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**PARASITE SPECIES RICHNESS OF FISH FROM FISH PONDS AND
FINGERLING SOURCES IN CENTRAL ETHIOPIA: IT'S IMPLICATION ON
AQUACULTURE DEVELOPMENT**

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Abstract

This study was conducted from October 2016 to January 2017 from Koftu Lake, Sebeta ponds and selected private fish farms in Wonchi area, Ethiopia. The main objective was identifying the major parasites of *O. niloticus* to the lowest possible taxa, quantitatively determining the prevalence, mean intensities and mean abundances of parasites and calculating parasite diversity in the three study sites. A total of 302 *O. niloticus* were examined using conventional parasitological examination procedures. From the sampled fish, 11 different genera of parasites were identified in the three study sites. One genus of Protozoa, one genus of Monogenea, unidentified larvae of Cestoda, four genera of Digenea, two genera of Nematoda, one species of Acanthocephala and one genus of Crustacea were recorded. The overall prevalence of parasites of *O. niloticus* covering all study sites was 83.4%. The prevalences of parasites in Koftu Lake, Sebeta ponds and private fish farms were found to be 100%, 71.0% and 82.2% respectively. There was statistical significant difference ($p < 0.05$) between the study sites in overall prevalence and mean intensity of the parasite infestations. Koftu Lake and Sebeta ponds have higher diversity indices and show the same community similarity coefficient. Values of prevalence, mean intensity and diversity of the parasites were especially high in Koftu Lake and there is a need to design control strategies especially when fingerlings from the lake are used for stocking in other water bodies and intensive and small scale fish farms. Two digeneans, *Clinostomum sp.*, *Euclinostomum sp.* and one nematode, *Contracaecum sp.* were identified as possible health risk for humans.

Key words: Koftu Lake, Fish farms, Parasite intensities, *O. niloticus*, Parasites, Prevalence, Diversity, Ethiopia

TABLE OF CONTENTS	PAGE NO.
Acknowledgements.....	ii
Abstract.....	iii
List of figures.....	viii
List of Tables.....	ix
1. INTRODUCTION.....	1
1.1 Background.....	1
1.2 Statement of the Problem.....	3
1.4 Research Questions.....	3
1.5 Hypotheses.....	4
1.3 Objectives.....	4
1.3.1 General Objective.....	4
1.3.2 Specific objectives.....	4
2. LITRATURE REVIEW.....	5
2.2 Parasites of fish.....	6
2.2.1 Protozoa (Goldfuss, 1808).....	6
2.2.2 Monogenea (Carus, 1863).....	7
2.2.3 Trematoda (Rudolphi, 1808).....	9
2.2.4 Cestoda (Rudolphi, 1808).....	10
2.2.5 Nematoda (Rudolphi, 1808).....	12
2.2.6 Acanthocephala (Koelreuter, 1771).....	13
2.2.7 Crustacea (Lamarck, 1801).....	14
2.3 Controlling fish parasites.....	16
2.4 Physico-chemical parameters.....	16
2.5 Factors influencing parasite communities of fish.....	17

3. MATERIALS AND METHODS	19
3.1 Study area.....	19
3.1.1 Koftu Lake	19
3.1.2 Sebeta ponds.....	20
3.1.3 Wonchi private fish farms	21
3.1.4 Water physico-chemical parameters of the three study sites.....	22
3.2 Sampling Design	22
3.2.1 Physicochemical parameters measurements.....	22
3.2.2 Fish Sampling.....	22
3.2.3 Examination of fish for parasites.....	23
3.2.4 Identification of parasites	24
3.3 Epidemiological and diversity calculations.....	24
3.3.1 Epidemiological parameters	24
3.3.2 Diversity indices calculations.....	25
3.4 Statistical Analysis	26
4. RESULTS	27
4.1 Identification of parasites occurring in <i>O. niloticus</i>	27
4.1.1 Protozoa.....	27
4.1.2 Monogenea	28
4.1.3 Cestoda	28
4.1.4 Digenea.....	29
4.1.5 Nematoda.....	31
4.1.6 Crustacean	32
4.1.7 Acanthocephala	33
4.2 Prevalence, intensities and abundance of parasites.....	34

4.2.1 Overall infection parameters	34
4.2.2 Infection parameters in Koftu Lake.....	35
*-Unavailable data.....	36
4.2.3 Infection parameters in Sebeta ponds.....	36
4.2.4 Infection parameters private farms in Wonchi	37
4.3 Comparison of common <i>O. niloticus</i> parasites among the three study sites.....	38
4.3.1 Protozoa	38
4.3.2 Monogenea	39
4.3.3 Cestoda	41
4.3.4 Digenean trematodes	42
4.3.5 Nematoda.....	44
4.3.6 Acanthocephala	46
4.5 Parasite diversity and species richness.....	46
5. DISCUSSION	48
5.1 Protozoa.....	48
5.2 Monogenea	48
5.3 Cestoda	49
5.4 Digenea.....	49
5.4.1 <i>Clinostomum sp.</i>	49
5.4.2 <i>Euclinostomum sp.</i>	51
5.4.3 <i>Tylodelphys sp.</i>	51
5.5 Nematoda	52
5.6 Acanthocephala	52
5.7 Crustacea	53
5.8 Diversity and richness	53

5.9 Frequency distribution of parasites within the fish host population	54
5.10 Public Health important parasites	55
6. CONCLUSTION AND RECOMMENATIONS	56
6.1 Conclusion.....	56
6.2 Recommendations	56
7. REFERENCES.....	57

List of figures	Page No.
Figure 1: Fish species sampled for parasite investigation: <i>O. niloticus</i>	5
Figure 2. The location of the study sites (map modified from Wubie A., 2015).....	19
Figure 3: Human activities in and around Koftu Lake.	20
Figure 4: Bird population observed in and around Koftu Lake.	20
Figure 5: Sebeta acclimatizing and spawning ponds.	21
Figure 6: <i>Trichodina spp.</i> on <i>O. niloticus</i> from Sebeta ponds (a: 100x and b: 400x).....	27
Figure 7: <i>Cichlidogyrus spp.</i> from the gills of <i>O. niloticus</i> in selected private fish farms (a) attached to gill-100x, b) detached from gill-400x).....	28
Figure 8: The hooks of plerocercoides of cestodes under microscope (400x).	28
Figure 9: <i>Clinostomum sp.</i> from brachial cavity of <i>O. niloticus</i> from Sebeta ponds (b- Microphotography).	29
Figure 10: <i>Euclinostomum sp.</i> metacercaria from kidney of <i>O. niloticus</i> from Sebeta (a- cyst in the kidney and b- microphotography).	30
Figure 11: <i>Tylodelphys sp.</i> metacercariae in the Vitrous humour of eye from <i>O. niloticus</i> in Koftu Lake (100x).	30
Figure 12: Blackspot metacercariae a) in the skin and b) gill of <i>O. niloticus</i> from Sebeta ponds.	31
Figure 13: <i>Contacecum sp.</i> from pericardial cavity (a) and unidentified nematode species from intestine (b) of <i>O. niloticus</i> in Koftu Lake.	32
Figure 14: <i>Dolops sp.</i> from the fin of <i>O. niloticus</i> fish in Sebeta ponds (a: ventral side and b: dorsal side).	33
Figure 15: <i>Acanthogyrus tilpiae</i> from intestine of <i>O. niloticus</i> in Sebeta ponds.....	33
Figure 16: Prevalence of <i>Trichodina spp.</i> on <i>O. niloticus</i> in the three study sites (n=302).	39
Figure 17: The a) prevalence (%) and b) mean intensities (\pm SDE) of <i>Cichlidogyrus spp.</i> from <i>O. niloticus</i> of Lake Koftu, Sebeta ponds and selected private fish farms (n=302). 40	40
Figure 18: Frequency distributions of <i>Cichlidogyrus spp.</i> a) Koftu Lake (n=101), b) Sebeta ponds (n=128) and c) Wonchi private farms (n=73).	41
Figure 19: Prevalence of cestode plerocercoides in Koftu Lake (n=101) and Sebeta ponds (n=128).	42

Figure 20: The a) prevalence (P) and b) mean intensity (MI) of <i>Euclinostomum sp.</i> from Koftu Lake (n=101), Sebeta ponds (n=128) and private farms (n=73).	43
Figure 21: The a) prevalence and b) mean intensity of <i>Tylodelphys sp.</i> in Koftu Lake (n=101) and Sebeta ponds (n=128).....	43
Figure 22: Frequency distribution of <i>Tylodelphys sp.</i> in Koftu Lake (n=101).	44
Figure 23: The a) prevalence and mean intensity of <i>Contracaecum sp.</i> in <i>O. niloticus</i> from Koftu Lake (n=101) and Sebeta ponds (n=128).	45
Figure 24: Frequency distribution of <i>Contracaecum sp.</i> from a) Koftu Lake (n=101) and b) Sebeta ponds (n=128)	45

List of Tables	Page No.
Table 1: Common physico-chemical values (mean \pm SE) of the study sites.....	22
Table 2: Overall prevalence of parasites in each sample site (n=302).	34
Table 3: Total prevalence (P), Mean intensities (MI), Mean abundance (MA) of <i>O. niloticus</i> parasites in the three study sites (n=101).	35
Table 4: Prevalence (P), Mean intensities (MI), Mean abundance (MA) of <i>O. niloticus</i> parasites in Koftu Lake (n=101).	36
Table 5: Prevalence (P), total parasite count, Mean intensities (MI), Mean abundance (MA) of <i>O. niloticus</i> parasites Sebeta ponds (n=128).	37
Table 6: Prevalence (P), total parasite count, Mean intensities (MI), Mean abundance (MA) of <i>O. niloticus</i> parasites in selected private fish farms (n=73).	38
Table 7: An index of parasite diversity and evenness.....	46
Table 8: Parasite component community similarity by Sorenson's coefficient.	47

1. INTRODUCTION

1.1 Background

Ethiopia has large water resources with estimated surface area of 733k km² of major lakes and dams, 275 km² of small water bodies and 7,285 km long rivers within the country (FAO, 2005). The numbers of constructed water dams are also in progress for hydroelectric power generation and irrigation purpose including the Great Renaissance Dam. As a result of these ecological variations, the country has been the home of highly diversified flora and fauna. More than 200 species of fish are known to occur in lakes, rivers and dams in Ethiopia (Mengesha, 2015). The country depends on its inland water bodies for fish supply for its population. The annual fish production potential of the country based on empirical methods on individual lake surface area and mean depth of major water bodies was estimated to be 94,500 tonnes (Tesfay and Wolf, 2014).

The Ethiopian fisheries sector contributes to the gross domestic product (GDP) and thus plays an important role in the national economy. This figure could be much higher if value addition is considered and the efforts to reduce postharvest losses are increased (Tesfay and Wolf, 2014). Fishing as an economic activity earns people a living, provides protein food, employment, job opportunities, fish traders, fish processors and fish farmers. Therefore, efforts by the government of Ethiopia for intensification of fish production have been in top gear. The government of Ethiopia has also established division in ministry level in 2015 as Ministry of Livestock Development and Fishery in an effort aimed at enhancing food security by intensifying aquaculture and fishery. With this expected growth in fish production, the quality and biosafety of the fish need to be guaranteed (Teshome *et al.*, 2014; Mengesha, 2015).

However, there has been a consistent and persistent decline in world fisheries and the Ethiopian is not exempt. Available data shows a significant decline in fish caught in Ethiopia due to various reasons such as destructive fishing, overfishing, biodiversity reduction, climate change, declining water levels, fish mass mortalities, pollution, eutrophication, and variability in macrophyte densities and general environmental degradation especially in the catchments (FAO, 2010; Tesfay and Wolf, 2014).

Parasites are important components of host biology, population structure and indeed ecosystem functioning. They can be found in any fish species and within any type of aquatic and culture system. They range from protozoans such as flagellates, ciliates,

and apicomplexans to metazoans including myxozoans, trematodes, cestodes, acanthocephalans, nematodes, and crustaceans (Marcogliese, 2004).

Over 40% of all known species on earth are parasitic with parasitism being ubiquitous in some taxa and either absent or rare in others. In some well-studied helminth taxa, the rate of discovery of new parasite species has grown linearly or exponentially. The knowledge of the status of parasite diversity in the tropics is still inadequate (Dobson *et al.*, 2008).

The Food and Agricultural Organization of the United Nations (2009) reported that, to satisfy an increasing demand in freshwater fish, extensive research must include studies of their parasites for optimal production levels. The knowledge of fish parasites is of particular interest in relation not only to fish health but also to understanding ecological problems in tropical Africa. Fish parasites have long been recognized as serious threats of fish both in aquaculture and fisheries (Paperna and Thurston, 1969). Because of this recognition, there has been in the recent past an increasing interest and an explosion of knowledge, reports and description of new species of parasites from the African continent (Řehulková *et al.*, 2013). However, much of the research has been mainly concentrated in Western and Southern African countries with very little work from Eastern Africa (Gillardin *et al.*, 2012).

The government of Ethiopia has in the recent past put emphasis on fisheries and aquaculture as an important sub-sector with potential to contribute to food security, employment creation and poverty reduction. Fishery and aquaculture was identified as one of the key sectors. With this expected growth in fish production, it is important if threats are identified for prevention and control. Most parasites are easily transferred from the wild stocks to cultured stocks due to their direct nature of transmission and lifecycle. Identifying parasites and studying their abundance will lead to developing prevention and control mechanisms.

Studies on fish parasites in Ethiopia are very scarce and very few research articles deal with parasites larger water bodies including a report of fish in Lake Tana, Lake Hawwasa, Lake Ziway and Koka Dame (Temesgen, 2003; Teferra, 1990; Shibru and Tadesse, 1997; Amare, 1986; Yimer, 2003). This indicates a slow progress in research in fish diseases and parasites. There are also some recent published articles on fish parasites of Ethiopian water bodies such as Lake Lugo, Small Abaya, Lake Ziway and

Lake Hawassa. (Florio *et al.*, 2009; Bekelle and Hussien, 2015, Amare *et al.*, 2014, Reshid *et al.*, 2015). Koftu Lake has commercially important fish species where Sebeta fishery research centre is collecting fingerlings and disseminating them for small scale fish farmers when there is demand. Most parasites are easily transferred from the wild stocks to cultured stocks due to their direct nature of transmission and lifecycle. But in spite of the high commercial value of fishes in Koftu Lake, there is no documented work for fish parasites in the lake and no previous study on the status of parasites along the chain of fingerling stocking line from source to farmers.

1.2 Statement of the Problem

External and internal parasites can be serious pathogens of fish which can cause morbidities and mortalities in fish. They attack the vital organs such as gills, heart, and intestine, skin and eyes which essential for normal body functioning. There is a lack of knowledge and past records of the species of internal and external parasites infecting fish from most Ethiopian water bodies and fish farms is no exception. There is no previous study and report on parasite status of fish from the selected study sites for this study. Koftu Lake is one of the main sources of fish fingerlings to restock small water bodies and small scale fish farms. But the potential risks associated with fish parasites that can easily be transmitted to these water bodies and farms are not known. Three cases of heterophide infections transmitted by eating raw fish were recorded out of the 150 children around Lake Hawassa which can be used to predict potential human health (Merid *et al.*, 2001). Therefore, the aim of this is done to gather baseline information and forward recommendations on parasites of the economically important fish *O. niloticus* in Koftu Lake, Sebeta fish farm and selected private fish farms in Wonchi area, Ethiopia.

1.4 Research Questions

1. What are the dominant fish parasites in Lake Koftu, Sebeta fish ponds and selected private fish farms?
2. Are there differences in epidemiological parameters of fish parasite between fingerling source (Lake Koftu) and cultured fish (Sebeta fish ponds and selected fish farm)?
3. Are there parasites of public health importance in the study sites?

1.5 Hypotheses

- There are no differences in prevalence, mean intensity and abundance of parasites fauna between fingerling source and private fish farms.
- There is no difference in terms of species richness of the parasite fauna of fish from Lake Koftu, Sebeta ponds and private fish farms.

1.3 Objectives

1.3.1 General Objective

The main objective of this study was to investigate the most common parasites of *Oreochromis niloticus* (Linnaeus, 1758) and to determine the epidemiological parameters and diversity of parasites in selected fish farms and its fingerling sources, Ethiopia.

1.3.2 Specific objectives

- ✓ To identify the major parasites of *O.niloticus* to the lowest possible taxa by using morphological parameters.
- ✓ To determine the prevalence, mean intensities and mean abundances of the external and internal parasites infecting the study fishes.
- ✓ To compare the parasite infection levels and parasites diversity from the wild fish source and in fish farms.
- ✓ To identify parasites having public health significant for consumers.

