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Some biological aspects and external parasitic infestation of fish species in Upper Awash River, West Showa Zone, Ethiopia

Bacha Temesgen G.[1]; Zenebe Tadesse S.[2]; Mathewos Temesgen K.[3*]

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Abstract

A fishery is one of the important agricultural sectors for food security in Ethiopia. The resources are however highly decreasing due to unwise use and lack of scientific information for management. This study was aimed to assess some biological aspects and the external parasite infestation of fishes collected from the upper Awash River, Ethiopia. Fish samples were collected from five randomly selected sites using Frame net of 3mm mesh size. Three fish species belonging to the Family Cyprinidae namely: Garra quadrimaculata, Garra dembeensis and Labeobarbus beso were identified. Dietary composition, GIS, fecundity, Length–Weight relationship, Fulton's condition factor, sex ratio and ectoparasite infestation were assessed for the sufficiently collected fish species. The dietary composition revealed that G. quadrimaculata and L. beso were omnivorous. There was a significant correlation between the fish length and weight (r2=0.915 for G. quadrimaculata and 0.912 for G. dembeensis) with an allometric growth. The mean K value of females and males of G. quadrimaculata and G. dembeensis ranged between 1.31-1.36, 1.23-1.27 and 1.26-1.91, 1.35-1.98, respectively. The GSI value significantly varied among the study months. Fecundity of G. quadrimaculata ranged from 273 to 1056 eggs per fish with mean value of 692±270 eggs. There was a preponderance of females over males in most of the study months. The overall prevalence of Neascus (black spot) parasite was 18.09% and the intensity was highest (22 per individuals) on females. Less animal based food composition, poor body condition, early arrival at L50, less fecundity and the high infestation of Neascus parasite were the major problems identified in this study. Thus, effective management of the river basin and stocking the species into the aquaculture is very imperative for sustainable utilization of fish resources.

Keywords: Upper Awash River; External parasite; Biology of garra; Fish species.

1-Department of Fisheries and Aquatic Sciences, Wollega University, Shambu Campus, Shambu, Ethiopia

2-National Fisheries and Aquatic Life Research Center, Sebeta, Ethiopia

3-Department of Biology, College of Natural and Computational Science, Ambo University, Ethiopia

*Corresponding author email: mathewos_temesgen@yahoo.com

Introduction

Rivers and streams show signs of surprising phenomena with physical, cultural and psychological expression in life of human beings; they bring life and death, civilization and devastation, opportunity and risks and also used as shelter and feeding opportunities for a wide range of organisms like fish, insects, plants, mollusks, birds and mammals (Tesfaye, 2006). They carry water from the mountains to the sea, fueling the water cycle coupling land, ocean and the atmosphere (Aufdenkampe et al., 2011). They are also the most suitable media to clean, disperse, transport and dispose of wastes from domestic and industries, drainage and irrigation to the water bodies and change the appearance and quality of water bodies (Meybeck et al., 1996). River systems throughout the world have been however disturbed by a wide variety of anthropogenic activities which ultimately affects the biological aspects of fish (Welcomme, 1995; Madejczyk et al., 1998).

Ethiopia is endowed with numerous water bodies including lakes, rivers, reservoirs and some wetlands. It has about 7334 km2 of major lakes and reservoirs, and about 8065 km length of rivers within the limits of Ethiopia (Tesfaye and Wolff, 2014). Though Ethiopia is presently separated from both East African and South Arabian mountains (Tudorancea et al., 1999), two main biogeography units called Nilo-Sudan and East Coast ichthyofaunal provinces are in contact with this region (Vijverberg et al., 2012). This made the freshwater fish fauna of Ethiopia to have a mixture of Nilo-Sudanic, East African and endemic fish forms (Getahun, 2005). Baro Akobo Basin is the richest in fish diversity, which could be attributed to the presence of diverse and rich habitats, and past and present connection with other systems (east and central Africa) (Getahun, 2002).

Fish species of the high mountains torrential streams are largely Cyprinidae, which adapted to live in the swiftly flowing floodwaters that occurs seasonally (Mostafavi et al., 2021; Zamani-Faradonbe et al., 2021). Abebe (2016) recently reported five Cyprinidae species from the Upper Awash River namely: G. quadrimaculata, G. dembecha, G. herticceps, large barbus and small barbus species. Of these, G. quadrimaculata and G. dembecha were the most abundant fish species in the basin (Temesgen et al., 2021).

Studies on the biology of fish are very important to obtain information on the quantity and quality of available habitat (Mirghaed et al., 2021). Moreover, information on length-weight relationship, sex ratio, size at sexual maturity, disease prevalence and condition factor provides basic knowledge for the proper management of the resources in a sustainable way (Abera et al., 2015). The studies focusing on the biological aspects of small riverine fisheries are very rare, probably due to the perception that fisheries of small rivers are not economically viable. Virtually, all studies of riverine fish fauna have focused solely on the patterns of fish species distribution and abundance in the rivers (Getahun and Stiassny, 1998; Golubtsov and Mina, 2003; Getahun, 2005; Tesfaye and Wolff, 2014; Abebe, 2016). In recent years, studies on the riverine fish have gotten little attention by some researchers and the resources are being exploited without enough knowledge on the biological aspects of the fish (Abebe, 2016; Garomsa, 2017).

Along with the growing interest in the development of fish industries, it is also important to increase the awareness on the importance of fish diseases in fish culture (Assefa and Abunna, 2018). The major diseases associated with fish are parasites, bacteria, viruses and toxic algae that reduce fish production by affecting the normal physiology (Ayotunde et al., 2007). It is usually the parasitic infections that predispose fish to secondary infections like fungal, bacterial and viral disease through inflicting different kinds of external or internal damages such as irritation, injury or atrophy of tissues, and occlusion of the alimentary canal, blood vessels or ducts (Iyaji and Eyo, 2008; Gebreegziabher et al., 2020). This damages in turn pave the way for different factors like introducing of toxic metabolic by-products that produce changes in the blood, enzyme, vitamin and/or hormone activity of the host, depriving the fish, acting as vectors of other pathogens and providing a point of entry for other pathogens through mechanical damage. They can also spoil the appearance of fish and usually affect the marketability of commercially important fish species, thus raising public health concerns especially in areas where raw fish is eaten (Gebreegziabher et al., 2020). Understanding the etiology of parasitic diseases is therefore very important as it determines the choice of a potential treatment (Bihonegn and Tilahun, 2017). The information on the fish health is however highly constrained and needs to be addressed for the improvement of the fishery sector in Ethiopia. This study was therefore initiated to fill the scientific gap on some biological aspects and external parasites infestation of fish species in upper Awash River, west Showa zone, Ethiopia.

Materials and methods

Description of the study area

The study was conducted at the Upper Awash River Basin in Dandi District, which is located 75 km away from the capital Addis Ababa toward the west direction in West Shoa Zonal Administration, Oromia Regional State, Ethiopia. Dandi District has a total area of 109,729 ha with an altitudinal range from 2000-3200 m.a.s.l. It has a tropical climate condition. The mean temperature and rainfall of the area is 16.5oC and 1409.5 mm, respectively (Dandi District RDO, 2010, unpubl. data). The rainfall distribution is bimodal: a short rainy season from March to April and the main rain season from June to September with mean annual rainfall of 1100 mm.

