, temperature, turbidity, transparency and vegetation cover; chemical factors such as ion concentration and dissolved gases in water as well as biological factors such as availability of food, competitors and predators (Ofoezie, 1999). Thus, a better understanding of the ecological factors that enhance the development of the parasite-intermediate host snail system is important to facilitate control interventions.

Although several epidemiological studies for intestinal schistosomiasis due to *S. mansoni* infection were carried out in different parts of Ethiopia, limited studies are available on the habitat suitability modeling of snail intermediate host in the country. Hence, this study aims to identify the most suitable habitat for freshwater sails to be targeted for snail control interventions in the Omo-Gibe river basin, Southwest Ethiopia.

1.3. Significance of the Study

The information obtained from the model of environmental and biotic suitability for freshwater snails an intermediate host for schistosomiasis transmission will:

- Help to identify environmental and biotic factors to be targeted for the snail control.
- Support measures to gain and sustain control of freshwater snails an intermediate host for schistosomiasis. This is particularly relevant as emphasis is shifting from morbidity control to interrupting transmission.
- Provide a useful tool to monitor the development of new hotspots of potential schistosomiasis transmission based on habitat data.

CHAPTER TWO: LITERATURE REVIEW

2.1. Schistosomiasis

Schistosomiasis is a chronic, debilitating parasitic disease caused by blood flukes of the genus Schistosoma. Freshwater snails, after being infected by schistosome "miracidiae," (larvae that emerge by the hatching of eggs found in human excreta, deposited in the water) act as intermediate hosts. The infected snails produce other larvae called "cercariae," which infect humans by entering the body through the skin during water contact. The disease, also known as "bilharzia," is endemic in 74 countries in Africa, South America and Asia. Worldwide, an estimated 200 million people are infected, of which 20 million is assumed to suffer from more or less a severe form of the disease creating 4.5 million daily lost. Schistosomiasis is endemic in 46 out of the 54 countries in the African continent. The disease may cause damage to various tissues (the bladder, liver or the intestines) depending on the species, and lower the resistance of the infected person to other diseases. There are 16 different known species of Schistosoma (S), of which five are infective to man -- S. mansoni, S. haematobium, S. intercalatum, S. japonicum and S. mekongi. The species differ according to their snail intermediate hosts, egg morphology, and final location of the adult worms in the human body, resulting symptoms, and their geographical distribution. The most common forms of the disease in Africa are: intestinal schistosomiasis, which is caused by S. mansoni; and urinary schistosomiasis, which is caused by S. haematobium. In sub-Saharan Africa, approximately 393 million people are at risk of infection from S. mansoni, of which 54 million are infected. Those numbers for S. haematobium are estimated to be as high as 436 million at risk, of which 112 million are infected (Boelee & Madsen, 2006).

The intermediate hosts of schistosomes in Africa are freshwater pulmonate snails, which belong to the "Planorbidae" family. The species belong to two genera, namely Biomphalaria, host for *S. mansoni*, and Bulinus, host for *S. haematobium* and *S. intercalatum* (Sturrock 1993). Both Biomphalaria and Bulinus species prefer water velocities below 0.3 m/s, gradual changes in the water level, a slope of less than 20 m/km, firm mud substrate, little turbidity, some organic pollution, partial shade and optimal water temperature between 18°C and 28°C (Boelee & Madsen, 2006).

The snails are considered to be intermediate hosts because humans harbour the sexual stages of the parasites and the snails harbour the asexual stages. People serve as vectors by contaminating the environment. Transfer of the infection requires no direct contact between snails and people. Freshwater snails are also intermediate hosts of food borne fluke infections affecting the liver, lungs and intestines of humans or animals. Schistosomiasis is a disease caused by infection with blood flukes of the genus Schistosoma. Transmission of, and exposure to, the parasite result from faecal or urinary contamination of freshwater containing intermediate host snails, and dermal contact with the same water (Grimes *et al.*, 2015).

2.2.Life cycle of Schistosomiasis

Schistosomiasis is a water-based parasitic disease, and the causative agents are blood flukes of the genus Schistosoma. There are six Schistosoma species that can infect humans. However, of particular public health importance are only three species, namely S. haematobium, S. japonicum and S. mansoni. Schistosomiasis has a fairly complex life cycle. In brief, the adult female and male worms live in permanent pairs in the perivesical (S. haematobium) or portal veins and mesenteric blood vessels (other species). Eggs produced by the female worms reach the bladder or intestine, respectively, from where they can be excreted with urine or feces. In case the eggs get in contact with freshwater, the parasitic first-stage larva, the miracidium, hatches. Miracidia infect species-specific intermediate hosts, freshwater snails of the genus Bulinus (S. haematobium, S. intercalatum and S. guineensis), Biomphalaria (S. mansoni), Oncomelania (S. japonicum) and Neotricula (S. mekongi), in which they multiply asexually. Snails shed hundreds to thousands of cercariae (4-6 weeks after infection), and this larval stage is infective for humans. If humans get in contact with natural freshwater, cercariae are able to penetrate their skin, are transported with the blood stream via the lungs to the liver, mature into adult worms in the portal veins and finally mate and migrate to their final destination. Although most Schistosoma species have humans as the only definitive host, S. japonicum also infects a large range of domestic and wild animals, including cattle, dogs, pigs, water buffaloes and rodents, thus contributing to disease transmission (Knopp et al., 2015).

Modeling running waters based on ecological knowledge and monitoring data has proven to considerably facilitate and improve the assessment of habitats and the determination of the relationship between environmental variables and the occurrence of certain organisms. Different modeling techniques have been applied to running waters, mainly focusing on the environmental responses of river communities to specific disturbances. Modeling the distribution of taxa as a function of the abiotic environment, often called habitat suitability modeling, has been recognized as a significant component of conservation planning (Hoang *et al.*, 2010).

2.2. Habitat/Ecology of freshwater snails

The planorbid intermediate hosts have become adapted to a wide range of environmental conditions. They are found in freshwater bodies-large and small, flowing and standing; in waters with pH varying between 5.8 and 9.0; in tropical forest regions and in arid situations; at low or at high altitudes; and at water temperatures from 20°C to 30°C (WHO, 1968).

Usually they inhabit the shallow waters-either still or only slightly flowing-of streams with moderate organic content, moderate light penetration, little turbidity, a muddy substratum rich in organic matter, submergent or emergent aquatic vegetation, and abundant micro- flora. They have been collected from rivers, lakes, marginal pools along streams, borrow-pits, marshes, flooded areas, irrigation canals, aqueducts, and water cress fields. They are not found in environments with high tidal fluctuations or in reaches of streams having a fall steeper than 20 meters per 1,000 meters of length, though protected pools or swampy areas alongside these steep streams often harbor them. They are generally not found in situations close to the sea, but in some areas fair-sized colonies have been discovered only about 200 meters up- stream (WHO, 1968). Snail-control can be an important component of integrated campaigns against schistosomiasis transmission. Chemicals (molluscicides) can be effective in the control of snails on the short term, but not as a long-term measure as they are too expensive, eco-toxic and unsustainable. Environmental control of schistosomes' intermediate hosts (freshwater snails) offers good alternative approaches, especially when they reduce man-water contact as well. The main options available are adapted system design, adequate water management and proper maintenance (Boelee & Madsen, 2006).

Snail habitats include almost all types of freshwater bodies ranging from small temporary ponds and streams to large lakes and rivers. Moreover, snail densities vary significantly with the season. In general, the aquatic snail hosts of schistosomes occur in shallow water near the shores of lakes, ponds, marshes, streams and irrigation channels. They live on water plants and mud that is rich in decaying organic matter. They can also be found on rocks, stones or concrete covered with algae or on various types of debris. They are most common in waters where water plants are abundant and in water moderately polluted with organic matter, such as faeces and urine, as is often the case near human habitations. Plants serve as substrates for feeding and oviposition as well as providing protection from high water velocities and predators such as fish and birds. Most aquatic snail species die when stranded on dry land in the dry season. However, a proportion of some snail species are able to withstand desiccation for months while buried in the mud bottom by sealing their shell opening with a layer of mucus (WHO, 1968).

Therefore, regression models confirmed the importance of the use of environmental variables to characterize the snail habitat in the endemic area (Guimarães *et al.*, 1984).

The alteration of the physical structure of the habitat is one of 5 major factors from human activities that degrade aquatic resources. Habitat, as structured by instream and surrounding topographical features, is a major determinant of aquatic community potential. Both the quality and quantity of available habitat affect the structure and composition of resident biological communities (Barbour, M.T., J. Gerritsen, B.D. Snyder, 1999).

