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Prevalence of cercarial infection in *Biomphalaria* snail hosts and *Schistosoma mansoni* infection among fishermen in the shore of Lake Hawassa and Tikur Wuha River, Southern Ethiopia

BY

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A thesis submitted to Jimma University, Institute of Health Sciences, Faculty of Public Health, Department of Environmental Health Science and Technology, in partial fulfillment of the requirements for the degree of Masters Science in Environmental Health

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Jimma, Ethiopia

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DECLARATION

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

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Approval Sheet for Submitting Final Thesis

As members of the Board of Examining of the Final MSc. thesis open defense, we certify that we have read and evaluated the thesis prepared by **Wolyu Korma** under the title **Prevalence of Cercarial Infection in Biomphalaria Snail Hosts and Schistosoma Mansoni Infection Among Fishermen in the Shore of Lake Hawassa and Tikur Wuha River, Southern Ethiopia** and recommend that, the thesis be accepted as fulfilling the thesis requirement for the Degree of Master of Environmental Health.

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Abstract

Background: Freshwater snails serve as intermediate hosts for different digenetic trematode species that cause snail borne diseases of humans and animals. Different environmental factors and human activities affect the abundance and infection rate of the snails. Environmental conditions in Africa favor the growth of snail and trematode which has the potential to become a public health problem in many countries. In addition to environmental conditions, there are other different factors that cause a high prevalence of schistosomiasis, these are water resources development projects, irrigation, fishing, population increase, and migration. These conditions favor infection of freshwater snail and increase the risk of schistosomiasis infection.

Objective: the main objective is to determine prevalence of cercarial infection in *Biomphalaria* snails and *S. mansoni* infection among fishermen in Lake Hawassa and Tikur Wuha River in 2020.

Methods and materials: The snails were collected from 26 sampling sites and examined for cercarial infection using natural shedding method. The cercariae were identified morphologically. In the surveyed sites the data on environmental factors, human disturbance, demographic and behavioral factors were collected. Stool samples from fishermen were examined for *S. mansoni* infection. To determine factors influencing the prevalence of snail infection PCA was used. Chi-square was used to assess the association between *S. mansoni* infection and the risk factors.

Results: The study indicate that only *Biomphalaria sudanica* snail species were infected with cercaria. Five different types of cercaria were recorded with a total prevalence of 12.03%. Of which *BAD* (human schistosome) cercariae were the dominant accounting 37.5 % of all recorded cercarial type. The prevalence of cercarial infection in snails positively influenced by BOD_5, turbidity, total suspended solids, human activities, and habitat condition. The total prevalence of *S. mansoni* infection among fishermen was 14.7% and significantly associated with frequency of swimming and educational status.

Conclusion and Recommendations: The prevalence of intestinal schistosomiasis was 14.7% among the fishermen which transmitted by the vector *Biomphalaria* intermediate host snails. The prevalence of cercarial infection in snails was high in this area which results high risk for the transmission of schistosomiasis. Therefore, an intervention programs should be focused on the snail control, provision of adequate sanitation, and health education to control the transmission of schistosomiasis.

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Acronyms and abbreviations

APHA: American Public Health Association

BAD: *Brevifurcate Apharyngeate Distome*

BOD: Biological Oxygen Demand

CopE: Coproparasitological Examination

DALYs: Disability Adjusted Life Years

DO: Dissolved Oxygen

EC: Electric Conductivity

EPG: Egg per Gram

FMoH: Federal Ministry of Health

GIS Geographic Information System

GPS: Global Positioning System

PCA: Principal Component Analysis

TSS: Total Suspended Solids

WHO: World Health Organization

Chapter one: Introduction

1.1. Background

Snails belong to phylum- Mollusca and class- Gastropoda, which accounts for a large and highly diverse group of invertebrates. Many freshwater snails serve as an intermediate host for different digenetic trematodes that cause schistosomiasis, fascioliasis and other snail borne diseases of humans and animals (Tigga *et al.*, 2014; Lu *et al.*, 2018). The eggs of the parasite enter into the environment through faeces or urine of the definitive host. Upon reaching to freshwater bodies, the eggs will hatch and release the first free-swimming larva of digenetic trematodes (miracidia) which then infect a freshwater snail host. The larval developmental stages, such as sporocysts, rediae, and cercariae are completed in the snail. The infected snails shed thousands of the second stage free-swimming larva called cercaria into the water. Then the cercaria enters into mammalian hosts through direct penetration of skin during contact with water bodies contaminated with human excreta containing parasite eggs. The infection with digenetic trematodes cercaria gives rise to a disease for mammalian hosts. Understanding the parasite lifecycle and transmission mechanism between freshwater vector snails and mammalian hosts is important to the control and elimination of human schistosomiasis (Colley *et al.*, 2014; WHO, 2018).

Schistosomiasis is caused by six species of trematodes from the genus *Schistosoma*: *Schistosoma mansoni, S. haematobium*, *S. japonicum, S. intercalatum, S. guineensis* and *S. mekongi.* The predominant causes of disease are *S. mansoni* and *S. haematobium* in tropical and subtropical regions, particularly in sub-Saharan Africa (WHO, 2018). *Schistosoma mansoni* and *S. haematobium* trematodes are transmitted by two primary snail species of the genus *Biomphalaria* and *Bulinus* respectively, which are widely distributed throughout African countries (Colley *et al.*, 2014).

In Ethiopia, the genus *Biomphalaria,* mostly *Biomphalaria pfeifferi* and *Biomphalaria sudanica* are the intermediate host for *Schistosoma mansoni* which are the causative agent for intestinal schistosomiasis (Kristensen *et al.*, 2001). The former is found in most parts of the country while the latter one is only found in limited areas specifically in the lakes of Ziway, Abaya, Hawassa and Tikur Wuha River (Erko *et al.*, 2006; Mitiku*, et al.*, 2010).

As schistosomiasis is transmitted by snail intermediate hosts, the transmission result is affected by the degree of freshwater body contact, the infection rate among the snails and social and cultural practice of the community in the area where schistosomiasis is endemic. Schistosoma and its snail vector are essential parts of the freshwater environment in which they are found (Geteneh *et al.*, 2017).

Environmental conditions in Africa favor the growth of intermediate host snail, *Biomphalaria* and *S. mansoni* which has the potential to become a public health problem in many countries. Different water bodies like lakes, rivers, dams, and irrigation canals are also important for schistosomiasis transmission and epidemiology, particularly in Africa (WHO, 2007). In addition to environmental conditions, there are other different factors that cause a high prevalence of *S. mansoni* in Sub-Saharan Africa these are water resources development projects, population increase or displacement, migration and competing priorities in the health sector (WHO, 2008).

In Ethiopia, there is an increment in water resource development and intensive population movement which results in widespread of *S. mansoni*. Similarly, activities like irrigation, fishing, recreation, swimming, washing and other human activities are common practices around Ethiopian rift valley lakes and rivers which can contribute to the production and discharge of human excreta and other waste into the water bodies and increase the extent of human-water contact. These conditions favor infection of freshwater snail and increase the risk of schistosomiasis infection (Erko *et al.*, 2002; Mesfin *et al.*, 2015).

The area around Ethiopian rift valley lakes and rivers where schistosomiasis is endemic and there is a high abundance of freshwater snails and human disturbance (anthropogenic activities) which increase the risk to schistosomiasis for the community who live around the shores of these rift valley lakes and rivers (Chala & Torben, 2018; Olkeba *et al.*, 2020). The prevalence of freshwater snail infection (proportion of snails that release cercariae) and the intensity of cercarial infection (the number of cercariae released from each infected snail) take part in significant roles in the transmission of cercarial from freshwater snail host to human beings (Tigga *et al.*, 2014). However, the infection rate of freshwater snails in the shore of Hawassa Lake and Tikur Wuha River was not determined.

1.2. Statement of the problem

There are different intermediate host snail species in the world freshwater bodies which cause snail borne parasitic diseases like schistosomiasis, paragonimiasis, fascioliasis, fasciolopsiasis, angiostrongyliasis, clonorchiasis, and opisthorchiasis. These diseases are the most important parasitic disease which remains crucial to public health issues worldwide, mainly in developing countries. Millions of people in 90 countries have suffered from snail borne disease, in which snails are intermediate hosts and transmitting vectors. These diseases also resulting in extensive socioeconomic burdens in many tropical and sub-tropical countries (Lu *et al.*, 2018).

Human schistosomiasis is one of the most prevalent parasitic infections in the world and found in 52 countries. A report from WHO indicated that 219.9 million people worldwide are estimated to be affected by schistosomiasis, of which it is estimated that at least 90.4% of those requiring treatment for schistosomiasis live in Africa. This disease caused a loss of 2.5 million disability-adjusted life years (DALYs) (WHO, 2018). It is the second most widespread parasitic disease after malaria and killing an estimated 300,000 people each year in the African region alone and 163 million population need treatment in sub-Saharan Africa (Olveda *et al.*, 2013; Lai *et al.*, 2015).

Specifically, *S. mansoni* is highly prevalent in sub-Saharan African countries, Sudan, Egypt, Venezuela, Libya, Brazil, Some Caribbean islands and the Arab peninsula. In sub-Saharan Africa, it has been estimated that about 54 million are infected and 393 million individuals are at risk of infection due to *S. mansoni* only (van der Werf *et al.*, 2003; Ismail *et al.*, 2016).

Most of the sub-Saharan African country has a favorable condition for the main causative agents (*S. mansoni* and *S. haematobium*) of schistosomiasis. But, in Ethiopia *S. mansoni* have a countrywide distribution, whereas *S. haematobium* has a limited distribution particularly in small foci in the Rift valley region, Somalia, and Gambella region (Hotez & Kamath, 2009; Chala & Torben, 2018). The anticipated values for the people who live in schistosomiasis endemic areas are 68.3 million. Among these 34.4million are preschool children, 12.3 million are school-aged children, and 21.6 million are adults (FMoH, 2016). Generally, the disease is recorded in all regions of Ethiopia and the prevalence is significantly high (Chala & Torben, 2018).

Regarding intestinal schistosomiasis, there is no summarized prevalence data of *S. mansoni* infection at national wide to facilitate the formulation of suitable intervention methods. But the pooled prevalence of *S. mansoni* among the Ethiopian population was 18.3% (Assegu & Shimelis, 2019). This indicates an endemicity and moderate prevalence of *S. mansoni* infection found in Ethiopia (WHO, 2002). However, several studies were conducted in different areas near to Ethiopia rift valley lakes showed that the prevalence of *S. mansoni* was high among the community. For instance, 59.9% in Wondo Genet (Aemero *et al.*, 2014), 73.7% in Bushulo village near Lake Hawassa (Terefe *et al.*, 2011), 43.2% in Ziway town (Legesse *et al.*, 2009), 35.7% in Bochessa Village near Lake Ziway (Teklemariam *et al.*, 2018) were recorded.

This parasitic disease will be transmitted if there are intermediate snail hosts and community water contact with freshwater bodies. Findings of the previous study on the ecology of the freshwater intermediate snail hosts in the Ethiopian Rift Valley region revealed that there is a high abundance of snails in habitats disturbed by human activities (Olkeba *et al.*, 2020). However, information on both snail abundance and their cercarial infection rate provide information on the probability of human becoming infected with parasites if there is human contact with freshwater bodies that contain schistosome cercariae released from specific snails. Clarifying the interaction between snail and schistosome cercaria, at a local/fine-scale level, is not only critical for our understanding and monitoring of schistosome transmission but also for the application of snail control interventions to support schistosomiasis control and elimination program (Rollinson *et al.*, 2013). Freshwater snail control is one of the most important preventive methods for the reduction of schistosomiasis transmission and it is a vital component to achieve the goal of control program. To implement a control measure against snail-borne diseases, identification of infected snails or snail hotspots is crucial (Ross *et al.*, 2017).

In our case, a human settlement close to Lake Hawassa and Tikur Wuha River with different environmental factors may influence the abundance and cercarial infection of freshwater snails which probably modify schistosomiasis transmission. However, there was no study carried out to determine the cercarial infection of intermediate host snails from these water bodies to elucidate the risk of acquiring schistosomiasis among these communities. Hence, this study aims to determine the prevalence of cercarial infection in *Biomphalaria* snails and *S. mansoni* infection among fishermen in the shore of Lake Hawassa and Tikur Wuha River.

1.3. Significance of the study

Determining cercarial infection in freshwater snail is important to predict the epidemiological situation of cercarial infection in human beings. It helps to undertake successful and sustainable control measures in the study area. Infected snail's detection is a crucial indicator of the presence of schistosomiasis in a given region, and the potential for transmission. Additionally, the detection of infected snail is crucial in detecting infection risk regions to guide surveillance and required interventions. Therefore, this study provides information on the prevalence of cercarial infection in snails and environmental factors that affect the prevalence of snail infection. And it also, provide information on the prevalence of intestinal schistosomiasis among fishermen. It could also be used for the development of effective and eco-friendly control measures that could be applied in communities living around the lakeshore of Hawassa and Tikur Wuha River and to disseminate this control measure to the other schistosomiasis endemic areas. The findings of this study may serve as a baseline for other researchers.

Chapter Two: Literature Review

2.1. Schistosomiasis

Schistosomiasis is one of the world's most common water-based diseases resulting from infection with parasitic trematode worm of the genus Schistosoma. It involves a complex of syndromes that are often acute but mainly chronic and disabling due to the parasitic infections (WHO, 2013). In 1851, German scientist Theodor Bilharz, while conducting an autopsy at a hospital in Cairo, found the parasite *Schistosoma haematobium* in a vesical plexus. Of note, the word bilharzia is still used today as a synonym for schistosomiasis (Utzinger *et al.*, 2011).

There are six Schistosoma organisms that parasitize humans, namely, *Schistosoma mansoni, S. haematobium, S. japonicum, S. intercalatum, S. mekongi,* and *S. guineensis*. The first three are the most significant and common from a public health viewpoint, triggering either urinary schistosomiasis (*S. haematobium*) or intestinal schistosomiasis (*S. mansoni and S. japonicum*). Although humans (and in the case of *S. japonicum*, various domestic and wild animal species) are the definitive host of schistosomiasis, some snails serve as intermediate hosts. *Bulinus* and *Biomphalaria* aquatic snails of the family are involved in the transmission of *S. haematobium* & *S. mansoni* respectively while amphibious snails of the genus *Oncomelania* serve as intermediate hosts for *S. japonicum* (Utzinger *et al.*, 2011).