The district is well known for its richest water resources like Awash River and Dandi Lake. Awash River Basin covers a catchment area of 110,000 km2 and serves as home for 10.5 million people. Based on physical and socio-economic factors, Awash Basin is divided into Upland (all lands above 1500 m a.s.l.), Upper Valley, Middle (area between 1500m and 1000m a.s.l.), Lower Valley (area between 1000m and 500m a.s.l.) and Eastern Catchment (closed sub - basin between 2500m and 1000m a.s.l.). The river is perennial, the water level and flow rate decline drastically during the dry season. The discharge fluctuates concurrently with the rainfall intensity. The maximum volume of the river is in the months from July to September; it reaches its peak in August (16.3m/s) and lowest in December (0.16m/s). Catchment of the river is entirely used for mixed agricultural activities (Shawul and Chakma, 2020).

Study design and sample site selection

Preliminary survey was made in December, 2017 in order to have the topography and anthropogenic activities information in the basin for sample site selection. Based on physical, chemical, biological and land-use information obtained during the observation, five sampling stations were selected for primary data collection. The sampling stations were selected along the longitudinal zonation of the river and designated as; Station I (Galessa), Station II (Arera), Station III (Anjory), Station IV (Walgata) and Station V (Osole). Sampling stations were classified following the rapid bioassessment protocol criteria based on major stressor around the river (Barbour et al., 1999).

1. Station I (S-I): this site is located at upper Awash River at the base of Galessa Stream where there is less human settlement and other activities and few domestic animals sometimes grazing in the area. There is natural vegetation around the station. The Galessa Stream is located in the Gare Arere peasant association in Chillimo forest. Activities such as grazing and deforestation are not common and water is comparatively less polluted in this area. The site is considered as a reference site.

2. Station II (Arera): this is located at the confluent point of Arera and Warabbo streams which are found in the Gare Arere peasant association. At this station, there are some natural vegetation and scattered eucalyptus trees which are planted at the river bank. There is a scattered human settlement nearby and natural and artificial forest. Activities such as agriculture, grazing and bathing are common in this site. This site is relatively less impacted as compared with the remaining three stations.

3. Station III (Anjory): this is located in Arere Kure peasant association, at the back of China camp close to Ginchi town below the point where Kerensa Stream joins Awash River. This site is totally surrounded by grazing lands. The structural habitat of the river is dominated by mesolithic and macrolithal stones at riffle, and by sand and mud at the pool area of the site. Anthropogenic activities such as farming and grazing, cattle watering, bathing, car and washing are common practices in the area. This area is highly impacted as compared to S-I and S-II.

4. Station IV (Walgata): this is located below the paper mill Wamara Saqo peasant association, below the town and is polluted by the effluent discharged from the paper mill industry and from Ginchi town. The area is highly covered with immense agricultural activities and no vegetation coverage at the river bank. Anthropogenic activities such as farming and grazing, herbicide spraying, washing clothes, cars and bathing are also common activities and the site is considered as highly impacted. The structural habitat of the river is dominated by mesolithal at riffle; and sand and mud at pool parts of the site.

5. Station V (Osole): this is located one to two km away from the fourth site, which is totally

surrounded by agricultural activities. It was characterized by high human settlement from the right side and Ginchi town from the left side.

Collection of fish samples

Fish samples were collected monthly between February and April, 2018 from each site (pools and riffles) using frame net mesh size of 3 mm with total area of 4.6m2. This frame net was pulled by two persons from each side of the net end from downstream to upstream to collect fish from the pool of each station. To collect fish from riffle, a frame net was fixed at the downstream of the selected riffle of each site held by two persons and the third person disturb the fish from upstream of that riffle chasing the fish towards the fixed frame net. The collected fish samples were preserved in 5% formalin and identified to the family and species level in Biology laboratory, Ambo University with the help of standard reference keys (Habteselassie, 2012). The fish samples were measured for their total length and total weight using measuring board and sensitive balance, to the nearest to 0.1 cm and 0.1 gm, respectively.

Collection and Analysis of Fish Guts

The live fish spacemen were dissected and the guts of fish were removed. G. quadrimaculata for its dominance and L. beso for its economic importance was considered for guts analysis in this study. The identification was made to the possible lowest taxa level using different identification keys (Belcher and Erica, 1978; Shiel et al., 1998,). Analysis of the gut contents was carried out using numerical and frequency of occurrence methods (Hynes, 1950; Hyslop, 1980; Ugwumba and Ugwumba, 2007) at the National Fishery and Aquatic life Research center (NFALRC) Laboratory, Sebata and Biology Department, Ambo University, Ethiopia.

Then, the relative abundance of each food items was calculated as:

$$\text{Relative Abundance} = \frac{\text{Total number of particular food item}}{\text{Total number of food items}} \times 100$$

$$\text{Frequency of Occerunce} = \frac{\text{Total number of guts with particular food items}}{\text{Total Number of guts with food items}} \times 100$$

Length-weight relationship and condition factor

The relationship between total length (TL) and total weight (w) of the two dominant fish species (G. quadrimaculata and G. dembeensis) was also calculated using the equation given below for separate and combined sex for each station and month (Gayanilo and Pauly, 1997).

$$TW = aTL^b$$

Where TW = Total weight of fish in g, TL = total length of fish in cm, a = constant (intercept) and b = the length exponent (slope)

Fulton's condition factor (K) was used to compute the fish condition factors (Htun-Han, 1978.) for G. quadrimaculata and G. dembeensis, which was calculated as:

$$K = \frac{TW}{TL^3} X100$$

Where K= is the Fulton Condition Factor or Coefficient of Condition; often referred to as the "K factor," TW= is the weight of the fish in g and TL= is the length of the fish in cm.

Sex ratio and Gonadosomatic index

Similarly, the gonadosomatic index of dominant species: namely, G. quadrimaculata was calculated monthly using Wooten (2012) formula as:

$$GSI = \frac{GW}{TW} * 100$$

Where: GSI = gonadosomatic index; GW = gonad weight; TW= Total weight in g

Sex ratio was also calculated for G. quadrimaculata and G. dembeensis using the following formula:

$$\text{Sex ratio} = \frac{\text{Number of females}}{\text{Number of males}} * 100$$

Length at first sexual maturity

The average length at first sexual maturity (L50) was calculated for G. quadrimaculata. First, the fish were grouped by length class, and the percentages of male and female fish with mature gonads were plotted against total length to estimate L50 (Tweddle and Turner, 1977).

Examination of fish fecundity

Fecundity was determined by counting all eggs in ripe ovaries that was preserved in Gilson's fluid (Hunter et al., 1985). For this purpose, only one dominant fish species: G. quadrimaculata was considered by selecting all ripe ovaries with the maturity level IV(maturity of female fish at which all eggs are ripe and larger in size than stage three which is a mixture of small and large size eggs). The relationship of fecundity rate with total length, total weight and ovary weight were calculated using Bagenal and Tesch (1978) method as:

$$F = aTL^b = aTW^b = aGWt^b$$

Where, F= Fecundity, TL= total length (cm), TW= Total weight (gm), a= constant, b= exponent

Examination of fish parasite

Live fish species were packed in polythene bags containing water and oxygen. The fish species were then transported to the National Fishery and Aquatic Life Research Center, Sebeta for investigation of parasitic infestation. The fish samples were examined for the presence of live external parasites in the infected organs for confirmatory diagnosis using stereo microscope for the whole fish species followed by compound microscope by crashing the scraped sample on the skin. Skin scraping was followed by wet smear preparation to identify the live metacercaria. Counting of parasites was done under stereomicroscope and unaided eye for the presence of the black spot metacercaria on the external surface of the fish's skin, fins, operculum and gills. The total number of parasites infected the fish species was counted to estimate the infestation intensity. Epidemiological parameters were made based on infestation parameters of the parasites such as prevalence and intensity (Bush et al., 1997).Prevalence (P) is the proportion of the number of infested hosts over the number of hosts examined.

$$\text{Percent of prevalence} = \frac{\text{Number of fish infected}}{\text{Number of fish examined}} \times 100$$

Mean intensity of the parasites (MI) which expressed in number was calculated as:

$$\text{Mean intensity} = \frac{\text{Total numberof parasites counted}}{\text{total number of infested fish}}$$

Data analysis

The data collected were filled in MS Excel. Descriptive statistics such as percentage, mean, and standard deviation were used to analyze descriptive data such as fish gut contents, condition factors, FCF, fish fecundity, and black spot metacercaria infestation level. Furthermore, inferential statistics such as regression analysis and the chi-square test were performed using SPSS version 21. The regression analysis was used to determine the relationship between the fish's length and weight, total length and fecundity, total weight and fecundity, and gonad weight and fecundity. The Chi-square test was also used to determine the significance difference in fish sex ratio at the 0.05 confidence level.