2.3. Factors that affect the distribution of Schistosomiasis in fresh water

Ecological niche is a term for the position of a species within an ecosystem, describing both the range of conditions necessary for persistence of the species, and its ecological role in the ecosystem. Ecological niche subsumes all of the interactions between a species and the biotic and abiotic environment, and thus represents a very basic and fundamental ecological concept. The tentative definition presented above indicates that the concept of niche has two sides which are not so tightly related: one concerns the effects environment has on a species, the other the effects a species has on the environment. In most of ecological thinking, however, both meanings are implicitly or explicitly mixed. The reason is that ecology is about interactions between organisms, and if persistence of a species is determined by the presence of other species (food sources, competitors, predators, etc.), all species are naturally both affected by environment, and at the same time affect the environment for other species. Why a species' range sometimes ends abruptly, even when the environment changes smoothly across space, has interested ecologists and evolutionary biologists for many decades (Polechov, 2014)

2.3.1. Biotic Factors

Biological parameters refer to aspects of the living environment, from microscopic algae and invertebrates to macrophytes and fish (Farrell-Poe, 2000). Aquatic vegetation and algae, which

are the main sources of nutrition for aquatic snails, flourish creating favorable habitats for intermediate snail hosts (Boelee & Madsen, 2006).

2.3.2. A biotic factors

The Planorbidae vectors of schistosomiasis possess certain peculiarities that need to be mentioned at the outset. Being freshwater organisms, they inhabit limited physical spaces, but they continue to grow throughout their life span. They are found in a wide variety of habitats, and in particular in shallow, lentic/still waters (lakes, ponds, or wetlands), and lotic/flowing bodies of water with weak currents. In general they are highly, or at least relatively, resistant to desiccation. They live under a narrowly defined range of conditions, in terms of temperature, salinity, and pH: the optimal temperature for their development is 20-26°C, and they maintain themselves at a lower limit of 18°C and an upper limit of 30-32°C; *B. glabrata* is inhibited at sodium chloride concentrations of 6,000 p.p.m., and the optimal pH for development is in the range of 7.0-8.0 (Barbosa & Barbosa, 1994).

In tropical regions, the factor that has the greatest effect on freshwater snail population dynamics is rainfall. Some natural habitats dry out for a substantial part of the year, leading to the death of the major part of their Planorbidae populations, notwithstanding the ability of these animals to resist drought. However, the extremely high reproductive capacity of these animals ensures that these habitats are quickly repopulated (within no more than six weeks) once the rains have arrived (Barbosa *et al*, 1994).

In permanent habitats, fluctuations in the distribution, size, and density of Planorbidae populations occur without any apparent cause (Barbosa *et al*, 1994).

2.4. Habitat suitability modeling

Water quality and habitat factors are used to develop habitat suitability modeling. Snail species assemblages are dependent on physical factors such as canopy cover, substrate type, water current, temperature, turbidity, transparency and vegetation cover; chemical factors such as ion concentration and dissolved gases in water as well as biological factors such as availability of food, competitors and predators (Ofoezie, 1999). Thus, a better understanding of the ecological factors that enhance the development of the parasite-intermediate host snail system is important to facilitate control interventions.

In general, the areas predicted to be unsuitable habitats for snail are the dry and hot areas of northern and north-eastern Uganda and for some of the snail species, also scattered parts of the cooler southwestern Uganda with low precipitation. Environmental parameters, such as water temperature, oxygen content, pH, stream flow, etc., potentially have a greater influence. Finally, the spatial scale or resolution of the model can also determine the patterns of association detected between the environmental variables and species presence/absence (Stensgaard et al, 2006).

Conceptual Fram	e work tl	ne Study
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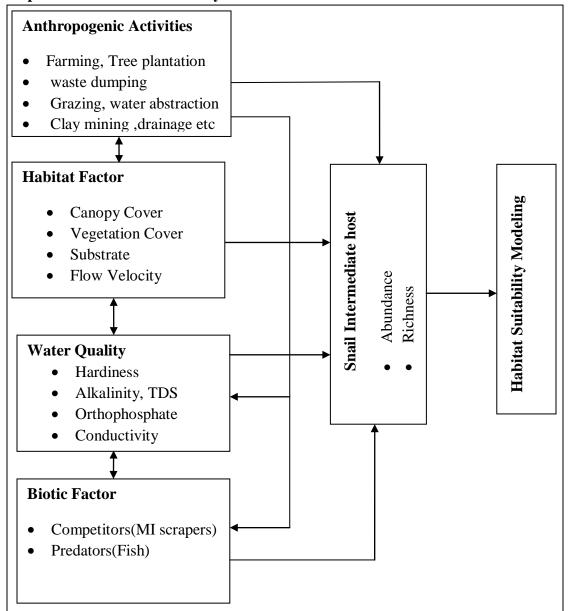


Figure 1: Conceptual Frame work of habitat suitability modeling of freshwater snail in Omo-Gibe river basin, Southwest Ethiopia.

CHAPTER THREE: OBJECTIVES

3.1. General objective

The main objective of this study was to identify and determine the most important factors that affect the occurrences and abundance of freshwater snails in Omo-Gibe river basin, Southwest Ethiopia.

3.2. Specific objectives

- To determine the occurrences of freshwater snails.
- To determine the abundance of freshwater snails.
- To identify the biotic and abiotic factors affecting the occurrences of freshwater snails.
- To identify the biotic and abiotic factors affecting the abundances of freshwater snails.

CHAPTER FOUR: METHODS AND MATERIALS

4.1. Study Area

The study was carried out in the Omo-Gibe river basin, southwest Ethiopia, extending from $4^{\circ}25'51.611"$ to $9^{\circ}22'28.047"N$ latitude and from $33^{\circ}0'24.434"$ to $38^{\circ}24'42.242"E$ longitude (Figure 2). The Omo-Gibe river basin has an areal coverage of 79561.2 km². Elevation data derived from ASTER DEM imagery shows that the basin has an altitude between 500 and 3000 m.a.s.l., with 57% of their total area below 1500 m.a.s.l. Altitude classification from DEM (based on altitude ranges to delineate Agro-ecological zones of Ethiopia, determined by (Hurni *et al.*, 2015) also indicate that four Agro-ecological zones are found in these watersheds; which, according to their dominance, are Kolla (38%), Weyna Dega (37%), Bereha (19%), and Dega (6%).

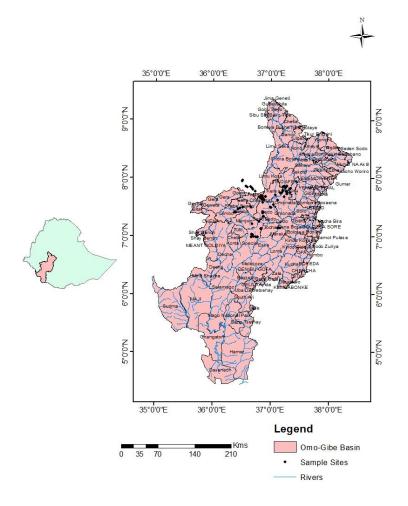


Figure 2: Map of Omo-Gibe river basin, Southwest Ethiopia.

Chebera Churchura National Park (CCNP) is one of the recently declared wildlife protected areas of the Southern Nations Nationalities and Peoples Regional State (SNNPRS) of Ethiopia(Megaze, Belay, and Balakrishnan, 2012). It is partly located within Dawro zone and in Konta Special Woreda, about 300 km and 580 km southwest of Awassa and Addis Ababa, respectively. It covers an area of 1250 km2 and lies between the coordinates 36°27'00''- 36°57'14''E and 6°56'05''-7°08'02''N. CCNP is bordered by Konta Special Woreda to the north, Omo River to the south, Dawro zone to the east and southeast, and Agare high mountains and Omo River to the west(W/Yohannes, 2006).

The mean annual rainfall of the area is 2,154 mm. Shoshema, Zigina, Mensa and Tikurwuha Rivers and their tributaries drain the area. These rivers join the Daworo zone of the Park, then flow to Omo River, that bounds the Park at the southern boundary. There are five lakes (Shita, Shasho, Keribela, Bahi, Koka and Chefre) located at the southeast, west, northwest and north of the Park. There are also several hot springs in different parts of CCNP. CCNP has four major vegetation types: savannah grasslands with scattered trees, woodlands, montane forest and Riverine forest (Megaze et al., 2012).