Since the parasite is transmitted by very unique intermediate-host freshwater snails, the perpetuation of the life cycle of the Schistosoma involves suitable environmental conditions as well as water pollution from human excreta. Transmission is therefore related to local environmental factors and also to underdevelopment and lack of sanitation (Bustinduy & King, 2014). Persistent exposure to reinfection is related to a lack of adequate water supply and recreational activities, a condition that is widespread in developing countries. As such, schistosomiasis is a poverty-stricken disease, with a growing prevalence in rural areas and unplanned peri-urban developments (Barbosa *et al.*, 2010; Ugbomoiko *et al.*, 2010).

2.1.1. Schistosomiasis transmission /lifecycle

The transmission process of the Schistosoma is complex and highly effective when the good environmental conditions are met. The egg of the parasite released via urine or feaces of those infected mammals. When an egg riches to the water bodies and hatches the first free-swimming stage called miracidia and infect the freshwater snails. After the infection of snail by miracidium,

the larva miracidium immediately undertakes development inside the intermediate snail (Humphries, 2011). The miracidia transformed into mother sporocyst (primary sporocysts) after undertaking morphological and physiological changes in the tissue of snail's cephalopodal region around the penetration site. This mother sporocyst produces daughter sporocysts (secondary sporocysts) through polyembryony after 2-3 weeks. Then the daughter sporocysts transfer from the cephalopodal to the digestive gland-gonad complex where they undertake multiplication. The daughter sporocysts release cercariae after four weeks of the infection and the cercariae released from the snails into the water bodies (Humphries, 2011).

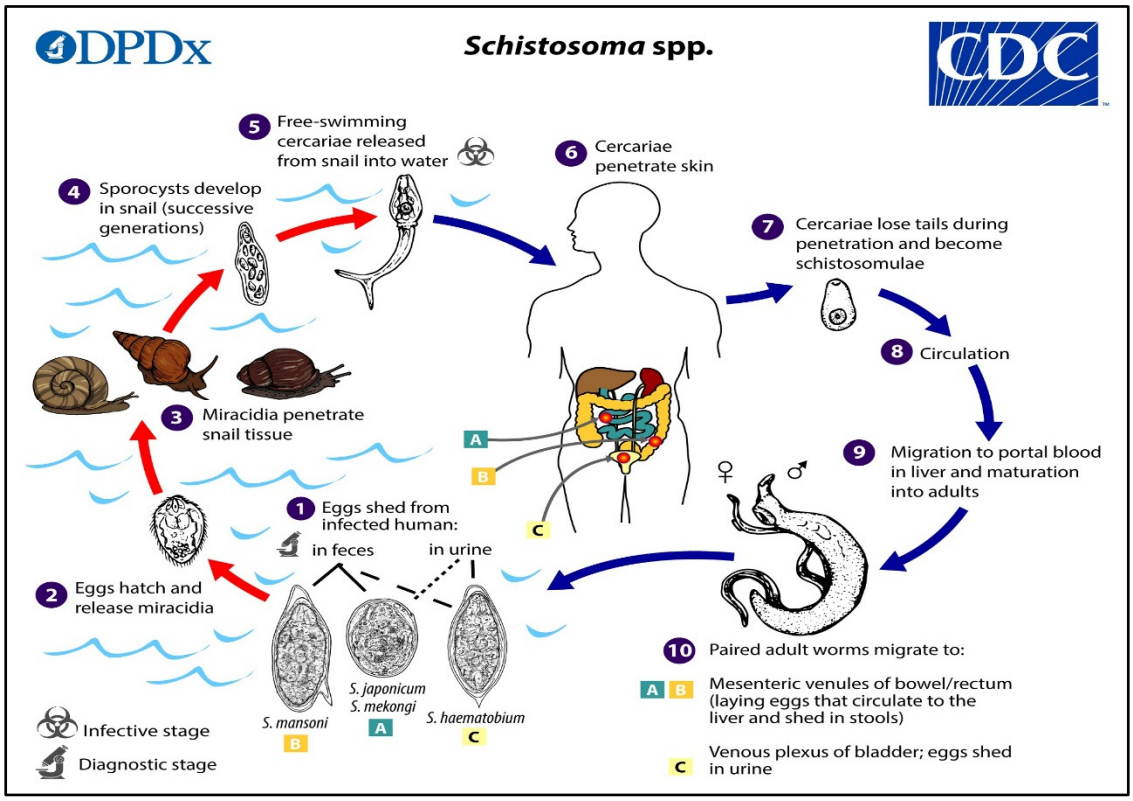

Figure 1:The life cycle of the three-common human Schistosomes (CDC, 2019)

All species have a similar route of sexual reproduction by adult schistosomes within the definitive human host's vascular system, an asexual process in the freshwater snail host and a return to humans via cercarial invasion of the skin or mucosa at the exposure of a host to cercaria infested water (Bustinduy & King, 2014).

The adult schistosomes are dioecious and have full separate sexes. They live as pairs inside capillary blood vessels, depending on the species in various anatomical locations: *Schistosoma mansoni, S. japonicum*, *S. mekongi,* and *S. intercalatum* in the mesenteric veins and *S. haematobium* in the vesical plexus. The slender, smooth females are carried inside the male's gynaecophoric canal, where they copulate. The adult worm's lifespan in humans isn't well understood. In the past, as recorded in a Madagascar migrant residing in France, stress was put on clinical evidence of longevity ranging from 18 years to 37 years. However, the average lifetime of the worm is estimated to be 3–5 years (Bustinduy & King, 2014).

Transmission takes place in a large variety of freshwater ecosystems. Transmission is achieved when the free-swimming cercariae shed by the intermediate host snails reach the human skin during water contact. Remarkably, the cercarial density in the freshwater body for *S. haematobium* and *S. mansoni* are highest around the noon, after the intermediate snails have been exposed to sunshine for several hours. Certainly, light is the major stimulus for the release of cercaria. The hours around noon period in Africa often coincide with the peak human activity at freshwater sites, emphasizing the intimate coevolution between parasite and human behavior. Human interaction with freshwater is typically induced by household activities (e.g. washing of clothes and dishes, fetching water), occupational practices (e.g. river crossing, farming, and fishing), and leisure activities (e.g. bathing, swimming, and water play) (Utzinger *et al.*, 2011).

2.2. Freshwater snails and digenean trematode

Freshwater snails act as an intermediate host for many digenean trematode parasites which causes human and animal diseases. In Africa, freshwater pulmonate snails in the "Planorbidae" family are the main intermediate host of digenean trematode parasites (schistosomes). Within this family, there are two genera, namely *Biomphalaria* and *Bulinus*, hosts for mainly two digenean trematode parasites: *S. mansoni* and *S. haematobium* respectively (Boelee & Madsen, 2006).

Digenean trematode parasites have a relationship with snails to undertake their life cycle as an intermediate host. The snails support the parasite by providing the nutrient and space for multiplication and the means to reach the next definitive host. In the interaction between trematodes and snails, specificity is one of the most important features. Even though the fact that freshwater body is usually occupied by different freshwater snail species, each trematode miracidia infects specific snail species found in the aquatic environment and never with others in spite of the

collection of different snail species found in the aquatic environment. This use of limited snail species as a host by trematode parasite miracidia is known as intermediate host specificity. This specificity is crucial for defining the geographical areas where trematodes are present, restricted to areas occupied by compatible intermediate snails, which is predominantly significant for medically significant schistosomes (Lockyer *et al.*, 2004). As a result for trematode parasite, *S. mansoni* transmission the presence of *Biomphalaria* snail species as a susceptible intermediate host are obligatory despite having an association with environmental and socio-economic conditions (Negrão-Corrêa *et al.*, 2012).

Classification of digenean trematode parasite is a complicated task, but the larval characters of the trematode can also be used in classification. Depending on the position and number of suckers, four types of cercariae are described namely, *monostome*, *amphistome*, *gasterostome,* and *distome*. The occurrence of other body structures such as the collar spine (*Echinostome*), anterior stylet (*xiphidiocercariae*), eyespots (*ophtalmocercariae*) are also being used for groping the trematodes (Jayawardena *et al.*, 2010). Different studies reveled that, naturally infected intermediate snail hosts were releasing various types of digenean trematode parasite. The study in Sudan shows four types of trematodes were released from the collected one species snail; namely human schistosome cercariae followed by *Amphistome*, *xiphidiocercariae*, and lastly by avian cercariae (Ahmed *et al.*, 2006). Similarly, the study in Sri Lanka eight cercarial types was identified from four snail species namely *Furcocercous*, *Gymnocephalous*, *Echinostome*, *Gymnophallus*, *Macrocercous, Distome, Xiphidiocercous,* and O*culopleurolophocercou* (Jayawardena *et al.*, 2010).

2.3. Identification of trematode parasites in freshwater snails and human

Determining the presence or absence of *Biomphalaria* snails to a large extent is important to know the prevalence and distribution of schistosomiasis (Brown, 1994). To control these disease there are different complementary public health interventions such as the provision of health education, safe water supply and sanitation, environmental management and snail control would be applied to reduce and control transmissions of human infection (WHO, 2013). Among these control measures, snail control is one of the most rapid and accurate means of reducing the transmission of schistosomiasis (Farghaly *et al.*, 2016).

For applying snail control program as a control measure first there should be infected snail monitoring methods. There are different known monitoring methods to identify cercaria from infected snails. Of these methods, cercarial shedding and crushing the snail methods are the most widely used method to identify cercaria from infected snails after exposure of the specimens to light and crushing the snail respectively (Abbasi *et al.*, 2010). A combination of morphological and biological characteristics of a cercarial specimen are used for identification of the released cercariae on the genus and species level (Frandsen & Christensen, 1984).

There is no single identification method optimal for all situations in the schistosome examination of human beings. Most of the currently used parasitological methods can be interpreted quantitatively or qualitatively based on the diagnostic goal. Quantitative methods are most often used in different research studies, in epidemiological surveys, or in the evaluation of allied intervention measures for disease transmission control. There are different techniques such as coproparasitological examination (CopE), molecular and immunological diagnostic methods (Gordon *et al.*, 2019). CopE methods rely on direct detection and visualization of parasite eggs in stool and include the Kato-Katz thick smear technique, which is recommended by World Health Organization for surveillance and epidemiological survey of schistosome and soil-transmitted helminth infections due to the relative ease of carrying out the examination at low cost, although it lacks sensitivity in low-intensity infections (Habtamu *et al.*, 2011; WHO, 2019). Sensitive and specific diagnostic methods are required for examination of schistosomiasis. However, the most sensitive methods, involving molecular or immunological techniques, can be expensive and require specialized facilities and equipment and trained personnel to perform the procedures (Gordon *et al.*, 2011; Leonardo *et al*., 2016).

2.4. Prevalence of freshwater snail infection

Freshwater snails were distributed in different water bodies like streams, rivers, lakes, and ponds and infected by digenetic trematode parasite if these water bodies are contaminated by the feces of humans, livestock, birds, and wild animals. Then the free-swimming cercaria released to the aquatic environment from the infected snail. The study in lakeshore of Tanganyika in western Tanzania revealed that the snails were distributed in all study sites but the distribution was limited in space and time. Of these, all snails were morphologically identified as *Biomphalaria pfeifferi*.

These snails were exposed to artificial light (60W) for 6 to 12 hours for cercariae shedding then 12.4% of snails were shed cercaria (Bakuza *et al.*, 2017).

In Lake Albert malacological analysis, 8.9% *Biomphalaria* snails were observed for shedding under natural condition. Among these snails 15.8% (87/551) of the snails were infected with human infective cercaria (*S. mansoni*) and 84.2% (464/551) of the snails were infected with non-human infective cercaria. The *Biomphalaria* infection was found in species *B. stanleyi* (69%), *B. sudanica* (25.3%), and *B. pfeifferi* (5.7%) for human infective cercaria were found along Lake Albert (Rowel *et al.*, 2015).

In similar study in Lake Victoria, 2.1% *Biomphalaria* snails were observed for shedding under natural condition. Among these snails 13.9% (39/280) of the snails were infected with human infective cercaria (*S. mansoni*), 85.7% (240/280) of the snails were infected with non-human infective cercaria and 0.4% (1/280) of the snails were infected with both human and non-human infective cercaria. The *Biomphalaria* infection was found in species *B. choanomphala*, (57.5%), *B. sudanica* (22.5%), and *B. pfeifferi* (20%) for human infective cercaria were found along Lake Victoria (Rowel *et al.*, 2015).

The study in Egypt, from 200 collected field snails 1(0.5%) and 3(1.5%) of the snails were positive by using shedding and crushing methods respectively (Farghaly *et al.*, 2016). A similar study in N'Djamena, Chad shows that a totally of 892 snails were collected. Of these 108 (12.1%) *B. pfeifferi*, 413 (46.3%) *B. truncates* and 371 (41.6%) *B. forskalii*. The snail composition was depending on seasonal variation. *B. pfeifferi* was present in a high number during the dry season. *Schistosoma mansoni* was determined in one of the 108 *B. pfeifferi* with an infection rate of 0.93% (Moser *et al.*, 2014).

The study in Ziway Lake revealed that the prevalence of cercaria infection in *B. sudanica* recorded 0.5 to 6% for snail sampling sites with the highest values (4–6%) equivalent to sampling dates (Erko *et al.*, 2006). During snail survey in Lake Hike and Ketie Stream, 31 live *B. pfeifferi* were collected from Ketie Stream but no live snail was found from Lake Hayk during the study period except *Melanoides tuberculata* and *B. pfeifferi*. Out of 31 *B. pfeifferi* surveyed from Ketie Stream, only one (3.2%) snail was shed the cercaria (Amsalu *et al.*, 2015). Jimma town's three rivers were observed to harbor snails of various genera and with varying degrees of snail infection. Among the rivers, in Chore River high (70.5%) number of *Biomphalaria* species was infected with human

schistosome followed by Awetu, (55.2%) and Kito (9.1%) river. The cumulative cercariae shading rate of *Biomphalaria* species was 325 (58.0 %) (Mengistu *et al.*, 2011).

A similar study in the Omo Gibe River basin shows that a total of 3107 snails were collected. Of these 3045 snails were examined for cercarial infection which is 3.6% infection rate. Most cercaria infection (85%) was detected from *B. pfeifferi* whereas the infection was not detected in *B. sudanica* snail species (Mereta *et al.*, 2019). But in Lake Ziway high prevalence of cercaria infection recorded in *B. sudanica* snail species coincided with raised *Biomphalaria* population density (Erko *et al.*, 2006). The study on the abundance of snails, *Biomphalaria sudanica* was not recorded from Lake Hawassa but 76 *Biomphalaria sudanica* were collected from Tikur Wuha River (Mitiku, *et al.*, 2010).