2. LITRATURE REVIEW

2.1 Nile tilapia (*Oreochromis niloticus* (Linnaeus, 1758)).

The Nile tilapia is a tropical fish species that prefers to live in shallow water and is a well-known tropical food fish native to Africa. It is a member of the Cichlids family and is one of the largest freshwater tilapia found in most tropical waters (Lamtane, 2008). The preferred temperature for *O. niloticus* growth ranges from 25 to 30 °C. The lower and upper lethal temperatures are 11-12 °C and 42 °C respectively. It is indigenous to the Nile basin and plays an important role in fishery and aquaculture globally (El-Sayed, 2006). Tilapia are produced most economically in tropical and subtropical countries which have favourable temperatures for growth. Tilapia species is the second most important group of farmed fish after carps and the most widely grown of any farmed fish (El-Sayed, 2006).

Nile tilapia is an omnivorous grazer that feeds on phytoplankton, periphyton, aquatic plants, small invertebrates, benthic fauna, detritus and bacterial films associated with detritus. It can filter feed by entrapping suspended particles including phytoplankton and bacteria on mucous in the buccal cavity although its main source of nutrition is obtained by surface grazing on periphytons (de Graaf, 2004).

Oreochromis niloticus is a fish which can be easily managed by farmers, highly prolific with indiscriminate appetite and is tolerant to poor water quality and hence it is the most common artificially raised species of fish and has a great market demand and economic interest in Ethiopia (de Graaf, 2004; FAO, 2005). The total annual yield of inland fisheries estimated at 94,500 tons are essentially dominated by Cichlidae (Bonaparte, 1835), notably *O. niloticus* which represents between 50 to 70% of the total catches in Ethiopia (Tefaye and Wolff, 2014).



Figure 1: Fish species sampled for parasite investigation: *O. niloticus*

2.2 Parasites of fish

Fish have a full range of diseases like all animals and many of these are due to external agents and other arises internally. External agents that causes fish disease include viruses, bacteria, fungi and parasites are known to affect fish while internally they suffer from almost all the common organic and degenerative disorders. Parasitic infestation frequently occurs in fish that causes retarded growth rate, reduced production, consumer rejection, low reproduction and mass mortality in fish (Claude *et al.*, 1998). The most common symptoms of parasitic infestations in fish are weight loss, disruption of reproduction or impotency, blindness, abnormal behaviour, epithelial lesions, deformities of gills and others. These all eventually cause an economic loss in the fish farming sector and hence parasites are among the important factors responsible for production losses but fish parasites may be present in or on fish in subclinical state or carrier state and do not always cause disease in fish (Barber, 2007).

Fish parasites include parasitic protozoans, acanthocephalans, nematodes, digeneans, cestodes and crustaceans which are the most important parasites of fish. Helminths are highly specialized parasites that require specific definitive hosts. They frequently occur within the body cavity and viscera of fish. Due to their location in host fish, they may affect one or more important organ systems (Amlacher, 2005).

2.2.1 Protozoa (Goldfuss, 1808)

Protozoans are single-celled organisms many of which are free-living in the aquatic environment. There are also several protozoans that act as external and internal parasites which infect fish, molluscs and amphibians (Martins *et al.*, 2015). They damage the skin and gills and cause reduced growth of the host fish, favouring secondary bacterial infections and mortality which lead to constraints in global aquaculture production. Parasitic protozoan diseases are responsible great losses to the commercial fishing industry and small scale aquaculture activities contributing to food production (Xu *et al.*, 2012).

According to Basson & Van Asv(2006), trichodinids are protozoan parasites with mobile ciliates having body covered by a slender membrane surrounded by ciliary spiral, a horse-shoe shaped macronucleus and an adhesive disc provided with a denticulate ring in which the denticles are found. They can be found in freshwater and marine fishes. Their predilection site in fish host is on the body surface, buccal cavity

and gills (Lom & Dykova, 1992). Deteriorated water quality and ecological aspects of the fish species favours the proliferation of the trichodinids in the environment. High stocking density, high organic matter contents and increased water temperature aggravates their reproduction in fish farms (Basson & Van As, 2006). According to Yemmen *et al.*, (2011), some trichodinid species were found to be suppressed with increased water temperature.

Trichodinids reproduce by binary fission and have direct life cycle on the host. They can reproduce rapidly in very short period of time and reach 100% prevalence when condition favours. Transmission can be by direct contact, contaminated water and contaminated fish farming utensils. (Martins *et al.* 2010). Diagnosis can be made from scraps of skin, fins and gills of diseased fish observed under a stereomicroscope under a microscope. Silver nitrate impregnation and staining by Giemsa or haematoxylin (Van As & Basson, 1989) can be for specific diagnosis. Attachment and rotating movements of trichodinids may cause serious irritation and damage to the epithelial or epidermal cells of fish. Martins *et al.*, (2015) stated that diseased fish may show darkness of the skin, whitish areas in the gills, hypoxia and flashing on the ponds or aquaria surface. But clinical signs are not specific to the parasites.

2.2.2 Monogenea (Carus, 1863)

The class Monogenea is one of the largest groups of Platyhelminthes. They parasitize fish and other aquatic animals throughout freshwater and marine habitats (Paperna, 1996). Monogeneans are subdivided into several major taxa; Dactylogyroidea (Yamaguti, 1963), are the most common monogeneans found in inland water fish. They usually have one or two anterior-dorsal pairs of eyes and are 0.3-2 mm long and a posterior-ventral attachment organ. Most dactylogyroids are gill parasites except Gyrodactylidae which are skin parasites (Paperna, 1996). Morphologically, Gyrodactylidae are viviparous where the parent worm contains a distinct well differentiated embryo. Intra-uterine embryos already contain second and often third generation embryos recognized by their developing marginal hooklets and anchors. They do not have eye spots and Gyrodactylus (Von Normann, 1832) is a common genus which parasitizes fish. All the remaining Dactylogyroidea reproduce by laying eggs. They usually have one to two pairs of pigmented eyes (Bakke *et al.*, 2007).

The life cycle of monogeneans is direct which involves only one host. Fish to fish contact and by infective stages (oncomiracidium) hatched from eggs shed in the environment is the mechanism of transmissions for viviparous species and oviparous ones respectively (Öztürk and Özer, 2014). They mostly spread by way of egg releasing and free swimming infective larvae. Monogeneans are often in equilibrium with their hosts in the natural environment but can cause serious morbidity and mortality when there is water quality deterioration and when fish are stressed in significant economic losses in aquaculture (Öztürk and Özer, 2014). Oviposition often accelerated by adverse living conditions and incubation time is temperature dependent. Larvae are either actively attached to the skin of the host fish and then migrate to the gills or become attached when washed with swallowed water through the gills. All monogeneans are hermaphrodites (Blahoua *et al.*, 2016).

Monogeneans are attached to the host body by special posteriorly positioned attachment organs called opisthaptor. Their anterior end contains apical sensory structures, a mouth with or without accessory suckers and special glands or clamps for attachment (Whittington *et al.*, 2000). Those preferring gills cause more damage to the host due to the thin epithelium and results in excessive production of mucus and hyperplasia of epithelium leading to fusion of gill lamellae, which eventually cause clubbing and fusion of gill filaments and necrosis of tissues. This can finally lead to parasite induced fish mortalities. They are commonly found on fish gills and skin. However, some monogenean species invade the rectal cavity, ureter, body cavity, nostrils, intestine, stomach and even the vascular system (Buchmann *et al.*, 2004).

Clinically, fish heavily infected with *Gyrodactylus* appear pale due to excessive mucus secretion and epithelial proliferation. There is skin erosion, desquamation of the skin epithelium, focal haemorrhagic lesions and loss of scales in the more heavily infected skin zones (Obiekezie and Taege, 1991). Species of dactylogyrids as well as gyrodactylids demonstrate different tolerances to water salinity. Taxonomical aspect, host specificity and biological cycle are mostly used for classification and identification purposes (Pariselle and Euzet, 2009). Some genera of monogenea demonstrate a high degree of host specificity and follow their respective specific fish hosts throughout their distribution range. Monogeneans are common and present in all inland waters of Africa (Paperna, 1980).

2.2.3 Trematoda (Rudolphi, 1808)

Trematodes also known as digeneans are flat worms morphologically characterized by a dorso ventrally flattened and oval body with a smooth. They have corrugated surface and a sucker around the antero-ventral mouth. Both suckers are used for attachment and locomotion (Schell, 1970). The digestive system consists of a pharynx connected to the mouth opening, a short oesophagus and two blind intestinal caeca. Most trematodes are hermaphrodite, containing both male organs (testes, adults and copulatory system) and female organs (ovary, vitelline glands, ducts and uterus). Some also contain a specialized copulatory organ (ovary, vitelline glands, ducts and uterus) which is useful for differential diagnosis. Eggs are evacuated to the genital opening and are usually oval and operculated (Schell, 1970).

Trematodes have a multiple host life cycle and most of them require molluscs as their first intermediate host. Fish can be an intermediate host harbouring metacercarial digeneans like *Diplostomum spathaceum* in eye lens, *Tylodelphys clavata* in vitreous humour of the eyes and *Postdiplostomum cuticola* ('black spot') on skin and fins. The final host are some fish eating birds like herons. Fish can also serve as definitive host harbouring adult worms like *Sanguinicola intermis* in blood, *Bunodera luciopercae* and *sphaerostoma bramae* in intestine. The larval stages of Digenea generally include miracidium (free living stage), sporocyst, redia and cercariae in the digestive gland of the intermediate host (snails) and metacercariae which is the encysted stage. Blood flukes (Sanguinicolidae) are slender, spiny, and lack anterior ventral suckers and pharynx. The intestinal caeca are short, X- or H-shaped. Eggs are thin – shelled and lack an operculum (Smith, 1972).

Clinostomum spp. infections are caused by metacercarial stage of the digenean trematods. The metacercariae are large, yellow or white giving the fish unattractive appearance for consumers (Yimer, 2000). Clinostomatids are very widespread in Africa (Yimer & Muluaem, 2003) and most of the researches on fish, were carried out in Southern, Western and Central Africa and just few in the North and East part of Africa (Aloo, 2002; Florio *et al.*, 2009).

The most common definitive hosts of Diplostomidae, Clinostomatidae and Heterophyidae encysting as metacercariae in fish are piscivorous birds. Mammalian hosts including dogs play an important part in dissemination of Heterophyidae.

Heterophyidae notably *Heterophyes heterophyes* are very versatile in their choice of definitive hosts and will develop to maturity in both mammals and birds. Crocodiles are definitive hosts to metacercariae of the Clinostomatid *Nephrocephalus* and *Pseudoneodislostomum thomasi* which infect Bagrus and *C. gariepinus* (Fischthal & Thomas, 1990).

Hérons are the common definitive hosts of Diplostomidae and natural infection of *B. levantinus* has been found in *Ardea purpurea*. Eggs of *D. spathaceum*, incubated at 29⁰C, hatched after 9-11 days, while infected snails (*Lymnaea peregra*) commenced shedding with 6-9 weeks. Cercariae of all diplostomatids are fork-tailed furcocercariae). The vector of Neascus causing blackspot disease in Lake Victoria cichlids is the local bulinid, *B. ugandae*. Hyperparasitism, i.e. a cyst within another cyst of an apparently different species of Diplostomatidae has been revealed in *Clarias gariepinus* muscles in Israel and in Uganda (Whyte *et al.*, 1988).

Definitive host for *Clinostomum spp.* and *Euclinostomum sp.* are herons, pelicans, cormorants and darters. In all of these the adult trematodes become attached to the wall of the posterior pharynx and in the laryngeal zone. Some species, however, may restrict their choice of hosts. Eggs shed by worms are either washed directly to the water habitat or swallowed and defecated. Eggs shed undeveloped and like those of diplostomids require oxygen and light for development. Miracidia of *C. tilapiae* hatches following 10 days incubation at 25-30⁰C (under constant illumination) and those of *C. marginatum* after 11-13 days (Paperna, 1996). According to the study carried out by Eshetu Yimer (2000), digenean trematodes of the genus *Clinostomum* were reported from *O. niloticus*, *T. zilli* and *C. gariepinus* in Lake Ziway. Although no evidence is available in Ethiopia, *Clinostomum complanatum* is known to cause laryngopharyngitis infection humans as was reported in the Near East resulting apparently from ingesting inadequately cooked infected fish (Paperna, 1980).

2.2.4 Cestoda (Rudolphi, 1808)

Cestodes also called tapeworms are ribbon like flat worms. They infect the alimentary tract, muscle or other internal organ of fish (Woo, 1995). The clinical signs when fish is affected by cestode parasite are variable degree of dropsy, distended abdomen and reduced in activity. Cestoda are all endoparasites of vertebrates with over 5000 species so far described. Most of them require at least one intermediate host and complete their

life cycle as adults in the definitive hosts. Two life cycle stages are represented in fish: adults inhabit the intestine, and plerocercoid larvae of the same or different species are found in the viscera and musculature; the first-stage larvae (procercoids) are generally found in aquatic crustaceans (Woo, 1995).

Morphologically the adult cestodes are strongly flattened dorsoventrally, the body consists of the scolex (head), neck, and strobila (body) and the latter generally made up of several serial sections (proglottids). Some unsegmented cestodes are also described from fish (*Caryophyllaeus*, *Khawia*, etc.). Scolex is an attachment organ used to fasten the parasite to the host's intestinal mucosa, so it is generally provided with holdfast structures such as suckers and bothria and additionally hooks and/or proboscids. Cestodes have no intestine, the nourishment being absorbed by the tegument covering the whole surface of the body. With a few exceptions, cestodes are hermaphroditic, each proglottid having its own set of male and female gonads (Woo, 1995).

Numerous cestodes cause disease in fish mainly due to the plerocercoid larval stage and in some cases they can be transmitted to humans as in the case of *Diphyllobothrium* spp. causing a serious fish-borne zoonosis called Diphyllobothriasis. Identification of the cestodes parasite can be made from wet mount of faecal contents having proglottides or organs. Identification of adult cestode parasite to species uses features of the scolex and organs of the mature proglottid; immature cestode might only be classifiable to order. A variety of adult and larval tapeworms (over 40 species occur in native African fish; unsegmented forms notably Caryophyllidae as well as one amphilinid representatives and the segmented pseudophyllideans and proteocephalidae (Scholz *et al.*, 2009).

Amirthalingamia macracantha is mainly characterized by its 20 hooks arranged in two rows in bilaterally symmetrical pattern (Bray, 1974). *Proteocephalus exiguus* (scolex with 4 lateral suckers), *Proteocephalus macrocephalus* and *Bothriocephalus claviceps* are some of cestodes fish as definitive host. Different fish species act as intermediate and pike as definitive host for *Triaenophorus crassus*. *Ligula intestinalis* uses fish as intermediate host (plerocercoids in body cavity of fish) and birds as final hosts. Plerocercoids of *D. latum* inhabit muscle and body cavity of fish (intermediate host) while the adults (broad ribbon worm or broad fish tapeworms) live in mammals. *Diphyllobothrium latum* is typically a parasite of human with a worldwide distribution,

mainly prevalent in cold lake regions where raw or undercooked fresh water fish is eaten (Merid *et al.*, 2001).

Although tapeworms are widespread in Africa, there is only a few records of tapeworms from cichlid fish, e.g. *Proteocephalus bivitellatus* and several gryporhynchid cestodes (Scholz *et al.*, 2009). *Ligula intestinalis*, *Proteocephalus* spp and *Bothriocephalus* spp. are those observed in natural fish habitat of Ethiopia (Yimer and Mulualem, 2003; Yimer *et al.*, 2001). There several species of tapeworms which can seriously affect wild and cultured fish populations among which *Ligula intestinalis*, *Diphyllobothrium* spp, *Diphyllobothrium acheilognathi* and *Kwawia sinensis*. They affect not only the organ they infect but also induce pathological and physiological changes on fish (Pike and Lewis, 1994). The prevalence of fish tape worms and other helminthic parasites were studied from 150 male children less than 15 years of age who are involved in fishery and fish processing in Lake Awassa, Ethiopia. This study confirmed three cases of heterophid infections caused by eating raw fish (Merid *et al.*, 2001).

2.2.5 Nematoda (Rudolphi, 1808)

The phylum Nematoda is one of the most common phyla of animals with over 80,000 different described species of which over 15,000 are parasitic and diffused in freshwater, marine and terrestrial environments. The phylum contains both free-living organisms and parasites of plants and animals, including fish (Grabda, 1991). They are also called “roundworms”, as they have an elongated, cylindrical in shape with 1 mm to 1m length and circular in section. Nematodes are unsegmented, bilaterally symmetric with a complete digestive system consisting of three sections: anterior (esophagus), middle (intestine), and posterior (rectum) ending with the anus (Grabda, 1991).