Figure 3: Some habitats from Chebera Churchura National Park; (A) Bulo/ Koka lake, (B) Keribela lake, Omo-Gibe river basin, Southwest Ethiopia.

Sampled habitats in Chebera Churchura National Park includes: Lakes (Bulo/Koka and Keribela), Rivers (Shoshema, Mery/Meri, Ajikolle, and Nechwuha), Hot springs (Hage and Filwuha), and Chebera irrigation ditch. These rivers are flowing into Zigna River which finally drains into Omo River. River habitats like Didiba, Shakta, Wegaye which flows into Gojeb and Gojeb river which drains into Omo river were sampled from downstream and upper stream along Tercha-Gojeb road (i.e., from Tercha Zonal seat of Dawro towards Dedo). Moreover, river habitats like Anchano, Keso, and Gonfa were sampled along the Dedo- Gojeb road. Bedesa, Dakache, Umech, Kishe, Fiturari, Meti and Gulufa river habitats were also sampled.

Gilgel Gibe watershed is part of the Omo-Gibe river basin which is one of the twelve main basins of Ethiopia. In this watershed, Gilgel Gibe River is one of the major tributaries of the Omo-Gibe River in south-western Ethiopia. The study area receives annual rainfall in the range of 1200-2800 mm. It is situated between the latitudes 7°25'00'' and 7° 55'00'' N and between the longitudes 36°30'00'' and 37° 22'00'' E, and the altitude ranges from 1096 to 3259 m above sea level. The river basin has an area of about 5371 km2 estimated with Arc view-GIS (Ambelu, 2009).

Along the road Jimma-Dedo-Omo Nada river habitats like Beyem, Kawa, Lote, Unta and Ofole which flows into Gilgel Gibe were sampled. River habitats that include Nedi, Kebela, Lanjebo, and Menisa were sites that had been sampled along the road Gibe town-Tiro Afeta towards Gilgel Gibe Dam1. Additionally, Yedi, Nada Guda, Chilelu, Arer, Bulbul, Merewa and Mendiwo river habitats along Jimma-Deneba main road were sampled.

4.2. Source and study population

4.2.1. Source population

All aquatic natural and manmade habitats such as wetlands, reservoirs/dams, streams, rivers, irrigation ditches and other temporary aquatic habitats, which are found in Gibe-Omo river basin, were the potential sites for the snail intermediate host breeding habitats.

4.2.2. Study population

The study population for snail habitat sampling included: Gilgel Gibe dam one, wetlands like Awetu, Kito, and Haro, major rivers and third order streams, and an irrigation ditch.

4.3. Study Design and Period

A cross-sectional study design was employed to collect data for this study and data were collected during dry season from February to May 2016 to identify the most important factors that affect the occurrence and abundance of freshwater snails in Omo-Gibe river basin, Southwest Ethiopia.

Study Variables

- ➤ Habitat and water related variables
 - Vegetation cover
 - Substrate type
 - Canopy cover
 - Habitat type
 - Flow velocity
 - Land use pattern
 - Water physico-chemical quality
 - Water and ambient air temperature
- Biotic Variables
 - Competitors presence/absence
 - Predators presence/absence (Fish)
- Snail Intermediate host related variables
 - Snail abundance
 - Snail presence/absence

4.4. Sampling technique

4.4.1. Snail habitats site selection

Snails were collected using scoop and rectangular frame net (20 x 30cm) with a mesh size of 300µm from each habitats. Within each habitat, all stones and vegetation were overturned, surfaces scraped, and the substrate was agitated by hand and snails were removed. The collected snails were transported to department of Environmental Health laboratory, Jimma University for identification at lowest possible taxonomic level and storage of voucher specimens. In the laboratory the snails were identified at species level and some freshwater snails like Sphaeriidae and Unionidae were identified at family taxonomic level according to their malacology based on

morphological structure of their shell contribution to the monograph (Mandahl-Barth, 1962 and Harrold, 2010).



Figure 4: Sampling of Freshwater Snails and their habitats, Omo-Gibe river basin, Southwest Ethiopia.

4.5. Data collection

Data was collected from 130 sampling sites from March 22, 2016 to May 05, 2016. At each sampling site data related to habitat and disturbance, water quality, and snail predator and competitor sampling was collected during the dry season only in one round.

4.5.1. Habitat assessment and disturbance scoring

Habitat characteristics were assessed at each sampling station over a 500 meter reach using the USEPA habitat assessment protocol (Baldwin *et al.*, 2005). Land-use patterns were classified as farming, waste dumping, clay mining, grazing, damming, tree plantation, vegetation clearance, ditching, filling and draining. The intensity of land-use was quantified an ordinal scale based on the protocol described by Hruby (2004), and modified by Mereta *et al.*, (2013) (0 = no disturbance, 1 = minimal disturbance, 2 = moderate disturbance and 3 = high disturbance). The overall intensity of land-use at each site was calculated by summing the values of the individual land-use categories. Data on the size of the water body (area), substrate type, vegetation cover, canopy cover and land use pattern was collected for each sampling site. Water and sludge depth was measured using a metal ruler at different points of each habitat and average depth was recorded. Substrate was classified into silt, sandy, gravel. The emergent, submerged and floating plant cover of a habitat was visually estimated as the percentage cover and categorized as very

low (<10%), low (10-35%), moderate (35-65%), high (65-90%) and very high (>90%)(Parsons et al., 2001). Canopy cover was defined as the amount of vegetation covering the water surface (Sodhi *et al.*, 2010)

4.5.2. Water quality sampling and analysis

Dissolved oxygen, conductivity, turbidity, pH and water temperature were measured in the field using a multi-probe meter (HI 9829-13102 Hanna, Romania). At each site one liter of water was collected and stored on ice until return to the Laboratory of Environmental Health at Jimma University, where the samples were analyzed for, hardness, chloride, nitrate and ortho-phosphate according to the standard methods as prescribed by (APHA, 1995). In addition, transparency was measured by a 30 cm diameter secchi disc. Physical variables such as sludge depth, and water depth were measured at each site using tape meter.

4.5.3. Snail predator and competitor sampling and identification

Macroinvertebrates were collected at each sampling station using a rectangular frame net (20 x 30cm) with a mesh size of 300 μ m. Each collection entails a 10-minute kick sampling over a distance of 10 metres (DNRE, 1999). Time was allotted proportionally to cover different meso-habitats such as open water and emergent vegetation. The bottom sediment was disturbed by foot during sampling in order to collect the benthic macroinvertebrates (Gabriels *et al.*, 2007). Macroinvertebrates were sorted in the field, stored into vials containing 80% ethanol and labelled. Afterwards, macroinvertebrates were identified to family level using a stereomicroscope (10 x magnifications) and the identification key of Bouchard (2004). Each family was categorized into one of the five functional feeding groups (FFG): gatherer-collector, filterer-collector, predator, scraper, and shredder (Tomanova *et al.*, 2006). The effects of competitors on intermediate snail hosts were evaluated by considering the abundance of scrapers. In addition the effects of predators on snail population were evaluated by considering the presence absence of fish. To this end, fyke nets were used for fish sampling. Fyke nets were put in each habitat during the day and retrieved after about 24 hours. Specimens of fish were identified using taxonomic keys (Holden and Reed, 1991).



Figure 5: Macroinvertebrates and Freshwater Snail and identification in the Environmental Sciences Laboratory of Jimma University

4.6. Data Analysis

4.6.1. Multivariate data analysis

To determine whether a linear or Unimodal type of response was present along environmental gradients, the data-set was first analysed using a Detrended Correspondence Analysis (DCA) in CANOCO4.5 (Braak & Prentice, 2004) in order to examine whether RDA or CCA was appropriate for data analysis.

When the gradient length is < 3SD Linear PCA was used; when the gradient length is > 3SD Unimodal CA was used. DCA is popular among practical field ecologists, presumably because it provides an effective approximate solution to the ordination problem for a Unimodal response model in two or more dimensions - given that the data are reasonably representative of sections of the major underlying environmental gradients (Jongman, 1995). Hence, since the gradient of environmental variable was greater than three (> 3SD), canonical correspondence analysis (CCA) was used for ordination plot. Based on stepwise forward selection, environmental factors were selected as predictor variables. Snail species and environmental data, except for pH, were log transformed [log(y+1)] prior to analysis for the purpose of obtaining homogeneity of variance (Smilauer, 2003).