2.5. Factors that affect the prevalence of snail infection

2.5.1. Environmental factors

2.5.1.1. Abiotic factors and habitat conditions

The study done in Lake Lindu, Indonesia suggests that environmental factors were the major determinant for cercarial infection of snails. The correlation between environmental factors and the high prevalence of snail infection was very strong with a correlation coefficient (R) of 0.95 and 0.99 in both study areas. Temperature (water and air), pH, dissolved oxygen, and availability of food were the major ecological factor causing the high prevalence of snail infection (Mardin *et al.*, 2018).

As the study done in Lake Albert and Lake Victoria revealed that the infection of *Biomphalaria* species had a positive relationship with water temperature, slight wave action and *Biomphalaria* abundance but, a negative relationship with electric conductivity, pH, and water depth along Lake Albert and Lake Victoria. Generally, according to this study water temperature, EC, pH, water depth and *Biomphalaria* population had an effect on the prevalence of snail infection (Rowel *et al.*, 2015). Poulin (2006), reported that temperature had a strong positive relationship with the production and the emergence of cercaria from freshwater snail species. And also, since cercarial stage is important in the parasite life cycle, it has been suggested that global warming might increase future disease transmission. In another study: pH, water temperature, dissolved oxygen,

water depth, and hardness were also influence the cercarial infection of snails (Pietrock & Marcogliese, 2003; Hamburger *et al.*, 2004).

Freshwater snails need calcium for shell hardening, egg production and unable to get it in the water with less than 2 mg/l calcium. The ratio of calcium to magnesium is significant for calcium uptake by the snails. The freshwater snail that lives in the water with a low concentration of calcium was highly infected than in high concentration calcium (Mostafa, 2007).

The study done in Omo Gibe River Basin revealed that physicochemical environmental factors like water temperature, pH, electric conductivity, dissolved oxygen, turbidity, hardness, and BOD_5 influence the prevalence of snail infection. Among these factors only dissolved oxygen was negatively affecting cercarial infection of intermediate snail species whereas the other factors had a positive relationship. Concerning habitat type pool, habitat type had a positive relationship with infection rate of snail but riffle type was negative (Mereta *et al.*, 2019).

Trematode-infected snails represent a small proportion of the total snail population in their native habitats and infected snails prefer to be spatially aggregated. Therefore, the" hot-spots" infection (i.e. where aggregates of infected snails occur) contributes greatly to the transmission of trematode. Factors that contribute to the development of the hotspot include vulnerability of snails to infection, probability of interaction with viable trematode eggs, and suitability of snail habitat (Tolley-Jordan & Owen, 2008).

The habitat condition has an influence on the infection of snails. It has positive relationship with abundance of trematodes since most freshwater snail species prefer slow moving water for living and breeding (Mereta *et al.*, 2019). The study done in Lake Albert shows that the prevalence of snail infection with *S. mansoni* was significantly different from different sampling sites. The highest infection rate was recorded from Piida whereas the lowest infection rate was recorded from the Booma study site. This shows the availability of suitable snail habitat differed markedly between the sites. The Piida site had a more suitable habitat to have the highest snail infection rate with *S. mansoni* than the other study site (Kazibwe *et al.*, 2006). In another study which was done in Landa Lake, USA shows that infected snails were aggregated into lentic habitat with the highest level of infection. The majority of infected snails were found in silt substrates, depth less than 0.3m, greater than 50% vegetation and 25% detritus coverage (Tolley-Jordan & Owen, 2008).

The study in Lindu Lake, Indonesia shows that there is a high prevalence of snail infection in both study sites due to ecological determinants. Both areas have an altitude of greater than 1500m above sea level that allows a great distribution of macro and micro fauna which have suitable habitat conditions for snails. The habitat is muddy/organic, shadow and have muddy grass-grown which highly favored by the snails (Mardin *et al.*, 2018).

2.5.1.2. Biological factors

In addition to abiotic factors, biological factors have an impact on the infection and abundance of freshwater snails. The study done by Yigezu *et al.* (2018), revealed that when there were greater than 40 individuals of predators and competitors, there was a low abundance of *Biomphalaria* species. The freshwater snails, including the intermediate hosts of human schistosomes, are preyed upon by different macroinvertebrate predators such as Belostomatidae (Younes *et al.*, 2017), Dytiscidae (Inoda *et al.*, 2015), Odonates (Turner & Chislock, 2007), Psychodidae (El Bardicy *et al.*, 2009) and Glossiphoniidae (Brönmark & Malmqvist, 1986).

Whereas the invertebrate belongs to Physidae considered as compotators of freshwater snails (Yigezu *et al.*, 2018). Under laboratory conditions, Belostomatidae showed a great ability to catch and feed on *Biomphalaria* species (Armúa de Reyes & Estévez, 2006). Even there are some invertebrates have been assessed as snail biocontrol agents for integrated schistosomiasis control programs (Appleton *et al.*, 2004).

The experimental study shows that two-third of the *Biomphalaria* species snails were distracted by the predators. And also, the predators consume the egg of these species' snails. The predators also consume *S. mansoni* miracidia, then the prevalence of *Biomphalaria* snail infection with *S. mansoni* was significantly (p-value <0.05) decreased (El Bardicy *et al.*, 2009; Yousif *et al.*, 2013).

The snail predators have an important impact on schistosomiasis transmission by reducing the abundance of infected snails. In addition to the importance of predators in patterns of schistosomiasis transmission, the effect of schistosome parasitism on snail anti-predator behavior which increases predation risk is crucial on the reduction of the abundance and occurrence of infected snails. Infected snails were preferentially consumed by predators that means the predators have a high attacking rate (20.3±4.12%: M ± SE) for infected snails than uninfected snails (10.4±1.67% M ± SE). And also, the infected snails were easily handicapped by the predators due

to the slower movement (25.2±3.1 mm min−1) than the uninfected snails (18.9±2.7 mm min−1) (Belgrad & Smith, 2014; Swartz *et al.*, 2015).

The experiment conducted in deep permanent lakes revealed that predation by fish and crayfish and food shortage can regulate snail abundance and regulate the structure of the snail distribution (Turner & Chislock, 2007).

2.5.2. Human disturbance and land use

An area which has more frequent water contact during different activities by the local community harbored a higher infection rate than the area which were less frequented (Kazibwe *et al.*, 2006). Human activities are the most important factor on prevalence of intermediate snail infection. The activities are open defecation, open field urination, livestock grazing, swimming, washing, irrigation, fishing, dumping, farming, and bathing which have to enhance the cercarial infection of intermediate snail species. Regarding land use, more cercarial infection of intermediate snail species was observed in cropland area whereas limited cercarial infection of intermediate snail species was observed in forest and shrubland (Mereta *et al.*, 2019).

2.6. Prevalence of *Schistosoma mansoni*

The study in Aladoas, Brazil revealed that the prevalence of *S. mansoni* among the fishermen was 13.8%. More than half (57.9%) of carriers of the infection comes from the rural environment. The fishermen have a relative risk of 3.6 times higher of getting infected with the schistosomiasis, for having a high frequency of contact with the water bodies. Among these respondents, the adults (29-49yr) and elderly (>60yr) were the most affected group. Regarding the educational status, the infection is prevalent in those who can't read or write (illiterate) and among those who incomplete basic school. The *S. mansoni* infection intensity among the Aladoas, Brazil were low to moderate infection, in which low infection intensity is the predominant among the fishermen (78.8%) (Melo *et al.*, 2019).

The study done in Lake Manzala; Egypt shows that 186(26.6%) fishermen were positive for *S. mansoni* among the examined fishermen. The infection is significantly higher (31.6%) in the age group of 20-40yr followed by age from 5-20yr with a prevalence of 24.7%. And also, males were significantly more infected (27.9%) by *S. mansoni* than the females (23.8%). The intensity of *S. mansoni* infection was relatively low (42.7±7.2) among the participant (Taman *et al.*, 2014).

According to the study done on Lake Hawassa fishermen revealed that the prevalence of *S. mansoni* among the fishermen was 29.22%. Of the total stool examination, 169(69.5%) were positive for one or more intestinal parasite infection. The identified parasites were *A. lumbricoides* (40.74%), *T. trichiura* (35.80%), and hookworm species (5.76%). Of the total positive stool sample for intestinal helminth, 43.78% had a single helminth infection, while 56.21% were infected with more than one intestinal helminth. The most predominant multiple intestinal helminth infections were a double infection of *A. lumbricoides* and *T. trichiura* (50.52%), followed by *S. mansoni* and *T. trichiura* (13.68%) (Menjetta *et al.*, 2019).

Regarding the infection intensity, *S. mansoni* infection was 158.8EPG. The level of *S. mansoni* infection intensity was light (52.11%), moderate (43.66%), and heavy (4.23%). The risk for *S. mansoni* infection among Lake Hawassa fishermen had a significant association with swimming, bathing, and other activities involving frequent water contact. But there was no statistically significant association between *S. mansoni* infection and the water sources used for drinking by fishermen (Menjetta *et al.*, 2019).

2.6.1. Demographic and behavioral factors

Demographic factors influence the transmission and spread of schistosomiasis significantly. Additionally, behaviors of the community decide the rate of transmission of schistosomiasis by influencing the local people's exposure level to schistosome parasite in endemic areas. As a behavior-related disease, the risk of schistosomiasis infection is associated with age, gender, and occupation of the individual. Males aged between 15 and 40 years who involve in farming, fishing, and herding is at greatest risk of parasite infection (Ross *et al*., 2001; Gordon *et al*., 2019).

Research of human water interaction to date has measured exposure to either direct observational studies or interviews at the population level. These studies have shown that infection severity is greatly affected by many aspects of human interaction with infectious water including the frequency, length, or time of day of interaction. For example, interaction with water is greatest in the middle of the day or early afternoon, when cercariae are released from the intermediate snail host (Azim *et al.*, 2008; Sow *et al.*, 2011).

2.7. Conceptual framework

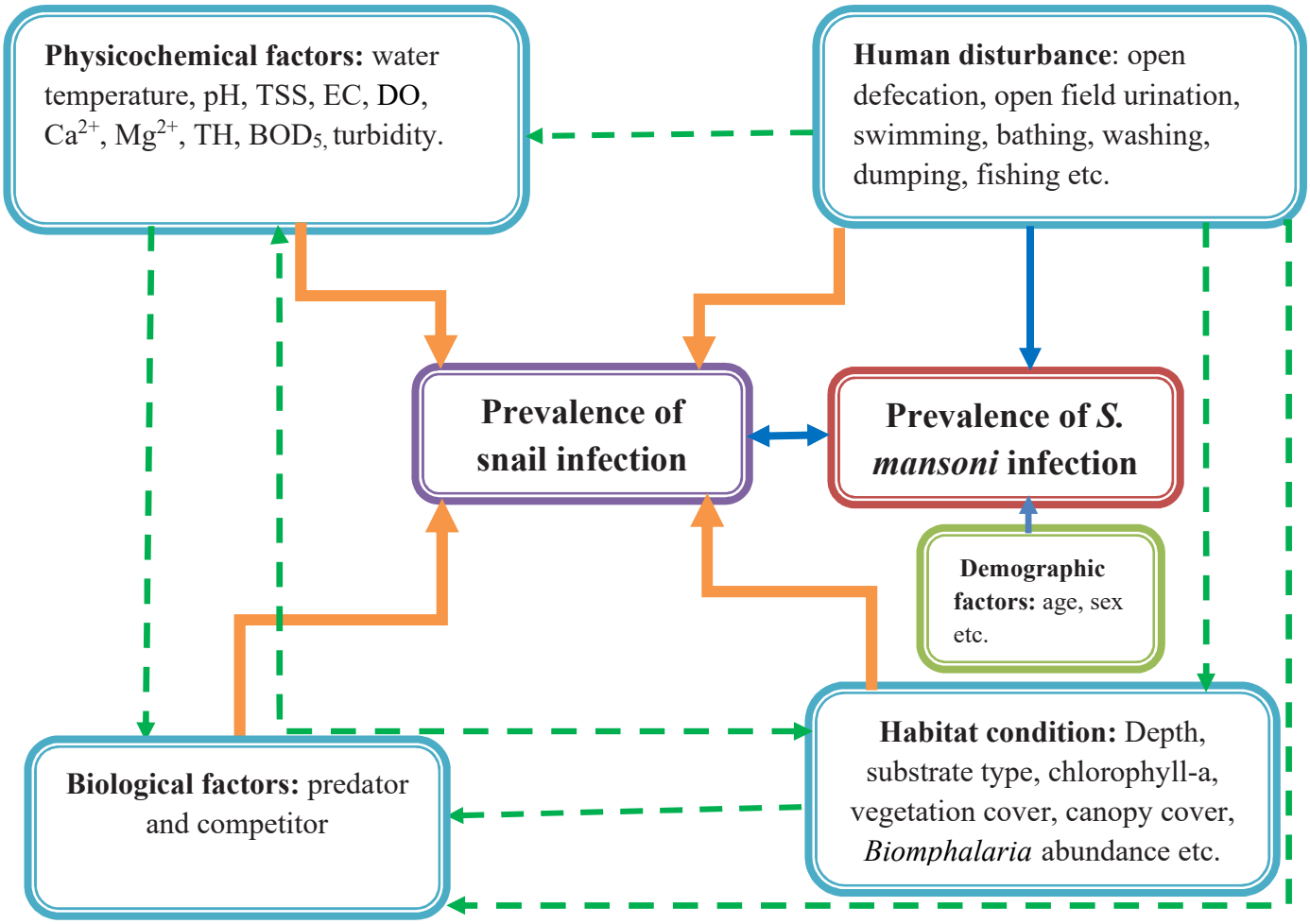

Figure 2: Conceptual framework for prevalence of freshwater snail infection and *S. mansoni* infection among fishermen in the shore of Lake Hawassa and Tikur Wuha River, March 2020.

Chapter three: Objectives

3.1.General objective

- To determine the prevalence of cercarial infection in *Biomphalaria* snails and *S. mansoni* infection among fishermen in the shore of Lake Hawassa and Tikur Wuha River in 2020.