Results

Gut content of Garra quadrimaculata

A total of 206 guts of G. quadrimaculata were examined. Of which, 87% (n=179) guts contained food items, whereas 13% (27) guts were empty. The gut of G. quadrimaculata contains different food types like detritus, sand grain, different genera of phytoplankton, zooplankton, and annelids. Of these, phytoplankton, detritus and sand grain were the most frequent and numerically abundant food items in the guts of most examined fish. Generally, a total of 35 phytoplankton taxa (20 genus of Bacillariophyceae, 9 genus of Chlorophyceae, five genus of Myxophyceae and 1 genus Euglenophyceae), 3 genus zooplankton and 1 genus of annelid were identified from the gut contents. Bacillariophyceaewas the most frequently occurring phytoplankton in the guts contents of G. quadrimaculata (80.11%) followed by Chlorophyceae (12.87%) and Myxophyceae (4.92%).Navicula (58.06%), Cymbella (50%), Nitzschia(43.55%), Cyclotella(33.87%), Pinnularia (27.42%) and Synedra (24.19%) comprised the most frequently occurred Bacillariophyceae in the guts contents. Similarly, Spirogyra (27.42%), Ankistrodesmus (17.74%) and Closterium (11.29%) were the most frequently observed genus of Chlorophyceae, whereas Anabaena (25.81%) and Microcystis (19.35%) were the most frequently occurred Cyanobacteria in the guts contents. Zooplankton like Copepoda, Rotifera and Daphnia were however rarely occurring in the guts contents and Oligochaete was the only annelid rarely occurring food item in the gut contents.

In terms of numerical abundance, detritus and sand grains comprised the highest total number of food items. Numerically, 80.11% of the food items in the gut were composed of different genera of phytoplankton where Bacillariphyceae takes the largest share. Among Bacillariophyceae, 20 diatom taxa were identified from the gut contents. Navicula, Cyclotella, Cymbella,Pinnullaria, Nitzschia, Diatoma, Synedrawere the most numerically abundant diatoms in the gut contents. Chlorophyceae was the second most abundant phytoplankton food (constituted about 12.87%) while Myxophyceae and Euglenophyceae were the least abundant (4.92 and 1.28% by number, respectively). Spirogyra and Anabaena were the most abundant genus of Chlorophyceae and Myxophyceae, respectively. Numerically,Oligochaete accounted for only 0.40 % of the gut content. Copepods, Rotifers and Daphnia were the zooplankton food items identified in this study, which takes the least abundance (0.45%) in the gut of G. quadrimaculata (Table 1).

Table 1: Frequency of occurrence and Numerical abundance of food items identified from the guts of G. quadrimaculata in Upper Awash River from February to April 2018.

Food items	Frequency	% of occurrence	Number	% of number	Abundance
Phytoplankton					
Bacillariophyceae-Diatoms				**80.11**	
Navicula	104	58.06[a]	12811	23.86	Abundant
Cymbella	89	50.00[a]	8403	15.65	Abundant
Nitzschia	79	43.55[a]	5656	10.53	Abundant
Cyclotella	61	33.87[a]	3430	6.39	Abundant
Pinnularia	49	27.42[c]	2672	4.98	Common
Synedra	40	24.19[c]	2864	5.33	Common
Gomphonema	37	22.58[c]	1233	2.30	Common
Fragilaria	17	20.97[c]	705	1.31	Common
Diatoma	29	16.13[c]	2427	4.52	Common
Tabelaria	20	11.29[r]	1020	1.90	Rare
Amphora	14	8.06[r]	539	1.00	Rare
Eunotia	12	6.45[r]	346	0.64	Rare
Cocconies	9	4.84[r]	166	0.31	Rare
Melosera	9	4.84[r]	85	0.16	Rare
Cymatopleura	9	4.84[r]	176	0.33	Rare
Surirella	6	3.23[r]	136	0.25	Rare
Rhizosolenia	3	1.61[r]	88	0.16	Rare
Aulacosera	3	1.61[r]	149	0.28	Rare
Astreonella	3	1.61[r]	5	0.01	Rare
Gyrosigma	3	1.61[r]	112	0.21	Rare
Chlorophyceae-Green algae				**12.87**	Rare
Spirogyra	49	27.42[c]	2693	5.01	Common
Ankistrodesmus	32	17.74[c]	1014	1.89	Common
Closterium	20	11.29[r]	816	1.52	Rare
Cladophora	17	9.68[r]	733	1.36	Rare
Microspora	14	8.06[r]	739	1.38	Rare
Zygnema	9	4.84[r]	149	0.28	Rare
Ulothrix	9	4.84[r]	502	0.93	Rare
Crucigenia	6	3.23[r]	79	0.15	Rare
Pediastrum	4	1.94[r]	185	0.34	Rare
Myxophyceae-Cynobacteria				**4.92**	
Anabeana	46	25.81[c]	1934	3.60	Common
Microcystis	35	19.35[c]	305	0.57	Common
Aphanizomenon	17	9.68[r]	328	0.61	Rare
Achananthes	14	8.06[r]	64	0.12	Rare
Oscillatoria	3	1.61[r]	9	0.02	Rare
Euglenophyceae				**1.26**	
Phacus	9	12.90[r]	674	1.26	Rare
Zooplankton				**0.45**	
Daphnia	17	9.68[r]	138	0.26	Rare
Copepods	9	4.84[r]	60	0.11	Rare
Rotifer	46	4.84[r]	42	0.08	Rare
Annelids				**0.40**	
Oligochaete	14	9.68[r]	216	0.40	Rare
Others					
Detritus	179	100[a]	Nd	Nd	Abundant
Sand grain	179	100[a]	Nd	Nd	Abundant

Abundant= indicates %occurrence> 30, common= indicates %occurrence 15-30), rare= indicates % occurrence ≤15, Nd= Not determined.

Labeoburbus beso

A total of 70 guts of L. beso were examined. Of these, 84.29% (n=59) contained food in their guts and the remaining 15.71% (n=11) were empty. L. beso also feed on a variety of food items including phytoplankton, zooplankton, detritus, sand grain and insects; from which, phytoplankton, detritus, and sand grain were the most frequently occurred and numerically abundant food items in the gut contents. Generally, a total of 32 taxa (29 taxa of phytoplankton, 1 taxa of zooplankton, 1 taxa of annelid and 1 taxa of insect) were identified from the gut contents.

Similar to G. quadrimaculata, detritus, sand grains and phytoplankton were the most frequently and numerically abundant food items consumed by L. beso. Bacillariophyceae (diatoms) was the most frequently occurring (79.63%) phytoplankton food item in the gut of L. beso followed by Chlorophyceae (12.09%) and Myxophyceae (Cyanobacteria) (7.44%). Genus Navicula (60.71%), Cyclotella (50.00%), Cymbella(39.29%) and Nitzschia (32.14%) from Bacillariophyceae (diatoms), and genus Anabaena (42.86) from Cyanobacteria were the most frequently occurring food items in the guts contents. Copepoda (21.43%) and Oligochaete (17.86%) were the only zooplankton and annelids rarely consumed food items by L. beso.