4.6.2. Classification and Regression Tree Models (CART)

Habitat and water quality variables were used to determine the most important factors for the prediction of snail species at different breeding habitats. Classification and regression tree models (CART) were applied to develop the models. The classification tree models were built

using the J48 algorithm (Quinlan, 1993), a java re-implementation of the C4.5 algorithm, which is a part of machine learning package WEKA (Witten and Frank, 2005). Regression tree models were built using the M5 algorithm in WEKA (Witten and Frank, 2005) and (Bouckaert et al., 2014) in order to relate the abundance of snail intermediate host to habitat and water quality variables. Default parameter settings were used to induce the trees.

Model training and validation was based on a three-fold cross validation procedure (Witten and Frank, 2005). The dataset was stratified into three sub sets, of which two subsets were used as training data and the remaining one subset was used for testing the model. The cross validation process was repeated three times each with one of the three subsets used once as the validation data set. In this way, three models were built. The results from the three models were averaged to produce a single prediction of the dependent variable.

The percentage of correctly classified instances (CCI) (Witten and Frank, 2005) and Cohen's Kappa statistic (K) (Cohen, 1960) was used to evaluate the predictive performance of the classification tree models. The CCI is the percentage of the true positive and true negative predictions, which is calculated based on a confusion matrix.

CCI is mathematically expressed as follows:

$$CCI = \frac{(TP + TN)}{(TP + FP + TN + FN)}$$

Cohen's Kappa statistic simply measures the proportion of all possible cases of presence or absence that are predicted correctly by a model after accounting for chance predictions. It is mathematically expressed as follows:

$$K = \frac{\left[(TP + TN) - (((TP + FN)(TP + FP) + (FP + TN)(FN + TN))/n) \right]}{\left[n - (((TP + FN)(TP + FP) + (FP + TN)(FN + TN))/n \right]}$$

Where; n = is the total number of instances.

TP = True positive, TN = True negative, FP = False positive and FN = False negative instances Models with K higher than or equal to 0.4 will be considered reliable (Dakou *et al.*, 2007; Gabriels *et al.*, 2007). CCI is affected by the frequency of occurrence of the taxon being modeled (Manel *et al.*, 2001). Unlike CCI, k takes a correction into account for the expected number of correct predictions due to randomness, which is strongly related to taxon prevalence (Manel *et al.*, 2001). The ranges of k recommended by (Landis and Koch, 1977) were applied for model performance evaluation: k < 0 (poor), 0-0.2 (slight), 0.2-0.4 (fair), 0.4-0.6 (moderate), 0.6-0.8 (substantial) and 0.8-1 (almost perfect). The determination coefficient (R^2) value was used to evaluate the performance of the regression tree models (Déath and Fabricius, 2000). The determination coefficient is a measure of the goodness of fit of the models (Kallimanis *et al.*, 2007). Its value is always between 0 and 1. The closer the value is to 1, the better the model predicts the training data.

4.7. Quality Control and Quality Assurance

Sampling equipment was rinsed by water from which the sample was taken after each sampling occasions to avoid contamination of the samples. Field and laboratory instruments and measurement were calibrated and standardized. Sample labels were properly completed, including the sample identification code, date, and stream name, sampling location, and collector's name, and placed into the sample container. The outside of the container was labeled with the same information.

After sampling was completed at a given site, all nets, pans, etc. that have come in contact with the sample were rinsed thoroughly, examined carefully, and picked free of organisms or debris. The equipment was examined again prior to use at the next sampling site (Barbour et al, 1999).

Specimens were properly labeled, preserved, and stored in the laboratory for future reference. Basic taxonomic literature is essential in aiding identification of specimens and was maintained. Identification was carried out by an experienced person (Barbour et al.; 1999).

4.8. Ethical and environmental considerations

Ethical clearance was obtained from ethical review board of Jimma University College of Health Sciences. Written consent was obtained from regional, zonal, district and kebele administrations.

4.9. Dissemination plan

The final study will be presented to Jimma University, College of Health Sciences, Department of Environmental Health Science and Technology. It will be disseminated to different relevant stakeholders (e.g. health program managers) who might have contribution for the implementation of the result of this study. Furthermore, efforts will be made to publish at national and international journals.

CHAPTER FIVE: RESULTS

5.1. Occurrences and abundances of freshwater snails

As shown in Table 1 a total of 2561 freshwater snails was sampled. Among freshwater snails sampled *Biomphalaria pfeifferi* showed the highest frequency of occurrence 51 (39%) of the sampling sites and *Lymnaea natalensis* were observed to occur in 38 (29%) of the study sites. A snail species *Bulinus globosus* occurred in 22 (16.8%) of the areas sampled. *Physa acuta* and *Lymnaea truncatula* were observed in 8 (6.1%) and 7 (5.3%) of the sampling sites respectively. *Biomphalaria sudanica* was observed in only 2 (1.5%) of the study sites. Among freshwater snails identified at family level Sphaeriidae occurred in 31 (23.7%) of the sampling sites and Unionidae only in 1 (0.76%) of the sites.

As indicated in Table 1, *B.pfeifferi* was the most abundant 906 (35.5%) freshwater snail species followed by *L.natalensis* 421 (16.4%) of the total snails collected. But, from snails identified at family taxonomic level Sphaeriidae was the most abundant 810 (31.6%) in abundance. *B.sudanica* and *Physa Spp.* were the least abundant accounting for 8 (0.003%) and 1 (0.003%), respectively which was less than 1% of the relative abundance in the study sites.

Species/Family	Number of positive sites	Snail Occurrence (%)	Snail Abundance (%)
L.natalensis	38	29	421 (16.4)
L.truncatula	7	5.3	18 (0.01)
P.acuta	8	6.1	336 (13.1)
P.Spp.	2	1.5	7 (0.003)
B. pfefferi	51	39	906 (35.5)
B.sudanica	2	1.5	8 (0.003)
B.globosus	22	16.8	53 (0.02)
T. hispida	1	0.76	1 (0.0004)
Sphaeriidae	31	23.7	810 (31.6)
Unionidae	1	0.76	1 (0.0004)
Total			2561(100)

Table 1: Occurrence and abundance of freshwater snails with their frequency in 130 sampling stations of Omo-Gibe river basin, Southwest Ethiopia.

5.2. Predictor variables

The totals of sixty-four types of environmental and biotic predictor variables were collected from 130 sampling stations to identify and model the suitable habitats for freshwater snails in Omo-Gibe river basin, Southwest Ethiopia. To this end, to identify factors affecting the occurrences and abundances of these freshwater snail species about 69 parameters were measured. The mean, standard deviation and range of these predictor variables are shown in Table 2.

Variables	Unit	Mean	SD	Range
Alkalinity (total)	mg/l	68.7	85.4	12-980
Altitude	Meter	1610.7	300.4	1032-2278
Ambient temperature	°C	26.24	3.5	18.8-35
Bathing	Minimal(1), Medium(2), High(3)	NA	NA	NA
BOD ₅	mg/l	13.0	4.8	2.0-28
Ca ²⁺	mg/l	13.9	10.7	0.8-67.2
Canopy cover	%	32.4	30.4	0-100
Car washing	Minimal(1), Medium(2), High(3)	NA	NA	NA
Chloride	mg/l	9.8	10.9	2-102
Clay mining	Minimal(1), Medium(2), High(3)	NA	NA	NA
Color	Absent(0), Present(1)	NA	NA	NA
Dissolved Oxygen	%	63.1	31.2	33-376
Disturbance score	Minimal(1), Medium(2), High(3)	NA	NA	NA
Dominant Riparian vegetation	Five types for rivers and Eight typ wetlands	es for NA	NA	NA
Dominant substrate	Eight types	NA	NA	NA
Draining	Minimal(1), Medium(2), High(3)	NA	NA	NA
EC	μS/cm	206.4	275.1	42-3107
Farming/cultivation	Minimal(1), Medium(2), High(3)	NA	NA	NA
Filling	Minimal(1), Medium(2), High(3)	NA	NA	NA
Fish	Absent(0), Present(1)	Absent(0), Present(1)	na NA	na NA
Fishing	Minimal(1), Medium(2), High(3)	NA	NA	NA
Grazing	Minimal(1), Medium(2), High(3)	NA	NA	NA
Grazing	Minimal(1), Medium(2), High(3)	NA	NA	NA
Habitat type	Six type	NA	NA	NA
Helodidae	Abundance	1.8	11.5	0-126
Heptageniidae	Abundance	15.1	25.7	0-114
Mg^{2+}	mg/l	3.1	9.0	0-28
NO ₃	mg/l	1.5	5.0	0-41.53
Oxygen Saturation	mg/l	4.2	31.2	1.72-5.74

Table 2: Predictor variables mean, standard deviation (SD), minimum and maximum values (range).