3.2. Specific objectives

- To determine the prevalence of cercarial infection in freshwater snails.
- To determine the prevalence of *S. mansoni* infection among the fishermen.
- To identify environmental factors affecting the prevalence of cercarial infection in freshwater snails.
- To identify human disturbance factors affecting prevalence of cercarial infection in freshwater snails.
- To identify factors contributing to *S. mansoni* infection among the fishermen.

3.3. Research questions

1. What is the prevalence of cercarial infection in freshwater snails?
2. What is the prevalence of *S. mansoni* among the fishermen?
3. How does the prevalence of cercarial infection in freshwater snails relate to environmental factors?
4. How does the prevalence of cercarial infection in freshwater snails relate to human disturbance?
5. Which human behaviors contributed to the *S. mansoni* infection among the fishermen?

Chapter Four: Methods and Materials

4.1. Study area

The study was conducted in Lake Hawassa and Tikur Wuha River. Lake Hawassa is one of the major Ethiopian rift valley lake which located Southwest of Addis Ababa. Lake Hawassa is found in the west of Hawassa town and lies between 06º 58′– 07º 07′ N and 38º 23′–38 º 28′ E. The total catchment area of the lake is 1250km^2. Only the overflow of Lake Cheleleka was draining into Lake Hawassa through Tikur Wuha River and has no surface outflow river. The climatic condition is a dry sub-humid climate with the mean annual rainfall of 950mm and a temperature of 19.8ºC (Ayenew & Gebreegziabher, 2006; Tilahun & Ahlgren, 2010). The lake watershed covers an altitudinal range of 1690 to 2700 meters above sea level. The area characterized by a dry season from October to April and the wet season from May to September. The land-use coverage was more than half of the watershed covered by woodland (Kebede *et al.*, 2014).

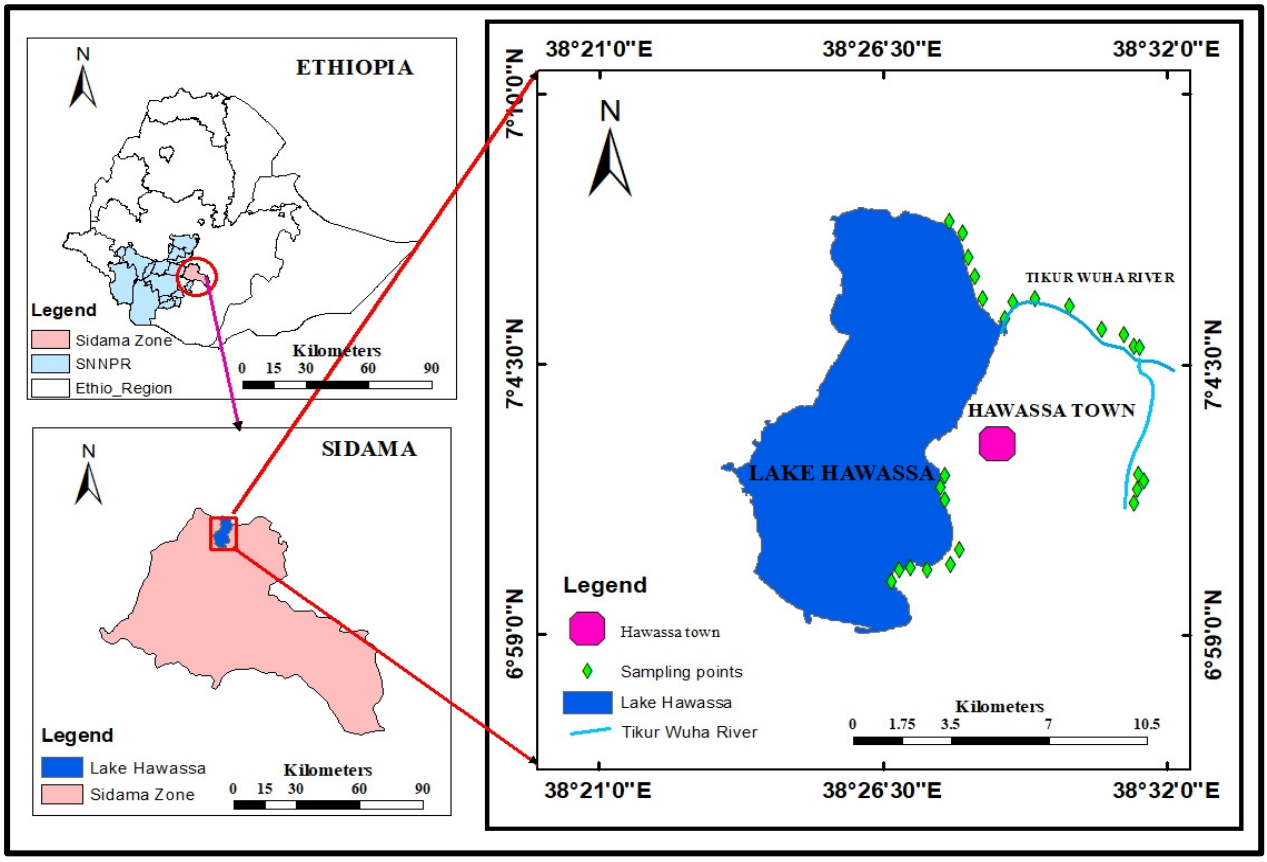

Figure 3: Map of the study area indicating sampling points, March 2020.

4.2. Study design and period

A cross-sectional study was conducted in Lake Hawassa and Tikur Wuha River in March 2020 in order to determine the prevalence of *Biomphalaria* snail infection and *S. mansoni* infection among fishermen.

4.3. Sampling and identification of snails

The sampling sites were selected based on easy access to the site and the presence of human activities (human-water contact) in and around the lake and river (Jayawardena *et al.*, 2010). Snails were collected from 26 sampling sites by scooping 10m stretch for 30 minutes using handled scoop and forceps. And also, dead decaying materials, floating objects, and stones were overturned in each sampling site for searching the snails by gloved hand where the scoop could not be favorable for usage (Kazibwe *et al.*, 2006; Furnish *et al.*, 2008).

Each collected snail was placed in labeled separate plastics buckets filled with water and natural vegetation from the same habitat (Jayawardena *et al.*, 2010). The samples were transported to the Hawassa University Department of Environmental Health Science laboratory. Snails were identified by using appropriate identification keys based on their morphological feature to the species level (Itagaki *et al.*, 1975; Brown, 1994).

In the laboratory, the water added in the plastic bucket was kept minimum for two days to evaporated chlorine gas. Snails from each sampling site kept by providing fresh lettuce in plastic bucket containers containing aged tap water which was replaced daily and the container placed at room temperature. During the changing of water, the plastic buckets repeatedly washed to remove debris and feces of the snail (Jayawardena *et al.*, 2010).

Figure 4: Collecting snails using a hand healed scoop in Lake Hawassa and Tikur Wuha River, March 2020.

4.4. Shedding and identification of cercariae

The snails were rinsed with dechlorinated tap water to remove the mud and others remain substrates from the shell. The rinsed snails were put individually in small beakers and petri-dish filled with dechlorinated tap water to half their size and exposed to natural light for a period of 4-6 hours to induce cercariae shedding (Frandsen & Christensen, 1984). The water in each beaker and petri-dish were then checked frequently for the presence of cercariae using a hand lens. If any beaker or petri dish confirmed the presence of cercaria, a sample of water was taken to observe the resting position and swimming behavior of cercaria before staining under a dissecting microscope. Then the sample was transferred to slides using a pipette and stained with iodine solution and covered by a coverslip. The preparation was observed using a compound microscope for detail morphological identification (Frandsen & Christensen, 1984).

Types of cercariae were described morphologically based on gross characteristics, swimming behavior, and resting position of the cercaria. Through the microscope the picture of cercariae was taken by digital camera fitting the eye lens of compound microscope. In the laboratory, snails that did not shed cercariae at first exposure was kept in plastic container and rechecked for cercarial shedding weekly for another consecutive thirty days (Mohammed *et al.*, 2016). Snails were feed lettuce during this period, and had no mortality among the snails (Frandsen & Christensen, 1984; Schell, 1985).

Figure 5: Putting snails individually in small beakers/petri-dish (a), lettuce feeding (b), shedding (c) and identification of cercariae (d) in Hawassa University Department of Environmental Health Sciences laboratory, March 2020

4.5. Environmental variables

The physicochemical parameters were measured onsite at each surveyed site include ambient temperature, turbidity, pH, water temperature, dissolved oxygen and electric conductivity. Ambient temperature was measured by a thermometer. Water temperature, pH, dissolved oxygen and electrical conductivity were measured by the portable multiparameter probe (HQ30d single input multi-parameter digital meter Hach). Turbidity was measured by using a turbidity meter (Wag-WT3020; Halma PLC Company, Amersham, UK). At each surveyed site water samples were taken by using 1000ml polyethylene plastic bottle, preserved in icebox and transported to Hawassa University Department of Environmental Health Sciences laboratory for analysis. The parameters such as total hardness, Ca^{2+} hardness, Mg^{2+} hardness, chloride, total suspended solids (TSS), and five day biological oxygen demand (BOD_5) were analyzed in the Hawassa University Department of Environmental Health Sciences laboratory by following APHA (2017) standard.

The habitat condition such as water depth, chlorophyll-a, transparency, substrate size, vegetation cover, canopy cover, and detritus cover were measured at each snail sampling location. Water depth was determined with the aid of calibrated rod which was dipped vertically into the lake and river to touch the lakebed and riverbed, thereafter withdrawn and then reading recorded. Chlorophyll-a and transparency were measured onsite using a handheld fluorometer (Aqua Fluor; Turner Designs, San Jose, USA) and Secchi disc 30 cm in diameter respectively. At each sampling site, the substrate type was measured visually and assigned either of the seven categories: mud/silt, sand, gravel, pebble, cobble, boulder, and bedrock; vegetation cover and detritus cover were visually evaluated. And also, the type of aquatic vegetation and soil were assessed (Tolley-Jordan & Chadwick, 2019). The canopy cover was estimated visually in each sampling site and other essential information were recorded in recording sheet (Giovanelli *et al.*, 2005). Latitude, longitude, and altitude were taken and recorded using a handheld Global Positioning System (GPS) instrument (Garmin GPS 60, Garmin International Inc. and Olathe, Kansas, USA) (Kazibwe *et al.*, 2006). The map of the study area showing the sampling sites location was created using the Geographic Information System (GIS) software ArcGIS10.5. And also, at each sampling site macroinvertebrates were collected by kicking 10m stretch for 10 minutes using rectangular frame kick net (20 x 30cm) (Mereta *et al.*, 2013). Macroinvertebrates were sorted and stored in labelled vials containing 70% ethanol. Subsequently, the macroinvertebrates were identified at family level

using dissecting microscope and identification key (Gerber & Gabriel, 2002; Bouchard, 2004) in Jimma University, Department of Environmental Health Sciences and Technology laboratory.

Figure 6: Macroinvertebrate sampling & sorting in the fieldwork (a, b & c) and its identification in Jimma University Department of Environmental Health Sciences and Technology laboratory (d), March 2020.

4.6. Human disturbance

The presence or absence of different human activities that affect snail's infection and schistosomiasis transmission like bathing, swimming, washing, fishing, open defecation, open urination, farming, grazing, dumping, filling, water abstraction, and clearance were observed during sample collection. Then the magnitude of each activity was measured based on ordinal scale following as zero was given for absent, one for minimal disturbance, two for moderate disturbance, and three for high disturbance (Mereta *et al.*, 2013).

Figure 7: Human activities in the shore of Lake Hawassa and Tikur Wuha River, March 2020.

4.7. Parasitological survey

4.7.1. Sampling population and sample size determination

The source population of the study was the people living around Lake Hawassa and Tikur Wuha River. Study participants were selected randomly from the fishermen association list during the data collection period. The sample size (n) needed for this study was determined using the statistical formula $n = \frac{Z^2\, p(1-p)}{d^2}$, Where n = sample size, Z is statistics for 95% confidence interval (1.96), p is the previous prevalence of *S. mansoni* infection (p = 29.22%), as a baseline prevalence in the study area (Menjetta *et al.*, 2019) and d is a margin of error (5%). The calculated sample size was 318. Since the total number (520) of the fishermen was less than 10,000, a population correction formula was used and by adding 10% for the non-respondent rate, the final sample size was determined to be 219.

4.7.2. Socio-demographic and practice data collection

Sociodemographic data which include sex, age, educational status, and other essential information were collected. Presence of *S. mansoni* infection risk factors were asked from the study participants using semi-structured questionnaire. Direct observations around the sampling areas were also undertaken on these risk factors.

4.7.3. Stool sample collection and examination

Fresh stool samples were collected using pieces of plastic containers distributed to each of the selected participants with an applicator stick. The collected stool samples were transported to the Hawassa University Department of Environmental Health Sciences laboratory. Then the stool samples were processed using the Kato-Katz thick smear method for microscopic identification. The egg count of *S. mansoni* and determination of the presence and absence of other intestinal helminths were undertaken after 24 hours of smear preparation. Since the template delivering 41.7 mg of faeces was used to prepare Kato-Katz slides, the number of eggs observed is multiplied by 24 to convert into eggs per gram of faeces (EPG) (WHO, 2019). The intensity of *S. mansoni* infection was classified according to the intensity classes recommended by WHO as light (1–99 EPG), moderate (100–399 EPG), and heavy infections (greater than/equal to 400 EPG) (WHO, 2002).

Figure 8: Parasitological investigation in Hawassa University Department of Environmental Health Sciences laboratory, March 2020.

4.8. Study variables

- **Dependent variables**
  - ✓ Prevalence of cercarial infection in *Biomphalaria* snail
  - ✓ Prevalence of *S. mansoni* infection among fishermen
- **Independent variables**
  - ✓ Water quality parameters**:** water temperature, pH, EC, DO, turbidity, total hardness, Ca^{2+} hardness, Mg^{2+} hardness, chloride, TSS, and BOD_5.
  - ✓ Biological factors: macroinvertebrate predator and competitor
  - ✓ Human disturbance: open defecation, open field urination, grazing, swimming, washing, fishing, farming, bathing, dumping, filling, water abstraction, and clearance
  - ✓ Habitat condition: water depth, substrate type, canopy cover, *Biomphalaria* abundance, chlorophyll-a, vegetation cover etc.
  - ✓ Socio-demographic factors

4.9. Data analysis

The raw data were entered into Excel 2016 and Epi data version 3.1 for malacological and parasitological study data respectively. These data were transported into SPSS version 26 and PAST version 4.03 for statistical analysis. The physicochemical parameter results were presented based on descriptive statistics by using table. At the same time, inferential statistics were analyzed to determine the factors that affect the prevalence of cercaria infection in snails and *S. mansoni* infection among fishermen.

