

Research Paper

Host-Parasite-Environment Interactions on the Infestation of *Argulus africanus* Thiele, 1900 on Nile tilapia in Lake Hawassa, Ethiopia

¹Endalkachew Daniel*, ²Natarajan Pavanasam

¹Department of Biology, Wolaita Sodo University, P.O.Box 138, Ethiopia

²Department of Aquatic Sciences, Fisheries and Aquaculture, Hawassa University, P.O.Box 05, Ethiopia

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Abstract

Argulus is one of the economically important ectoparasite of Nile tilapia (*Oreochromis niloticus* L. 1758); however, it poses a serious problem both in culture and capture fisheries globally. This investigation was done, from May 2022 to January 2023, to evaluate the infestations and effects of *Argulus africanus* on *Oreochromis niloticus*, in Lake Hawassa, Ethiopia. A total of 270 Nile tilapia were taken from the fish landing site of the lake. Distinctive body shape and morphological features were used to identify *A. africanus*. To evaluate the parasite effects, prevalence, mean intensity, mean abundance and infestation index were determined. The infestation was highest and lowest in January and August, with a prevalence of 56.67 and 26.67% and an abundance of 1.0 and 0.3, respectively. However, the intensity was highest and lowest in July and September, with values of 1.82 and 1.08, respectively. Higher prevalence of *Argulus* was found in dry season than the wet season. Considering the part of the body infected, just over half of *A. africanus* was detected from gills. The size of *O. niloticus* and the presence of *A. africanus* were positively correlated. The water quality parameters showed statistically significant differences in temperature and nitrite among the study months; however, ammonia and nitrate showed insignificant difference. In conclusion, *A. africanus* infestations significantly impact *O. niloticus* depending on body size, season and physicochemical parameters. It is suggested to monitor the discharges of any organic and inorganic wastes into the lake.

1. Introduction

Conditions in aquatic environments, combined with fish density and stress levels, play a substantial role in the occurrence of parasites and diseases in fisheries catching as well as cultivating fish (Mishra et al., 2017). The presence of parasites in fish poses significant threats to aquaculture in specific and fishing industry, in general (Kaleem & Sabi, 2021). The incident can be linked to poor environmental conditions and low-quality feed. Pollution, overcrowding, and rapid changes in water quality contribute to environments where parasites can thrive. Parasites have vital functions in the

biology, population management, and ecosystem operation of their hosts; however, a fish disease caused by them can result in loss of production (Yunikasari & Mahasri et al., 2020). The parasite group includes metazoans, such as myxozoan, trematodes, cestodes, acanthocephalans, nematodes, and crustaceans, as well as protozoans, including flagellates, ciliates, and apicomplexans (Marshet & Dawit, 2021). Significant mortality rates, substantial economic losses, and reduced market value are all consequences of parasites

*Corresponding author, e-mail: endalkdan74@gmail.com

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in both aquaculture and wild fisheries (Giari et al., 2022).

One of the key contributors to changing populations in a natural setting and fish abundance are parasites and diseases. *Argulus* species, particularly those in the family Argulidae, are significant obligate ectoparasites that infect various fish species, leading to considerable economic losses in aquaculture. *Argulus* parasites exhibit diverse host preferences and can cause severe health issues in infected fish, including inflammation and tissue damage, collectively termed argulosis (Dekari et al., 2024; Haridevamuthu et al., 2024). Depending on the species, they are found all over the world and can invade both freshwater and saltwater fish (Radkhah, 2017; Brahmchari et al., 2023). Numerous freshwater fish species are infected by the parasite *Argulus*, also known as fish lice. The ectoparasite results in tissue damage and secondary infections. It takes blood from its host by puncturing the skin, and then injecting a toxin (Alom et al., 2019).

Argulus species are found in a diverse array of geographic locations, including North and South America, Africa, Eurasia, and certain regions of Asia, specifically India and Japan (Shukla et al., 2022; Amriana et al., 2021; Brahmchari et al., 2023; Dekari et al., 2024). Approximately 150 species within this genus have been documented (Rayamajhi & Kunwor, 2017). Saha & Bandyopadhyay (2015) identified 15 species of freshwater fish, among which some were found to infect marine fish. The global distribution encompasses species such as *A. foliaceus*, *A. coregoni*, and *A. japonicus* (Ambu, 2017; Nagasawa, 2023). Infected fish surrender to illness and death due to *Argulus* (Shukla et al., 2022). The generation of different toxins and enzymes that causes damage to host tissues and organs triggers inflammation, bleeding, and ulceration (Amriana et al., 2021).