NA

pH	_	7.2	2.6	5.26-9.26
PO ₄ ³⁻	mg/l	0.23	5.1	0-2.121
Pool	%	54	32.8	0-100
Predators	Absent(0), Present(1)	NA	NA	NA
Protection of riparian vegetation(left) Protection of riparian	Absent(0), Present(1)	NA	NA	NA
vegetation(right)	Absent(0), Present(1)	NA	NA	NA
Rainfall	Absent(0), Present(1)	NA	NA	NA
Riffle	%	46.0	33.0	0-100
Settlement	Minimal(1), Medium(2), High(3)	NA	NA	NA
Smell	Absent(0), Present(1)	NA	NA	NA
Swimming	Minimal(1), Medium(2), High(3)	NA	NA	NA
Total Hardness	mg/l	42.4	30.5	2-180
Tree plantation	Minimal(1), Medium(2), High(3)	NA	NA	NA
TSS	mg/l	89.5	65.1	6.67-322.3
Turbidity	NTU	63.1	93.5	1.5-545
Vegetation removal	Minimal(1), Medium(2), High(3)	NA	NA	NA
Velocity	m/s	0.288	0.304	0-1.2
Washing	Minimal(1), Medium(2), High(3)	NA	NA	NA
Waste dumping	Minimal(1), Medium(2), High(3)	NA	NA	NA
Water abstraction	Minimal(1), Medium(2), High(3)	NA	NA	NA
Water depth	Meter	0.358	0.644	0.06-1.3
Water temperature	°C	24.6	4.3	18.9-47.5
Weather condition	Clear(0), Cloudy(1), Rain(2)	NA	NA	NA
Width riparian vegetation	Meter	278.7	1405.1	0-13000

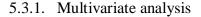
NB: NA = Not applicable

5.3. Multivariate Analysis

The result of effects of gradients of environmental factors on freshwater snail habitat preference using multivariate analysis, by CANOCO for windows version 4.5 showed that the first and second canonical axes explained 32.8% (eigenvalue of 0.328) and 20.4% (eigenvalue of 0.204) of the variation in species data, respectively. From the CCA ordination plot (Fig. 6) the first axes was positively correlated with the presence/absence of most of the snail species like *L. natalensis, B.sudanica, B.globosus, B.pfeifferi* with pool habitat and negatively correlated with water velocity, canopy cover, and competitors. That means, these species preferred to inhabit open habitat system.

From CCA ordination plot, it was also noted that the second axes positively correlated the presence/absence of *L.truncatula* with DO saturation, pH, and predator as well as negatively

correlated with water depth, chloride and altitude. On the other hand, *P.acuta* was positively correlated with water depth and negatively correlated with DO saturation, predators, and pH. Sphaeriidae was positively correlated with altitude and was also negatively correlated with DO saturation, predators and pH like *P. acuta*.



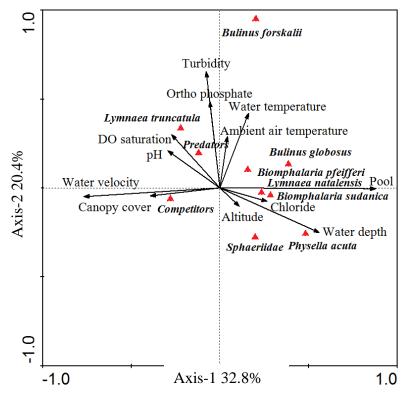


Figure 6: Ordination plot of snail larvae and predictor variables based on the canonical correspondence analysis (CCA).

The result of effects of gradients of anthropogenic activities on freshwater snail habitat preference using multivariate analysis (Fig. 8), by CANOCO4.5 showed that the first and second canonical axes explained 27.6% (eigenvalue of 0.276) and 15.3% (eigenvalue of 0.153) of the variation.

From the ordination plot using Canonical Correspondence Analysis (CCA), relating the effects of gradients of anthropogenic activities on freshwater snail habitat preference by multivariate analysis, in the first axis most snail species like *L.natalensis*, *P.acuta, and B.pfeifferi* were positively correlated with human activities (e.g. waste dumping, grazing, farming, vegetation

clearance, settlement, bathing, swimming, etc.). But, these snail species were negatively correlated with biotic factors like competitors and predators (Fig. 7).

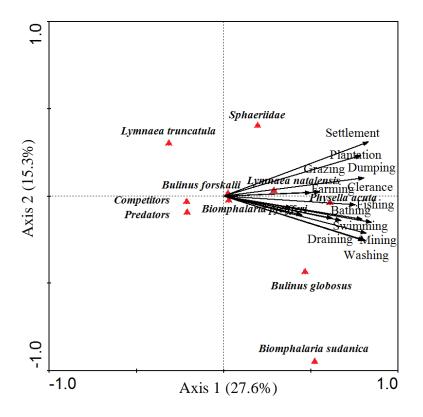


Figure 7: Ordination plot of snail larvae and human disturbances based on the canonical correspondence analysis (CCA).

5.4.Classification tree

To develop the classification tree (CART) model using predictor variables for freshwater snail habitat preference, variables which were with greater than 10% of frequency of occurrence were selected.

5.4.1. Frequency of variables selection

Sixty-four environmental variables (Table 2) were used as predictors to determine the presence / absence of three freshwater snail species (*L.natalensis*, *B.pfeifferi*, *B.globosus*) and one freshwater snail family (Sphaeriidae) (Table 2). Figure 6 indicates the average frequency of selection of these environmental variables and biotic variables by the classification tree models. Three models were developed for each freshwater snail species and one taxon (i.e. Sphaeriidae); because the training and validation were based on three-fold cross validation. Totally, 12 models

(three models for each of the three freshwater snail species and three models for family (Sphaeriidae)) were constructed.

The most frequently selected predictors were predators (75%), canopy cover (58%), habitat type (50%), pH (50%), pool (42%), competitors and waste dumping (33%) each. Furthermore, predators and habitat types were mostly selected as root of a classification tree implying that these variables were the most important informative attributes to determine the presence/ absence of freshwater snail species and family.

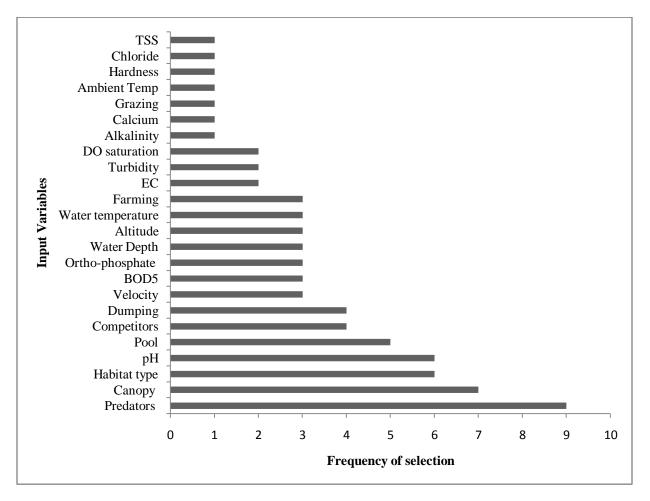


Figure 8: Overview of the average frequency of selection of the different input variables used in the decision tree models to model the presence or absence of each freshwater snail species/family.

5.4.2 Classification trees

The classification tree model for *B.pfeifferi* freshwater snail species depicted in Fig 9, for example, has seven leaves and eleven branches. The classification tree shows that Predator,

appear at the root of the tree, is regarded as the most informative attribute/variable to explain the occurrence of *B.pfeifferi*. This freshwater snail species were commonly absent when predators were present, farming was absent, velocity was higher than 0.54m/s and electric conductivity was less than 77μ S/cm. But, *B.pfeifferi* were present in sites where there were no predators, farming activity was practiced at medium and high level. The classification tree for *B.pfeifferi* had a good overall predictive performance, with a CCI of 82% and a Kappa statistics of 0.55.