Nematodes have acoelomate (false body cavity) and covered with a solid cuticle. Because of their resistant cuticle, these worms last longer than flatworms in post-mortem conditions. They have tubular digestive and reproductive organs to sustain hydrostatic pressure. The sexes are separate (dioecious) and mostly males are smaller than the females. Nematodes have mostly indirect life cycle including mammals, fish, copepods and piscivorous birds depending on their species. They have four moults in the life cycle; eggs hatch to produce first stage larvae (L₁), L₁ moult to L₂ then L₃, L₄ and final the fourth stage larvae moult to become adults (Moravec, 1974).

Copepods are first intermediate hosts to Camallanidae, Cucullanidae, Philometridae and Anguillicolidae. Larval stages of the *Contracaecum* species of the family Anisakidae that infect freshwater fish are usually found as adults in fish-eating birds such as cormorants and pelicans. Larvae in copepods or other invertebrate intermediate hosts will develop to fourth stage larvae and further into adult males and females when ingested by a suitable definitive host. Larvae ingested by “wrong” piscine hosts often survive as waiting stages (fourth stage larvae) in the gut or other tissues for a variable length of time and continue development into the adult stage if their carrier host (paratenic host) is predated by the compatible host. Nematodes occur worldwide in all animals. Larval nematodes, in addition to adults, occur either encysted in tissue or free in body cavities most often in pericardial cavity. Most notorious larval nematodes existent in our country lakes include are the genera *Amplicecum*, *Contracecum* and *Porrocaecum* (FAO, 2012). The clinical symptoms of Nematodiasis include anaemia, emaciation and untherifitness (Yimer and Mulualem, 2003).

In Africa, 40 species of adult nematodes which are representatives of 9 families from fish were reported by Khalil and polling (1997). Infections of the pericardia in cichlid fish by larvae of nematode *Contracaecum sp.* occurs in Ethiopia (Yimer, 2000; Yimer and Mulualem, 2003), in Lake victoria (Malvestuto & Ogambo ongoma, 1978) and in South Africa (Basson *et al.*, 1993). *Amplificaecum spp.* was reported from Sudan (Khalil, 1969) and Ethiopia (Yimer, 2000). Eustrongylides has only been found in the East African lakes, including Tanganyika (Paperna, 1996), Chamo and Tana (Yimer *et al.*, 1999; Yimer and Mulualem, 2003).

2.2.6 Acanthocephala (Koelreuter, 1771)

Acanthocephalans also known as spiny or thorny-headed worms which belong to the separate distinct phylum with about 1200 species divided into three classes namely: Archiacanthocephala, Eoacanthocephala and Palaeacanthocephala. All are intestinal parasites of vertebrates including fish, amphibians, birds and mammals. They are cylindrical worms from few mm to 70 cm long with the anterior part provided with an eversible hooked proboscis, without digestive system. They absorb nutritive materials with the whole surface of the body (Grabda, 1991).

The worms have sac-like containing lemnisci connected to the proboscis and genital organs opening posteriorly. The sexes are separate and the male opening is within a

membranous bursa. The number and arrangement of the hooks on the proboscis are the main criteria for differentiation of species. A wider range of anatomical details are considered for determination of higher taxa (Kabata, 1985).

They develop via one or more intermediate hosts (heteroxenous). Adult acanthocephalans are all gut parasites. Eggs are laid into the intestinal lumen and evacuated with faeces. First intermediate hosts of piscine acanthocephalan are amphipods, isopods, copepods or ostracods. The first larvae (acanthor) hatch from eggs after being swallowed by a suitable invertebrate host. Some species will develop to the adult stage when their larvae in the invertebrate host are ingested by the definitive vertebrate host (Madanire-Moyo and Barson, 2010). The pathogenic effects of acanthocephalans are strictly related to the damage caused by the proboscis in the intestinal wall and to the infection intensity. Attachment of the adult acanthocephalans in the digestive tract and also to the encapsulation of larval stages in the tissues causes pathogenic effects on fish. In low to moderate infections, pathological effects are localized around the attachment of the adult worm. The extent of damage is proportional to the depth of penetration of the proboscis. The depth of penetration of some species may vary in different host fish (Madanire-Moyo and Barson, 2010).

2.2.7 Crustacea (Lamarck, 1801)

Crustaceans are a large group of arthropods comprising almost 52,000 described species and are usually treated as a subphylum. The majority of them are aquatic, living in either marine or freshwater environments. Most of crustaceans are motile, moving about independently, although a few taxonomic units are parasitic and live attached to their hosts such as sea lice, tongue worms and anchor worms (Grabda, 1991).

Over 80 species of copepod and Argulid ectoparasites have been recorded from freshwater fish in Africa. The two most serious crustacean parasites which may become problematic under intensive aquaculture conditions in Africa are *Lernaea cyprinacea* and *Argulus japonicus* both of which have been introduced into South Africa together with carp and goldfish and *Dolops ranarum*. Most of the crustacean ectoparasites are found on the skin, mouth and on the gills. The erosion and degradation processes through external digestion cause lesions which can become secondarily infected by bacteria and fungi. Under natural conditions the rate of infection are low but can become chronic and acute under poor water quality and crowded conditions. Crustacean

parasitic infections are particularly lethal to early juvenile fish (Avenant and van As, 1985).

Argulids ('fish lice') are dorso-ventrally flattened mite-like and covered dorsally by a rounded or horseshoe shaped carapace. Ventrally positioned head appendages are developed for attachment, four thoracic segments each bear a pair of bifid swimming legs. The compound eyes are prominent and the mouth parts and antennae are modified to form a hooked, spiny proboscis armed with suckers as an adaptation to parasitic life. They leave their hosts for up to three weeks in order to mate and lay eggs and reattach afterwards behind the fish's operculum where they feed on mucous and sloughed-off scales, or pierce the skin and feed on the internal fluids. Twenty nine endemic species under family Argulidae occur in Africa in fish of diverse families. *Argulus africanus* and *Dolops ranarum* are opportunists and occur in diverse fish in all major systems of Africa (Paperna, 1996). Allied species, *A. rhiphidiophorus* and *A. cunningtoni*, replace *A. africanus* in some East African lakes connected to the upper Nile and co-exist in others due to later artificial introductions of fish. The largest number of species was reported from the Congo basin followed by that of Lake Tanganyika (Fryer, 1968). In South African fish, *D. ranarum* is widespread as is the ubiquitous Eurasian species in addition to a few locally endemic species. *A. japonicus*, introduced apparently with carp (Avenant-Oldewage, 2001). Only one species of the genus Dolops, *Dolops ranarum*, is present in Africa. It differs from *Argulus* sp. in having the second maxilla armed with a hook rather than a sucker, characteristic of the latter. Both genera Dolops and Argulus are in the family Argulidae (Paperna, 1996).

Ergasilidae (Copepoda) are common in fish of all major African water systems and only sub-adult and adult females of Ergasilidae occur on fish, mostly on the gills. The cephalothorax constitutes half or more of body length, the first of four thoracopods occurs at about mid-length. Segmentation of the thorax (except the first segment, fused with the head) and of the abdomen is distinct. The second antenna terminal segment is hook-like in Ergasilus and three clawed in Paraergasilus. Eggs are clustered in a bunch rather than arranged in a single line (Paperna, 1996).

In the family Lernaeidae (copepod) rod-shaped, unsegmented, or partly segmented parasitic stage lernaeid female is anchored with the aid of a specialized holdfast organ to the host skin or buccal mucosa. Larval stages, copepodites and copepod-shaped

males are attached to the gills. Differentiation to lernaeid genera and to species in the genus *lernea* is based mainly on the morphology of the holdfast organ (anchors) of the parasitic females. Among crustaceans, *Dolops ranarum* was found on caudal fins of African catfish in Lake Tana, Ethiopia which was reported by Yimer and Mulualem (2003).

2.3 Controlling fish parasites

The reliable way to manage parasites is to avoid introducing parasites to a new system which can be done by following a quarantine protocol. Another simple way to minimize the introduction of parasites and other external parasites is to dip fish in fresh or salt water depending on the fish species (Peggy *et al.*, 2012). Dipping saltwater fish in freshwater will reduce the number of many single-celled external parasites and freshwater fish can be dipped in sea water to accomplish the same goal. Dipping fish will not completely eliminate the risk of introducing parasites to an established tank or system but it may help minimize the numbers brought in. Unfortunately, the sticky eggs of monogeneans are resistant to changes in salinity and are easily transported into the new facility even when fish have been appropriately dipped (Hirazawa *et al.*, 2004).

Fish should be quarantined for at least four weeks before they are placed into a new system. The design of a quarantine system should be very simple so that fish are readily accessible for observation and handling and so that water can be easily changed and treatments easily administered (Hirazawa *et al.*, 2004; Peggy *et al.*, 2012).

2.4 Physico-chemical parameters

For sustainable production of fish in aquaculture, healthy fish fry and fingerlings availability is one of the most critical requirement. The sanitary conditions in the water where the fish is living is related to the occurrence and magnitude of parasite infestations and hence there should be good relationship between the environment the fish is living and the fish itself (Delwar *et al.*, 2011). A study conducted by Hossain *et al.*, 2007 indicated that physicochemical factors such as water temperature, alkalinity, ammonia, dissolved oxygen (DO), pH and total hardness have strong influence on fish health and their resistance against the disease causing agents. According to Aguilar *et al.*, 2005, disease transmission can be greatly favoured by environmental conditions such as reduced oxygen concentration, overcrowding, poor hygiene, temperature variations, and availability of intermediate and final hosts in fish farms.

The physical and chemical characteristics of a water body have been recognized as valuable limiting factors in the biological productivity of aquatic ecosystems. Maintenance of a healthy aquatic ecosystem is dependent on physicochemical parameters of water. The physical and chemical character of a water body could influence and determine the parasitic fauna (Kennedy, 1990). The nature of parasitic infestation of fish population in any confined body of water is affected by a variety of biotic and abiotic factors. The common abiotic factors are temperature, pH, DO content, alkalinity, conductivity, etc. The effect of temperature on the fishes is an important factor for infestation rate of parasites (Gaffar, 2007). For example, different species of fish parasites have a different response and react differently to changes in water pH where they live. According to Marcogliese and Cone (1996), the composition and quantity of the parasite community of eel (*Anguila rostrata*) depended on water PH. When the PH of water increase, the snails which are the intermediate host of digenean trematods get affected and the trematode parasites could prohibited from proliferatio. On the other hand, gammarids which is an intermediate hosts of acanthocephalan developed more intensely and more acanthocephalan parasites could prevail (Dzika & Wyzlic, 2009). A study conducted by Halmentoja *et al.*, (2000) on the reactions of parasites of perch to acidified water in two artificial lakes in Finland showed that a decrease in the number of metazoan parasite species and in their abundance in the acid waters. However, greater species diversity was observed in more alkaline waters as compared to lakes with PH approximating neutral.

2.5 Factors influencing parasite communities of fish

Biotic and abiotic factors affects the parasites distribution and community structure in aquatic systems. Biotic factors such as distribution of infected hosts, immune status of the host, spatial and temporal distribution of infective stage and intermediate hosts are important determinants of parasite exposure (Byers *et al.* 2008). The distribution of infection can also be influenced by host diet or trophic position in a food chain (Valtonen *et al.* 2010).

Among the abiotic factors, water quality issues explained in section 2.4 can be an important determinant of parasite species diversity and infection intensity (Karvonen *et al.*, 2013). High and water temperature may directly influence release of infective stages and aggravating transmission in the population and vis versa in cold water and which

consequently leads to seasonality of parasitism (Poulin, 2006). In addition, climate change, eutrophication and pollution are strongly affect the parasite community structure (Karvonen *et al.* 2013).

3. MATERIALS AND METHODS

3.1 Study area

The study was conducted in Koftu Lake which is the source of fingerlings for the center and to dispatch to private and governmental fish farms, Sebeta ponds and selected private fish farms in Wonchi area, Ethiopia.

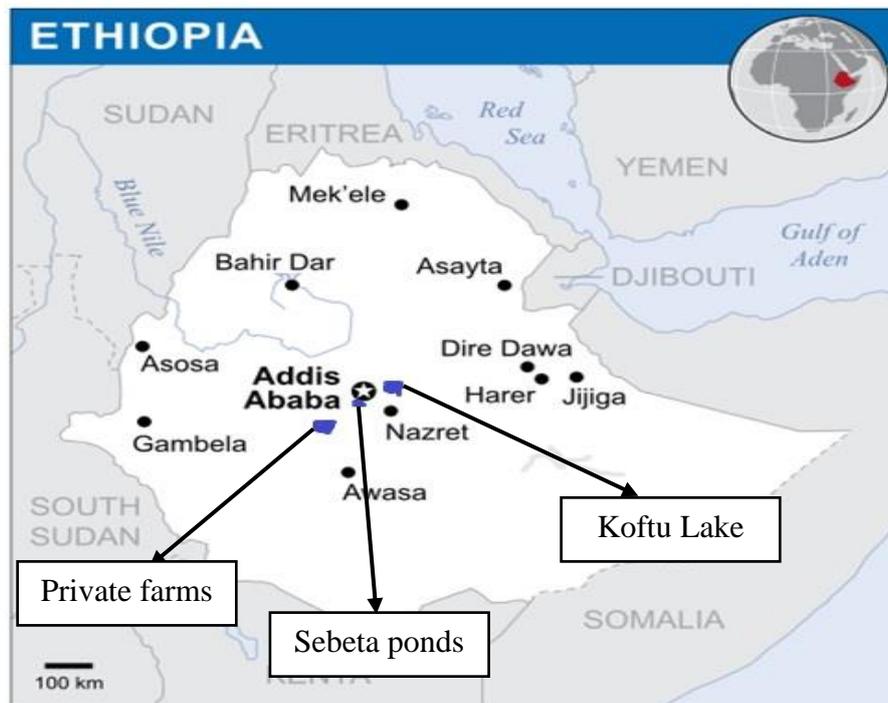


Figure 2. The location of the study sites (map modified from Wubie A., 2015).

3.1.1 Koftu Lake

Lake Koftu is located 50 km west of the capital Addis Ababa. The lake is situated at an altitude of approximately 1800 meter above sea level in the North-Western highlands of the country $8^{\circ}.82'N$, $39^{\circ}.06'E$ (Google Earth). There are many human activities being done at the shoreline of Koftu Lake including animal watering and washing, cloth washing. The surrounding area is intensive agricultural land and farming is done to the shore of the lake. The land is not well vegetated and there is evidence of runoff in the lake which may affect the animal fauna serving as intermediate hosts for many fish parasites.



Figure 3: Human activities in and around Koftu Lake.

During this study, fish eating birds (final host for many helminth parasites) were observed around Koftu Lake eating fish from the lake and fish left overs. The common bird in Koftu Lake was Egyptian goose.



Figure 4: Bird population observed in and around Koftu Lake.

3.1.2 Sebeta ponds

Sebeta fish Farm is located in Oromia regional state in Sebeta city administration. It is 24 km far from the capital city Addis Ababa. Sebeta fish farm has established in 1977 under the ministry of agriculture now called National Fishery and Aquatic Life Research Centre. Now it is under Ethiopian Institute of Agricultural Research with a mandate of conducting research related to fish and other aquatic life resources, source of fish fingerlings and training of personnel's from different institutions of the country.

The centre has 32 fish rearing and experimental ponds constructed from earthen ponds, concrete ponds and lined ponds. The fish species in the centre include Nile tilapia (*O. niloticus* (Linnaeus, 1758), *Clarias garipinus* (Bruchell, 1822) *Cyprinus carpio* (Linnaeus, 1758), and *Tilapia zilli* (Gervais, 1848) and gold fish *Carasius auratus* (Linnaeus, 1758). It is situated at 2200m above sea level and an altitude of 8°55'N

38°37'E covering a total area of 16 hectare. The area is characterized by a moderately warm climate with annual mean temperature of about 21°C. The area gets annual rainfall of about 866–1200 millimetres. The annual minimum and maximum temperature is 18 °C to 25 °C respectively (Sebeta meteorology department).

Hammerhead and African fish eagle were frequently observed fish eating bird species in Sebeta ponds. But the fish ponds are protected by thin nets placed over the ponds to protect the fish from being eaten by fish-eating (piscivorous) birds. The ponds in Sebeta are fenced, water quality parameters are being monitored every second days, continuous flow of water to the ponds, and no human activities in and around the ponds which can deteriorate the fauna and flora of the ponds is observed. The farm is kept for fish fingerling acclimatization and experimental purpose.



Figure 5: Sebeta acclimatizing and spawning ponds.