The distribution and infestation levels of *A. africanus* exhibit considerable variation, with geographical location and environmental factors significantly impacting both. *A. africanus* is mainly discovered in African freshwater environments. The intensity of an *Argulus* infestation can vary significantly and is determined by a combination of both environmental factors and the host's overall health condition (CABI, 2022). The clearness of the water and its high

temperature enable *Argulus* life cycles to intensify, resulting in more severe infections (Taylor, 2004).

Research into fish parasites is crucial due to the fact that infestations in fish not only compromise fish health and reduce fish yields, but also results in substantial economic losses, ultimately impacting the livelihoods of fish farmers (Barber, 2004). A comprehensive investigation into the occurrence, prevalence, and interconnections between hosts, parasites, and their environments is crucial in order to guarantee food security. This study was conducted from May 2022 to January 2023 to examine the infestation and consequences of the highly destructive branchiuran ectoparasite *Argulus africanus* on *Oreochromis niloticus* in Lake Hawassa, Ethiopia.

2. Research Methods

2.1 Description of the study area

The study was conducted in Lake Hawassa is an endorheic basin in Sidama Region of Ethiopia, located in the Main Ethiopian Rift south of Addis Ababa, the capital city of the country and it lies to the west of the Hawassa City. The Lake is located at 06° 58' - 07° 14' North latitudes and 38° 22' - 38° 28' East longitudes. The level of the lake varies considerably from year to year and a dyke has been built to prevent the town from flooding. The surface area ranges between 85 and 90 km² and the maximum depth is 22 m. Hawassa is the smallest of the Rift Valley lakes, but is highly productive. It has a rich phytoplankton (over 100 species have been identified) and zooplankton that support large populations of six fish species. The most important commercial species is *Oreochromis niloticus*, but there are also good populations of catfish and Barbus. Lake Hawassa, despite its lack of an outflow, is essentially a freshwater lake (conductivity is variable, but less than 1,000) indicating that it must have a subterranean outlet.

2.2 Sampling design and fish sampling

Nile tilapia was chosen for the study because it is the most common edible fish and potential aquaculture species. The required sample size was calculated with a 5% expected occurrence rate and a 95% confidence interval for infinite populations (Ossiander & Wedermeyer, 1973). Thus, 270 Nile tilapia specimens, with different sexes and sizes, were bought from fishermen who had caught them with gill nets. These

fish were transported to Hawassa University Fisheries Laboratory in appropriate containers. Measuring board was used to obtain morphometric data, which includes total and standard lengths, and a digital weighing balance was used to measure total weight, to the nearest centimetre and gram, respectively (Dadebo et al., 2005).

2.3 Identification of *Argulus* species

The distinctive body shape and morphological features (Figure 1) of *A. africanus* were used for its identification (Mousavi et al., 2011). Any local variations of appendages on the described species were noted as remarks for discussion. The collected parasites were washed in saturated sodium bicarbonate solution to clear the parasites and after repeated washings in pure tap water, they were preserved in 70% alcohol (4% formalin solution). The specimens were left in glycerine-ethanol for 5-7 days to enhance their transparency for observation under a stereo microscope (Woo & Buchmann, 2012).

2.4 Examination of *O. niloticus* for parasites

The external surface, namely scales, gills, fins, and operculum, of freshly killed Nile tilapia were examined for *Argulus africanus* infestation and the accompanying pathological features. A hand lens was used to examine the *A. africanus* on the skin and fins of fish in real time. Larvae of *A. africanus* were detected upon skin examination. 1,350 samples were taken from the Nile

tilapia's dorsal head region and ventral area, from the head to the anal point, including fins, and from the gills. A clean microscope slide was smeared with the mucus sample with a drop of water. The sample was examined under a compound microscope with magnifications of 10X, 40X, and 100X after covering with a coverslip. 540 gills were transferred to a Petri dish with tap water. Forceps were used to separate gill rakers for microscopic examination of *A. africanus*.

2.5 Parameters used to evaluate the parasite effects

To evaluate the parasite effects, prevalence (*P*), mean intensity (*MI*), mean abundance (*MA*) and infestation index (*II*) were determined by the following formulas (Bush et al., 2001).