The principal component analysis was performed for different environmental factors to reduce the original independent variables and to describe interrelation patterns between the factors and the prevalence of snail infection. Before analyzing the PCA, the data which needs standardization was standardized by log transformation [log (x+1)]. The principal components were selected based on the criteria of eigenvalue greater than one. The variables in each environmental parameter were also checked for correlation with each other, and one selected if indeed there was a lot of greater than 0.3 correlation coefficient then after included in the factor analysis. After performing PCA, the variable which has a loading value of less than 0.3 was suppressed (Umar, 2005; Abdi *et al.*, 2013).

The data was not satisfying the assumption of one-way ANOVA and two independent sample tests. Prior to use the equivalent non-parametric test, the data was subjected to log transformation [log (x+1)]. Then after the data also not valid for one-way ANOVA, the equivalent Kruskal-Wallis test was analyzed to compare the prevalence of snail infection between the habitat conditions (canopy cover, substrate type and soil type). Similarly, the data was not valid for two independent sample tests, the equivalent Mann-Whitney test was analyzed to compare the snail distribution between the lake and river.

The prevalence and intensity of infection with *S. mansoni* were reported in percentage and mean egg count respectively. Chi-square test had been used to determine the association between *S. mansoni* and risk factors (age, sex, educational level, and frequency of water contact). The analysis was analyzed by using SPSS version 26 and PAST version 4.03 statistical software. The p-value and 95% confidence interval were calculated and a p-value of less than or equal to five was considered as significant for all statistical tests.

4.10. Quality control and assurance

During the sampling process, we were followed by sampling protocols for both snail and water sampling procedures. Expert on the identification of snail species was consulted to identify snails in species-level to prevent misidentification. Calibrate instruments and ensure that instrument measurements do not drift. Standard laboratory procedures were followed strictly in order to assure quality assurance of laboratory investigation.

4.11. Ethical consideration

Before starting the study, ethical clearance was obtained from institutional review board of Jimma University, Institute of Health. A formal letter was written to all concerned bodies and permission was secured at all levels. It was conducted by taking the permission letter from the Department of Environmental Health and Technology of Jimma University for any activity during the study period. Verbal consent was obtained from area residents to conduct the malacological study.

For parasitological study permission to perform the test was obtained from the manager of the fishermen association. The study's goals were clarified to the manager and fishermen, during which the fishermen participating in the study received verbal informed consent. The confidentiality of the information was assured and the respondent's privacy was preserved. Those fishermen who found the *Schistosoma mansoni* positive were linked to the local health administration for treatment service.

4.12. Dissemination of the study findings

After data analysis and interpretation, the result of this study will be submitted and presented to Jimma University, Institute of Health Science, Department of Environmental health science and technology. It will be disseminated to different concerned bodies (like SNNPR Health Bureau, Oromia Region Health Bureau, Health program manager) to control and prevent schistosomiasis by undertaking snail control programs. Finally, the effort will be made to present in different workshops and for publication in an international or national journal.

4.13. Limitation of the study

The limiting factor in this study was the outbreak of the COVID-19 pandemic that limit to get the planned sample size for parasitological investigation. Because of the restriction of direct contact with the participant to collect the data and some of the participant lacks willingness to provide sample fear of the COVID-19 pandemic disease. The anticipated sample size was 219, but the samples were collected only from 109 participants.

4.14. Operational definition

Soil type

- Organic: Leaves, decaying organic matter
- Mineral: Clays, silicates, inorganic components of biological origin (e.g. skeletal material)

Aquatic macrophytes:

- Very sparse = <10%
- Sparse = 10-35%
- Moderate = 35-65%
- Heavy = 65%- 90%
- Very Heavy = >90%

Substrate type:

- Bedrock: - Smooth surface rock/hardpan (>4000 mm –bigger than a car)
- Boulder: - Basketball to car (250-4000 mm)
- Cobble: - Tennis ball to basketball (64-250 mm)
- Pebble: - Marble to tennis ball (16-64 mm)
- Gravel: - Ladybug to marble (2-16 mm)
- Sand: - Gritty – up to ladybug (0.06-2 mm)
- Silt (Including Clay & Muck): - Fine - not gritty (<0.06 mm).

Human activities

- Absent: - Any activities in a distance of greater than 100m from the water bodies
- Minimal: - The presence of activities in a distance of between 50m and 100m
- Moderate: - The presence of activities in a distance of less than 50m from the water bodies
- High: - The presence of activities inside the water bodies

Fishermen practice

- Always: practicing a minimum of once a week.
- Seldom: practicing less than once a week.
- Never: hadn’t practice

Chapter Five: Results

5.1. Malacological investigations

5.1.1. Distribution of snail's population

A total of 108 freshwater snails were collected from 26 sampling sites. Snails belonging to two different species in the genera of *Biomphalaria* namely; *B. pfeifferi* and *B. sudanica* which are the intermediate host of *S. mansoni.* In the shore of Lake Hawassa, both types of snail species were collected and it accounts for 9.3% (10/108) of the total collected snails. But in the tributary of Lake Hawassa (Tikur Wuha River), only *B. sudanica* was recorded and it accounts for 90.7% (98/108) of the total collected snails. The most abundant species was *B. sudanica* constituting 93.5% of the entire sample and collected from 30.8% of the surveyed sample sites.

The output of the Mann-Whitney U test indicates that there is a significant difference ($P = 0.0297$) in the distribution of *Biomphalaria* snail abundance between the Lake Hawassa and Tikur Wuha River.

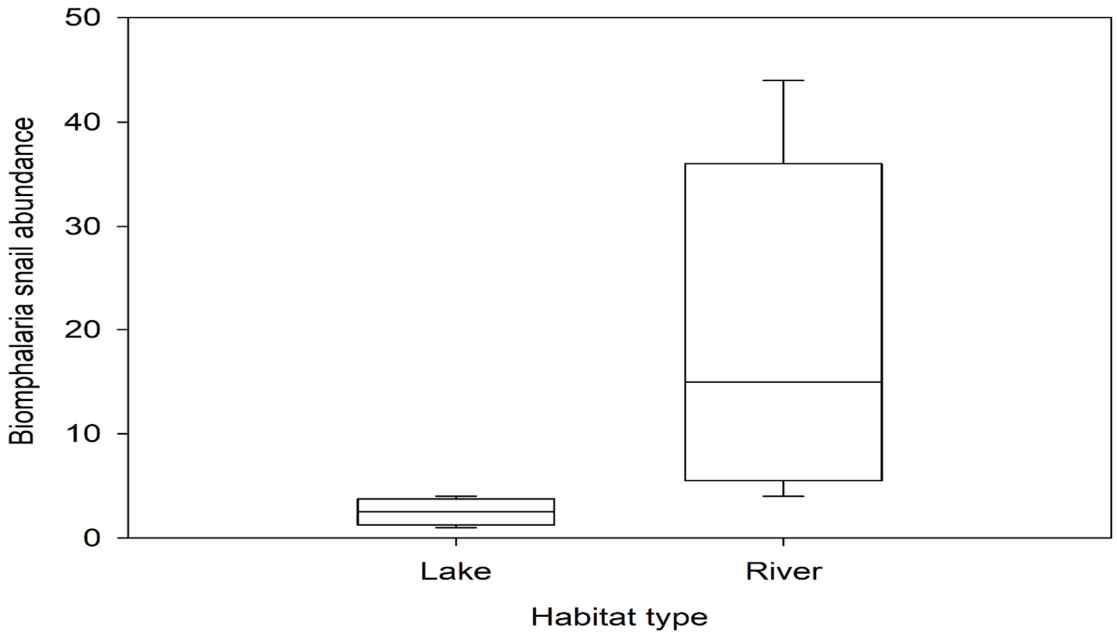

Figure 9: Box-plot showing *Biomphalaria* abundance based on habitat type, March 2020.

5.1.2. Prevalence of cercarial infection in freshwater snails

Overall, 13 snails from 4 (15.4%) sampling sites were found to be infected with cercaria. The total prevalence of cercaria infection in the snails was 12.03% (13/108). The infection was recorded only from *B. sudanica* snail species. The highest, 13.3% (13/98) cercaria infection was recorded from the inflow of Lake Hawassa (Tikur Wuha River). But there was no cercarial snail infection in Lake Hawassa.

5.1.3. Types of cercarial infection in freshwater snails

A total of five different types of cercariae were released by *B. sudanica* species (see Table 1, and Figure 10); no infections were recorded in the *B. pfeifferi* snails. The released cercariae were categorized into individual groups based on morphologically differentiable structures according to Frandsen and Christensen (1984). The type of cercaria harvested from these study sites were *Echinostome* cercariae, *Brevifurcate apharyngeate distome (BAD)* cercariae, *Amphistome* cercariae*, Gymnocephalous* cercariae, and *Ornatae xiphidiocercariae* cercariae.

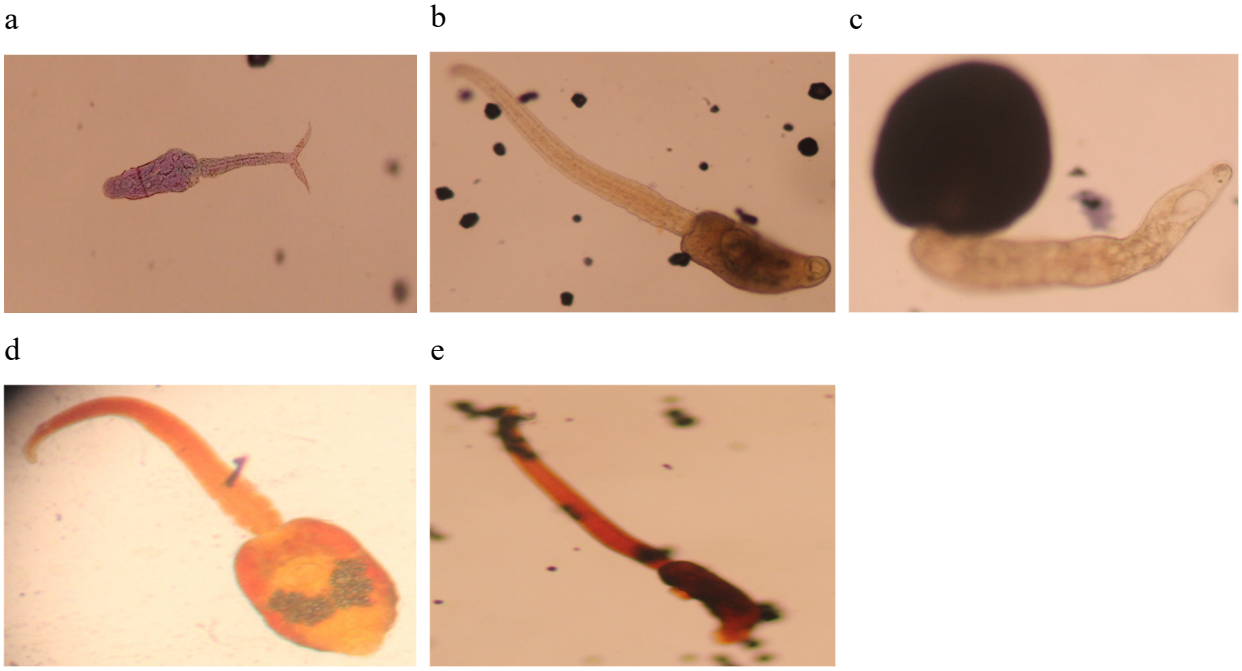

Figure 10: Morphotype of cercariae recovered from *Biomphalaria* snails in the shore of Lake Hawassa and Tikur Wuha River, March 2020. The types are: a. *BAD* cercariae, b. *Echinostome* cercariae c. *Amphistome* cercariae d. *Gymnocephalous* cercariae e. *Xiphidiocercariae* cercariae

Double infections were recorded from this snail species. The three *B*. *sudanica* snails were found to be simultaneously shedding *BAD* cercariae, and *Amphistome* cercariae*, Echinostome* cercariae *and Amphistome* cercariae*, Echinostome* cercariae, *and Ornatae xiphidiocercariae* cercariae. The highest prevalence of infection was recorded by *BAD* (human schistosome) cercariae, which accounted for 37.5 % followed by *Echinostome* cercariae (25 % of all infections), *Amphistome* cercariae (18.75%), *Gymnocephalous* cercariae (12.5%) lastly by *Ornatae xiphidiocercariae* (6.3%) cercaria (see Tables 1).

Table 1: Number of infections recorded in *B. sudanica* snail species collected from Hawassa lake shore and Tikur Wuha River, March 2020.

Cercariae type	*B. sudanica*
Gymnocephalous cercariae	2(12.5%)
*Echinostome**	4(25%)
*Brevifurcate apharyngeate distome (BAD)**	6(37.5%)
*Amphistome**	3(18.75%)
Ornatae xiphidiocercariae,	1(6.3%)
Total	16

✍ * Double infection

5.1.4. Effects of environmental factors

5.1.4.1. Physicochemical parameters

The mean and standard deviation of the altitude of the study sites was 1680.46m and 3.7 respectively. It ranged from 1669 to 1688m. The ambient temperature ranged from 20.4 - 31.0ºC with a mean and standard deviation of 26.1ºC and 2.54 respectively. Whereas the temperature of the water ranged from 23.7-29.5ºC with a mean and standard deviation of 26.46ºC and 1.7 respectively. The range of pH was 6.23 - 9.6 with a mean of 7.78. The mean and standard deviation of DO saturation were 88.35% and 66.8 respectively and it ranges from 7.8% to 267.5%. For total hardness, the mean and standard deviation was 42.92mg/l and 9.36 whereas for calcium hardness 29.85 & 6.9 respectively. The mean, standard deviation, and range of all measured water physicochemical parameters and some habitat parameters were presented in Table 2.