3.1.3 Wonchi private fish farms

The small scale fish farm selected for this study in South-West Shoa zone of Oromia region, Wonchi woreda (Senkole Kebelle), Ethiopia. The area is characterized by mixed farming system where crop and livestock are integrated (Teshome *et al.*, 2016). It is located 157 km far from Addis Ababa and at an altitude of 2,063 m above sea level with coordinates of 8°32'N 37°58'E. In the Wonchi area, farmers are organized by group and have more than 21 small scale fish farmers in the district. The ponds are constructed with 15m by 20m. The source of water is the river passing across the district and serving the community for domestic use, irrigation and fish farm ponds. The fish for these small scale fish farmers were introduced by the National Fishery and Other aquatic Life research Centre after collecting it from Koftu Lake and acclimatized in Sebeta ponds. The fish species introduced include *O. niloticus* and *T. zilli*. The ponds are protected by fence and continuous supply of river and stream water. During the sampling period,

observation was made if there are fish eating birds around and they were not observed in and around the farms.

3.1.4 Water physico-chemical parameters of the three study sites

The physico-chemical parameters were measured during the study and their mean value were within the normal and tolerable range required for normal physiological activity of *O. niloticus* (Table 1). The desirable ranges of water dissolved oxygen, PH, temperature and conductivity for Nile tilapia are 4.5-8 mg/L, 6.5-9, 20-30°C and 30-500 µc/cm respectively. A change from the desirable ranges of the physico-chemical factors may cause significant stress to the fish hence increasing its susceptibility to disease (Bhatnagar, 2013).

Table 1: Common physico-chemical values (mean ± SE) of the study sites.

Parameters	Koftu Lake	Sebeta ponds	Private farms
DO(mg/l)	5.62±0.26	7.10±0.66	4.15±0.78
DO (%)	78.03±2.93	104.00±12.63	57.10±10.79
PH	7.18±0.05	8.13±0.67	7.19±0.05
Temperature (°C)	18.50±0.65	20.33±0.74	18.03±0.66
Conductivity(µc/cm)	266.00±4.78	157.10±24.38	260.33±8.37
Salinity	146.50±4.63	79.90±11.99	144.93±6.29

*DO=Dissolved Oxygen

3.2 Sampling Design

3.2.1 Physicochemical parameters measurements

Water quality parameter data including water temperature, dissolved oxygen (DO), conductivity and pH were measured in-situ using a portable multi-parameter probe a (Model HQ40D, HACH Instruments) before sampling the fish for parasitological investigation.

3.2.2 Fish Sampling

The fish species included in the current study was *O. niloticus*. It is the most cultured and economically important fish as it is preferred by many farmers and consumers. A

cross sectional study was conducted from October 2016 to January 2017 to identify the most abundant parasites and determine the prevalence, intensities and abundance of *O. niloticus* parasites species in the study sites. A total of 302 *O. niloticus* were investigated from the three study sites. The number of fish sampled from Koftu Lake, Sebeta ponds and selected private fish farms were 101, 128 and 73 respectively.

Fish were collected mostly by fishing with seine nets in all the study sites. Fish were put in a fish tank with lake water and oxygen filled polyethylene bag and transported alive to the National Fishery and Aquatic Life Research Centre, Sebeta. They were killed by severing the spinal cord. All fish were weighed and the total length of the fish were taken using digital weighing balance and meter rule respectively and recorded.

3.2.3 Examination of fish for parasites

The external body surface including scales, gills, fins and operculum of freshly caught fish specimens were examined for external parasites. Any abnormalities on the fish were recorded. A hand lens was used for quick identification of ectoparasites on the skin and fins of the fish sample. Skin was also checked if there were capsules with metacercariae of trematodes in black dots and yellowish cysts which were sliced off the skin for further investigation. Scrapings from the fish skin were taken with a cover slip on dorsal part of the head and ventral region of the fish from head to just after the anal point and from fins. The mucus sample is then smeared onto a clean microscope slide along with a drop of pond/lake water. The sample was then covered with a cover-slip and examined under compound microscope on 100x and 400x magnification. The opercula were removed using scissors and gills were removed and then placed in Petri dish containing normal pond water. Gill rakers were detached apart by forceps and examined under stereomicroscope for the presence of worms.

For internal examination, the fish were dissected from anus ventrally along the middle of abdomen to mouth with care not to damage the internal organs and parasites in the body cavity. Then the fish was opened by cutting from anus up to the lateral line, then further along the lateral line up to operculum and the detached part was removed. Pericardial cavity, mesentery, liver, gonads, body cavity, sites behind the gills and other internal organs were checked for helminths by naked eyes and microscopically. The digestive tract was taken out together with pharynx, cleaned of adipose tissue and mesenteries; dissected along using scissors and investigated by parts. The inside part of

the gut were examined by stereomicroscope and macro parasites were taken out using forceps. The 'gut wash method' (Cribb & Bray, 2010) in saline was used to examine the intestinal contents. The eye balls were taken out using scissors and forceps. It was then crushed and examined under the stereo microscope and compound microscope. The kidneys and the liver were also examined for the presence of parasites.

3.2.4 Identification of parasites

The identification of parasites collected were based on the distinctive body shapes and the morphological features of the collected specimen and those described in literature (Florio *et al.*, 2009; Woo, 1995). A key to identification modified from Paperna (1996) for identification of the major taxa of adult parasites of fish was exhaustively used. Taxonomic identifications were mostly limited to genus level except *Acanthogyrus tilapia* because the fish harbours mostly larval stages of nematode and trematode parasite and could not be distinguished to species level morphologically. The adult stage which is easy to identify is usually in the bid final host. Larval forms without visible diagnostic characteristics, such as sex organs, cannot be differentiated with certainty among taxa (Hoffman, 1967).

Protozoans and monogeneans were identified mainly based on the morphological characteristics of the fresh specimen referring standard manuals and scientific papers. The metazoan and crustacean parasites were preserved and taken to Natural History Museum (Austria) for detail morphological identification in the laboratory while they are in 80% Ethanol alcohol. In the lab further identification of crustaceans, acanthocephalans and digeneans, the specimens were stained in glycerine-ethanol for few days to remove the alcohol in the specimen and make it more visible under stereomicroscope.

3.3 Epidemiological and diversity calculations

3.3.1 Epidemiological parameters

Prevalence (P) is the proportion of the number of infected hosts over the number of hosts examined (Bush *et al.*, 1997).

$$P = \text{Number of infected fish} / \text{Total number of fish examined} * 100$$

Mean Intensity (Mi) is the mean number of parasites of one taxonomic group found in an infected host (Bush *et al.*, 1997).

MI= Number of parasites/Number of infected fish

Mean abundance (Ma) is the mean number of parasites of one taxonomic group found on hosts that were examined (Bush *et al.*, 1997).

MA= Number of parasites/ Number of Examined fish

Dispersion Spectrum: explains how parasite individuals of one species are aggregated among the host population and is determined by the relationship between prevalence and mean intensity. There are three different distribution patterns: under dispersion, overdispersal and random dispersion (Anderson, 1993). When a small number of infected hosts which harbour parasites even at high infection levels, aggregation for fish parasites is considered as an over dispersion (Poulin, 2007).

3.3.2 Diversity indices calculations

Simpson's index (D) is the probability that any two individuals randomly drawn from an infinitely large community will belong to the same species and is calculated as:

$$D = \sum p_i^2,$$

Where p_i is the proportion of individuals in the i -th species (Magurran, 2004). As D increases, diversity decreases. The Simpson's index measures the evenness, and is a high number when there is an equal number of individuals per species (Morris *et al.*, 2014).

The Shannon Wiener Index (H'): is also probability measuring tool calculated from the equation:

$$H = - \sum_{i=1}^s p_i * \ln p_i,$$

Where p_i is the proportion of individuals belonging to the i -th species in the dataset and represents the relative abundance of a species (Magurran, 2004). If both the number of individuals and the species relative abundance are increasing, the value of the index gets higher. The maximum is reached when a given number of species indicate the same evenness (Morris *et al.*, 2014).

Evenness: is the equal number of specimens per species in a given ecosystem (Magurran, 2004).

Richness: Species richness is the number of species that are known to exist (Whittaker, 1972). It is one of the commonly used and simple metric to express species diversity (Morris *et al.*, 2014).

Sorenson's Coefficient is used to calculate the species component similarity in a community.

$$SC= 2C/S1+S2,$$

where C is the number of species the two communities have in common, S1 is the total number of species found in community 1, and S22 is the total number of species found in community 2 (Sørensen, 1948). Sorenson's coefficient gives a value between 0 and 1. When the value of the index is closer to 1, the different communities will have more fauna in common. Complete community overlap is equal to 1; complete community dissimilarity is equal to 0.

3.4 Statistical Analysis

The collected raw data was entered in to Microsoft excel data sheets and analyzed using SPSS-21 statistical software. Descriptive statistics, percentages and 95% confidence intervals were used to summarize the proportion of the infested. Statistical significance was set at $p<0.05$. Prevalence, intensity and abundance were analyzed by using the calculations formulated by Bush *et al.* 1997. U-test (Mann-whiteny test) were used to compare intensities in the different study sites. Variance to mean ratio was calculated with excel to plot the distribution pattern of parasite infestation in the hosts. Shannon and Simpson's species diversity indices were used to compare the parasite diversity of each study site.

4. RESULTS

4.1 Identification of parasites occurring in *O. niloticus*

In this study, a total of 302 *O. niloticus* were sampled from October 2016 to January 2017 for parasitological examination in Koftu Lake, Sebeta ponds and selected private fish farms in Wonchi area all of located in central Ethiopia. From the sampled fish, 11 different genera of external and internal parasites were identified in the three study sites. One genus of Protozoa, one genus of Monogenea, unidentified larvae of Cestoda, four genera of Digenea, two genera of Nematoda, one species of Acanthocephala and once genus of Crustacea were found from the three study sites (Table 2).

4.1.1 Protozoa

Trichodina spp. (Ehrenberg, 1831) was the only external protozoan parasite found from *O. niloticus* fish sampled for this study. This parasite was found in Koftu Lake, Sebeta ponds and selected private farms. It is an external parasite and was found on the skin and scale scrapings and gills of *O. niloticus* fish. Under microscope examination, *Trichodina spp.* were round ciliates with disc like and hemispherical shape parasites. They have cilia on the edge of the disk and were observed moving freely under microscope (Fig. 6).

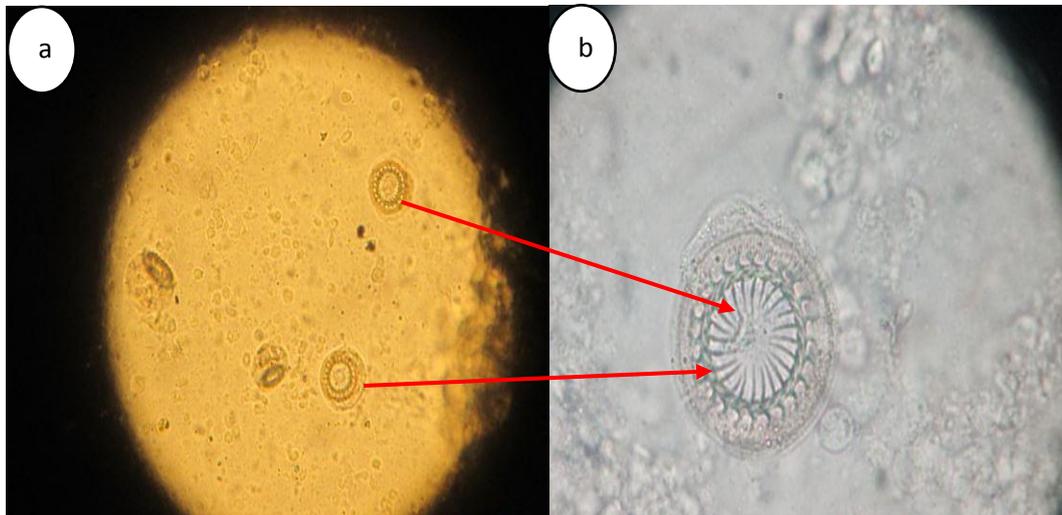


Figure 6: *Trichodina spp.* on *O. niloticus* from Sebeta ponds (a: 100x and b: 400x).

4.1.2 Monogenea

Cichlidogyrus sp. (Paperna, 1960) was found attached to the gill filaments and gill arch and moving in position of *O. niloticus* (Fig. 7). It is the only adult stage monogenean parasites found in Koftu Lake, Sebeta ponds and selected private fish farms in Wonchi area.

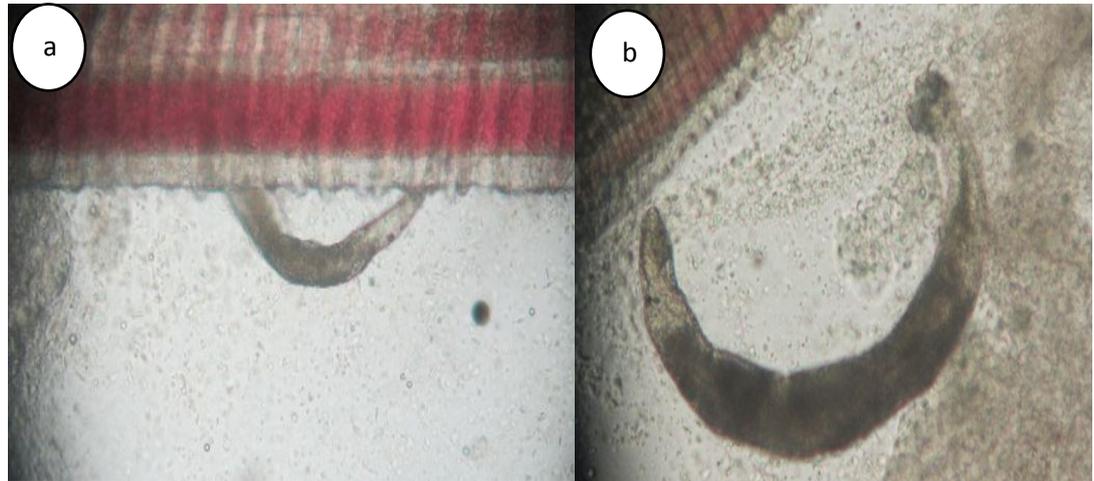


Figure 7: *Cichlidogyrus spp.* from the gills of *O. niloticus* in selected private fish farms (a) attached to gill-100x, b) detached from gill-400x).

4.1.3 Cestoda

The larval stage of cestodes were observed in *O. niloticus* of fish in Koftu Lake and Sebeta ponds. No cestode parasite was not recorded in fish of selected private farms. These plerocercoides were found encysted as white patches spread all over the liver and intestinal wall of the fish. The larvae has flat shape with visible protruding hook on the anterior part.

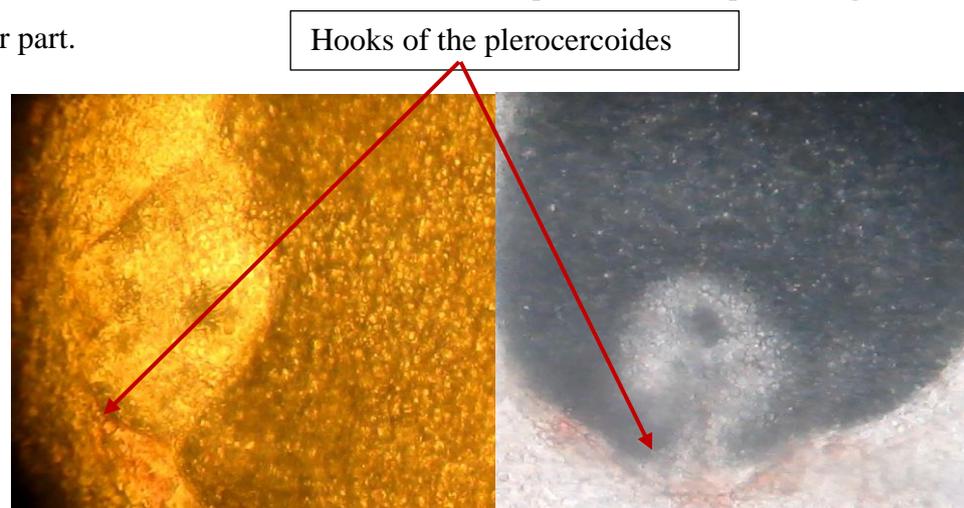


Figure 8: The hooks of plerocercoides of cestodes under microscope (400x).