Prevalence (P) =

$$\frac{\text{No. of infected fish with a parasite species}}{\text{Total No. of host examined}} * 100\%$$

Mean Intensity (MI) =

$$\frac{\text{Total No. of parasite species in host}}{\text{Total No. of infected host species}}$$

$$\text{Mean Abundance (MA)} = \frac{\text{No. of parasites}}{\text{No. of examined fish}}$$

Infection Index (II) =

$$\frac{\text{No. of infected host} * \text{No. of parasites collected}}{\text{Total No. of samples examined}}$$

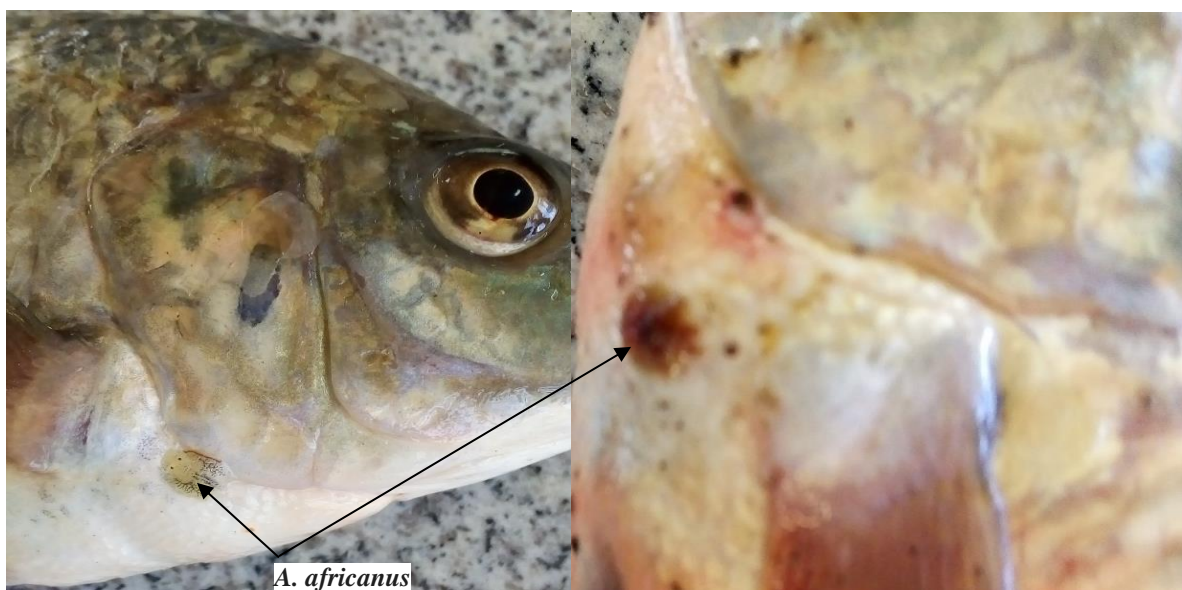


Figure 1: Photograph showing the presence of *A. africanus* on *O. niloticus* body

2.6 Determination of water quality

250 mL water samples were collected early in the morning from three distinct locations in Amora Gedele part of the lake, close to the area where fish are landed. A total of 27 water samples were gathered monthly, on the 15th day, from May, 2022 to January 2023. One large composite bottle and three small sampler bottles were used to collect the samples. Before collecting the sample, both the sampler and the composite bottles were rinsed by the lake water. At a slow rate, the sampler bottles were lowered vertically into the water until they reached a depth of two meters, at which point they were vertically pulled up. Then, the water samples were poured to the composite bottle and mixed well. The mixed water sample was put into a polyethylene bottle, and one ampule of sulfuric acid (1 mL of H₂SO₄ for a 250 mL polyethylene bottle) was added to lower the pH and stop reactions in the sample until it could be analysed. The polyethylene bottle was stored in insulated coolers containing ice and transported to Hawassa University Environmental Engineering Laboratory and kept in a refrigerator at 4 °C until analysis.

Temperature and dissolved oxygen (DO) were measured by using DO-810 model DO meter. The pH was measured using pen type pH meter of model P301. The ammonia concentrations were determined by acid-base titration method by using sulphuric acid and

sodium hydroxide. Nitrate and nitrite were analysed by using nitrate nitrogen comparator test kit and nitrite nitrogen test kit, respectively.