Table 2: Mean, standard deviation, and range of environmental factors in Lake Hawassa and Tikur Wuha River, March 2020

Variables	Unit	Mean	Std. Deviation	Range
Altitude	m	1680.46	3.7	1669 – 1688
Ambient temperature	℃	26.1	2.54	20.4 – 31.0
pH	-	7.78	1.16	6.23 – 9.60
Water temperature	℃	26.46	1.7	23.7 – 29.5
DO concentration	mg/l	5.75	4.22	0.51 – 16.66
DO saturation	%	88.35	66.8	7.8 – 267.5
EC	µS/cm	582.69	314.7	124.9 – 892.0
Turbidity	NTU	17.00	18.9	4 – 78
Transparency	m	24.54	17.03	0 – 50
Chlorophyll-a	µg/l	33.7	15.95	17.89 – 76.11
Calcium hardness	mg/l	29.85	6.90	16 – 44
Magnesium hardness	mg/l	13.08	6.15	0 – 24
Total hardness	mg/l	42.92	9.36	24 – 60
Chloride	mg/l	35.06	12.5	15.99 – 47.98
TSS	mg/l	22.04	18.01	0 – 64
BOD5	mg/l	47.03	53.15	0 – 140

The principal component analysis output shows two factors had an eigenvalue of greater than one, that includes: first factors with the eigenvalue 6.14 there were BOD_5, chloride, electric conductivity (EC), pH, turbidity, DO concentration, and total hardness, second factors with the eigenvalue 1.23 comprise only total suspended solid (Figure 11).

The aforementioned principal component analysis result shows that in the study area variability in the first and second axis was 81.8%. This means that 81.8% of the analytical data can be explained by the two axes. The first and the second component explains 68.2% and 13.6% of the variation in the prevalence of *Biomphalaria* infection, respectively.

The PCA tri-plot shows that the prevalence of *Biomphalaria* infection was positively correlated with BOD_5, total suspended solids (TSS), and turbidity but negatively correlated with dissolved oxygen concentration, pH, electrical conductivity, chloride, and total hardness (Figure 11).

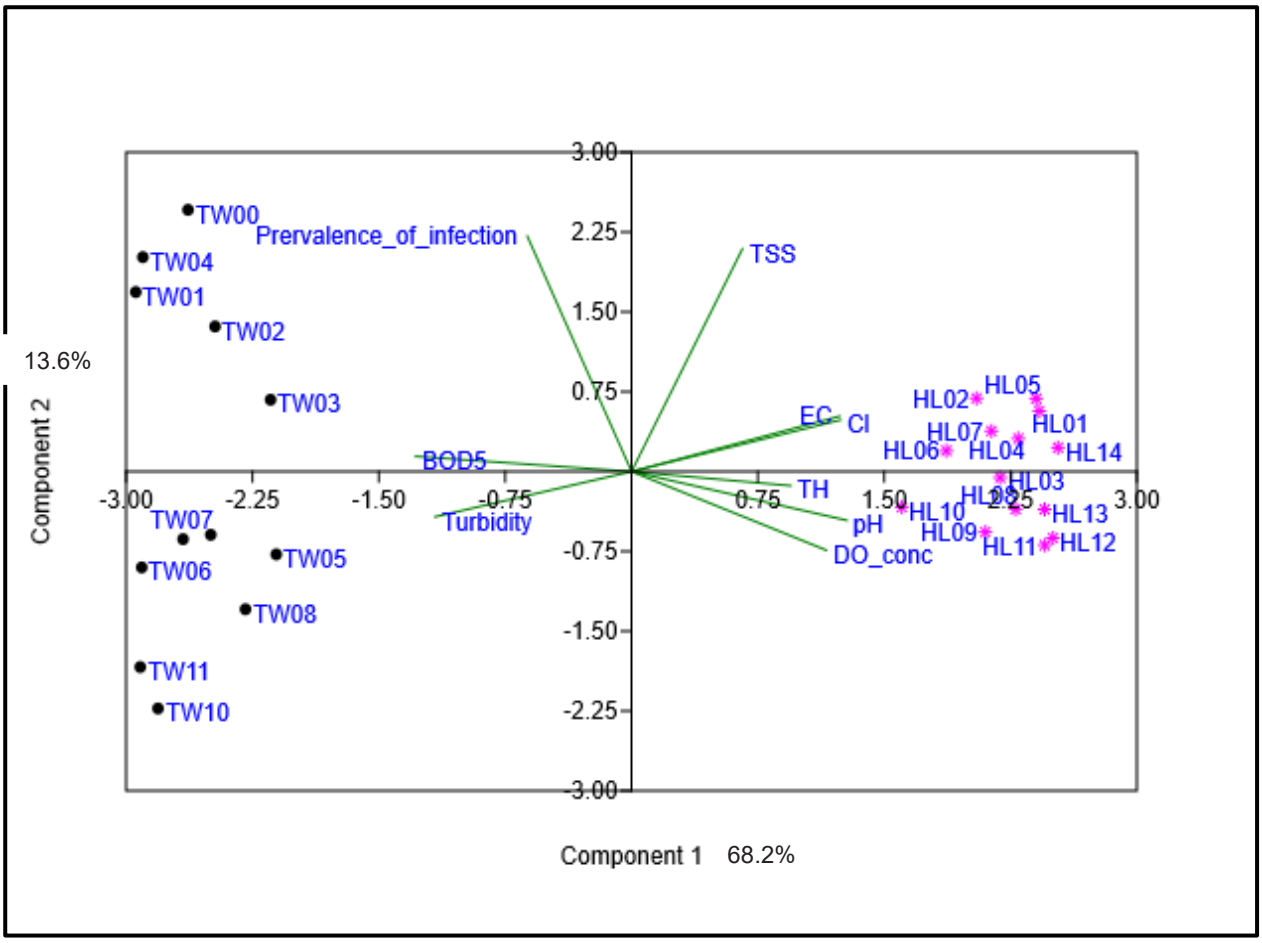

Figure 11: PCA tri-plot for some selected water physico-chemical parameters with the prevalence of *Biomphalaria* infection, March 2020.

5.1.4.2. Habitat conditions

The substrate type, soil type, adjacent land use, riparian vegetation cover and canopy cover of the sampling sites were assessed. In twelve (46.2%) of the sampling sites, the substrate type was silt followed by sand (26.1%). Regarding the soil type, 50% of the sampling site was organic followed by mineral (38.5%). The majority of the sampling sites found in water depth less than 0.3m and riparian vegetation (tree greater than 10m, tree less than 10m, shrubs, grass, and bare land) less than 25%. Out of the total, 80.8% of the sites were having partial canopy cover.

The result of macrophyte coverage shows that 42.3% moderate emerged macrophyte, 80.8% very sparse submerged macrophyte and 42.3% very sparse floating macrophyte. Whereas the filamentous algae coverage, periphyton coverage, and detritus coverage were 96.2% very sparse, 100% very sparse and 57.7% spars respectively.

Most of the adjacent land uses in the study areas were pasture, agricultural tilled, native vegetation and residential area. Cercarial infection of snails was present in residential areas and agricultural land use areas. However, near or around the pasture and native vegetation land use areas infected snails were absent in the sampling sites.

The Kruskal-Wallis test shows that, there was no statistically significant difference (*P*-value>0.05) in the distribution of prevalence of snail infection among substrate type (H = 3.86, df = 3, *P*-value = 0.277), soil type (H = 4.5, df = 2, *P*-value = 0.105) and canopy cover (H = 1.184, df = 2, *P*-value = 0.553). Regarding macrophyte coverage there was no significance difference in the distribution of prevalence of snail infection among all macrophyte types except in floating macrophyte (H = 11.6, df = 4, *P*-value = 0.020).

Based on PCA output, component one and two had an eigenvalue greater than one, these accounts 3.2 and 2.2 respectively. These two components in combination, explained 67.6% of the total variability in the data. The PCA tri-plot shows that the first component was positively correlated with silt, organic, chlorophyll-a, and grass. In contrast, canopy cover, riparian vegetation greater than 10m, and *Biomphalaria* abundance were negatively correlated with the first component. And almost all these factors were positively correlated with the *Biomphalaria* infection except grass (Figure 12).

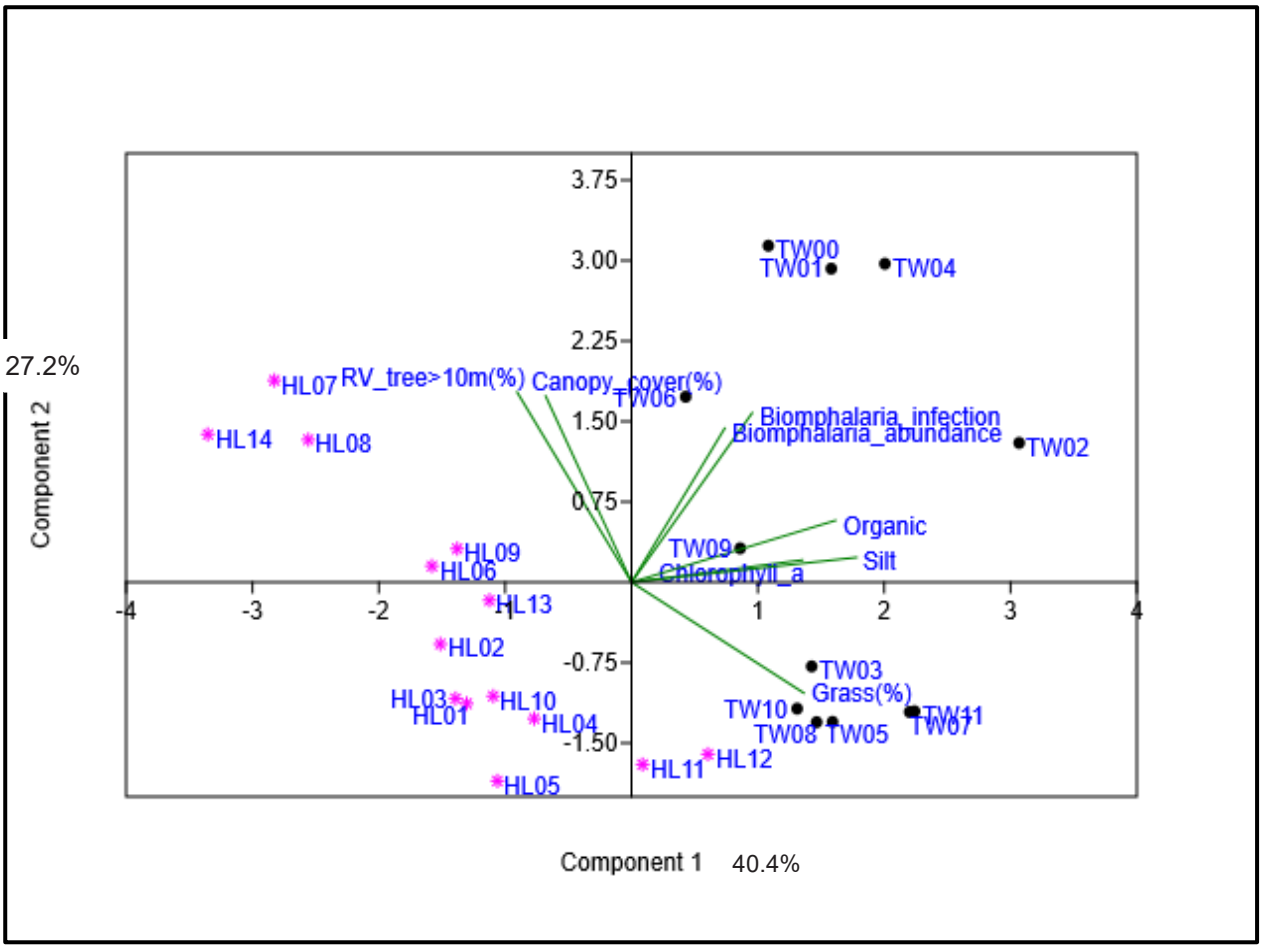

Figure 12: PCA tri-plot for some selected habitat conditions with *Biomphalaria* infection, March 2020.

5.1.4.3. Biological factors

The frequency of occurrence and abundance of collected macroinvertebrates are summarized in Table (3). A total of 32 macroinvertebrates taxa representing 10 orders were collected from 26 sampled sites, during the study period; included Hemiptera, Odonata, Diptera, Coleoptera, Basommatophora, Neotaenioglossa, Ephemeroptera, Tricladida, Megaloptera, and Hirudinea. Generally, the most dominant macroinvertebrates families were Dytiscidae, Thiaridae, Agriidae, Belostomatidae, and Physidae, which recorded 477, 306, 250, 231, and 206, respectively.

Table 3: Frequency of occurrence and abundance of macroinvertebrates along the shore of Hawassa Lake and Tikur Wuha River, March 2020.

Order	Family	Frequency of occurrence (%)	Abundance	Relative abundance (%)
Hemiptera	Belostomatidae	57.7	231	11.3
	Nepidae	23.1	21	1.02
	Pleidae	15.3	58	2.8
	Naucoridae	19.2	34	1.7
	Corixidae	7.7	4	0.19
	Notonectidae	3.8	2	0.1
Diptera	Culicidae	11.5	15	0.7
	Psychodidae	7.7	6	0.3
	Syrphidae	7.7	7	0.34
	Chironomidae	19.2	14	0.68
Odonata	Coenagrionidae	15.4	8	0.4
	Gomphidae	34.6	44	2.14
	Platycnemidae	3.8	18	0.9
	Aeshnidae	53.8	87	4.2
	Chlorolestidae	3.8	2	0.1
	Calopterygidae	3.8	1	0.05
	Agriidae	50	250	12.2
	Corduliidae	11.5	8	0.4
	Lestidae	3.8	12	0.58
	Chlorocyphidae	3.8	4	0.19
Coleoptera	Hydrophilidae	34.6	99	4.8
	Dytiscidae	53.8	477	23.3
	Gyrinidae	27	72	3.5
Basommatophora	Planorbidae	15.4	14	0.68
	Physidae	50	206	10
	Lymnaeidae	27	20	0.97
Ephemeroptera	Heptageniidae	3.8	2	0.1
	Baetidae	7.7	4	0.19
Neotaenioglossa	Thiaridae	46.2	306	14.9
Arhynchobdellida	Hirudinidae	23.1	23	1.12
Tricladida	Planariidae	3.8	1	0.05
Megaloptera	Corydalidae	3.8	2	0.1
Total		653.4	2052	100

The PCA output revealed that three factors had an eigenvalue 6.4, 2, and 1.4 for component one, two, and three respectively. These three components together explained 68.5% total variability of the data. The pollution tolerant family upper right cluster (Culicidae, Hydrophilidae, Planorbidae, and Coenagrionidae) had a strong positive correlation with the prevalence of *Biomphalaria* infection. But the left cluster competitors (Thiaridae, Physidae, and Lymnaeidae) and lower right cluster predators (Belostomatidae, Naucoridae, Nepidae, Gomphidae, and Gyrinidae) were negatively correlated with the prevalence of *Biomphalaria* infection (Figure 13).