4.1.4 Digenea

Clinostomum sp. metacercariae identified from *O. niloticus* were mostly encysted in the brachial cavity just behind the gills, on the inside surface of the operculum, and also observed as yellow cysts on the skin below the scales (Fig. 9). The encysted *Clinostomum metacercariae* have a slightly oval shape when released from the cyst. Their size varies from 3mm to 5mm long and appears yellow in colour. They are encountered in Koftu Lake and Sebeta ponds but not found in selected private fish farms in Wonchi area. They give unsightly appearance when found in high infestation level which consequently may cause the consumers to reject the fish. *Clinostomum sp.* is zoonotic parasite with public health significance and might be transmitted to consumers if they consume uncooked.

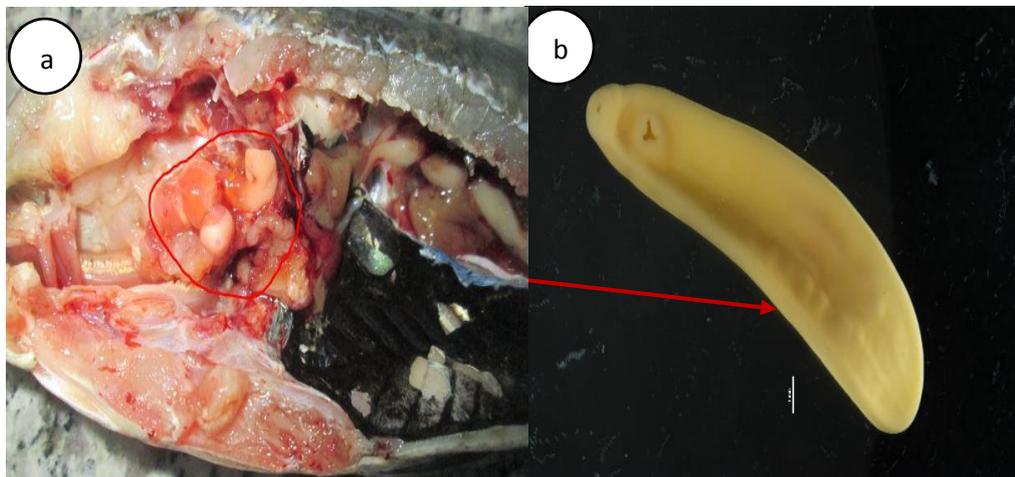


Figure 9: *Clinostomum sp.* from brachial cavity of *O. niloticus* from Sebeta ponds (b-Microphotography).

Metacercariae of *Euclinostomum sp.* were found dominantly inhabiting the kidney as large black round cysts infesting anterior and posterior regions of kidney. They are observed covering and degenerating the whole kidney. This metacercariae were also found encysted in liver, gonads and behind the gills. The encysted *Euclinostomum metacercariae* has a slightly oval shape when released from the cysts and appear pale to dark in colour (Fig. 10). It has a zoonotic parasite which causes illness in human beings when they are eaten raw or undercooked.

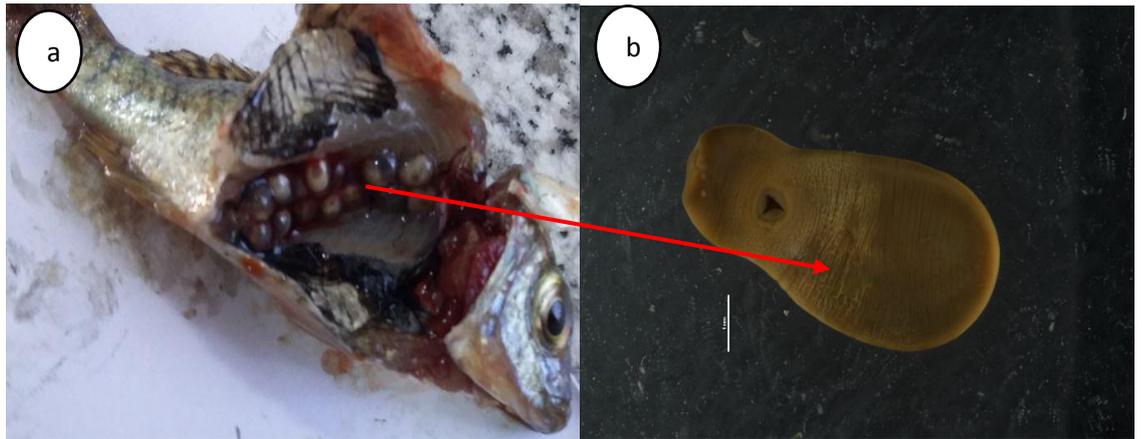


Figure 10: *Euclinostomum sp.* metacercaria from kidney of *O. niloticus* from Sebeta (a- cyst in the kidney and b- microphotography).

The other abundant digenean trematode parasite found commonly in *O. niloticus* was *Tylodelphys sp.* It was diagnosed by wet mount preparation of the crushed eye and predominantly found free swimming in the vitreous humour of the eyes of *O. niloticus* (Fig. 11).

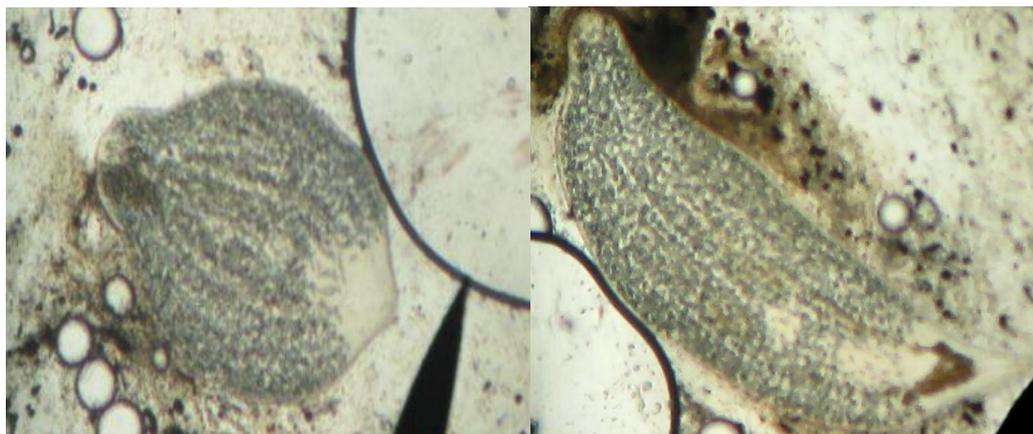


Figure 11: *Tylodelphys sp.* metacercariae in the Vitreous humour of eye from *O. niloticus* in Koftu Lake (100x).

Black spot digenean trematodes were found from *O. niloticus* of Koftu Lake and Sebeta ponds. Small, multifocal, slightly raised black spots were found scattered all over the fish external body surfaces including skin, scales, operculum, fins, gills and head of the fish. It was also detected in gills. Wet mount preparation of the skin scrapings revealed encysted and moveable metacercariae. Identification of the metacercariae to genus level was not possible morphologically (Fig. 12).

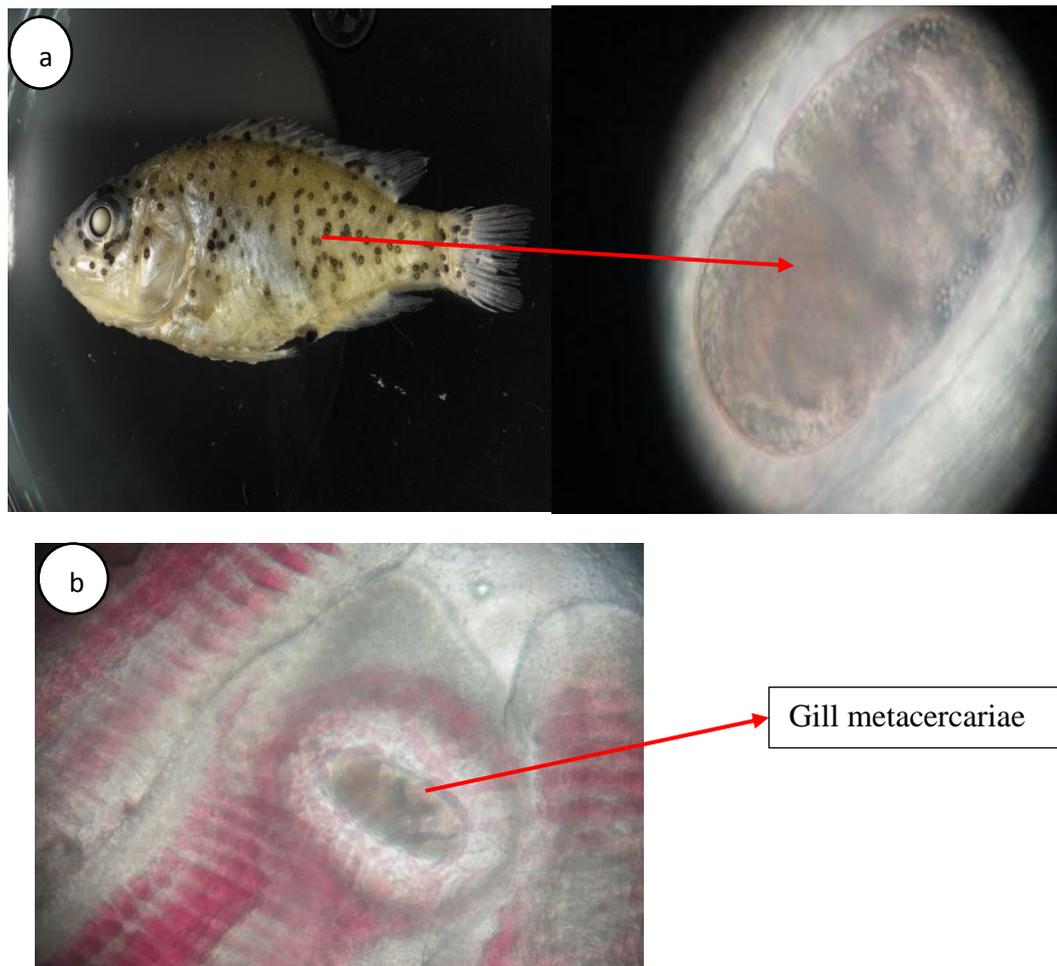


Figure 12: Blackspot metacercariae a) in the skin and b) gill of *O. niloticus* from Sebeta ponds.

4.1.5 Nematoda

The larval nematode *Contracaecum sp.* (Railliet & Henry, 1912) from Nile tilapia was found coiled in the pericardial cavity and also sometimes found dispersed in the abdominal cavity (Fig. 13a). It was also found inserted and coiled in the mesentery and gonads. Larvae are covered with a thick cuticle and it is the most common genus in *O. niloticus* of fish sampled in Koftu Lake and Sebeta ponds. It was not encountered from Wonchi private fish farms. *Contracaecum sp.* are responsible for human infections which can be caused by eating raw or undercooked fish. Another unidentified nematode species was observed in the intestine and mesentery of *O. niloticus* (Fig. 13b).



Figure 13: *Contoacecum sp.* from pericardial cavity (a) and unidentified nematode species from intestine (b) of *O. niloticus* in Koftu Lake.

4.1.6 Crustacean

The genus *Dolops* was the only external copepod parasite found in this study. It was found attached and sometimes crawling on fins and the skin on *O. niloticus* (Fig. 14). It was encountered in fish of Sebeta ponds. The entire body is covered by a wide carapace and are morphologically flattened and oval body shape with a prominent compound eyes. It has translucent body surface and was difficult to investigate on fish surface as it is attached to surface and difficult to identify. The mouthparts and the first pair of antennae are hooked with an associated spines and proboscis armed with suckers to attach to host. Their size varies from 20mm to 30mm. The difference between *Dolops sp.* and *Argulus sp.* is that *Argulus sp.* has a pair of suction discs which is a modified first maxillae where as *Dolops sp.* has hooks and associated spines.

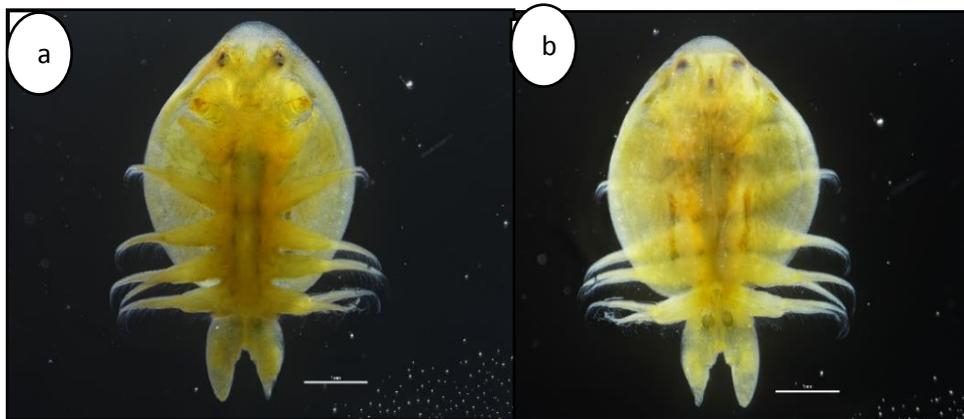


Figure 14: *Dolops* sp. from the fin of *O.niloticus* fish in Sebeta ponds (a: ventral side and b: dorsal side).

4.1.7 Acanthocephala

In this study, one species of Acanthocephala, *Acanthogyrus (Acanthosentis) tilpiae* was found in the intestine of *O. niloticus*. This parasite is characterized by the presence of an eversible hooked proboscis at the anterior part (Fig. 15). The proboscis is armed with spines which are used to pierce and attach to the gut wall of its host. Fish are final hosts for acanthocephalans harbouring it in the intestine. The parasite has a complex life cycles, involving at least two hosts. Arthropods especially crustaceans are the first intermediate hosts and invertebrates, fish, amphibians, birds, and mammals may serve as final host.



Figure 15: *Acanthogyrus tilpiae* from intestine of *O. niloticus* in Sebeta ponds

4.2 Prevalence, intensities and abundance of parasites

4.2.1 Overall infection parameters

In this study, a total of 302 *O. niloticus* fish caught randomly from the study sites were examined for the presence of parasites from October 2016 to February 2017. Out of the 302 *O. niloticus* examined in the three study areas, 251 (83.4%) of them were infested with single or multiple parasites belonging to different genera. The fish from Koftu Lake showed 100% prevalence followed by private fish farms with a prevalence of 82.2%. Private fish farms have relatively lower prevalence than others (71.09%).

Table 2: Overall prevalence of parasites in each sample site (n=302).

Study site	Sampled (n)	Number infected	Prevalence (%)
Koftu Lake	101	101	100
Sebeta ponds	128	91	71.1
Private farms	73	60	82.2
Total	302	252	83.4

Different types of parasites both from internal organs and external body surfaces of the both fishes were identified. The most prevalent parasites species were the external parasites protozoan *Trichodina spp.* and the monogenetic trematode *Cichlidogyrus spp.* with a prevalence of 52.96% and 52.63% respectively followed by the digenean trematode *Tylodelphys sp.* from the eye of the fish (Table 3).

Table 3: Total prevalence (P), Mean intensities (MI), Mean abundance (MA) of *O. niloticus* parasites in the three study sites (n=101).

Parasite species	Location Host	Developmental stage	P (%)	MI	MA
<i>Trichodina spp.</i>	Gills	Adult	52.96	-	-
<i>Cichlidogyrus spp.</i>	Gills	Adult	52.63	10.38	5.46
<i>Clinostomum sp.</i>	Brachial cavity	Larvae	20.07	3.51	0.70
<i>Euclinostomum sp.</i>	Gills/Kidney	Larvae	16.78	5.82	0.98
<i>Tylodelphys sp.</i>	Gill cavity	Larvae	36.51	7.32	2.67
Blackspot metacercariae	Skin/gills	Larvae	19.08	-	-
Cestoda larvae	Gut wall	Larvae	9.87	-	-
<i>Contracaecum sp.</i>	Pericardial cavity	Larvae	25.33	5.45	1.38
Unidentified Nematode	Body cavity	Larvae	0.99	5.00	0.05
<i>Acanthogyrus tilapiae</i>	Intestine	Adult	5.26	7.00	0.37
<i>Dolops sp.</i>	Skin	Adult	3.29	0.20	0.01

* Data not available

4.2.2 Infection parameters in Koftu Lake

A total of 101 *O. niloticus* were sampled and investigated for the presence of parasites from Koftu Lake. All fish were found infested with single or multiple parasite infections hence the overall prevalence was 100%. Nine genera of different parasites were identified in Koftu Lake and their prevalence, mean intensity and mean abundance were determined (Table-3). Accordingly, the most dominant parasite in this lake was the digenean trematode *Tylodelphys sp.* with a prevalence of 93.2% and mean intensity of 8.42 per infected fish. *Trichodina spp.* and *Cichlidogyrus spp.* were the other most common external parasites with prevalence values of 70.6% and 77.5%. *Euclinostomum sp.* was the least prevalent parasite (0.98%) (Table 3).