2.7 Statistical analysis

The collected raw data were entered in to Microsoft excel-13 data sheets and analysed using SPSS-25 statistical software. 95% confidence intervals were used to summarize the proportion of infested fish. Infestation on sex difference of *O. niloticus* was analysed by chi-square (χ^2) test. One way analysis of variance (ANOVA) and F-test were used to test the difference in the physicochemical parameters among the study months.

3. Results and Discussion

3.1 Infestation of *Argulus* species on *O. niloticus*

115 (42.59%) of the 270 *O. niloticus* examined were infested by *Argulus* parasites. The prevalence of *A. africanus* was highest and lowest in January 2023 and August 2022, respectively (Table 1). The highest and lowest mean intensity of *Argulus* sp. were recorded in July and September, with values of 1.82 and 1.08, respectively. Highest mean abundance of *Argulus* sp. was recorded in January (1.00); and lowest mean abundance of *Argulus* sp. was noted in August (0.3) (Table 1).

Table 1: Prevalence, mean intensity, mean abundance and infestation index of *Argulus* sp. on *O. niloticus* at Lake Hawassa (30 fish examined each month)

Month	No. of fish infected	Prevalence (%)	No. of <i>A. africanus</i>	Mean Intensity	Mean Abundance	Infection Index
May 2022	14	46.67	24	1.71	0.80	11.20
June 2022	12	40.00	21	1.75	0.70	8.40
July 2022	11	36.67	20	1.82	0.67	7.33
Aug. 2022	8	26.67	9	1.12	0.30	2.40
Sep. 2022	12	40.00	13	1.08	0.43	5.20
Oct. 2022	11	36.67	15	1.36	0.50	5.50
Nov. 2022	14	46.67	22	1.57	0.73	10.27
Dec. 2022	16	53.33	25	1.56	0.83	13.33
Jan. 2023	17	56.67	30	1.76	1.00	17.00
Total	115	42.59	179	1.56	0.66	76.26

The general prevalence of *A. africanus* on *O. niloticus* from Lake Hawassa, is extremely higher than values reported from cage culture at Mpakadam, Ghana (Alhassan et al., 2018). On the other hand, Marshet et al. (2018) reported the mean intensity of *A. africanus* on *O. niloticus* at the Gilgel Gibe-I dam to be 1.7, surpassing the mean intensity observed in the current study. Research by Anshary et al. (2022) found that *Argulus* sp. was relatively rare and had a low level of infestation on *O. niloticus* from Towuti Lake, with an incidence of 2.5% and a severity rating of 1.0. In contrast, at the Ujirpur carp hatchery pond in Bangladesh, the incidence of *Argulus* sp. was 79.17% and its severity was 41.34 (Alom et al., 2019). It is worth noting that Saravanan et al. (2017) found a 100% prevalence of *Argulus* sp. on *Catla catla* and *Labeo rohita* from the Andaman and Nicobar Islands, a rate much higher than what was observed in the current study. In general, the prevalence is case dependant.

In terms of parasite distribution on fish body, half of the 179 *A. africanus* parasites were found on gills. 52 (29.05%) and 37 (20.67%) were detected on body surface including the fins and the buccal cavity (mouth), respectively. *A. africanus* is exclusively external and has a non-specific distribution pattern across the body of its host, occurring in a variety of locations. The distribution found in this study is in agreement with the results of Dhanya & Amina (2017), who found the gills to have the highest infestation rate. A large number of fish ectoparasites typically attach themselves to the gills. For the larval stage of *Argulus* sp., gills provide a suitable environment that helps mitigate the risks associated with predators and water current challenges.

Similarly, the current research outputs support the conclusions of Omeji et al. (2022), which noted that the ectoparasite, like *Argulus* sp., displayed variation in levels of infestation on cultured and wild *O. niloticus*, with the gills being more heavily infested than the skin. The primary function of fish gills as both filter feeding organs and gas exchange sites may contribute to the higher prevalence of ectoparasites in these areas. The gill rakers' improved sieving capability may contribute to the capture of certain parasitic organisms. The parasitic community is primarily composed of ectoparasites, with *Argulus* sp. being prevalent, mainly located on the gills of the host fish. Freshwater fish

species are infested with ectoparasites, which are typically found on the skin, fins, or gills (Sriwongpuk, 2020). In this study, the number of parasites observed on the body surface was lower than from gills; many parasites detach from the body surface as fishermen try to pull fish out of the gill net. A study by Anshary et al. (2022) reported the presence of *A. indicus* on the surface of the fish body. The specificity of parasitic sites can be attributed to physiological factors and morphological adaptations that favour a particular location (Aloo et al., 2004).