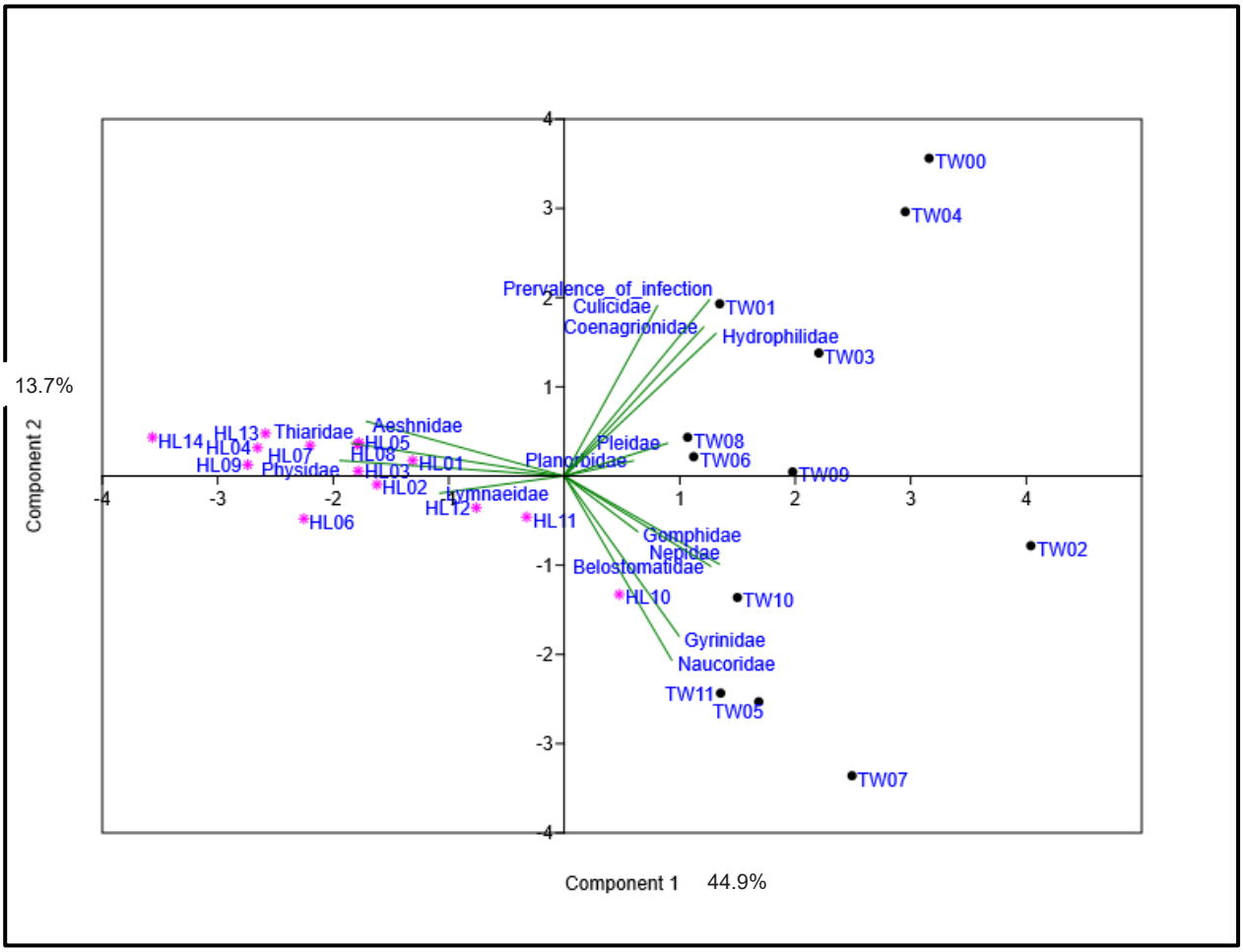

Figure 13: PCA tri-plot for macroinvertebrate with the prevalence of *Biomphalaria* infection, March 2020.

5.1.4.4. Human disturbance

Human activities such as open field defecation, open field urination, farming, fishing, grazing, washing, dumping, bathing, water abstraction, and swimming were practiced in almost all sampling sites, but draining, filling, and clearance were absent in the sampling sites. Even though these activities were present their intensity varied among the sampling sites. In relation to the presence of cercarial infection of snails, almost all human activities that were present in the sampling sites (open field defecation, open field urination, fishing, grazing, washing, dumping, bathing, and swimming) had majorly moderate and high intensity of disturbance.

The output of PCA shows that the first and second components were recorded with an eigenvalue of 2.1 and 1.3 respectively. The first component explains 34.2% variation and the second component explains 22.2% variation. These two components combined explain 56.4% total variation of the analytical data. The PCA tri-plot revealed that almost all human disturbance such as open field defecation, fishing, washing, dumping, bathing, and swimming were positively correlated with *Biomphalaria* infection (Figure 14).

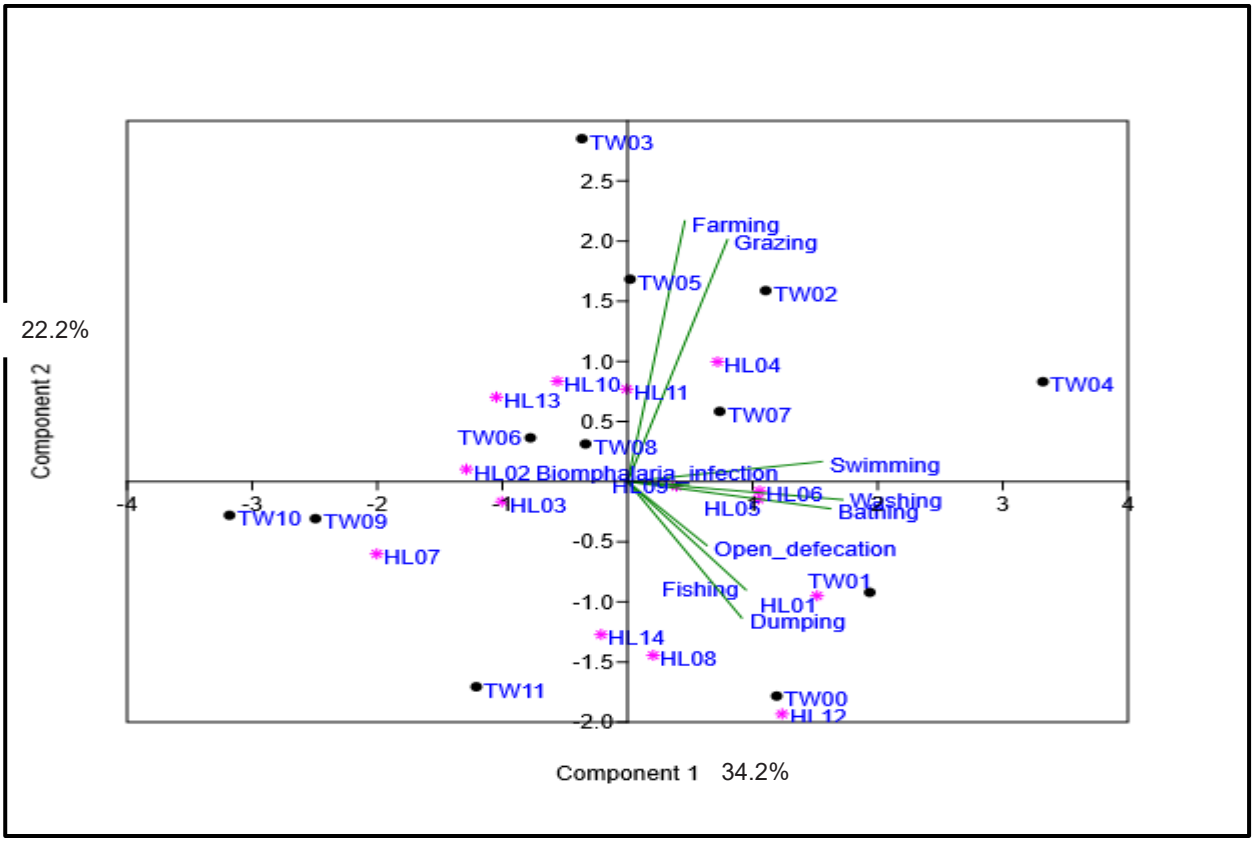

Figure 14: PCA tri-plot for a human disturbance with *Biomphalaria* infection, March 2020

5.2. Parasitological investigations

A total of 109 stool specimens were collected from fishermen and microscopically examined for *Schistosoma mansoni* infection. The prevalence of *Schistosoma mansoni* in this study found to be 14.7% (16/109). The range of intensity for *S. mansoni* infection was 24 and 672 EPG with a mean of 139.5 EPG. The levels of infection intensities for *S. mansoni* were 68.8%, 25%, and 6.3% for light, moderate and heavy infection intensities, respectively. The incidence of *S. mansoni* infection among the fishermen was found to be significantly associated with the frequency of swimming (χ^2 (1) = 12.68, P = 0.006) and educational status (χ^2 (1) = 17.11, P = 0.009). On the other hand, there is no significant association between the incidence of *S. mansoni* infection and sex and the age of fishermen.

Chapter Six: Discussion

The findings of this study found that the prevalence of *S. mansoni* infection among fishermen was 14.7%. The presence of *Biomphalaria* snail intermediate host infected with cercaria was likely contributed to the *S. mansoni* infection among fishermen in the study area. This study demonstrated that the prevalence of *S. mansoni* significantly associated with the frequency of swimming and educational status. The results are in agreement with the study done by Menjetta *et al.* (2019) in Lake Hawassa. The main reason for this association could be due to the high degree of water contact and lack of awareness regarding the transmission of the disease.

In this study*, Biomphalaria sudanica* was the most dominant species which observed 93.5% of the total collected snails in the study area. There was a statistically significant difference ($P = 0.0297$) in the distribution of *Biomphalaria* snail species between the lake and river. This difference may be due to the high presence of fish, and other snail species like Physidae*,* Thiaridae and Lymnaeidae in Lake Hawassa. Fish and other snail species were prey and compete on the food of *Biomphalaria* snail species respectively (Turner & Chislock, 2007). And also, may be due to the association with rich in organic matter in Tikur Wuha River habitat which support aquatic plants providing protection, egg-laying site and food sources for the snails. In addition to substratum difference, it could be due to the high-water depth in the lake than a river. This could be because of greater food availability in shallow sites than the deeper (Rowel *et al.*, 2015). Our finding was in accordance with the study done by Mitiku *et al.* (2010) which shows high dominance was observed in the Tikur Wuha River, but low in Lake Hawassa.

Based on our findings, both the human and non-human infective cercaria was released by *Biomphalaria* snails in the study area. The prevalence of cercarial infection in *Biomphalaria* snails observed (12.03%) was high compared to the previous study done in Omo-Gibe River Basin and in Lake Albert which reported 3.6% and 8.9% respectively (Mereta *el al*. 2019; Rowel *et al*., 2015). This high prevalence may be the difference in snail species and high parasite pressure, simply making high contact between miracidia and snails in the current study area. The other reason could be due to slow-flowing and low level of the river flush off the fecal matter increasing the chance of *Biomphalaria* getting the cercaria infection.

Pfukeni *et al.* (2005) reported that the prevalence of cercaria infection in snails in the diverse areas was different. This was because of the difference in environmental and management conditions. The result of this study shows that snail infection in the river was higher than the lake. This difference could be due to the presence of high fecal contamination and abundance of *Biomphalaria* snails in the river. The high prevalence in this area indicated that the environmental conditions are more suitable for the breeding of *Biomphalaria* snails. As there are suitable substratum, aquatic plants, and increased anthropogenic activities so there was a high incidence of cercaria infection in the *Biomphalaria* snails in the Tikur Wuha River (Mardin *et al.*, 2018).

In the current study, five cercarial types were recorded from one *Biomphalaria* snail species. *Brevifurcate apharyngeate distome (BAD), Gymnocephalous, Echinostome*, *Amphistome*, and *Ornatae xiphidiocercariae* cercariae were recorded from *B. sudanica*. Among these cercariae, *BAD* cercariae were the most commonly recorded type of cercariae which accounted for 37.5% of all recorded cercarial type, where all the examined *BAD* cercariae were human *Schistosome* cercaria (Frandsen & Christensen, 1984). This may indicate the area around the Tikur Wuha River and the shore of Lake Hawassa are highly affected by the transmission of *Schistosome* parasite.

This result was higher when compared with the study conducted in Uganda, Lake Albert accounting 15.8% of the *Biomphalaria* snails were infected with human infective cercaria *S. mansoni* and 13.9% of *S. mansoni* in *Biomphalaria* were recorded in Lake Victoria (Rowel *et al.*, 2015). The reason for the high prevalence of human s*chistosome* cercariae infection than other cercarial types in the current study could be due to human high-water contact behavior observed during data collection and might be due to most of *Biomphalaria* snail species infected by *schistosome* miracidia. However, low prevalence of cercarial infection was recorded in this study when compared to Chore River (70.5%) and Awetu River (55.2%) (Mengistu *et al.*, 2011). This difference might be due to habitat unsuitability, low snail abundance and low deposition rate of *Schistosoma* eggs in the study area water bodies.

Human schistosome (*BAD*) followed by *Echinostome* (25%), *Amphistome* (18.75%), and *Gymnocephalous* (12.5%) cercariae. The *Echinostome* cercariae are intestinal parasite causing echinostomiasis in human and animals. The infection are associated with common sociocultural practices of eating raw or insufficiently cooked snails and fishes (Sah *et al.*, 2018). The *Amphistome* cercariae are a group name for all members of the superfamily Paramphistomoidea

which are an intestinal fluke that cause amphistomiasis. These cercariae groups represent the major economic damage by infecting domesticated livestock worldwide. And occasionally the disease was reported in humans in different times and places Whereas, *Gymnocephalous* cercariae are a liver-fluke of domestic animals (Devkota *et al.*, 2011; Toledo & Fried, 2014).