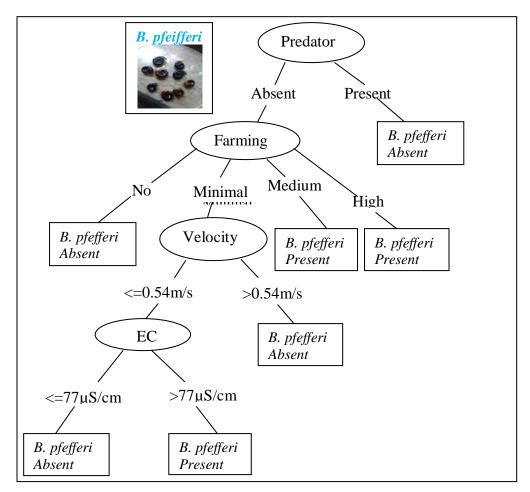
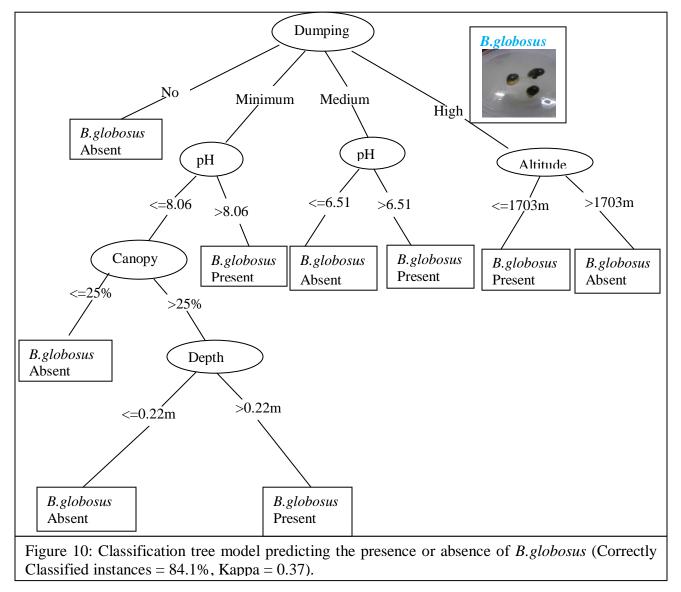


Figure 9: Classification tree model predicting the presence or absence of *B. pfeifferi* (Correctly Classified instances = 82%, Kappa = 0.55).

The classification tree model for *B.globosus* of the freshwater snail species has nine leaves and fifteen branches. This model indicates that waste dumping, appeared as the root of the tree, and is considered as the most informative attribute to predict the occurrence of *B.globosus*. *B.globosus* were generally absent when there was no waste dumping, waste dumping activity was minimal it was canopy cover that determines the occurrence of this species. That means *B.globosus* were absent when canopy cover is below 25%, provided that pH was below 8.07.

B.globosus were absent at an altitude higher than 1703m.a.m.s.l when waste dumping activity was high and where water depth is below 0.22m. On the contrary, *B.globosus* were present in sampled sites where there was minimal waste dumping activity at pH higher than 8.07, medium waste dumping activity at pH higher than 6.51, high waste dumping activity at an altitude below 1703m.a.m.s.l. they were also present where canopy cover is higher than 25% and water depth is higher than 0.22m. This classification tree had a good overall predictive performance, with a CCI of 84.09% and Kappa Statistic of 0.37.



The presence absence of predators was the most important variable that influences the occurrence of *L.natalensis* (Figure 11). The second most informative attribute to predict the occurrence of *L.natalensis* in this classification tree was waste dumping. Hence, *L.natalensis* was absent where canopy cover is higher than 10% and when there is no waste dumping. It was also absent where sites are with below 9.5mg/l of BOD₅ at minimum waste dumping activity, and where DO saturation is with 64.5%. However, *L.natalensis* were present in sites where there was medium and high waste dumping activity, BOD5 was higher than 9.5mg/l, and DO saturation was below 64.5%. This classification tree had a good overall predictive performance, with a CCI of 79.55% and Kappa Statistic of 0.47.

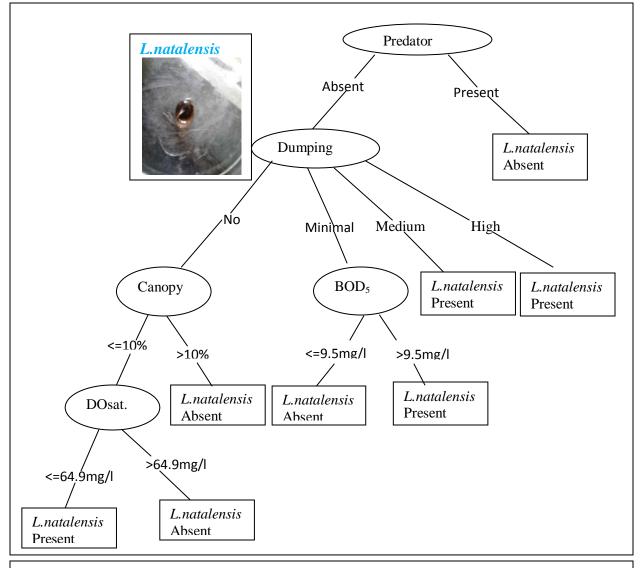


Figure 11: Classification tree model predicting the presence or absence of *L.natalensis* (Correctly Classified Instances = 79.6%, Kappa = 0.48).

The classification tree indicated in Fig. 12 has eleven leaves and seventeen branches. Predators were found to be at a base/ root of the classification tree, implying the most informative attribute to predict the occurrence of Sphaeriidae. Sphaeriidae were, therefore, generally absent when predators were absent. The second most informative attribute in this classification tree model to predict the occurrence of Sphaeriidae was habitat type. Sphaeriidae were absent when the habitat type is lake, dam, irrigation ditch, and hot spring. They were also absent in sites where water temperature was higher than 23.24°C and turbidity was greater than 30.5NTU. On the other hand, Sphaeriidae were present in sites where there was greater than 65% pool habitats of river, and turbidity was less than 30.5NTU. This classification tree had a good overall predictive performance, with a CCI of 86.4% and Kappa Statistic of 0.50.

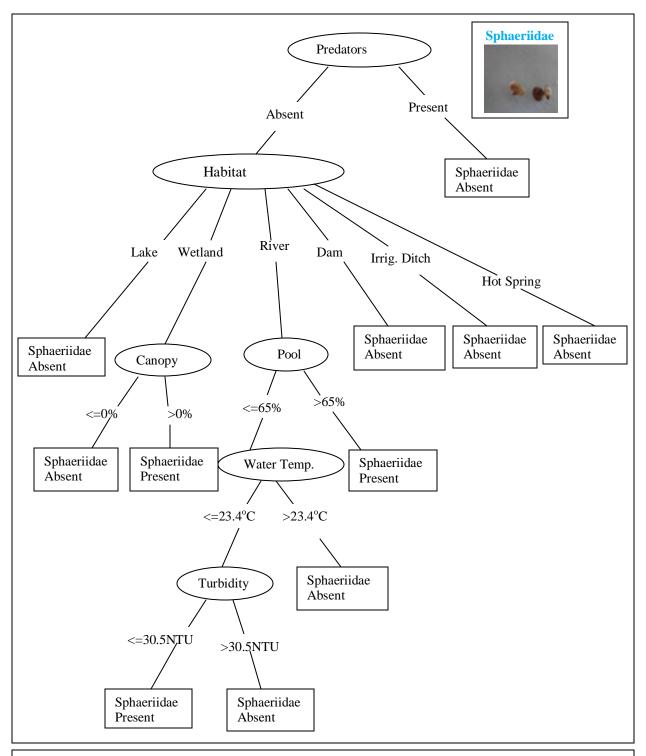


Figure 12: Classification tree model predicting the presence or absence of Sphaeriidae (Correctly Classified Instances = 86.4%, Kappa = 0.5).

5.4.3 Model Performance Evaluation

The performances based on the CCI and Cohen's Kappa Statistic of the three-fold cross validation for three freshwater snail are shown in Fig. 13A and B. Error bars give the variation between the subset models constructed. The CCI varied between $64\pm9\%$ and $86.4\pm11.2\%$. Based on CCI, the model predicted with a good reliability (CCI \geq 70.2%). Based on Kappa, *B.pfeifferi* had highest model predictive performance with a K value of 0.396 ± 0.15 , indicating a good model performance. On the other hand, *B.globosus* had the lowest K value (0.27 ± 0.089), showing slight to fair model performance. Sphaeriidae had a K value (0.233 ± 0.252) which is with largest standard error.