Table 4: Prevalence (P), Mean intensities (MI), Mean abundance (MA) of *O. niloticus* parasites in Koftu Lake (n=101).

Parasite species	Location Host	Developmental stage	P (%)	MI	MA
<i>Trichodina spp.</i>	Gills	Adult	70.59	-	-
<i>Cichlidogyrus spp</i>	Gills	Adult	77.45	12.56	9.73
<i>Clinostomum sp.</i>	Brachial cavity	Larvae	50.98	3.35	1.71
<i>Euclinostomum sp.</i>	Gills/kidney	Larvae	0.98	5.00	0.05
<i>Tyloodelphys sp.</i>	Gill cavity	Larvae	93.14	8.42	7.84
Blackspot metacercariae	Skin/gills	Larvae	27.45	-	-
Cestode larvae	Gut wall	Larvae	20.59	-	-
<i>Contracaecum sp.</i>	Pericardial cavity	Larvae	53.92	5.27	2.84
Unidentified nematode	Body cavity	Larvae	1.96	7.50	0.15

*-Unavailable data

4.2.3 Infection parameters in Sebeta ponds

In Sebeta ponds, 128 *O. niloticus* were sampled for during this study and out of them 91(71.09%) were infested with one or multiple parasites. Eleven genera of both external and internal parasites were identified in this study site. *Trichodina spp.* and *Cichlidogyrus spp.* were the most prevalent external parasites in Sebeta ponds with prevalence of 37.50 and 33.59%. Sebeta ponds has higher parasite richness than Koftu Lake and fish private farms (Table 5).

Table 5: Prevalence (P), total parasite count, Mean intensities (MI), Mean abundance (MA) of *O. niloticus* parasites Sebeta ponds (n=128).

Parasite taxa	Location Host	Developmental Stage	P (%)	MI	MA
<i>Trichodina spp</i>	Gills	Adult	37.50		
<i>Cichlidogyrus spp</i>	Gills	Adult	33.59	13.02	4.38
<i>Clinostomum sp.</i>	Brachial Cavity	Larvae	7.03	4.44	0.31
<i>Euclinostomum sp.</i>	Gills/kidney	Larvae	9.38	5.58	0.52
<i>Tyloodelphys sp.</i>	Gill cavity	Larvae	12.50	0.81	0.10
Blackspot metacercariae	Skin/gills	Larvae	23.44	-	-
Cestod larvae	Gut wall	Larvae	7.03	-	-
<i>Contracaecum sp.</i>	Pericardial cavity	Larvae	17.19	5.91	1.02
unidentified nematode	Body cavity	Larvae	0.78	-	-
<i>Acanthogyrus tilapiae.</i>	Intestine	Adult	11.72	7.33	0.86
<i>Dolops sp.</i>	Skin	Adult	7.81	0.20	0.02

*-Unavailable data

4.2.4 Infection parameters private farms in Wonchi

Out of the 73 *O. niloticus* sampled in private farms, 60 (82.19%) of them were found harbouring single or multiple parasitic infestation. *Trichodina spp.*, *Cichlidogyrus spp.*, *Euclinostomum sp* and Acanthocephala were the four parasitic genera encountered in private fish farms. *Trichodina spp.* and *Euclinostomum sp.* were the dominant external and internal parasites respectively (Table 6). This study site showed the lowest number of parasite taxa of the three study sites.

Table 6: Prevalence (P), total parasite count, Mean intensities (MI), Mean abundance (MA) of *O. niloticus* parasites in selected private fish farms (n=73).

Parasite taxa	Predilection site	Developmental stage	P (%)	MI	MA
<i>Trichodina spp.</i>	Gills	Adult	53.25	-	-
<i>Cichlidogyrus spp.</i>	Gills	Adult	49.35	2.84	1.40
<i>Euclinostomum sp.</i>	Gills/Kidney	Larvae	49.35	5.92	2.92
<i>Acanthogyrus tilpiae</i>	Intestine	Adult	1.30	2.00	0.03

*-Unavailable data

4.3 Comparison of common *O. niloticus* parasites among the three study sites

4.3.1 Protozoa

Trichodina spp. was the only protozoan parasite encountered in Lake Koftu, Sebeta ponds and Wonchi farms. The overall prevalence of *Trichodina spp.* in the three study sites was 52.96%. The highest prevalence was observed in Koftu Lake with prevalence of 71.29% and the lowest was in Wonchi private fish farms with a prevalence of 37.50%. Infestation of the protozoan external parasite *Trichodina spp.* was higher in the natural water body (Koftu Lake) than the in cultured fish in Sebeta ponds and selected private ponds in Wonchi area.

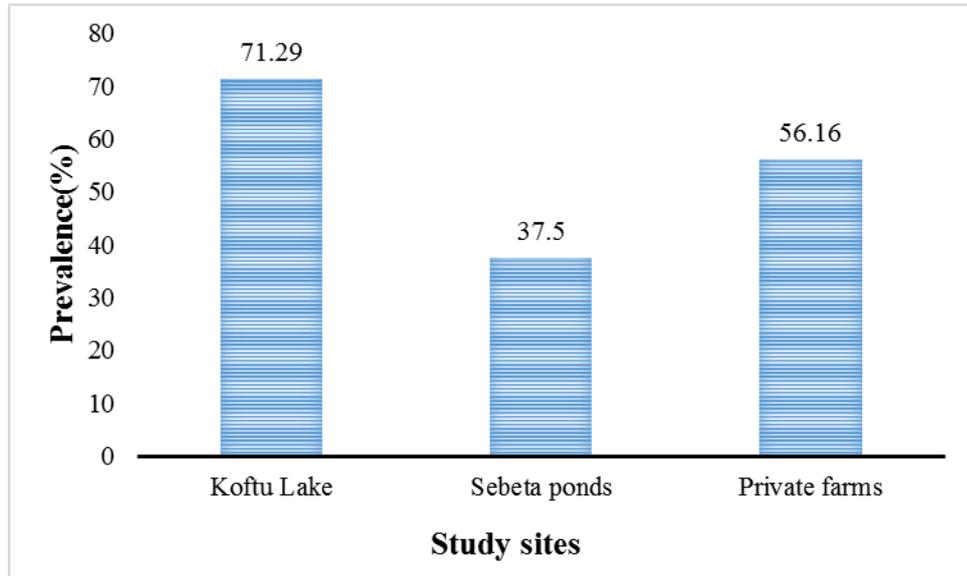


Figure 16: Prevalence of *Trichodina spp.* on *O. niloticus* in the three study sites (n=302).

4.3.2 Monogenea

Cichlidogyrus sp. was the only monogenetic trematode found in this study. The parasite occurred in *O. niloticus* in all three study sites with a total prevalence of 52.62. The highest values were recorded in Koftu Lake with a prevalence of 78.2% with a mean intensity of 12.5 worms per infected fish and the lowest was recorded in Sebeta fish ponds with a prevalence of 33.6% and mean intensity of 13. The mean intensity from *O. niloticus* of private farms was the least among the three study sites with 2.84 parasites per infected fish. Mann-Whitney U-test for intensities of *Cichlidogyrus sp.* showed that the intensity of *Cichlidogyrus sp.* in Koftu Lake is significantly different from Sebeta ponds and Wonchi private fish farms ($Z=-7.721$, $p=0.00$). A significance difference between the three study sites could be observed. ($P<0.05$).

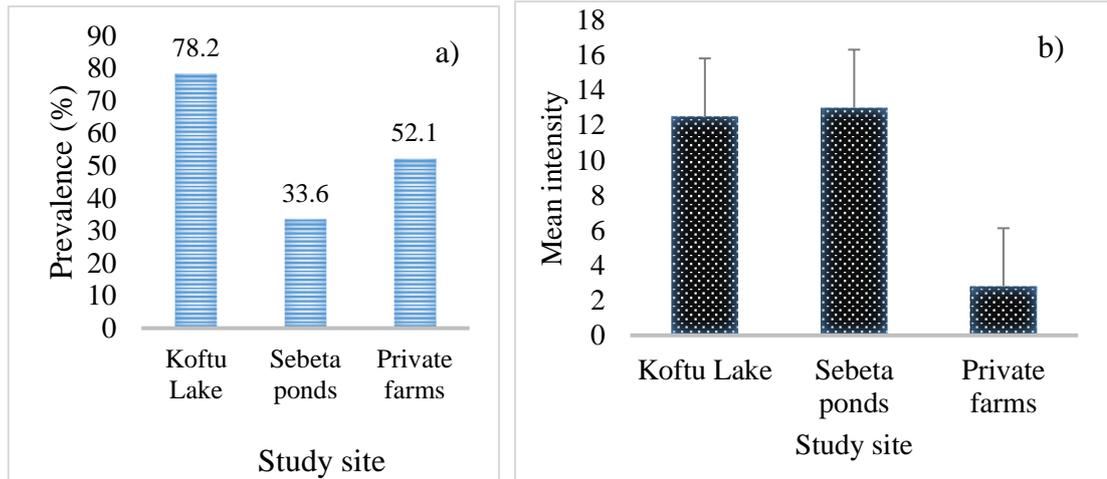


Figure 17: The a) prevalence (%) and b) mean intensities (\pm SDE) of *Cichlidogyrus sp.* from *O. niloticus* of Lake Koftu, Sebeta ponds and selected private fish farms (n=302).

Figure 11 below shows the frequency distribution of *Cichlidogyrus sp.* in the three study sites. The index of dispersion was calculated for the infestation level and its frequency distribution of parasites which intensity was counted and calculated. The variance to mean ratios (v/m) of the *Cichlidogyrus sp.* in Koftu Lake, Sebeta ponds and selected private fish farms in Wonchi area are greater than one. High infestation of *Cichlidogyrus sp.* was on few number of fish sampled for the investigation and this shows over dispersion of the parasite with in the host population. The fish from Koftu Lake had relatively lower dispersion than the other two and most fish were infested by higher number of parasites. On the other hand, high number of infestation was only on few number of fish and the number of uninfected fish was high in Sebeta ponds and the private farms.

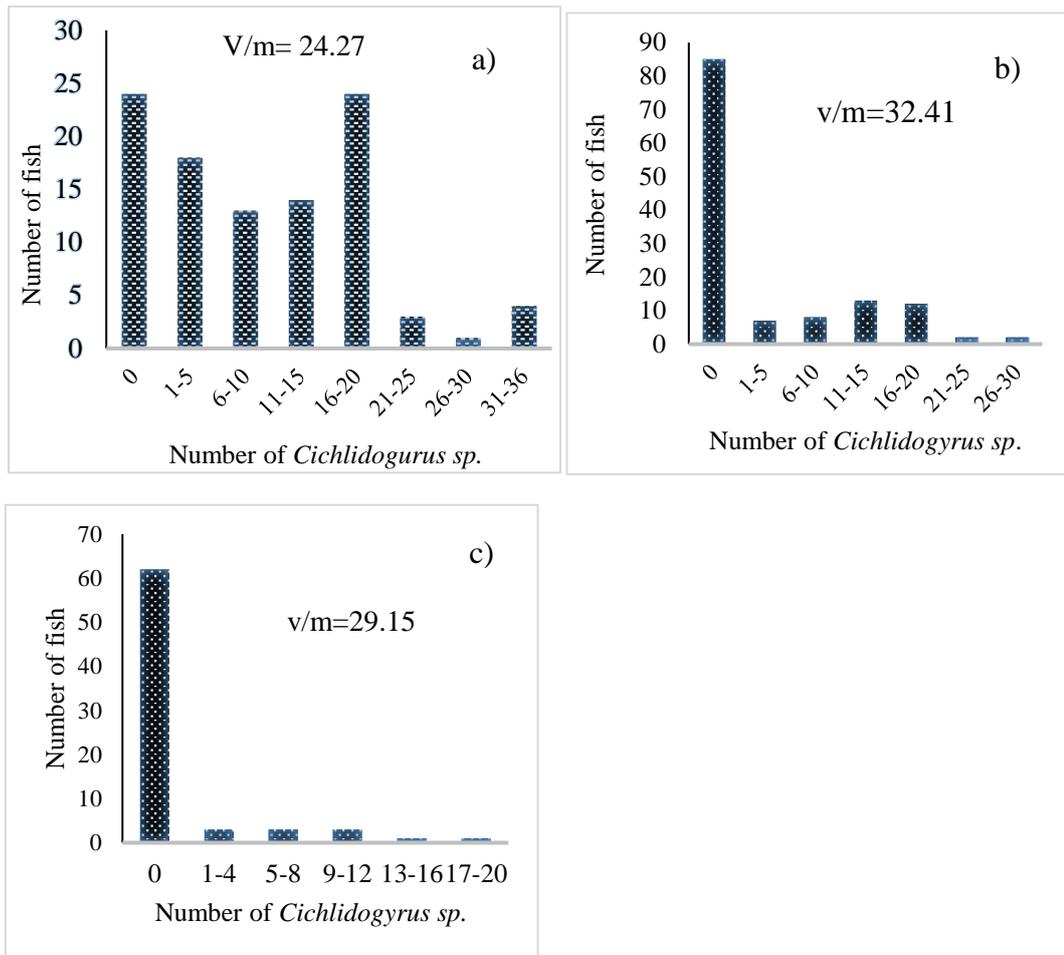


Figure 18: Frequency distributions of *Cichlidogyrus* spp. a) Koftu Lake (n=101), b) Sebeta ponds (n=128) and c) Wonchi private farms (n=73).

4.3.3 Cestoda

Plerocercoides of cestodes were encountered from Koftu Lake and Sebeta ponds with a prevalence of 20.79% and 7.03% respectively. The larval cestodes were found encysted in the liver and the wall of intestine but cestodes were not found in selected private fish farms.

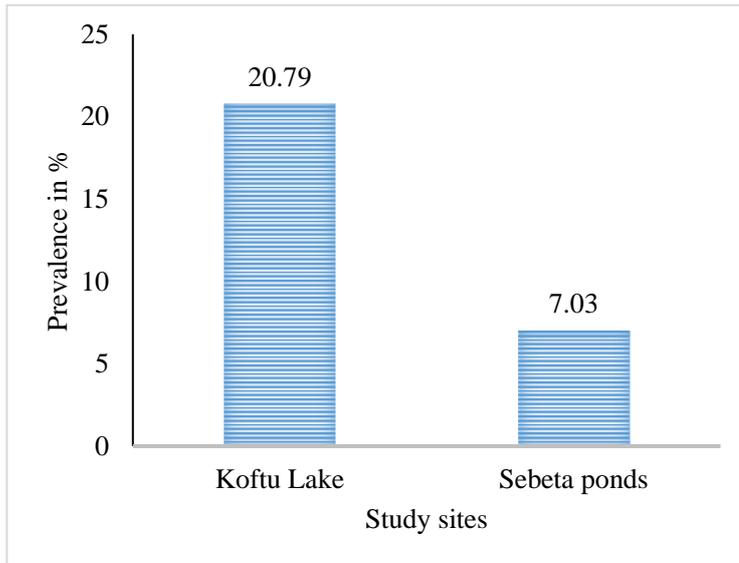


Figure 19: Prevalence of cestode plerocercoides in Koftu Lake (n=101) and Sebeta ponds (n=128).

4.3.4 Digenean trematodes

Three genera of trematodes were identified from the three study sites including *Clinostomum spp*, *Euclinostomum spp.* and, *Tylodelphys sp.* Back spot metacercariae were also found but was difficult to identify to genera level. *Clinostomum sp.* was found in Koftu Lake with a prevalence of 51.49% each infected with a mean intensity of 3.35 worms per fish. It was also found in Sebeta ponds with prevalence of 7.03% and mean intensity of 4.4 worms. *Clinostomum sp.* was not encountered in selected private farms in Wonchi area.

In this study, *Euclinostomum spp.* was one of the common parasites found in the three study sites but most dominant one in selected private fish farms with prevalence of 52.0% and the least was from Koftu Lake having prevalence of 0.98%. Mann-Whitney U-test for intensities of *Euclinostomum spp.* showed that the intensity in Sebeta ponds is significantly different from Wonchi fish farms ($Z=-6.717$, $p=0.00$).