Detritus and sand grains were the most numerically abundant food items in the gut contents followed by the phytoplankton. Of them, Bacillariophyceae was the most abundantly consumed food item. Among the Bacillariophyceae, the genera such as Navicula 27.57% (n=7506), Cymbella 16.58% (n=4515), Diatoma 9.48% (n=2580), Cyclotella 8.54% (n=2325), and Nitzschia 4.75% (n=1292), and from Myxophyceae genus Anabaena (4.23%) were the most numerically abundant food items in the gut contents of L. beso. The rest of the food items were the occasionally ingested food items (Table 2).

Table 2: Frequency of occurrence and Numerical abundance of food items identified from the guts of L. beso in Upper Awash River from February to April 2018.

Food items Group/genus	Frequency	% of occurrence	Number	% of number	Abundance
Phytoplankton					
Bacillariophyceae-Diatoms				**79.63**	
Navicula	36	60.71	7506	27.57	Abundant
Cyclotella	29	50.00	2325	8.54	Abundant
Cymbella	23	39.29	4515	16.58	Abundant
Nitzschia	19	32.14	1292	4.75	Abundant
Eunotia	17	28.57	614	2.26	Common
Diatoma	15	25.00	2580	9.48	Common
Synedra	13	21.43	573	2.10	Common
Pinnularia	13	21.43	593	2.18	Common
Gomphonema	10	17.86	384	1.41	Common
Fragilaria	8	14.29	140	0.51	Rare
Tabelaria	8	14.29	602	2.21	Rare
Melosera	13	14.29	47	0.17	Rare
Amphora	6	10.71	89	0.33	Rare
Rhoicospenia	5	7.14	50	0.18	Rare
Aulacosera	4	7.14	108	0.40	Rare
Cocconies	4	7.14	51	0.19	Rare
Uronema	4	7.14	61	0.22	Rare

Cymatopleura	4	7.14	57	0.21	Rare
Gyrosigma	4	7.14	93	0.34	Rare
Chlorophyceae-Green algae				**12.09**	
Spirogyra	17	28.57	1576	5.79	Common
Ankistrodesmus	13	14.29	381	1.40	Rare
Cladophora	8	14.29	715	2.63	Rare
Closterium	6	10.71	104	0.38	Rare
Zygnema	6	10.71	472	1.73	Rare
Pediastrum	4	7.14	43	0.16	Rare
Myxophyceae-Cynobacteria				7.44	
Anabeana	25	42.86	1151	4.23	Abundant
Microcystis	17	28.57	288	1.06	Common
Oscillatoria	10	17.86	379	1.39	Common
Achananthes	2	3.57	23	0.08	Rare
Euglenophyceae				**0.68**	
Phacus	6	10.71	184	0.68	Rare
Zooplankton				**0.36**	
Copepods	13	21.43	98	0.36	Common
Annelids				**0.33**	
Oligochaete	10	17.86	89	0.33	Common
Insects					
Batidae	4	7.14	44	**0.16**	Rare
Detritus	59	100	Nd	Nd	Abundant
Sand grain	59	100	Nd	Nd	Abundant

Abundant=indicates % occurrence> 30, common= indicates %occurrence; 15-30, rare= indicates %occurrence≤15, Nd= Not determined

Length-Weight relationship

The length-weight relationships of the two numerically dominant fish species; namely, G. quadrimaculata and G. dembeensis were calculated based on their total length and total weight. The total length and total weight of the G. quadrimaculata ranged from 4.4 to 13 cm and 1 to 26 g, respectively, whereas the total length and total weight of G. dembeensis ranged from 4.4 to 12.8cm and 1 to 21 g, respectively. The length-weight relationships were curvilinear and highly correlated for both species (r2= 0.915 and r2=0.912, respectively). The equations derived for G. quadrimaculata and G. dembeensis were well fitted for both species. Length-weight relationships of both sexes were also calculated separately for the two species. The b values of both species were less than three (b<3), which indicates that both fish species exhibited negative allometric growth (Fig. 1 & 2).