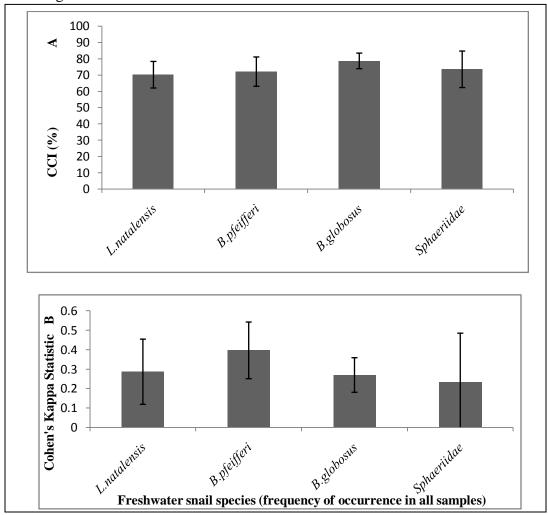


Figure 13: Overview of the average predictive performance of each classification tree model and the variation on the model based on (A) Correctly Classified Instances, (B) Cohen's Kappa Statistic of three freshwater snail species and one freshwater snail

5.5. Conditional Analysis

Taking into account the effect of competitors on the freshwater snails, the regression tree model showed that the occurrence of *B.pfeifferi* freshwater snail species decreased when the abundance of competitors (Helodidae and Heptageniidae) macroinvertebrates (Fig. 14A). The effect of competitors' abundance on *L.natalensis* species was also manifested from the regression tree model (Fig. 14B) in such a manner that *L.natalensis* abundance increased when competitors' abundance decreased below 40 and decreased when competitors' abundance was geater than 40. The abundance of *B.globosus* also decreased because of the effects of competitors' abundance when they exceeded 40 and slightly seemed remain competing afterwards (Fig. 14C). but their abundance become increased when competitors' abundance is below 40.

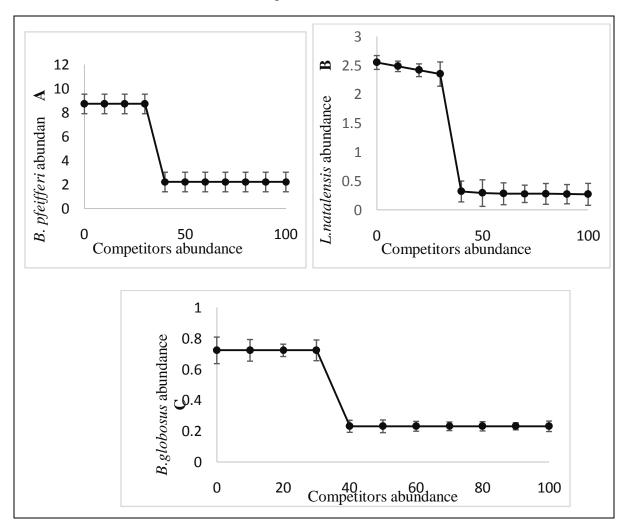


Figure 14: Effects of competitors on (A) *B.pfeifferi*, (B) *L.natalensis*, (C) *B.globosus* abundance of freshwater snail species.

The conditional analysis signified that the abundance of *B.pfeifferi* increased with decreasing percentage of canopy cover (Fig. 15A) and increased with an increase in altitude (Fig. 15C). Abundance of *B.pfeifferi* showed sharp increase after an altitude of 1750m a.m.s.l. The abundance of this species of freshwater snail showed an increase when water flow velocity is below 0.4m/s and showed decline in abundance when water flow velocity is greater than 0.4m/s.

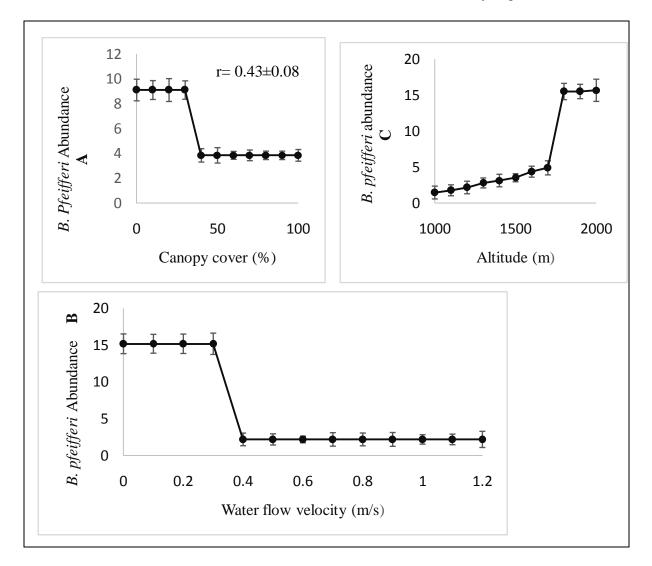


Figure 15: Effects of Canopy cover (A), Altitude (B), and Water flow velocity (C) on the abundance of *B.pfeifferi*.

The abundance of *P.acuta* increased with decreasing percentage of Dissolved Oxygen starting from 60% to 50% and become more or less stable afterwards (Fig. 16B). *L.natalensis* species of freshwater snail abundance increased slightly upto 300µS/cm of conductivity and showed sharp

increase between 300μ S/cm and 350μ S/cm and indicated slight increase from 350μ S/cm afterwards (Fig. 16A).

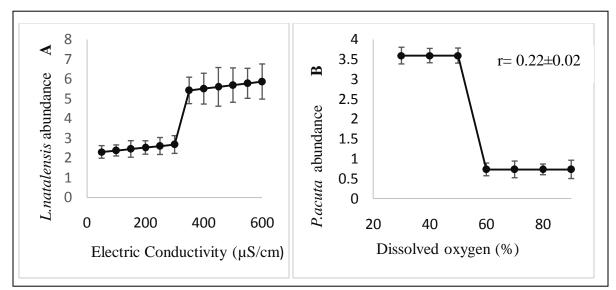


Figure 16: Effects of Electric conductivity (μ S/cm) (A) and Dissolved Oxygen (B) on abundance of freshwater snail species.

CHAPTER SIX: DISCUSSION

In this study, the results from multivariate analysis using canonical correspondence analysis (CCA) indicated that most of the freshwater snail species were positively correlated with pool habitat environmental types variable and negatively correlated with environmental variables like canopy cover, water flow velocity, and with biotic variables like competitors and predators (Fig. 6). Similarly a research done by (Anyona et al., 2014) showed that *L*.natalensis were mainly confined to streamside pools particularly at Tenwek falls sampling site-a site that was near the turbine feeders and accessible to people and livestock, along Nyangores tributary on the Kenyan side of the Mara River. This might be due to water overflow and human activities, as well as many livestock contact points around this area probably influenced the snail abundance by contributing organic wastes. Moreover, a research conducted by (OKAFOR, Fabian Chukwuemenam and NGANG, 2004), on fresh water snails from Niger-Cem, Nkalu with associated population and habitat attributes showed that, B.globosus prefers marshy pools, B.pfeifferi prefers quarry lakes, and Bulinus forsikali prefers pond habitat at Ezilo. According to this study *B.globosus* appear to prefer the less eutrophic zone. But, the bulinids were most often found immersed and attached to submerged vegetation and decaying organic matter, the lymnaeids seemed to prefer peripheral sites of the water bodies.

From the ordination plot of CCA analysis *L.truncatula* was positively correlated with dissolved oxygen saturation, predators, and pH and negatively correlated with water depth. This might be due to the species' preference of high dissolved oxygen for survival. This finding is supported by the result, the majority of the snails were found to be free floating in areas without any vegetation, of which *L.spp*. accounted for 87.1%, of the floating snails, while *B. pfeifferi* and *B. africanus* accounted for 9.7 and 3.2%, respectively. Consequently, *L.spp*. were the most dominant (88.9%) of the snails found attached to freely floating dead logs, while *B. africanus* and *B. pfeifferi* accounted for 7.4 and 3.7%, respectively (Anyona et al., 2014). But, the finding of (Michalik-kucharz, 2008) asserted that a comparison of environmental associations shows that the majority of common freshwater gastropods (e.g. *L.truncatula*, preferred dam reservoirs and old river beds while they avoided the fish ponds in characterizing their habitat preference related to predators.

