Ethiop. J. Agric. Sci. 30(3) 119-128 (2020)

The Prevalence of Fish Parasites of Nile Tilapia (*Oreochromis niloticus*) in Selected Fish farms, Amhara Regional State

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የዚህ ጥናት ዓላማ በአማራ ክልል በተመረጡ የዓሳ ግብርና በሚሰራባቸው አካባቢዎች የቆሮሶ ዓሳ ጥገኛ በሽታዎችን ለመለየት እና ሽፋናቸውን ለማወቅ የተካሄደ ነው። በዚህ ጥናት ውስጥ ሦስት አነስተኛ መጠን ያላቸው የግል ዓሳ ርባታ ማዕከላት እና አንድ በመንግስት ስር ያለ የዓሳ ዘር ማባዣ ማዕከል በጥናቱ ተካተዋል። የቆሮሶ ዓሳ ናሙናዎችም ከተመረጡት የዓሳ ኩሬዎች ውስጥ የተሰበሰቡ ሲሆን ወደ ባህር ዳር ዓሳ እና ሌሎች የውሃ ውስጥ ሕይወት ምርምር ማዕከል ላብራቶሪ ተወስደው ምርመራ ተደርጓል። የጥገኞች ዓይነትም በፓራሲቶሎጅ ላብራቶሪ ሂደቶች እና የመለያ ቁልፎች በመጠቀም ልየታ ተደርጓል። በዚህ መሠረት ስምንት ዓይነት ጥገኞች ማለትም ክሊኖስትቶመም፣ ዲፕሎስቲም፣ ኒያስከስ፣ ኮንትራሴከም፤ ሲክልዶጋይረስ፣ አክንቶሴፋላ፣ አርጉለስ፤ እና ትራይኮዲና የተባሉ ተለይተዋል። ከተሰበሰቡት 198 ናሙናዎች መካከል 73.2 በመቶ የቆሮሶ ዓሳዎች በተለያዩ ውጫዊ እና ውስጣዊ ጥገኛ ተህዋሲያን የተያዙ ናቸው። በክልሉ ውስጥ የዓሳ ጥገኞችን ቦታ እና ጊዜን መሠረት በማድረግ እንዲሁም ህይወት ያላቸው እና የሌላቸው አባባሽ ሁኔታዎችን በማካተት ተጨማሪ ጥናቶች ሊደረጉ ይገባል።

Abstract

This study was conducted to assess the prevalence of fish parasites collected from selected small-scale fish farms in selected zones of Amhara regional state. Three small scale private fish farms and one government owned fish seed multiplication farm were considered in this study. Nile tilapia (Oreochromis niloticus) samples were collected from selected fish ponds in using seine nets and were transported live to the Bahirdar Fishery and Other Aquatic Life Research Center laboratory. Parasites were identified to the genera level with parasitological laboratory procedures and identification keys. Accordingly, eight genera of fish parasites belonging to trematodes (Clinostomum spp, Diplostomum spp., and Neascus), nematode (Contracaecum spp.), monogenea (Cichlidogyrus spp.), Acanthocephala spp., crustacean (Argulus spp.) and protozoan (Trichodina spp.) were identified. Among 198 sampled O. niloticus, 73.2% of the fish were infected by different external and internal parasites. Further studies of fish parasites in the region should be done the considering spatial and temporal or biotic and a biotic factor in disease causations in aquaculture.

Keywords: Fish farms, Parasite, *O. niloticus*, Prevalence, Amhara regional State

Introduction

As an alternative means of achieving food security and poverty reduction in the rural area, aquaculture is recognized and considered as an integral part of rural agricultural development policies and strategies in Ethiopia. Promotion of integrated fish pond farming with agriculture has been considered as the most feasible approaches suggested to improve food and nutrition security in the rural areas (Aschalew *et. al*., 2017). However, much remains to build institutional capacity in the areas of research, technology and training which requires external assistance. Developing aquaculture to its full potential to contribute to food and nutrition security, economic growth, trade and improved living standards is the main emphases in Ethiopia (Shibru Tedla, 2017). This can be achieved by considering all the factors including feed, seed, health, policy considerations for aquaculture development in the country.