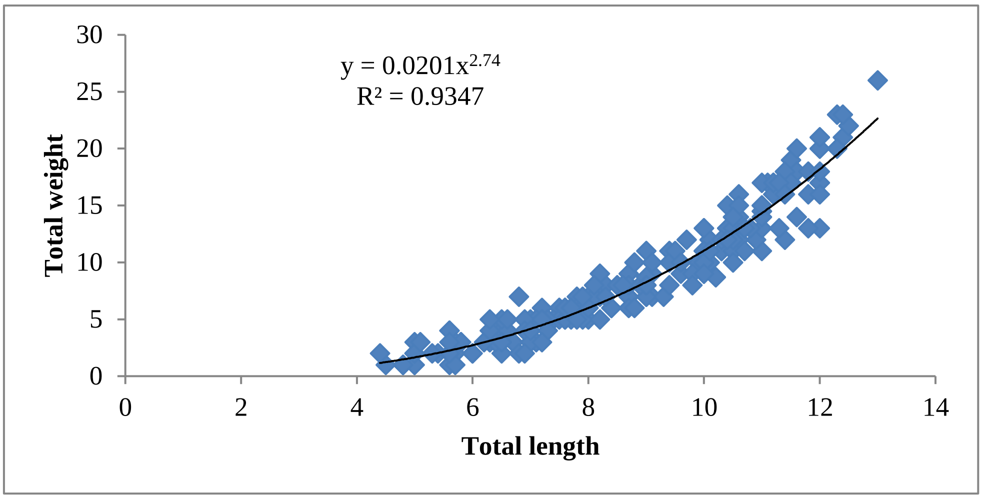


Figure 1: LWRs for combined sexes of G. quadrimaculata in the upper Awash River

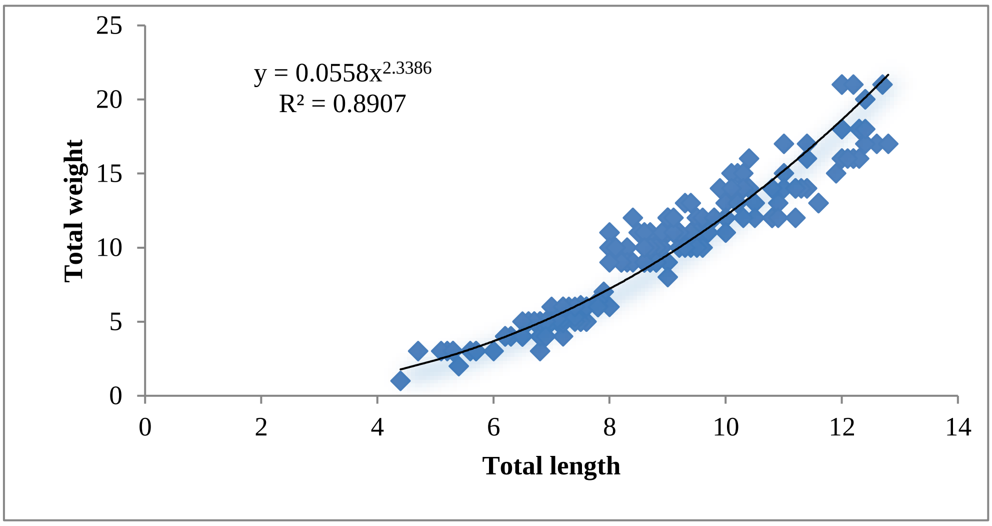


Figure 2: LWRs for combined sexes of G. dembeensis in the upper Awash River

Condition Factor

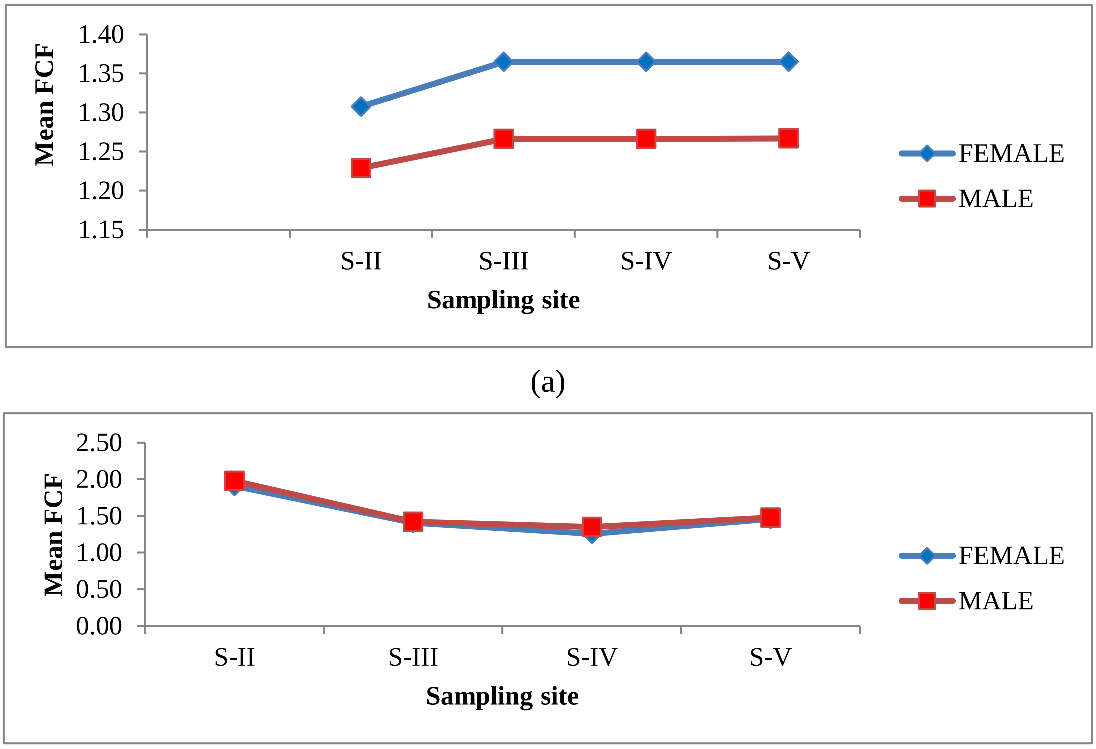
The FCF of G. quadrimaculata ranged from 0.53 to 3.98 with a mean K value of 1.23±0.39. The highest K value was recorded at S-II followed by S-V, but the lowest mean value was measured at S-III. In other ways, the mean condition factors of G. dembeensis ranged from 0.54 to 3.77 with the mean k value of 1.34±0.38. The mean condition factor for G. dembeensis was highest at S-II then slightly decreased up to S-IV and showed the sign of increment at S-V (Table 3). The result showed a significant difference of FCF among the study sites for both fish species (p=0.000).

Table 3: Number of fish collected, mean, maximum, minimum and standard deviation of K of G. quadrimaculata and G. dembeensis in the upper Awash River from February to April 2018.

Sites	Number of fish	mean of K	Min	Max	SD of K
G.quadrimaculata					
S-II	180	1.26	0.66	3.99	0.42
S-III	338	1.17	0.57	2.71	0.41

S-IV	73	1.20	0.75	2.35	0.35
S-V	129	1.24	0.54	2.26	0.37
G. dembeensis					
S-II	12	1.74	1.44	2.97	0.55
S-III	122	1.28	0.75	2.71	0.29
S-IV	128	1.29	0.51	2.26	0.32
S-V	95	1.42	0.79	3.77	0.49

Females of G. quadrimaculata had the higher FCF at all stations (p= 0.002) (Fig. 3a), whereas males seem to have higher FCF for G. dembeensis but the difference is not significant (p= 0.82) (Fig. 3b).

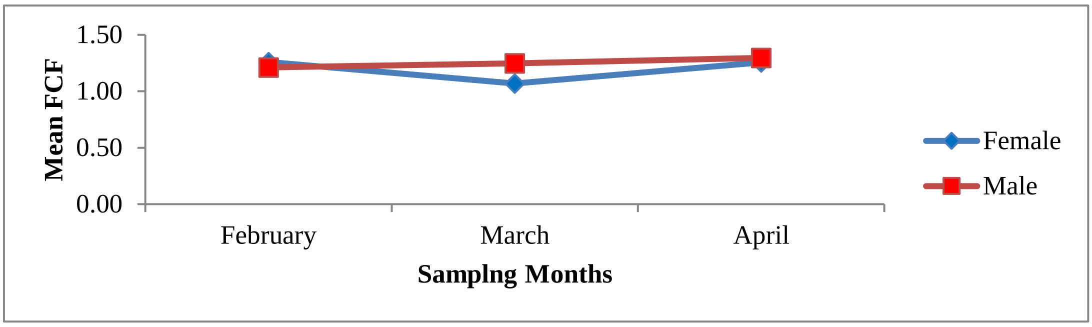


(a)

(b)

Figure 3: The mean Fulton condition factor of females and males of (a) G. quadrimaculata and (b) G. dembeensis among the study sites.

The result also showed that the mean FCF of the two species were higher in February, declined in March and slightly increased in April for both sexes (Fig. 4 a & b).



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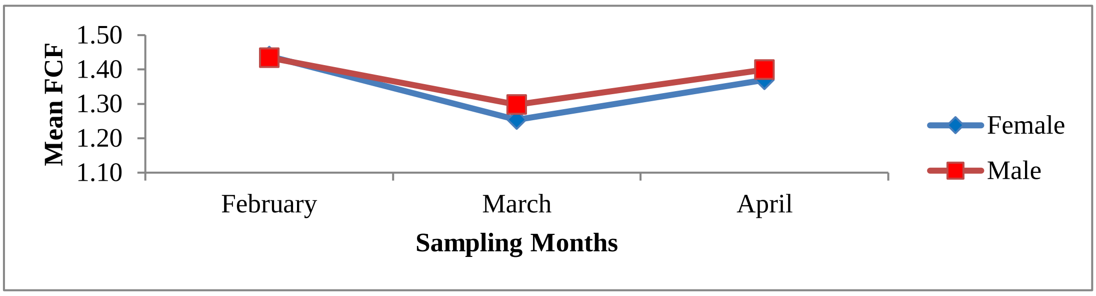

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Figure 4: Variations in mean Fulton condition factor of females and males of (a) G. quadrimaculata and (b) G. dembeensis among the study months at Upper Awash River.

Gonadosomatic Index (GSI), Sex ratio and L50

Gonadosomatic Index

Gonado-somatic index was calculated for 456 of G. quadrimaculata (244 females and 212 males). All fish with maturity levels of two to five were considered for GSI calculation. Comparatively, the highest GSI value was observed in February and there was a considerable decrease in March and slightly increased in April for both sexes (Table 4).

Table 4: Mean monthly GSI values of G. quadrimaculata at upper Awash River from February to April 2018.