Moreover, the ordination plot from the multivariate analysis using CCA (Fig. 7) revealed that most of the freshwater snail species (*L.natalensis, P.acuta, and B.pfeifferi*) were positively

correlated with most of the variables associated with anthropogenic activities like waste dumping, grazing, farming, vegetation clearance, settlement, bathing, swimming, etc.

From the conditional analysis using regression tree models, environmental variables signified that the abundance of *B.pfeifferi* increased with decreasing percentage of canopy cover (Fig. 16A) and increased with an increase in altitude (Fig. 16C). Abundance of *B.pfeifferi* showed sharp increase after an altitude of 1750m a.m.s.l. Previous study by (Michalik-kucharz, 2008) confirmed that geomorphologic conditions also belong to the factors affecting the diversity of snail species; thus, altitude above sea level influenced the distribution and diversity of freshwater gastropods in the Czech Republic.

In this study, the classification tree models allowed identifying important variables structuring the presence and absence of freshwater snails. Based on Kappa Statistic, a model developed for *B.pfeifferi* had good predictive performance. Predators occurrence was selected as the most important predictor variable, which appeared, as the root of the classification tree. This might be because of the presence of molluscivorous fish in the habitats that prey upon freshwater snails. This is supported by (Turner, 1996), freshwater snails are confronted by an array of potential predators (e.g. fish, leeches, crayfish and birds), and they face the challenge of evaluating both the level and the type of risk posed by each predator.

Based on this study, environmental variable like percentage of canopy cover was observed from the conditional analysis in determining the abundance of *B.pfeifferi* freshwater snail species. Other studies concluded that human-mediated riparian canopy loss can generate hotspots of snail biomass, growth, and nitrogen excretion along tropical stream networks, altering the impacts of an invasive snail on the biogeochemical cycling of nitrogen (Moslemi et al., 2012). Moreover, as identified by (Anyona et al., 2014) The snails were either found attached to aquatic weeds, dead logs, and open puddles/rock pools or floating freely on water.

Moreover, the habitat preferences of the *B.globosus* group as a whole were reviewed by (Malan et al., 2009) that the habitat preferences of all the snail hosts are similar, in that they prefer a permanently-inundated aquatic habitat, with slow flowing, or standing water.

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CHAPTER SEVEN: CONCLUSION AND RECOMMENDATION 7.1. Conclusion

The ordination analysis indicated that pool habitat condition favors the abundance of most of the snail species such as *L.natalensis*, *B.sudanica*, *B.globosus*, and *B.pfeifferi*. In contrary, the abundance of these snail species were negatively associated with water flow velocity, canopy cover, competitors' abundance and predators' occurrences. The classification tree also demonstrated that presence absence of predators was the most prominent biotic variable influencing the presence or absence of freshwater snails. This variable was selected in 75% of the classification tree models. Canopy cover, habitat type, pH were the most important variables determining the presence or absence of freshwater snail species. These variables were selected in more than 50% of the classification tree models. In addition the conditional analyses clearly indicated that the abundance of most of the snail species was decreased when the abundance of competitors' macroinvertebrates namely Heptageniidae and Helodidae abundance was greater than 40 individuals.

7.2. Recommendation

The monitoring strategy including health program managers and other related stake holders should take measures to gain and sustain control of freshwater snails an intermediate host for schistosomiasis through diversifying the ecosystem by introducing competitors and predators of freshwater snails. It should moreover work on maximizing percentage coverage of canopy along most habitats of freshwater snail species. The results from ordination analysis of this study also indicated that most of the freshwater snail species preferred area related to different anthropogenic activities. Hence, it is recommended that, human activities should be reduced in areas where freshwater snails are present or abundant to minimize risk related to snail which is an intermediate host of schistosomiasis.

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nex I: River Assessment Form				
1. DD/MM/YYY				
2. Site Code				
3. Altitude(m)				
4. Previous days rainfall history				
Physico-Chemical Parameters				• • • • •
5. Ambient Temperature(°C)	Water to	$mn_{aratura}(^{0}C)$)	
7. Velocity (m/s)Water dept				
8. TurbidityColor	ſ	Smell		
Habitat Assessment				
9. River bank width (m)	Bank heigh	ıt (m)		
10. River bed (%)				
a. Bed rock	e. Gravel		i. Sticks NLSM	ЛE
b. Boulder	f. Sand		j. Branches NI	LSN
c. Cobble	g. Silt		k. Logs NLSM	1E
d. Pebble	h. Detritus N		e	
11. Riparian vegetation				
a. Trees $> 10m$	d Grass			
b. Trees < 10m				
c. Shrubs		u		
		I a A		
12. Width riparian vegetation Right				
13. % Canopy Cover				
14. Protection riparian vegetation Rig				
15. % Pool				
16. % Riffle				
17. Sinuosity				
18. Slope				
19. List the available anthropogenic d	listurbances			
20. Upstream Land use				
21. Adjacent Land use Right	I			
22. Farming distance from the river b				
23. River habitat assessment	winit 1 ci 8-ivi			
Parameter	Optimal	Suboptimal	Marginal	P
Epifaunal substrate			gmml	
Pool substrate characterization				1
Pool variability				
Sediment deposition		1		
Channel flow status				
Channel alteration				
Channel Sinuosity				
Bank stability (LB, RB)				
Vegetative protection (LB, RB)				
Riparian vegetative zone width (LB, RB)				

 Riparian vegetative zone width (LB, RB)
 24. Take picture (Picture Number).....

Annex II: Wetland Assessment Form

General Information

1.	DD/MM/YYY		Time	
2.	Name of wetland	Sampling sta	tion	
3.	Altitude (m)	Coordinates		
4.	Weather conditionSunny/Clear	Cloudy	Rainy	Others
5.	Previous day rain history			
6.	Photo number			
7.	Size of site under assessment (ha)			
8.	Size of total wetland complex (ha)			

Notes and/or sketch of the site

Physico-Chemical parameters (Field)

9. Ambient Temperatur	re (°C)pH	
	(°C) DO (mg/l)	
11. Turbidity (NTU)	Transparency (cm)	·····
12. Chlorophyll a (ABS))(0.1309*ABS+11.	274)(µg/l)
13. Color	Ödor	
14. Water Velocity (m/s	ec)	

Physico-Chemical parameters (Laboratory)

15. COD	NO ⁻ 2
16. Chloride	.NH4
17. TSS	
18. BOD ₅	
19. NO ⁻ 3	

Hydrogeomorphological Assessment

20. Wetland geomorphic setting

a.	Reverine
b.	Depressional

	Meandering flood plain Other			
21. Po	ond/degree of isolation from	n other wetlands		
	Natural Artificial			
a. b. c. d. e. 23. Fro a. 24. Sh	te Setting/degree of isolation The site is connected up The site is only connecte The site is only connecte Other wetlands are nearly The wetland site is isolat ee water depth (cm) Minimum	steam and downstream d upstream with other d downstream with other y (within 0.25 mile) bu ed .b. Maximum	wetlands er wetlands t not connected c. Avera	ge
25. Ap a. b. c. d. e. 26. Hy a. b.	Minimum oparent hydro period Permanently flooded Seasonally flooded Saturated (Surface water Artificially flooded Artificially drained ydrological modification Ditch inlet and outlet Drainage Storm water input	seldom present) d. C e. F	ulverts	ng
a. b. c. d. Habitat A	Ijacent land use pattern Agriculture tilled Pasture Native vegetation Residential area	f	Commercial	
a. b.	vdrophytic vegetation cove Woody plants Water grasses Emerged macrophytes Submerged macrophytes		.f. Periphyton g. Filamentous al	gae

29. Name of dominant macrophytes

a.	
b.	
c.	
d.	

- 30. Wetland Fauna
 - a. Birds (ducks)d. Anurans
 - b. Fish.....e. Hippopotami.....
- c. Invertebratesf. Others...... 31. Disturbance scale

Disturbance		Score = 1	Score = 2	Score = 3
Habitat	Grazing			
Habitat alteration	Vegetation removal			
alteration	Tree plantation			
	Farming/Cultivation			
Land Use	Clay mining			
	Waste dumping			
I Inducio ai col	Damming/Draining			
Hydrological modification	Filling			
modification	Water abstraction			
	Car washing			
	Bathing			
Other human activities	Swimming			

32. Other potential threats

- a. Agricultural biocides
- b. Point source pollutionc. Others

33. Wetland ecological state

a.	Unmodified, natural
b.	Largely natural with few modification
	Moderately modified
	Largely modified
e.	Seriously modified
	Critically/Extremely modified
34. An	y additional comments