The dry season (Nov., Dec. and Jan.) had relatively high parasite prevalence of 52.22%, as 47 of the 90 infected, than the rainy season (June, July, and August), in which only 31 infected, which was statistically significant difference with p-value of 0.026. The dry season had slightly a higher parasite incidence (1.64) than the rainy season (1.61), but it was not statistically significant different, with p-value of 0.848. Seasonal fluctuations in environmental temperatures significantly impact the distribution and spread of parasites. Climate change has a significant impact on the life cycle of the crustacean ectoparasite *Argulus* sp., a parasite that often causes severe issues in fish populations. Development, hatching, and growth of *Argulus* sp. are all dependent on temperature. At temperatures of 28°C, egg hatching can occur in 8-10 days, whereas eggs laid in temperatures below 10°C are stored until they can be moved to warmer conditions above 10°C. Studies by Sahoo et al. (2013) found that successful egg hatching results in the recruitment of juvenile *Argulus*, ultimately increasing the infestation level.

Environmental factors, particularly temperature, exert an influence on the population dynamics of *Argulus* sp. (Hakalahti et al., 2006). The frequency and severity of infestations by the crustacean parasite *Argulus* sp. on *O. niloticus* are at their peak during the hottest months, with a subsequent decline during rainfall. Higher temperatures lead to increased prevalence and severity of *Argulus* sp. infestations (Walker, 2008); Saha & Bandyopadhyay, 2015).

The life cycle of fish lice is significantly influenced by environmental temperature, and an increase in temperature corresponds to a rise in infestations. The current research findings are in line with that of Koyun (2011), who found that an increase in temperature will

be accompanied by an increase in the number of fish lice and thus, higher damage to the fish. The primary recruitment of juvenile *Argulus* sp. took place in early summer, with egg hatching persisting until September (Hakalahti & Valtonen, 2003). Seasonal fluctuations were observed in juvenile *Argulus* sp. recruitment, with a notable drop during cooler periods and a corresponding rise as water temperatures rose (Webb, 2008).

3.2 Physicochemical parameters of Lake Hawassa

The spatial water temperature ranged from 20.5°C in August 2022 to 26.8°C in January 2023, with a mean temperature of 24°C. The mean temperature differed significantly between months ($F = 6.352$, $p = 0.04$; (1, 7)). As shown in Figure 3, January had the highest temperature (26.8°C), while August had the lowest (20.5°C). The pH level ranged from 6.9 to 10.1, with a mean value of 8.3. Variations were observed between

the months, but it was not statistically significant, ($F = 3.31$; $p = 0.112$; (1, 7)). Dissolved oxygen levels in Lake Hawassa ranged from 3 to 7.2 mg/L with a mean value of 5.1 mg/L. There was variation in mean dissolved oxygen concentrations between sampling months ($F = 3.39$; $p = 0.108$; (1, 7)). However, the difference was not statistically significant.

The value of ammonia in Lake Hawassa ranged from 0.82 to 2.37 mg/L with a mean value of 1.64 mg/L. However, the differences were statistically insignificant in the ammonia concentration ($F = 0.55$; $p = 0.482$; (1, 7)). Nitrite levels were between 0.013 and 0.08 mg/L, with a mean value of 0.038 mg/L, showing significant differences in concentrations among the months ($F = 8.152$; $p = 0.025$; (1, 7)). Nitrite and nitrate concentrations were between 0.08 and 0.013 mg/L and 3 and 4.4 mg/L, respectively. However, the mean nitrate did not differ significantly across the months studied ($F = 0.784$; $p = 0.405$; (1, 7)).