Recently, the government of Ethiopia has given emphasis on fisheries and aquaculture as an important sub-sector with potential to contribute to food security, employment creation and poverty reduction. For aquaculture activities, basic inputs are required including fish seed, fish feed, suitable environment, modest capital and market (Aschalew *et al*., 2017). In addition, fish pathogens are one of the factors which affect aquaculture production by causing mass mortalities, reducing market value and posing public health risk. One of the detrimental factors affecting the growth performance of fish in ponds are diseases and specifically parasites (Florio *et al.,* 2009). Except few studies carried in selected small-scale fish farms (e.g. Florio *et. al*., 2009; Marshet *et. al*., 2018), fish parasites are among the least studied in Ethiopian fish farms.

Fish can serve as definitive and intermediate hosts in the life cycles of many species of protozoan, helminthes and crustacean parasites. The parasites usually exist in equilibrium with their hosts as a survival strategy (Bush *et. al*., 1997). Parasitic diseases can spread very rapidly and cause gross mortalities in instances where the hosts are overcrowded in fish farms (Paperna, 1996). Studies indicated that the major productivity loss in aquaculture are caused by parasites (Lester R.J.G., 1988; Shariff M., 1989). Moreover, they can also spoil the appearance of fish and usually affect the marketability of commercially produced fish. Spoiled fishes have no market value and they pose public health concerns especially in areas where raw or undercooked fish is eaten (Paperna, 1996).

The impact exerted by parasites on host could be mechanical, chemical or physical. Effects of parasitic infection on fish are of notable importance, for instance respiratory function of the skin and gills of fish are disturbed by genera G*yrodactylus, Dactylogrus* and argulosis infections, causing the fish to become dull, feeble, frequently swimming to water surface with erratic movement and may die of exhaustion (Hoffman, 2019).. Metacercaria of the trematode *Clinostomum marginatum* are known to cause considerable damage to the viscera and musculature of many fish species both wild and cultivated (Hoffman, 2019). Therefore, the objective of the this study was to determine the prevalence of parasite disease of selected fish farms of Amhara region.

Materials and Methods

Study area

Three small scale fish farms and one government owned fish seed multiplication and research farm were selected. Yewula fish farm consists of 6 fish ponds and the stocked fish include *Tilapia zillii* and *O. niloticus.* The farm is located in the East Gojam zone some 25km far from Debremarkose city. It is government owned farm run under the farmers training center of Amanuel, Yewula woreda agricultural office. Kelti fish farm is a private farm consisting of three earthen ponds and three geo-membran lined ponds. Each pond has an average area of $200m^2$. It is located in west Gojam zone in Durbetie woreda owned privately for production propose.

Mamusha fish farm ponds are located in South Gonder Zone, Dera woreda and each pond has an average size of $150m^2$. The pond area is covered by trees and the tree leaves fall in the ponds continuously. High number of frogs and tadpoles were observed in these ponds. The Bahidar Fishery and Other Aquatic Life Research center (BFALRC) has different ponds for research and seed multiplication purposes. The fish ponds selected for this study serve as are tilapia seed multiplication ponds in the center.

Measurement of Physico-chemical Parameters

The water physico-chemical parameters were measured during the study. Water quality parameter 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 collecting the fish for parasitological investigation.

Fish Sampling

A total of 198 fish specimens were collected from all ponds considered in the study. Fish collected with seine nets were kept in fish tanks filled with pond water and oxygen to transport the fish alive to Bahirdar Fishery and Other Aquatic Life Research Centre laboratory, Bahirdar. In the lab, the fish 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.