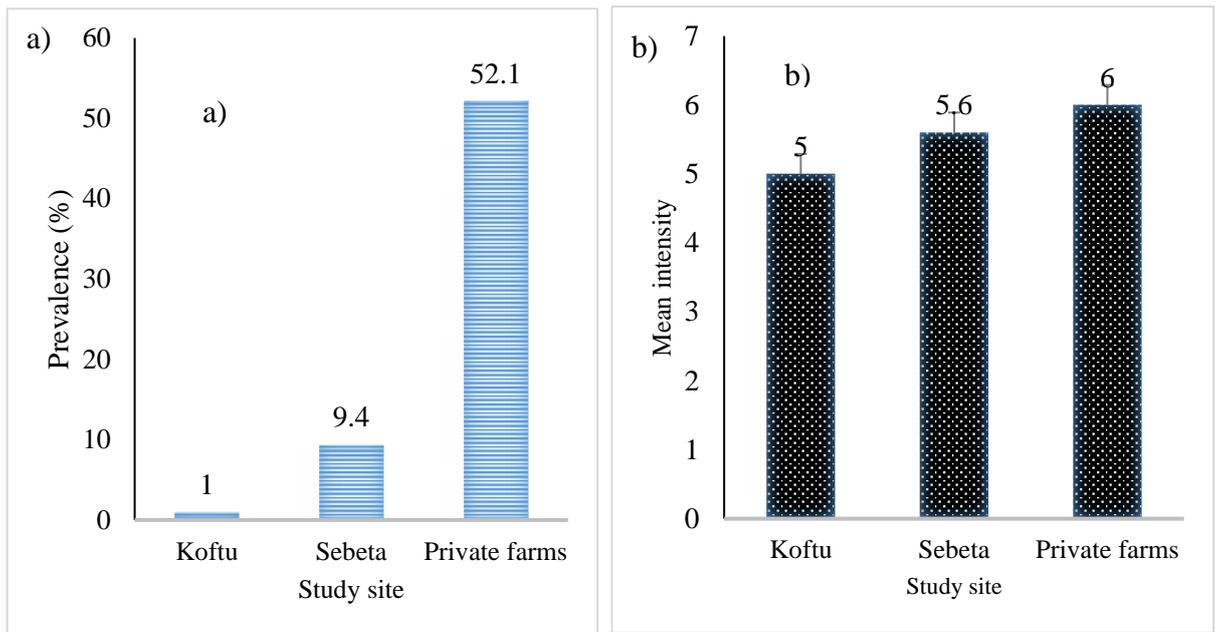


Figure 20: The a) prevalence (P) and b) mean intensity (MI) of *Euclinostomum sp.* from Koftu Lake (n=101), Sebeta ponds (n=128) and private farms (n=73).

The digenean trematode *Tylodelphys sp.* was found in *O. niloticus* of Koftu Lake with a prevalence rate of 94.06% and mean intensity 8.42 parasites per infected fish. The prevalence and mean intensity in Sebeta ponds was 12.50% and 0.81 respectively which is much lower than Koftu Lake. The parasite was not found in private farms in Wonchi area. Blackspot metacercaria were found in koftu Lake and Sebeta ponds with a prevalence of 27.72% in Koftu Lake and 23.44% in Sebeta ponds.

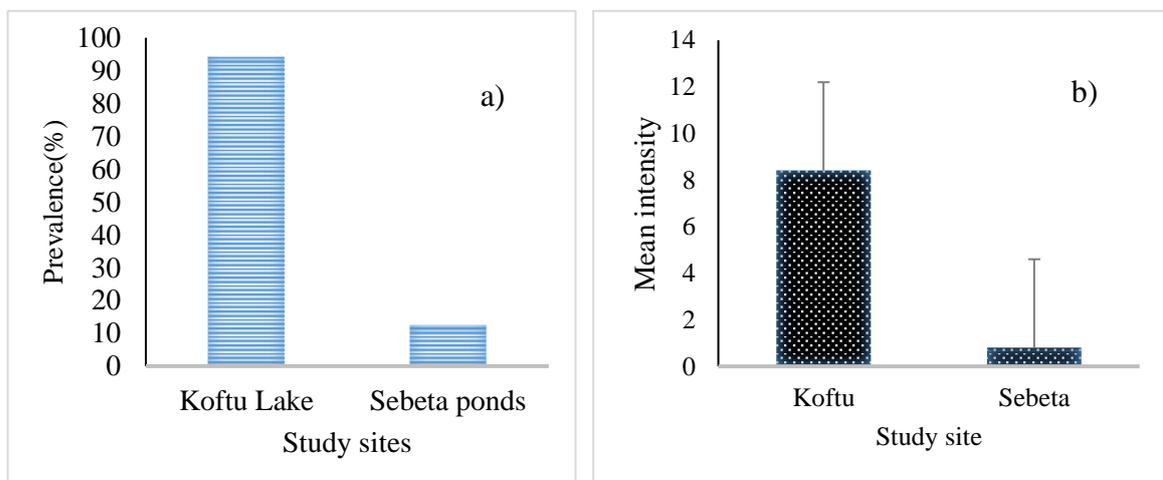


Figure 21: The a) prevalence and b) mean intensity of *Tylodelphys sp.* in Koftu Lake (n=101) and Sebeta ponds (n=128).

The index of dispersion (variance to mean ratio) of *Tyloodelpys sp.* in Koftu Lake *O. niloticus* fish had also a value greater than one and it showed over dispersion in its distribution. There are few number of fish which are not infested by the parasites.

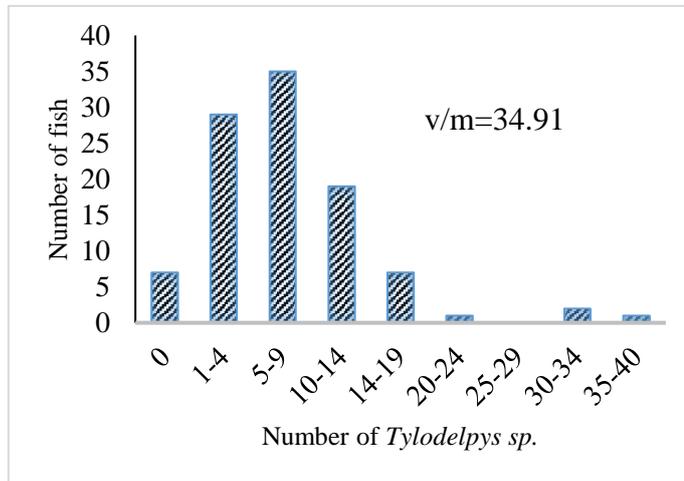


Figure 22: Frequency distribution of *Tyloodelpys sp.* in Koftu Lake (n=101).

4.3.5 Nematoda

Among the nematode parasites, *Contracaecum spp.* and unidentified intestinal nematode were identified from *O. niloticus* of Koftu and Sebeta ponds. Higher prevalence of *Contracum spp.* was found from Koftu Lake which is 54.46% but it was lower in Sebeta with prevalence of 17.19%. But the mean intensities from the two study sites are not that much different being 5.27 in Koftu Lake and 5.91 in Sebeta ponds. The prevalence of unidentified nematodes were 1.98% and 0.78% in Koftu Lake and Sebeta ponds respectively. Mann-Whitney U-test for intensities of *Contracaecum spp.* showed that the intensity in Koftu Lake is significantly different from Sebeta ponds ($Z=-7.335$, $p=0.00$).

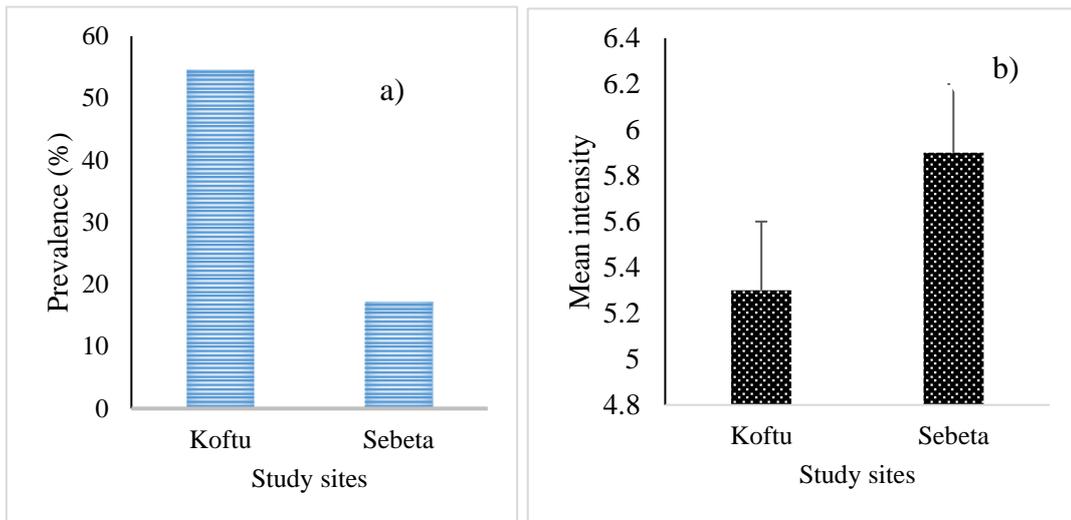


Figure 23: The a) prevalence and mean intensity of *Contracaecum sp.* in *O. niloticus* from Koftu Lake (n=101) and Sebeta ponds (n=128).

Most of the fish were not infested by *Contracaecum sp.* and the pattern was over disperses in Koftu Lake and Sebeta ponds. In Koftu Lake, there are some fish which are infested by parasites with lower intensity and very few were infested by high number of parasites.

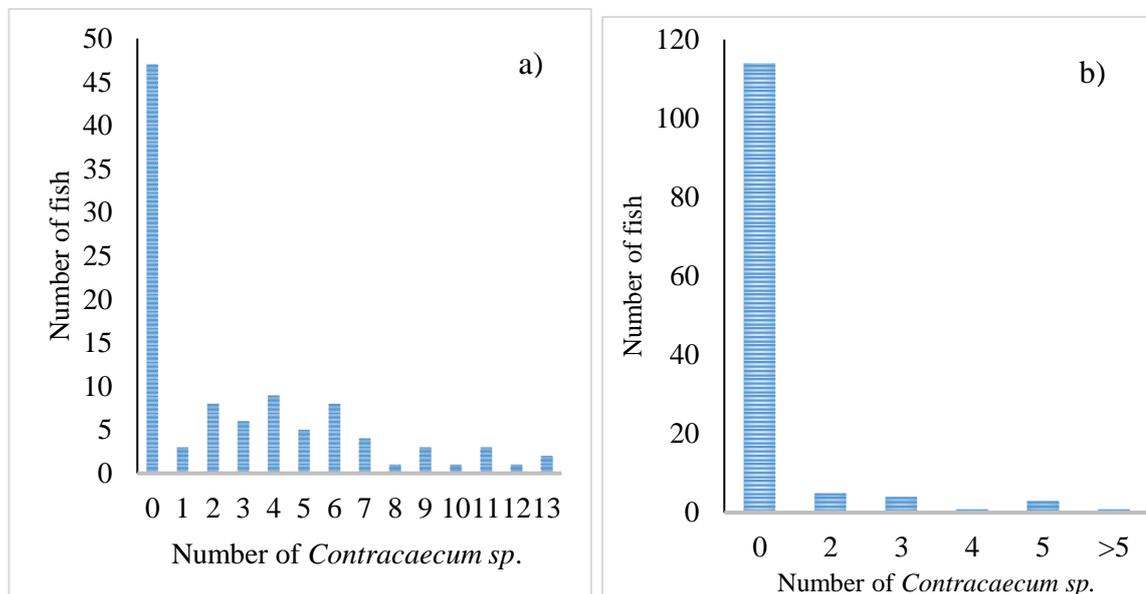


Figure 24: Frequency distribution of *Contracaecum sp.* from a) Koftu Lake (n=101) and b) Sebeta ponds (n=128)

4.3.6 Acanthocephala

All the Acanthocephalans found in this study were identified as *Acanthogyrus tilapiae*. The location of the parasite was in the intestine of the fish. The prevalence of *A. tilapiae* in *O. niloticus* of Sebeta ponds was 11.72% and mean intensity was 7.33 worms per infected fish. It was lower in private farms with a prevalence of 1.37% and mean intensity of 2 but it was not found in Koftu Lake fishes.

4.5 Parasite diversity and species richness

The parasites fauna of fish from Koftu Lake is more diverse than Sebeta ponds and Wonchi fish farms as shown by the Simpsons Diversity Index and Shannon index. The parasites fauna of private fish farms are least diverse than others (Table 7). The closer the Simpsons Diversity index (1-D) and Shannon index (H) to 1, the more diverse is the parasites fauna of fish.

Table 7: An index of parasite diversity and evenness.

Study Site	Simpsons Diversity Index(1-D)	Shannon Index(H) + Evenness	Parasite richness	Dominant group of parasites
Koftu	0.66	1.24 + 0.20	6	Digenea
Sebeta	0.59	1.23 + 0.63	7	Protozoa
Farms	0.44	0.66 + 0.60	3	Protozoa

Parasite community similarities were calculated by the Sorenso's coefficient. Table 8 shows the community similarity of parasites in relation to the respective study sites. Sorenson's community similarity coefficient between Koftu Lake and Sebeta ponds was 0.76. This value is close to 1 and showed that the most species found in Koftu Lake were also found in Sebeta ponds. In random sampling of individuals, there was a probability of 76% to find the same species from Koftu Lake and Sebeta ponds. But the probability to of finding individual parasites in random sampling from Kofu Lake and selected private fish farms in Wonchi area is 46% and that of Sebeta ponds and fish farms is 44%. Hence, the parasite community is more dissimilar in Sebeta ponds and private fish farms communities than the other two combinations.

Table 8: Parasite component community similarity by Sorenson's coefficient.

	Koftu Vs Sebeta	Koftu Vs Farms	Sebeta Vs Farms
Sorenson's coefficient	0.76	0.45	0.4

5. DISCUSSION

5.1 Protozoa

The protozoan *Trichodina spp.* was found in Koftu Lake, Sebeta ponds and private fish farms in Wonchi area. Previous reports show that *Trichodina spp.* was recorded on fish in Kenya, Uganda and Ethiopia in cages, ponds and natural water bodies. The prevalence showed lower values in natural water bodies and higher in cultured fisheries. For example in Uganda, a comparative study conducted in ponds, cages and wild *O. niloticus* fish showed a prevalence of 34.6%, 22.2% and 1.8% respectively (Florio *et al.*, 2009). Tadesse, (2009) also reported higher prevalence of *Trichodina spp.* in cultured systems in Yemlo and Wonji ponds with a prevalence of 56.67 and 46.70 % respectively, but lower prevalence in natural water bodies of Lake Awassa and Lake Babogaya with prevalence of 10% and 14.4% was reported. In contrast the present investigation reveals different results than the above mentioned studies. The prevalence in Koftu Lake (70.59%) which is a natural water body is much higher than from cultured fish in Sebeta ponds (37.59%) and private fish farms (53.25%). The continuous change and inflow of stream water in to Sebeta ponds and fish farms may probably be the reason for the low infestation of *Trichodina spp.* in cultured Nile tilapia. The human activities in and around Koftu Lake might cause deterioration of the water quality due to high organic matter load which favors the proliferation of protozoan parasites. Further studies should be done considering biotic parameters such as fish density, abundance and reproductive status and abiotic factors including nutrients and other water quality parameters. Most of protozoan parasite are ubiquitous in aquatic systems and can cause great loss in fish farms by parasite induced host mortality. This might be supported by poor water quality and presence of external damage on fish body which lead to stress and favour the multiplication of the parasite on fish (Paperna, 1996).

5.2 Monogenea

Among the monogeneans, *Cichlidogyrus sp.* was observed from all the three study sites in gills of *O. niloticus*. It is an external monogenetic trematode common in African water bodies reported from Uganda and Ethiopia (Florio *et al.*, 2009). High prevalence of *Cichlidogyrus sp.*, 77.45% was found in Koftu Lake and the lowest being in Sebeta ponds with prevalence of 33.59%. This agrees with reports from Uganda where the highest prevalence was found in wild fish with prevalence of 63.35% compared to

cultured fish which is 31.7% (Florio *et al.*, 2009). This result also contradicts the idea of Martines *et al.*, (2015) who explains that external parasites are more successful in fish farms where they spread easily to multiple hosts because of the overcrowding. They have the ability to reproduce faster and transmit from fish to fish in aquaculture than in natural conditions. Low water exchange and poor bottom hygiene as well as stocking density are the main risk factors favouring spread of infestation and may represent a further conditioning factor leading to heavy gill infestation in farmed fish (Florio *et al.*, 2009). The higher prevalence in Koftu Lake and lower prevalence in Sebeta ponds might probably due to the fact that water in Sebeta ponds is regularly regulated and there is continuous discharge of water to the ponds. The agricultural activity around the shore of Koftu Lake may also contribute to the water quality deterioration and high reproduction of the parasites.