This level of *S. mansoni* infection in *Biomphalaria* in the current study was sufficient to maintain intense transmission disease to human beings (Mohammed *et al.*, 2016). It has been noted that the prevalence of cercarial infection in snails coincides with *S. mansoni* infection among fishermen in our study area. There was a high prevalence of school children infected with *S. mansoni,* even though there were very few *Biomphalaria* infected with these trematodes in the river, stream, and irrigation canals (Abdien, 2006; Alemayehu *et al.*, 2017).

For the successful transmission of the infective stage of the parasite (miracidia) depends on different factors. These free-living stages miracidia rely on its own stored energy and are directly exposed to environmental factors including disturbance resulting from pollution and human activities (Pietrock & Marcogliese, 2003). In the current study, each environmental factor (physicochemical, biological, habitat condition, and human disturbance) was observed independently as an impact on the prevalence of cercaria infection in the *Biomphalaria* snails. The physicochemical factors result indicated BOD_5, total suspended solids, and turbidity had a positive association with the prevalence of *Biomphalaria* snail infection. These might be due to the presence of organic pollution which increases these parameters (BOD_5, total suspended solids, and turbidity) and subsequently increases the infection of *Biomphalaria* snail. Whereas dissolved oxygen concentration had negative association. This result is in agreement with the study done by Mereta *et al.* (2019) in Omo-Gibe River Basin. The low dissolved oxygen was an indication of the presence of organic pollution in the aquatic environment (De Troyer *et al.*, 2016). The study shows organic pollution contributed to the cercaria infection of snails. This organic pollution is beneficial and expands the habitat of *Biomphalaria* snail host and subsequently, more snails might present. And also, it might have schistosome egg in the waste. With the high availability of *Biomphalaria* snail hosts and schistosome egg, the probability of a miracidium searching and infecting a snail is higher. As a result, the cercarial infection in the *Biomphalaria* snail host could be increased (Grimes *et al.*, 2015).

Regarding the pH, in our work it had a negative association with the prevalence of cercarial infection. The more hydrogen ion concentration in the aquatic habitat could have an effect on the maturation and physiology of the parasitic stage (miracidia), leading to impaired survival and reduced infectivity (Pietrock & Marcogliese, 2003; Singh *et al.*, 2012). This result is similar to the study done by Rowel *et al.* (2015) which shows *Biomphalaria* infection had a negative relationship with water pH. In this work total hardness also had a negative impact on the prevalence of snail infection. The hardness of the water results for shell hardening of snails subsequently leads to low infection of snail by miracidia. The freshwater snail that lives in the water with a low concentration of calcium was highly infected than in high concentration calcium (Mostafa, 2007).

In this study, some habitat conditions like silt, organic, chlorophyll-a, canopy cover, riparian vegetation and *Biomphalaria* abundance had a positive association with *Biomphalaria* infection. These factors may be contributing to the development of the hotspot include the vulnerability of snails to infection, probability of interaction with viable trematode eggs, and suitability of snail habitat (Tolley-Jordan & Owen, 2008). This study is in line with the study done by Mardin *et al.*, (2018) which shows the habitat with silt, organic, shadow, and muddy grass-grown highly favored by the snails and the study done by Tolley-Jordan and Owen (2008) which shows the majority of infected snails were found in silt substrates, and greater than 50% riparian vegetation. And also, the prevalence of *Biomphalaria* infection showed a positive relationship with *Biomphalaria* abundance, this was in line with the study done in Lake Albert (Rowel *et al.*, 2015). This may be due to the high abundance of *Biomphalaria* snails that could promote cercaria transmission as the access distance for the miracidia is reduced as a result more snails get infected, which could be because of why the site with lowest *Biomphalaria* abundance had a low infection in *Biomphalaria.*

The prevalence of *Biomphalaria* infection in this study also influenced by biological factors. The predators including Belostomatidae, Naucoridae, Nepidae, Gomphidae, and Gyrinidae were prey on the *Biomphalaria* species and consume *S. mansoni* miracidia (El Bardicy *et al.*, 2009; Yousif *et al.*, 2013). As a result, the prevalence of *Biomphalaria* snail infection with *S. mansoni* might be reduced. And also, the prevalence might be indirectly influenced by competitors like Thiaridae, Physidae, and Lymnaeidae (Armúa de Reyes & Estévez, 2006; El Bardicy *et al.*, 2009). This work is in accordance with the study reported by Swartz *et al.* (2015) which shows the abundance of infected snails were decreased in the presence of predators and competitors.

In the present study again the prevalence of cercaria infection in Biomphalaria snails had a positive association with human disturbance such as open field defecation, fishing, washing, dumping, bathing, and swimming. This finding is similar to the study done in Omo-Gibe River Basin which shows cercarial infection had a high correlation with human activities (Mereta *et al.*, 2019). In our study area, water body habitats are commonly used for open defecation, swimming, bathing, washing, farming, fishing, and dumping which are risk factors for transmission of schistosomiasis (Mitiku*, et al.*, 2010; Tadege & Shimelis, 2017). The role of sanitation in the control of schistosomiasis is to control the contamination of freshwater with faeces. Schistosomiasis transmission might be expected to be amenable to control through adequate sanitation since the parasite eggs leave the definitive host in the faeces. By preventing eggs in the feaces from entering freshwater bodies inhabited by freshwater intermediate host snails, this should prevent cercaria infection in the snail. A reduction in cercaria infection in the snail, in turn, might be expected to minimize the concentration of cercariae, and hence, the risk of human infection (Grimes *et al.*, 2015). The faeces contamination may result from open defecation and washing following defecation in and around the aquatic habitat. The other source could be including bathing, swimming, fishing, laundering the clothes contaminated by faeces and cleaning of children perinatal area after defecation is carried out by women. These activities contaminate the freshwater with the *Schistosoma* egg in the feaces, subsequently the hatch upon entry into freshwater, and release miracidia. These miracidia infect the freshwater intermediate snail host and releases cercaria (Grimes *et al.*, 2015).

The dumping and farming activities in our study area might increase the organic pollution of freshwater bodies. This organic pollution is beneficial and expands the habitat of *Biomphalaria* snail host and consequently, more snails might present. With the high availability of *Biomphalaria* snail host, the probability of the miracidium searching and infecting a snail is higher. As a result, the cercarial infection in the *Biomphalaria* snail host could be increased (Grimes *et al.*, 2015).

Chapter Seven: Conclusion and Recommendations

7.1. Conclusion

There was moderate endemicity (14.7%) of intestinal schistosomiasis among the fishermen living along the shores of Lake Hawassa and Tikur Wuha River. The transmission was significantly affected by the frequency of swimming and educational status. The *Biomphalaria* intermediate host snails were infected by five types of cercariae with a total prevalence of 12.03%. The snails were dominantly infected by human schistosome cercariae. The cercarial infection of the snails was influenced by total suspended solids, turbidity, BOD_5, human disturbances (open defecation, swimming, bathing, washing, and fishing) and habitat conditions (organic, silt, chlorophyll, and *Biomphalaria* abundance) of the aquatic environment. This finding suggests that, it is necessary to focus on snail population control mainly around the hotspot sites to control the transmission of snail-borne diseases in the study area.

7.2. Recommendations

Based on the finding of this study, the following recommendations are forwarded:

- For the national schistosomiasis control program, responsible bodies or partners to provide adequate sanitation facilities. And also, focus on snail control by biological method and environmental management to gain dandier and sustainable control over the snail, especially on the vector of *S. mansoni*.
- Enhancing of the water quality by controlling the entrance of pollutants.
- Provision of regular health education for the local community on the prevention and transmission way of schistosomiasis.
- For the researcher to use molecular method for cercarial identification in the snails.

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Annex I: Field assessment form

General Information

1. DD/MM/YYY----------Time----------
2. Type of water body ----------
3. Name ----------Sampling station (Code)----------
4. Altitude (m)----------Coordinates ----------N, ----------E
5. Weather condition ----------
6. Previous day rain history ----------
7. Size of site under assessment (ha)----------
8. Size of total water body (ha)----------
9. Photo number ----------

Notes and/or sketch of the site

Physico-chemical parameters

10. Ambient Temperature(°C) ---------
11. pH ----------
12. Water temperature (°C) ---------
13. DO (mg/l) ---------%----------
14. EC (µS/cm) ----------
15. Turbidity (NTU)----------
16. Transparency (cm)----------
17. Chlorophyll-a (ABS)--------
    (0.1309*ABS +11.274) ------(µg/l)

Hydro-geomorphological assessment

18. Geomorphic setting
    a. Riverine----------
    b. Depressional ----------
    c. Meandering flood plain ----------
    d. Man-made----------
19. Substrate type (%)
    a. Bed Rock----------
    b. Boulder ----------
    c. Cobble ----------
    d. Pebble ---------
    e. Gravel ---------
    f. Sand ----------
    g. Silt ----------
20. Sinuosity----------
21. Free water depth (cm)
    a. Minimum ----------b. Maximum----------Average ----------
22. Soil type
    a. Organic----------
    b. Mineral ----------
    c. Both organic and mineral ----------
23. Apparent hydroperiod
    a. Permanently flooded
    b. Seasonally flooded
    c. Saturated (Surface water seldom present)
    d. Artificially flooded
    e. Artificially drained
24. Hydrological modification
    a. Ditch inlet and outlet ----------
    b. Drainage ----------
    c. Storm water input ----------
    d. Culverts ----------
    e. Filling or bulldozing
    f. Others (Specify)--------

Habitat Assessment

25. Depth (m)----------
26. Riparian vegetation
    a. Trees >10 m ----------
    b. Trees <10m----------
    c. Shrubs ----------
    d. Grass----------
    e. Bare land----------
27. Width riparian vegetation Right----------Left ----------
28. % Canopy Cover ----------
29. Periphyton, and macrophyte coverage
    a. Filamentous algae coverage <10% __10-35%___35-65%___65-90%____>90%____
    b. Periphyton coverage <10% ___10-35%___35-65%_____65-90%____>90%_____
    c. Detritus coverage <10% ___10-35%___35-65%_____65-90%____>90%____
    d. Emerged macrophytes <10% ___10-35%___35-65%_____65-90%____>90%____
    e. Submerged macrophytes <10% ___10-35%___35-65%____65-90%____>90%____
    f. Floating macrophytes <10% ___10-35%___35-65%_____65-90%____>90%_____
30. Protection riparian vegetation Right ---------- Left ----------

Land use

31. Adjacent land use pattern
    a. Agriculture tilled---------- f. Road ----------
    b. Pasture---------- g. Commercial----------
    c. Native vegetation---------- h. Industrial ----------
    d. Residential area---------- i. Recreational ----------

Human disturbance

32. Human activities	Present			Absent (0)
	Intensity			
	Minimal (1)	Moderate (2)	High (3)	
Open Defecation				
Open field urination				
Farming				
Grazing				
Fishing				
Swimming				
Washing				
Bathing				
Water abstraction				
Damming				
Draining				
Dumping				
Filling				
Clearance/deforestation				

33. Other Potential threats

a. Agricultural biocides_____

b. Point source pollution _____

34. Description of the site and other comments: _____

_____

_____

_____

Annex II: Laboratory data recording sheet

Sampling station (Code)_____

A. Physico-Chemical parameters

a. Ca^{2+} (mg/L) ----------
b. Mg^{2+}(mg/L) ----------
c. Chloride (mg/L) --------
d. TSS (mg/L) ----------
e. Total hardness (mg/L) -------
f. BOD_5 (mg/L) ----------

B. Number of collected snail species

a. *B. pfeifferi* ----------
b. *B. sudanica* ----------

C. Number of infected snails

a. *B. pfeifferi* ----------
b. *B. sudanica* ----------

D. Prevalence of snail infection

a. *B. pfeifferi* ----------
b. *B. sudanica* ----------

Annex III: Consent form for Questionnaire

Jimma University, institute of Health Science, college of Public Health

Research questionnaire for assessment of practices of fishermen on schistosomiasis transmission, prevention, and control.

Greeting

My name is……...……. Address……

I am working as a data collector with institute of Health, Jimma University, which is conducting a study to assess practices of fishermen on schistosomiasis. The main objective of this study is to identify the risk factors associated with schistosomiasis. The questionnaire will consist of variables including demographic information, and practices of fishermen on the transmission, prevention, and control of the schistosomiasis. Your answer will be recorded on a survey questionnaire. No personal identifiers will be recorded to the interview. All the data obtained kept strictly confidential by using only code numbers. Your participation in the study is upon purely voluntary basis. What we learn from this study will be used to generate information necessary for the prevention and control of schistosomiasis in our country.

The interview will take 10-20 minute. During the interview period, if you fill inconvenient, you can interrupt and clarify inconvenience, appoint to other time or even withdraw any time after you get involved in the study. Your honest and genuine participation in responding to the questions prepared is very important and highly apricated. If you agree to participate in this study, I will interview you. There is no any risk and benefit in involving in this study. However, the findings of this study could be used for development of decision support recommendations for the prevention and control of schistosomiasis.

Would you be willing to participate?

- If yes, proceed.
- If no, thank and stop here. Go to the next respondent.

Signature ……... Date……... (Signature of interviewer certifying that respondent has given informed consent verbally).

Name and contact address of the principal investigator: …….…….

Questionnaire developed to assess practices of fishermen on prevention and control schistosomiasis.

General information

1. Name of respondent _____
2. Code _____
3. Age _____
4. Sex _____
5. Educational status
    a. Illiterate
    b. Read and write
    c. Grade 1 – 4
    d. Grade 5 – 8
    e. Grade 9 – 10
    f. Grade 11 – 12
    g. Above 12
6. Residence
    a. Urban
    b. Rural
7. Type of activities involved
    a. Fishing
    b. Fish processing

Practice to prevent and control the spread of schistosomiasis

1. How often do you swim or bath in a Lake or River?
    a. Never b. Seldom c. Always
2. Do you defecate in the water?
    a. Never b. Seldom c. Always
3. Do you use the water from the Lake or River for domestic use?
    a. Never b. Seldom c. Always
4. Do you boil water for drinking?
    a. Never b. Seldom c. Always
5. Do you use protective water proof clothes when in contact with the water?
    a. Never b. Seldom c. Always

6. Do you go to health facility when you defecate and/or urinate blood?
    a. Never b. Seldom c. Always
7. Do you take anti-schistosomiasis deworming tablets?
    a. Never b. Seldom c. Always
8. Do you pass stools in the bush or river?
    a. Never b. Seldom c. Always
9. Do you pass blood in stool/urine?
    a. Never b. Seldom c. Always
10. Explain to me, what do you do when you pass blood in stool/urine ……...

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