Month	Female	GSI	Male	GSI
February	83	5.29	78	3.09
March	80	4.33	64	2.81
April	81	5.17	70	2.86
Total	244		212	

Sex ratio

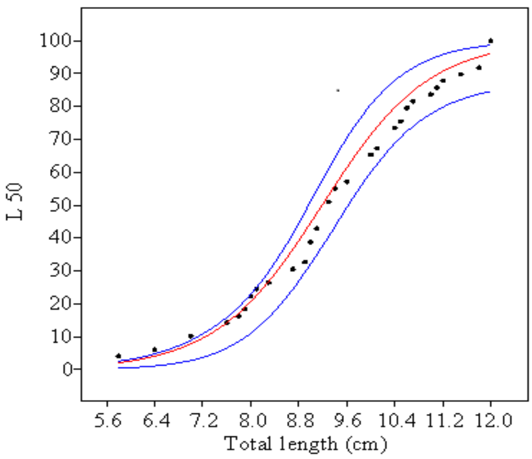
The sex ratio of 456 and 294 individual G. quadrimaculata and G. dembeensis was determined on monthly basis between February and April, 2018. In all sampling months, the number of female G. quadrimaculata was relatively higher than that of males, but it was not significantly deviant from the theoretical sex ratio (1:1) ($\chi2$, p> 0.05). The sex ratio computed for G. dembeensis was also very close to the theoretical ratio (1:1) ($\chi2$, p> 0.05) (Table 5).

Table 5: The sex ratio of G. quadrimaculata and G. dembeeensis in the Upper Awash River from February to April 2018 (mean significant at 5% level).

Species	Month	Female	Male	Sex ratio	χ^2	p- value
	February	85	82	1.05:1	0.81	0.38
	March	76	76	1.01:1	0.34	0.06
G. quadrimaculata	April	71	67	1.09:1	0.37	0.54
Total		232	225	1.05:1	**0.14**	**0.76**
	February	45	45	1.01:1	0.04	0.84
G. dembeensis	March	34	39	0.87:1	0.054	0.82
	April	70	61	1.15:1	1.09	0.29
Total		149	145	1.03:1	0.05	0.82

Length at first sexual maturity of fish (L50)

The L50 was calculated for G. quadrimaculata. The smallest sexually mature male and female of G. quadrimaculata considered for this study was 5.8cm and 4.5 cm TL, respectively. The estimated L50 was 9.4 cm for males (Fig. 5a) and 9.2 cm for females (Fig. 5b), which indicates that females appeared to attain sexual maturity at a relatively smaller size than males.



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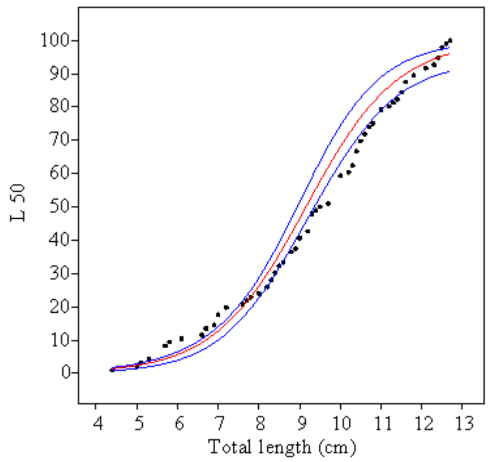
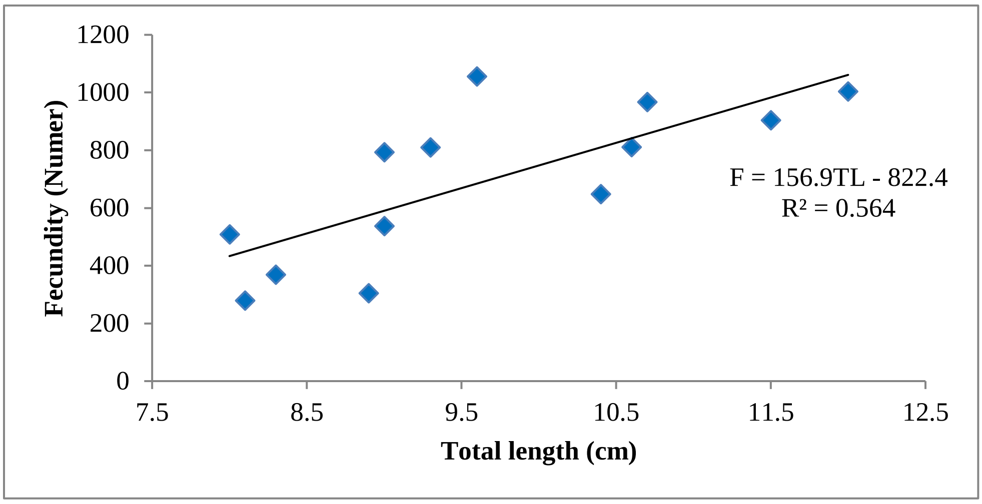


(b)

Figure 5: L50 of males (a) and females (b) of G. quadrimaculata

Fecundity

Fecundity was also estimated for 13 matured female G. quadrimaculata. The total length and total weight of the fish considered for this study ranged between 8 to12cm and 6 to 21g, respectively. The fecundity of the fish ranged from 273 to 1056 eggs per fish with the mean fecundity of 691.69± 269.69 eggs. Fecundity showed linear relationship with the total length (Fig. 6a), total weight (Fig. 6b) and gonad weight of the fish (Fig. 6c). Except for gonad weight ($p<0.05$), the relationship was statistically not significant ($p>0.05$).



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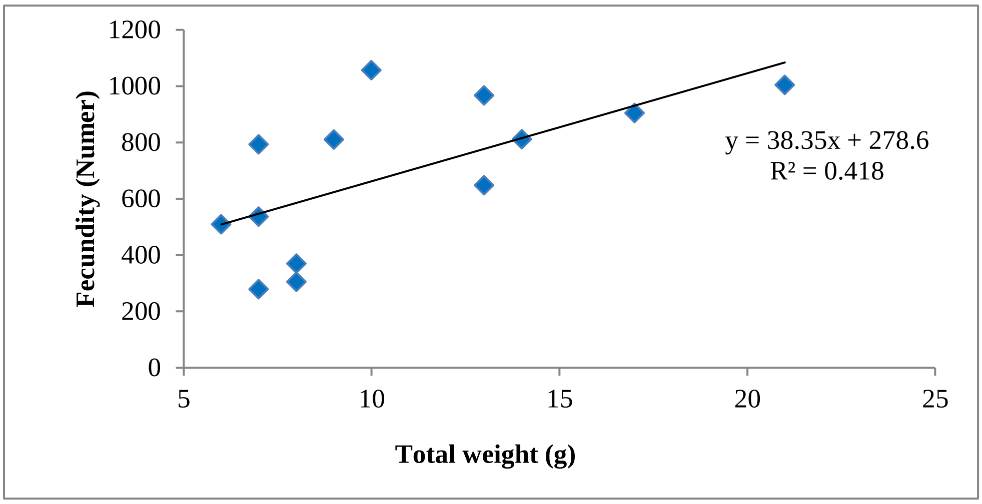

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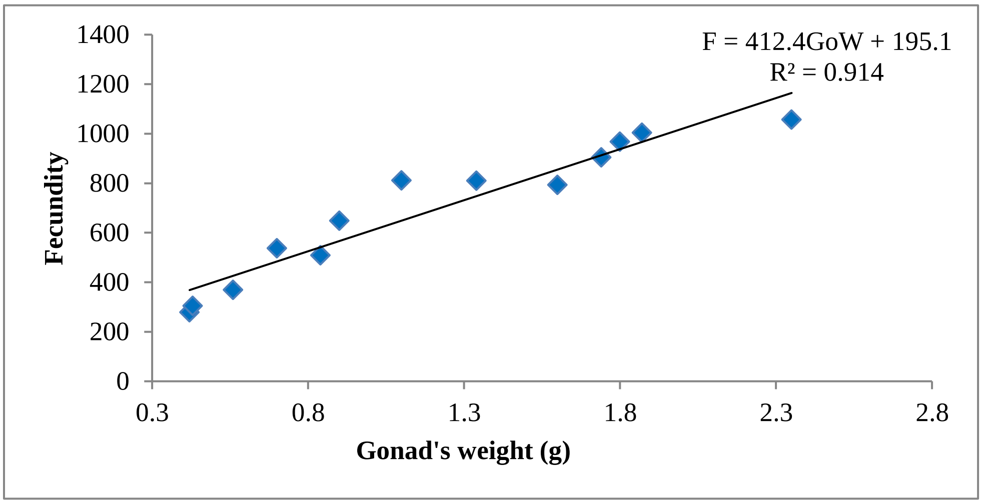

(c)

Figure 6: Relationship between total length and fecundity (a), total weight and fecundity (b), Gonad weight and fecundity (c) of G. quadrimaculata at Upper Awash River.