5.3 Cestoda

In the present study, plerocercoides of cestodes were found encysted as white patches spread all over the liver and intestinal wall of the fish. The fish serves as intermediate host in this case. At least one intermediate host is required to complete their life cycle. Fish can serve as both intermediate and sometimes final host (Woo, 1995). Identification of the plerocercoid larvae to genus level was not possible morphologically. The parasite was investigated in *O. niloticus* of Koftu Lake and Sebeta ponds with a prevalence of 20.59% and 7.03% respectively. Tadesse, (2009) reported encysted plerocercoids of the cestode *Amirthingamia macracantha* in the liver of Nile tilapia from pond of Wonji, Yemlo and Lake Babogaya with prevalence of 3.33%, 6.67% & 11.43% respectively, in Ethiopia. The prevalence of cestode parasites was 10% in wild fish and 6.1% in caged Tilapia in Ethiopia during parasitological survey (Florio *et al.*, 2009). Outbreaks of cestode parasites could occur in lakes or reservoirs where copepods or *Tubificid oligochaetes* are often abundant and intensities and prevalence of most cestodes have seasonal peaks (Roberts, 2001).

5.4 Digenea

5.4.1 *Clinostomum sp.*

The digenean *Clinostomum sp.* was one of the most common parasites identified in Koftu Lake and Sebeta ponds. This parasite species was also identified in many water bodies in Ethiopia including Tana Lake (Yimer, 2003), Yemlo and Wonji pond,

Babogaya Lake and Awassa Lake (Tadesse, 2009), Lugo Lake (Amare *et al.*, 2014), Koka reservoir (Gulilate *et al.*, 2013), Small Abaya (Reshid *et al.*, 2015) and Ziway Lake (Bekelle and Hussien, 2015).

In this study, the prevalence of *Contracaecum sp.* in Koftu Lake was 50.98% and mean intensity was 3.4. The results are similar to reports of *Contracaecum sp.* From *O. niloticus* from in Awassa Lake with a prevalence of 50%, but the mean intensity was higher in Awassa with 7 worms per fish than Koftu Lake which is 3.4. This could be attributed to the size of the fish sampled for the study. The size of the fish sampled in this study were smaller ranging from 6.5cm to 18cm in Koftu Lake. Smaller fish harbor lower numbers of parasites than bigger fish as there is a longer duration of exposure to parasitic agents in the environment which increases the chance of acquiring more parasites. Small fish also provide smaller surface area for the parasite availability on the host than larger fish (Roberts, 1978; Amare *et al.*, 2014).

Prevalence of the parasites in Koftu Lake was higher than reported in Lugo Lake with a prevalence of 33.8% (Amare *et al.*, 2014), Koka reservoir with prevalence of 27.39% (Gulilate *et al.*, 2013), Lake Ziway with a prevalence of 8.60% (Bekelle and Hussien, 2015) and Small Abaya Lake with a prevalence of 18.8% (Reshid *et al.*, 2015). The prevalence in Sebeta ponds was 7.03% with 4.4 worms per infested fish which is lower than Yemlo pond with a prevalence of 23.3% and Wonji cages with a prevalence of 20% (Tadesse, 2009, Florio *et al.*, 2009). This variation in prevalence of *Clinostomum sp.* could be due to the fact that it has an indirect life cycle with snails as first intermediate and fish eating birds as final host and the fish itself as the second intermediate host. Therefore, lower and higher abundance of the snail intermediate hosts and the fish eating birds as final host in the different study sites might play a contributing role in the variation of the prevalence.

Clinostomum sp. from Sebeta ponds might be carried by fingerlings from Koftu Lake during stocking the ponds. Its prevalence and mean intensity in Koftu Lake is 51.0% and 3.3 where as in Sebeta ponds prevalence was 7.3% and mean intensity was 4.4 parasites per fish. This difference in prevalence might be attributed to the presence of high numbers of fish eating birds in Koftu Lake (observation during the study period). In addition, the ponds in Sebeta were fenced and protected by nets placed on top of the ponds to hinder birds from eating fish. This could cause the breaking of the

Contracaecum sp. life cycle which consequently might lead to lower prevalence than in Koftu Lake where the fingerlings are brought from (observational study). Kabunda & Sommerville, (1984) stated that fish heavily infested by *Clinostomum sp.* are often rejected by consumers when marketed whole fish because it gives unsightly appearance for the whole fish and can have economic importance. *Clinostomum sp.* has also a potential of public health importance as some human cases have been reported by Clinostomids (Kakizoe *et al.*, 2004).

5.4.2 *Euclinostomum sp.*

In this study, *Euclinostomum sp.* was found primarily in the kidney but also reported from the gonads and brachial cavity of *O. niloticus*. *Euclinostomum sp.* was identified in Koftu Lake, Sebeta ponds and selected private fish farms in Wonchi area. It was also recorded in Ethiopia and Kenya from BOMOSA cage fish (Florio *et al.*, 2009) like Machakose and Sagana farms (Otachi, 2009) and Wonji cages and Awassa Lake (Tadesse, 2009). The presence of *Euclinostomum sp.* was highest in selected private fish farm with a prevalence of 49.35% and a mean intensity of 5.9. This prevalence is much higher than reported by Florio *et al.*, (2009) in Kenya Machakose and Sagana BOMOSA cages which was 1.4%, and BOMOSA cages in Ethiopia with a prevalence of 9.3%. It is also higher than reported in Lake Awassa and Wonji Cages with prevalence of 10% and 6.67% respectively. It shows also higher values of mean intensity with 5.9 worms per fish than Awassa nad Wonji cages where mean intensity was 1.0 (Tadesse, 2009).

5.4.3 *Tylodelphys sp.*

The digenean *Tylodelphys sp.* was found in *O. niloticus* of Koftu Lake and Sebeta ponds in the present study but not in private farms. This parasite was reported in Kenya from fish of Machakose and Sagana fish farms (Otachi, 2009). In Ethiopia, it was reported in Wonji ponds, Babogaya Lake and Awassa Lake (Tadesse, 2009). It was the most dominant parasite in Kofu Lake with a prevalence of 93.2% and mean intensity of 8.4 worms per fish. This result is much higher than the prevalence recorded in Babogaya Lake, Wonji pond, and Awassa Lake in Ethiopia with prevalences of 2.8%, 6.6% and 6.7% respectively (Tadesse, 2009). Florio *et al.*, (2009) also reported 52% prevalence of *Tylodelphys sp.* in ponds and 50% in wild fish in Kenyan water bodies which is lower than the present study. The higher prevalence of *Tylodelphys sp.* in Koftu Lake could

be associated to higher seasonal peaks of the intermediate hosts in the lake and availability of high numbers of fish eating birds which increase the abundance of the parasites. It could also be associated with the human activity in and around the lake which could lead to water quality deterioration. This subsequently causes stress for fish and can lower the immune status. Therefore, detailed studies on seasonality of the parasite and its developmental stages as well as water quality issues related to human activities should be done to assess the risk of infection and to take preventive measures.

When there is high infection burden of *Tylodelphys sp.*, it settles between lenses and retina causing partial blindness of fish. Severe infection may also lead to abnormal protrusion of the eye ball, blurred vision and total blindness (Paperna, 1996; Florio *et al.*, 2009). This hinders the fish from seeing food and is therefore leading to stunted growth which can lead to economic losses. Moreover fish may also be easily exposed to predators as they cannot escape from their enemy.

5.5 Nematoda

In this study, the nematode *Contracaecum sp.* was found in Koftu Lake and Sebeta ponds but not encountered in selected fish farms. It was reported in many African countries including in Nigeria, Uganda, Kenya (Otachi, 2014; Kaddumukasa *et al.*, 2013; Okoye *et al.*, 2016 and Gumpinger, 2016). It was also recorded from many Ethiopian water bodies such as Koka reservoir, Yemlo ponds, Babogaya, Tana Lake and Small Abay Lake (Yimer and Eniyewu, 2003; Tadese, 2009; Florio *et al.*, 2009; Gulilat *et al.*, 2013; Reshid *et al.*, 2014). The prevalence of *Contracaecum sp.* in Koftu Lake was 53.92% with mean intensity of 5.27 and a prevalence of 17.19 with mean intensity of 5.91 worms per infested fish in Sebeta ponds was. There was no record of this parasite in selected private fish farms. There is a significance difference ($p < 0.05$) in prevalence values of this parasite in the different study sites. This might be attributed to differences in the diversity and availability of zooplankton which serves as intermediate host and fish eating birds to complete its developmental cycle. From our observation, there were many fish eating birds in Koftu Lake and fish in Sebeta ponds were protected by fences and nets over the ponds.

5.6 Acanthocephala

In this study, *A. tilapiae* was recorded in Sebeta ponds and selected private fish farms in Wonchi area. This is could be the first report of the parasite in Ethiopian water bodies.

It was recorded from some of African countries such as Nigeria, Uganda, Kenya and Malawi (Amin and Hendrix, 1999; Otachi, 2009 Florio *et al.*, 2009 and Uhuro *et al.*, 2014). The prevalence of *A. tilapiae* in Sebeta ponds is higher than the prevalence in private fish farms. The results from Sebeta ponds show almost similar values of prevalence compared to 10.2% prevalence of wild tilapia in Uganda and pond fish (7.9%) and wild tilapia (7.1%) from Kenya. which is and respectively. The mean number of *A. tilapiae* per fish is 7.3 which much higher than the one reported by Florio *et al.*, (2009) in Uganda and Kenya which was 1.6 in Ugandan wild tilapia and 2.1 and 2.9 in Kenyan caged and pond tilapia respectively. This difference in mean intensity of the parasites may be explained by the differences in the water quality where the fish is living and the immune status of the fish in each study sites. It may also be associated with abundance of the crustacean, which are intermediate hosts for acanthocephalans. High infestation of fish with acanthocephalans lead to perforation of the gut wall and to wide spread inflammations and peritonitis. It also causes systemic clinical changes in the fish host. Therefore, the pathogenesis of acanthocephalan is caused by the attachment of adult parasites in the gut wall and the larval encapsulation in the fish (Uhuro *et al.*, 2014). A fish infested by high number of parasites may show pathological changes including granuloma and fibrosis of the intestinal wall and may cause obstruction of the gut especially in fingerling fishes which in turn causes retarded growth of the fish (Paperna, 1996).

5.7 Crustacea

Among the crustaceans, *Dolops sp.* was recovered from *O. niloticus* of Sebeta ponds with a prevalence of 7.8 % and mean intensity of 1.0. It was reported in Lake Tana, Lake Awasa, and Lake Babogaya in Ethiopia. A report done by (Tadesse, 2009) indicated that *Dolops ranarum* was recorded from African catfish with intensities of 85 parasites per host in Lake Awassa. This species of crustaceans were also reported from Lake Tana on African catfish (Yimer and Mulualem Enyew, 2003). They were also observed on Nile tilapia from Wonji out of cages, Lakes Babogaya and Awassa but with lower prevalence and intensities ranging from 3.0 to 8.6% (Florio *et al.*, 2009).

5.8 Diversity and richness

The highest parasite diversity was recorded from *O. niloticus* of Kofu Lake with Simpson diversity index of 0.66 and Shanon index of 1.24. There were 6 parasite genera

identified in this study area. The indices in Sebeta ponds are almost similar compared to Koftu Lake with Simpson diversity index of 0.596, Shanon index of 1.23 and 7 parasite groups. This similarity in diversity and richness of parasites in Koftu Lake and Sebeta ponds may probably be the reason that fish are collected and stay in the Sebeta ponds for experiment and acclimatization for short time. The parasite diversity may not be affected by this short duration of stay and not affected by environmental factors which are not different from Lake Koftu. A similar taxonomic parasite species richness in Koftu Lake and Sebeta ponds has been observed when compared with other research carried out in Kenya, Uganda and Ethiopia, (Akoll *et al.*, 2012; Otachi, 2009; Tadesse, 2009). A similar Shanon and Simpson's diversity index was reported in Ethiopia in Babogaya and Wonji cagesby (Tadesse, 2009) in Kenya. Similar diversity index results were also obtained in Baringo Lake, Kenya (Gumpinger, 2016). The diversity index and species richness in selected private fish farms in Wonchi area were lower than in Koftu Lake and Sebeta ponds. This might be due to the availability of higher numbers of fish eating birds which are final hosts especially for the presence of parasites with indirect life cycles. Most of the parasites discovered in the farms were external parasites with direct life cycles. The most dominant parasite in Koftu Lake was the digenean trematode *Tylodelphys sp.*, but in Sebeta ponds and private fish farms, the protozoan *Trichodina spp.* was the dominant one. This might be associated with the higher abundance of piscivorous birds in Koftu Lake. *Trichodina spp.* and *Cichlidogyrus sp.* have direct life cycles and most common in culture condition than natural water bodies.

5.9 Frequency distribution of parasites within the fish host population

Frequency distributions help to explain the dispersion concept of parasites in the host species population. Typically, there are three dispersion patterns: under dispersed, random and over dispersed distributions. These various patterns help to indicate how parasite and host populations interact between each other (Esch & Fernandez, 1993). Fish parasites are usually highly aggregated within their host population, showing overdispersion and a negative binomial distribution. This means, that only a small number of hosts harbour nearly all parasites, showing very high infection rates (Pennycuick, 1971). This variability is caused through heterogeneity in the behaviour and immunity of the host, environmental elements and spatial distribution of development stages of the parasite group. Some species also adopt direct reproduction ways in their hosts (Anderson, 1993). Underdispersed distributions are rarely occurring

in nature conditions, creating different theories. One theory implies a lower possibility of infection due to changes of the environment of a host. This is causing a interrupted host biology, with altered food and habitat selection (Esch & Fernandez, 1993). Nevertheless, many other theories consist, which are explaining underdispersion: parasite mortality, homogeneity and host mortality due to parasite infection (Anderson, 1993).

The distribution patterns for *Cichlidogyrus spp.*, *Tylodelphys sp.* and *Contracaecum sp.* *O. niloticus* for all the study sites showed and over dispersion. This over distribution pattern may be attributed to the special heterogeneity in the distribution of the parasites which consequently be associated with presence or absence of the intermediate and final hosts. This also could affect the distribution of the infective stages of the parasite among the three different study areas (Anderson, 1993). This study agrees with the study conducted in Yemlo, Wonji, Babogaya Lake and Awwassa Lake which showed over dispersion patterns of parasites for *O. niloticus* and *C. gariepinus* (Tadesse, 2009).

5.10 Public Health important parasites

In this study, one genus of nematodes (*Contracaecum sp.*) and two genera of digenean trematodes (*Clinostomum sp.* and *Euclinostomum sp.*) were identified having a potential public health risk. Anisakiasis is a human infection caused by accidental ingestion of the nematodes of anisakid genera such as *Anisakis*, *Pseudoterranova* and *Contracaecum* (Florio *et al.*, 2009). The parasite can migrate to the gastrointestinal tract, peritoneal cavity and various organs. They have potential public health concern and cause serious diseases causing stomach pain, diarrhoea, vomiting and fever (Adams *et al.*, 1997). Some human cases caused by clinostomids have been reported representing public health risk of the parasites (Kakizoe *et al.*, 2004). The digenean metacercariae of *Clinostomum complanatum* has been found to infect human organs following consumption of infected and raw fish food (Aohagi *et al.*, 1992; Park *et al.*, 2009). The infection in human shows a strong pain in the pharyngeal region when the parasites settle at the mucous membrane in the throat (Paperna, 1996). *Clinostomum* infections are more often reported in Asian countries, where fish is prepared raw more often (Chung *et al.*, 1995).

6. CONCLUSION AND RECOMMENATIONS

6.1 Conclusion

A total of 11 genera of external and internal parasites were found in *O. niloticus* of in three study sites. Lake Koftu and Sebeta ponds have almost similar parasite species richness which could suggest the introduction of parasites from natural water bodies to cultured condition through fingerlings. All *O. niloticus* from Lake Koftu harboured single or multiple parasite infestations and had higher parasite diversity than Sebeta and selected private fish farms. Ecto parasites such *Trichodina spp.* and *Cichlidogyrus spp.* were found on fish in all the three study sites but the Digenea *Tylodelphys sp.* was the most dominant parasite in Koftu Lake as there are many fish eating birds around the lake. Generally there was low species richness with high dominance of few parasite groups. *Clinostomum sp.*, *Euclinostomum sp.* and *Contracaecum sp.* were identified having a public health risk by eating raw fish. Results are similar compared to other studies on fish parasites in Ethiopia and East Africa.

6.2 Recommendations

- Fingerlings from Koftu Lake could be a risk to use for stocking small water bodies and fish farms and the hatchery system should be strengthened to avoid the problem.
- Further studies to identify parasites to species level by molecular techniques and parasite genomics of culture fish species.
- Biotic factors like stocking density and abiotic factors like water chemistry and water quality which can influence the abundance of parasites should be taken into consideration in surveys of fish parasites.
- Awareness should be raised about fish born zoonotic parasites and consumers should not eat raw or undercooked fish.

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