Examination and identification of fish for parasites

The presence of parasites was examined from all external and internal organs of fish in the laboratory. The external body surface including scales, gills, fins and operculum of freshly caught fish specimens were examined for the presence of external parasites. Scrapings from the fish skin were taken and smeared onto a clean microscope slide along with a drop of pond water. The sample was then examined under compound microscope on different magnifications (10X, 40X, 100X). 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 fish parasites.

Internally, the fish were dissected and pericardial cavity, mesentery, liver, gonads, body cavity, sites behind the gills and other internal organs were checked for parasites with naked eye and microscope. The inside part of the gut was examined by stereomicroscope and macro parasites were taken out using forceps. The eye balls were taken out using forceps, crushed and examined under the stereomicroscope and compound microscope. The kidneys and livers were also examined for the presence of parasites.

Identification of parasites

Identification guideline based on morphological features of parasites were used for identification in this study (Paperna, 1996, Florio *et al*., 2009); Taxonomic identifications were mostly limited to genus level as the fish harbors mostly larval stages of many of the nematode and trematode parasite and could not be distinguished to species level morphologically (Hoffman, 2019).

Statistical Analysis

The collected raw data was entered in to Microsoft excel data sheets and analyzed using descriptive statistics and percentages were used to summarize the proportion of the infested and uninfected fish. Prevalence was analyzed following the calculations formulated by Bush *et al.* 1997. Prevalence (P) is the proportion of the number of infected hosts over the number of hosts examined (Bush *et al*., 1997).

P= Number of infected fish/Total number of fish examined * 100

Results and Discussion

The results of water quality measurements indicated that the mean values were within the normal and tolerable range required for normal physiological activity of *O. niloticus* (Table 1). All the measured water quality parameters in all the studied pods were within the acceptable range of the cultured *Oreochromis niloticus*. But water temperature recorded was below the recommended range (27-31^{o}C) required for normal good growth performance (Santos *et al*., 2013).

Table 1. Some physico chemical parameters determined from ponds considered in this study

Parameters	Amanuel_Yewula fish farm	Durbetie_kelti fish farm	Hamusite_Mamusha Fish farm	BFALRC_ponds
DO(mg/l)	5.62±0.26	6.90±0.57	4.15±0.78	7.10±0.66
DO (%)	78.03±3.0	102.00±10.60	57.10±10.79	104.00±12.63
pH	7.18±0.05	8.13±0.67	7.19±0.05	7.73±0.80
Temperature (°C)	22.50±0.65	20.33±0.74	18.03±0.66	22.50±0.74
Conductivity(µS/cm)	166.00±4.78	-	260.33±8.37	148.10±18.2
Salinity(mg/L	146.50±4.63	-	144.93±6.29	74.80±10.00

*DO=Dissolved Oxygen

During the study period, a total of 198 *Oreochromis niloticus* were examined in all the study sites. Out of the fish examined, 145 (prevalence=73.2%) were found infested different internal and external parasites with a total of 75 (prevalence=37.9%) fish infected by single parasite genera and 70 (prevalence=35.3%) by mixed parasite (two or more parasites in a single *O. niloticus* host) genera.

Trichodina spp. was identified in all study farms except the BFALRC research ponds and it was previously identified in different fish farms including NFALRC (National Fishery & Aquatic Life Research Center) seed multiplication ponds in Sebeta (37.5%) and Wonchiarea small scale fish farms (53.25%) (Marshet *et al*., 2018).

Trichodinids are the most common ecto-parasites of cultured fish and in some cases cause inflicting heavy damages to stressed host resulting mortality (Woo, 2006). Heavily infected fish may show hypermelanosis for mucus secretion and epithelial sloughing off, with fin erosion frequently due to secondary bacterial infections, and respiratory distress in the case of heavy gill infections. Anyway, massive infections were never detected and infection intensity was always low (Florio *et.al*., 2009).