Infestation and intensity of fish parasite in the Upper Awash River

The result showed that the skin, fins, operculum and gills of G. quadrimaculata, G. dembeensis and L. beso were infested with black spot metacercaria (Neascus-like metacercaria) (Fig. 7).

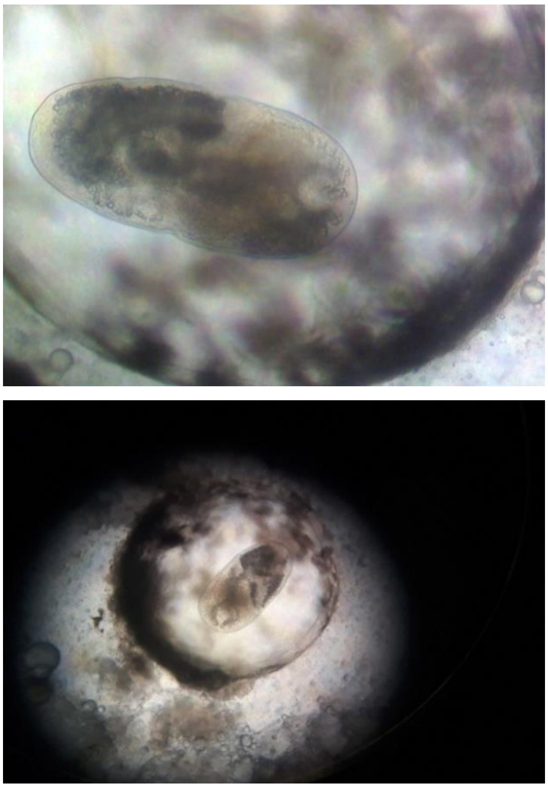

Figure 7: Black spot metacercaria on the skin of G. quadrimaculata observed under (10X) microscope in wet mount preparation.

The metacercaria of black spot parasites was found distributed all over the skin of fish species especially at the belly side from pictorial fin to anal fin (Fig. 8a& b).

Figure 8: Occurrence ofblack spot metacercaria on the ventral (a) and dorsal (b) side of G. quadrimaculata

Epidemiological parameters

The highest infestation of metacercariae infection (50%) was recorded at S-V (Osole) followed by S-IV (Walgata) (28.18%), S-III (Anjory) and S-II in descending order (Table 6). G. quadrimaculata was the most widely infested fish species with Neascus parasite followed by G. dembeensis. The overall prevalence of this parasite was found to be 18.09% in this study, which was most likely due to the river's moderate pollution.

Table 6: Infestation of black spot metacercaria on G. quadrimaculata, G. dembeensis and L. beso at the different sampling stations in the Upper Awash river

Study stations	Fish species	Number sampled (n=853)	Number infected (n=153)	Prevalence (%)
S-I (Galessa)	G. quadrimaculata	_	_	
	G. dembeensis	_	_	

S-I (Arera)	G. quadrimaculata	99	9	9.09
	G. dembeensis	9	_	
S-III(Anjory)	G. quadrimaculata	156	20	12.82
	G. dembeensis	100	10	10.00
S-IV(Walgata)	G. quadrimaculata	110	31	28.18
	G. dembeensis	114	6	5.26
	L. beso	24	5	20.83
S-V (Osole)	G. quadrimaculata	104	52	50.00
	G. dembeensis	77	14	18.18
	L. beso	24	3	12.50
Total		853	153	**18.09**

Generally, the infestation of black spotswas higher in females (25.2%) than males (22.27%) of G. quadrimaculata. On the contrary, the infestation was higher in males than females of G. dembeensis and L. beso, but statistically the difference was not significant ($\chi 2$, p> 0.05). The intensity of the black spot was highest on the G. quadrimaculata (22.34%) followed by G. dembeensis (18.07%) and L. beso (6.67%) (Table 7).

Table 7: Infestation of black spot metacercariae on the external parts of three fish species in the Upper Awash River.

Fish species	Sex	No. examined	No. infected	Prevalence (%)	Intensity
G. quadrimaculata	F	258	65	25.20	22.6
	M	211	47	22.27	22
	Total	496	112	22.58	22.34
G. dembeensis	F	150	14	9.33	21.43
	M	150	16	10.67	16.31
	Total	300	30	10	18.7
L. beso	F	25	2	8	6
	M	40	4	10	7
	Total	65	6	9.23	6.67
p-value				**0.052**	

Discussion

Food and feeding habits of fish species

Fish show variations in the type of food they consume (Cabana, 1994). Some scholars stated that food and feeding habits of fish can be affected by different factors like fish size, maturity level, water volume, depth, velocity, latitude, longitude, habitat types and others (Persson and Crowder, 1998). Diversity and abundance of fish diet can also vary significantly with the water level fluctuation (Cabana, 1994). Ingestion of food items depends on the availability of food (Werner, E1988). The same species inhabiting different ecological environments may not have similar food items in their guts because of the variation in their feeding habits (Desta et al., 2006; Shrestha and Shreshta, 2009). For instance, small fish prefer shaded habitat as a means to escape from external predators which attack them and prefer to feed on benthic organisms (Desta et al., 2006). Some scholars also related the food composition in the gut of fish to the seasonal availability of food items in the habitat (Dadebo et al., 2013).

The result of the present study revealed that G. quadrimaculata and L. beso feed on different types of food items including phytoplankton, detritus, sand grain and zooplankton (Table 1 & 2). Similar results were also reported from different water bodies in Ethiopia (Alemayehu, 2009; Tekle Giorgis et al., 2016; Urga et al., 2017). Most of the guts examined contained a variety of food items including the decomposed materials like plant fragments mixed with periphyton, diatoms and green algae. Presence of large amounts of detritus (plant tissues) and sand grains in their guts indicates that G. quadrimaculata and L. beso are bottom feeders (Ayotunde et al., 2007; Ayoade, 2011)and the presence of various food items showed the omnivorous feeding styles of the fish (Begum et al., 2008). Urga et al. (2017) and Garomsa (2017) also reported similar results from Debbis and Guder River, respectively. Admasu and Dadebo (Admassu and Dadebo, 1997) and Desta et al. (2006) also reported that L. intermedius feed on phytoplankton, insects, detritus, macrophytes, gastropods and fish in Lake Hawassa, which is very similar with our finding.

The relationships between total length and total weight of G. quadrimaculata and G. dembeensis recorded in the present study was curvilinear and strongly correlated (r^2 =0.915, r^2 = 0.907, respectively). The b value of females and males as well as the combined sexes (Fig. 1 & 2) of both species was lower than the theoretical cubic law (b= 3), which reveals the negative isometric growth of fish (Bagenal and Tesch, 1978). This indicates that fish become thinner as their length increases. The calculated b value for G. quadrimaculata (2.74) agreed well with the report from Debbis River (b=2.614) (Urga et al., 2017), but lower than the value recorded from Cholle Stream (b=2.9382) (Garomsa, 2017) for the same species. The difference could probably be because of the differences in the number of fish samples, the differences in food availability, gonad development and spawning period of the fish species (Bagenal and Tesch, 1978).