Table 2. The distribution and prevalence of parasites in different sites of fish ponds in Amhara region

Study sites	No. of fish Total Examined	Total infected	Site prevalence %	Parasites genera	Developmental stage	No. infected_specific parasites	Prevalence (%)	Predilection site/s	Observations
Yewula fish farm	34	2	5.8	*Trichodina* spp	Adult	2	5.8	Gills, skin, Fins	
				Mixed infection	-	-			
kelti fish farm	30	15	50	*Trichodina* spp.	Adult	12	40	Gills, Skin, Fins	mild infestations
				Cichlidogyrus spp.	Adult	9	30	Gills	
				Mixed		6	20		
Mamusha Fish farm	32	32	100	*Neascus* spp.	Larval	32	100	Skin,Gills	Shaded with tree and infested by frogs
				*Cichlidogyrus*spp.	Adult	6	18.8	Gills	
				Trichodina spp.	Adult	2	6.2	Skin, Gills	
				Mixed infection		8	25		
BFALRC_ponds	102	96	94.10	*Clinostomum* spp.	Larval	33	32.4	Brachial Cavity	Multiple infestation is common
				Contracaecum spp.	Larval	60	58.8	Pericardial cavity	
				*Diplostomum*spp.	Larval	7	6.9	Eye/Brain	
				Argulus spp.	Adult	12	11.8	Skin	
				*Acanthocaphala*spp.	Adult	9	8.8	Intestine	
				Cichlidogyrus spp.	Adult	3	2.9	Gills	
				Neascus spp.	Larvae	78	76.5	Skin, gills, fins	
				Mixed infection		56	54.9		
Total	198	145	73.20						

In present study, *Cichlidogyrus* spp of the monogenean dactylogyroids was identified in all sampled ponds except the Yewula fish farm with prevalence of 30%, 18.8%, 2.9% in Kelti, Mamusha and BFALRC fish farms respectively (Table 2). The prevalence is relatively lower than the one reported by Marshet *et al.,* 2019 in NFALRC ponds, Sebeta (33.6%) and Wonchi area small scale fish farms (49.35%). It was identified in from *Oreochromis niloticus Cichlidogyrus cirratus* (Paperna, 1964), C. halli (Price & Kirk, 1967), C. thurstonae (Ergens, 1981) and Scutogyrus longicornis from Lake Tana (Paperna & Thurston, 1969). as cited by (Moges Beletew *et al*., 2016). A study conducted by Florio *et al*. (2009) indicated that Dactylogrids were identified in reservoirs (Koka reservoire) and tilapia cages (Yemlo pond) of Bomosa plots in Ethiopia. Monogeneans are small flattened ectoparasites (Platyhelminthes) attached by characteristic organs (opisthaptor) to the host skin, fins or gills. The genus *Cichlidogyrus*, infecting the gills, is host more specific to diverse cichlid fish species from Africa (Paperna, 1996). According to Mdegela, *et.al*., (2011), poor environmental and management factors including overcrowding of fish into culture ponds or tanks promote heavy infestations consequently lead to productive losses, tissue damages and in some cases mortality.

Black spot metacercaria of the digenetic trematode was the most prevalent parasite in Mamusha fish farm and BFALRC fish ponds with 100% and 76.5% respectively (Table 2). The prevalence of this trematode is much lower than study conducted by Marshet *et al*., (2018). This variation may be attributed to the presence of intermediate and definitive hosts (Paperna, & Dzikowski, 2006) and water quality parameters in the study water bodies. Black spot metacercaria of the digenetic trematode was not identified in fish sampled from Yewula and Kelti fish farms. The high prevalence in BFALRC fish ponds might be due the fact that the vicinity of the farm is close to Lake Tana where piscivorous birds are more prevalent. In addition, the farm is located at the shore of Lake Tana where fishermen landed their fish and gutting activity is conducted. From our observation, fish ponds were infested by snails which are known to be intermediate hosts of the trematode parasites. Fish examined at the Mamusha fish farm were all infested by the digenean Black spot metacercaria. This high prevalence might be due to presence of high number of snail intermediate hosts in the ponds. The snails were abundant due to high organic load of the ponds from leaf droppings of the trees shading the ponds. The parasite mainly attack skin, gills and fins of the fish. Black spot metacercaria were identified in tilapia cages of BOMOSA plots in Ethiopia with low prevalence and intensity (Florio *et. al*. 2009). *Neascus* sp., *Posthodiplostomum* sp. and *Bolbophorus* sp. metacercariae are the most frequently reported in Africa forming “black spots” in fishes. The skin, fins, muscle, in visceral districts and gills are the predilection sites where black spot metacrcaria parasites encysted on the fish. The black spot appearance is the result of cysts consolidation around these metacercariae incorporate dermal melanophores and other chromophores. Even if infestations of these parasites causes relatively little damage to the fish, there is some evidence that heavily infected juvenile fish may suffer stress, weight loss and even death (Khan, 2012).

The digenetic trematode *Clinostomum* spp. metacercariae were found in BFALRC ponds with prevalence of 32.4%. This parasite is very commonly encountered in many natural water bodies including Lake Tana, Lake Lugo, Lake Koka, Gibe Reservoir, Lake small Abay (Eshetu & Mulualem, 2001; Amare, 2014; Yewubdar *et al*., 2009; Marshet *et. al.*, 2018; Reshid *et al*, 2016). As the distribution of clinostomids in fish follows the diffusion of the suitable first intermediate host (gastropods) and definitive host (several piscivorous birds), it was encountered in BFALRC which is close to Lake Tana but not identified in other studies fish farms. High infestation of fish by metacercarial parasites have sometimes resulted in mortalities of young fish and cutaneous infections are reasons of most of consumer rejections and lower market value because of the low asethetic value from the infestation (Florio *et al*., 2009). In addition, according to Baird *et al*., *(*2014), reports of human infections from clinostomids have been pointed out the potential zoonotic role of these parasites.

Only 6.9% of the Nile tilapia was found infected with Diplostomum species in BFALRC ponds but not in other study farms. This could also be attributed to the vicinity of the farm to Lake Tana where high fauna of intermediate and definitive host are found. Nile tilapia from Gilgel Gibe reservoir and Lake Koftu were found infected by this parasite (Marshet *et al*. 2018). Studies indicated that *Diplostomum* spp is most common and high intensity parasites in *Clarias garipinus* of Lake Tana (Zhokhov *et al*., 2010). Eye infections by Diplostomatid metacercariae have impact on feeding activity of the fish that consequently inhibit productivity and growth rate.

The nematode parasite *Contracecum* spp. was identified in BFALRC fish seed multiplication ponds with prevalence rate of 58.8%. The result is relatively higher than the one studied by Necho & Awake, (2018) and Shigut, & Arefaini (2014). with prevalence of *Contraceacum* spp. being 49.5% & 339.9% respectively in Nile tilapia of Lake Tana. This indicates that the parasites are intensified when it is in culture condition (BFALRC fish ponds) than in the natural water condition (Lake Tana). According to Florio *et al*., 2009, prevalence of pericardial *Contracaecum* infection among tilapia in a contaminated pond often reaches 100%. The larval nematode *Contracacum sp*. (Railliet & Hennry, 1912) from Nile tilapia was found coiled in the pericardial cavity and also sometimes found dispersed in the abdominal cavity.

In conclusion, a total of eight genera of external and internal parasites were identified in *O. niloticus* specimens collected from the study fish ponds. Most of the fish has infected by mixed parasites which could negatively affect the health and productivity of the fish in the farms. The authors recommend further detailed studies which consider the source water for the farms and the stocking density of the fish combined with identification of parasites to species level by molecular techniques are required for determining the impact of parasite on fish farms.

Acknowledgments

The Authors would like to acknowledge the Ethiopian Institute of Agricultural Research (EIAR), National Fishery and Aquatic Life Research Center (NFALRC) for funding this study. We also appreciate and thank the research staff of the Bahirdar Fishery and Other Aquatic Life Research Center (BFALRC) of the Amhara Region Agricultural Research Institute (ARARI) for collaborating in data collection and providing research facility.

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