The result of the present study showed that females G. quadrimaculata have slightly higher mean FCF than males in all stations which could be attributed to the higher GSI index than males. The mean FCF for both sexes were higher in S-II and S-III and the lowest value at S-IV (Table 3). This could be related to the less-impacted nature of the habitat compared with the downstream stations, and it is covered with canopy and vegetation which provides better habitat for the fish, while in S-III the high water volume and high nutrient availability could favor the fish growth and condition (Sarkar and Deepak, 2009). In contrast, the lowest mean value of FCF in S-IV may be attributed to the impacts of wastes from the paper mill factory and the discharges from Ginchi town as well as mining practices taking place around S-V. In general, the mean condition factor recorded for both female and male of G. quadrimaculata in this study (1.31-1.36 and1.23-1.27) agreed well with the report from Debbis River for the same species (1.2-1.23 and 1.14-1.31 for females and males, respectively) (Urga et al., 2017). It was, however, higher than result reported from Cholle Stream (tributaries of Guder River) (1.12-1.18) (Garomsa, 2017) and lower than report from Lake Hayq for G. dembecha (0.8-1.73 and 0.82-1.84 for females and males, respectively) (Alemayehu, 2009). Similarly, FCF of G. dembeensis decreased from S-II to S-IV (at which least FCF was recorded) and slightly increased in S-V. Even though the variation is not significant ($p<0.05$), males seem to have better mean FCF than females (Fig. 3 a & b). The increasing trend of FCF in April might be attributed to the rainy seasons of the area due to availability of food and suitable environmental condition in this time (Sarkar and Deepak, 2009) (Fig. 4 a & b). Similarly, Alemayehu (2009) reported that males of G. dembecha were in better body condition than females in Lake Hayq, Ethiopia.

Generally, the measure of fish condition can be associated with the general health of fish, prey or food availability, reproductive potential, environmental conditions and water level fluctuations (Berie, 2007). Moreover, the higher condition factor is associated with the higher energy (fat) content; increased food base, reproductive potential, or more favorable environmental conditions (Pauker and Roger, 2004). The low FCF of fish species in this study could probably be because of the less quantity and quality of food, water level (volume) fluctuation, high flow rate and high water temperature.

Various factors including temperature, number and availability of prey, life history and reproductive style play crucial roles for the development of fish gonads (Pankhurst and Munday, 2011). Some scholars also stated that the development of gonads could be initiated by the onset of rain that stimulates the pituitary glands to send messages to the gonad development (Bowley et al., 2010). Further, these authors stated that availability and abundance of food can negatively or positively influence the development of fish gonads which in turn are affected by the season. The present finding also shows that the higher GSI was recorded in February then decreased in March and slightly increased in April (Table 4). This variation could be attributed to availability of short rain and availability of food in this month. The study made on different rivers of west Shoa Zone also showed the decreasing trend of GSI for Garra species from January to April (Abebe, 2016; Garomsa, 2017).In contrary, the GSI of G. quadrimaculatain the present study decreased in March and slightly increased in April (Garomsa, 2017).

The sex ratio for G. quadrimaculata shows the dominance of females over males in February and April, but the ration is close to the theoretical value (1:1) (Table 5), and this is very similar with the report from Cholle stream (Garomsa, 2017). The result of G. dembeensis also shows dominance of females over males in February and April, and preponderance of males in March. The variation may be a coincidence with the type of gear used for sampling in the present study (Frame net) and the reproductive season. This was also supported by Temesgen et al. (2018) who stated that the difference between sexes could be linked with vulnerability to fishing gear type used, fishing stations, habitat, segregation during spawning and feeding.

The length at first maturity level also showed that females of G. quadrimaculata appeared to attain sexual maturity at smaller size than males (Fig. 5 a & b). The studies indicate that the length at first maturity in many fish species depends on demographic conditions, and is determined by genes and the environment interactions (Hunter et al., 2015). Similar results were reported from Cholle Stream of Guder tributary for the same species (Garomsa, 2017).

Present finding revealed that G. quadrimaculata was less fecund (273 -1056 eggs/ fish) than G. dembecha (320-1130) from Borkena and Mille rivers (Tessema et al., 2012) and the Cyprinids from Lake Tana (1215-1229 eggs/ fish) (Geremew, 2007). The lower fecundity of G. quadrimaculata could be related to the sampling of fish during the non-spawning season. The spawning period of most Cyprinids in Ethiopia is commonly associated with the rainy season (between June and September) (de Graff et al., 2004). Moreover, a few numbers of fish samples used in this study could also miss the highly fecund fish and this could result in biased fecundity estimation. Skelton et al. (1991) also stated that fecundity of Labeobarbus in African Lakes is moderately high during the rainy season.

The total weight and the total length of fish species in the present study also exhibited a weak relationship with fish fecundity ($r2=0.418$ & 0.564, respectively), whereas the gonad weight has a strong relationship with fish fecundity ($r2= 0.94$). The number of fish (n=13) specimens used for the fecundity estimation in this study might be a factor for the weak correlation observed in this study (Fig. 6a, b &c). This is in agreement with the finding of Khan (2012) who reported that small sample size could affect the statistical significance of the results.

Black spot of genus Neascus, order digenea was the common parasite that invaded the body of all fish species (Fig. 7 & 8). G. quadrimaculata was the most affected fish species in the study area in general and at S-IV and S-V in particular (Table 6). This could be related to the high pollution of the two stations with the discharges from the paper mill factory and domestic debris from Ginchi town. High infestation of the female's G. quadrimaculata and males of L. beso could be due to the susceptibility of this particular sexes or species to the parasite. Some scholars stated that fish species have different immunity levels to defend against parasites which could relate to different factors like feeding, environmental impacts and genetic makeup which could vary even between siblings (Khan, 2012).

The infestation rate of black spot recorded in this study area (18.09%) was comparable with the report made from Koftu Lake (19.08%) on O. niloticus (Adugna, 2017). This black spot was distributed on the external body of fish including skins, gills, operculum, and head of G. quadrimaculata and G. dembeensis and on the belly side for L. beso. High infestation of this parasite on the belly side of L. beso could be related to the bottom dwelling habit of the fish close to the mud and gravels in the river due to the bottom feeding behavior of the fish (Graff et al., 2004). The high infestation of this parasite could cause the poor condition factor of fish, which could directly (making the wound on the body) or indirectly (inhibit feeding) affect the fish (Sumuduni et al., 2014). If uncontrolled, it could increase in number and cause the death of fish or expose them to opportunistic diseases like bacteria, viruses and fungi (Khanum et al., 2008).

Availability of phytoplankton and zooplankton in the guts of G. quadrimaculata and L. beso indicates that both species are categorized as omnivorous while abundance of diatoms in their guts shows prefer of phytoplankton over the others. There were no variations in the diet composition between the sampling months, stations and sexes. The results of Length-Weight relationships, mean Fulton's condition factor, Gonado Somatic Indices (GSI) and sex ratio indicated that there are variations between sampling stations and months resulting from the reproductive conditions of the fish. The poor body condition, less fecundity and arriving at the L50 also reveals that fish species are living in the poor habitat. Moreover, the infestation of fish ectoparasites in the studied area showed that the area is highly affected with anthropogenic activities such as municipal wastes, industrial wastes and agricultural activities. Therefore, appropriate conservation measures should be taken in the upper Awash River to sustain the fish resources in the area.

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Conflict of interest

The authors declared that we have no conflict of interest.

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