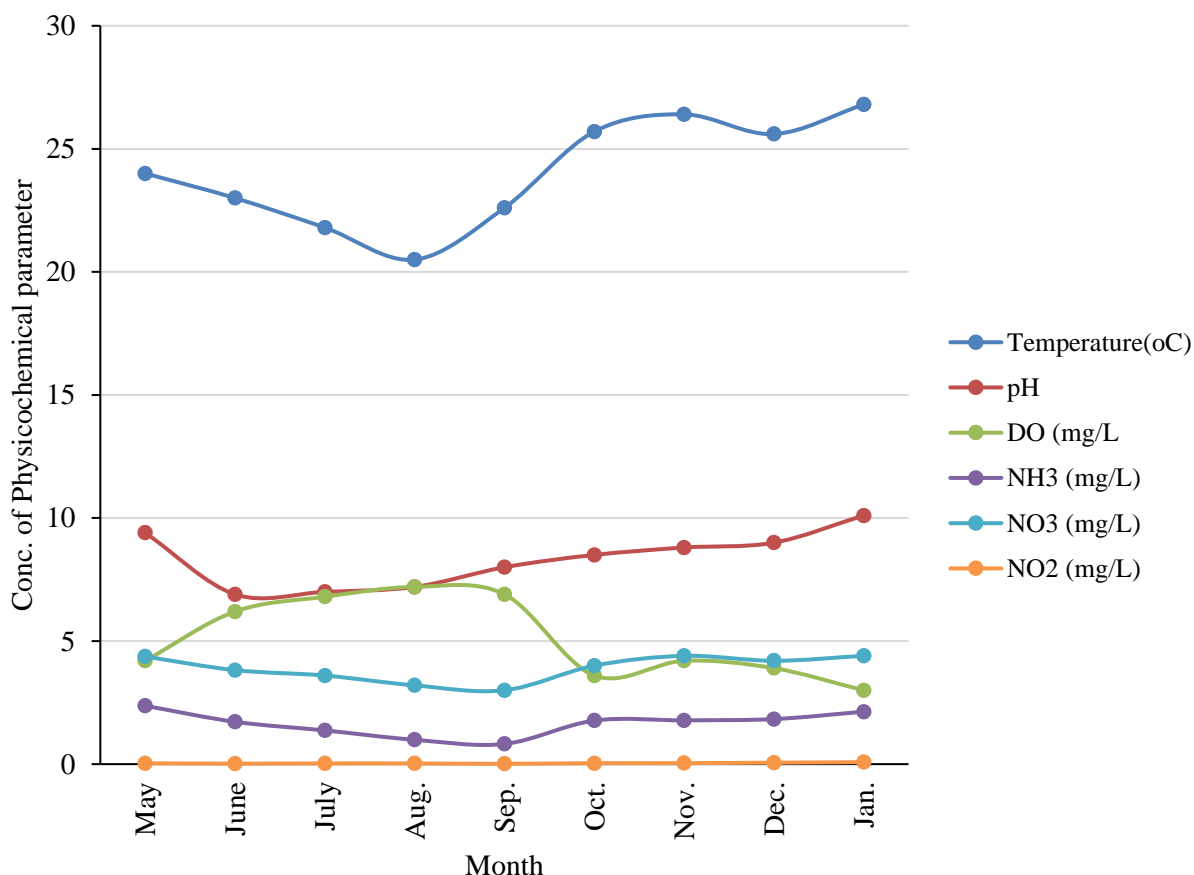


Figure 2: Monthly physicochemical parameters of Lake Hawassa, from May 2022 to January 2023

Argulus species are prevalent parasites in both freshwater and marine fish, primarily due to their rapid growth rate (Hanson et al., 2011). Khan et al. (2017) reported that for the parasite to survive, it must be capable of identifying and attaching itself to a host. The biological characteristics of the host and the water quality have a considerable influence on the prevalence of the parasites and their capacity to endure on the host (Tak et al., 2014). The abundance of *Argulus* sp. of this study (0.66) is significantly higher than the 0.08 reported by Amriana et al. (2021) for *O. niloticus* from Lake Towuti, Indonesia. At high environmental temperatures, there was a significant rise in the abundance and the infestation level of *Argulus* sp. A significant association was detected between low water transparency, a slow rate of stock replenishment, and high temperatures, and an abundance of the parasite *Argulus foliaceus* (Taylor et al., 2009). All risk factors for *Argulus* infestation include chemical stressors such as diet composition, water quality, and pollution, physical stressors such as salinity and temperature, and biological stressors including population density and the presence of other macro or microorganisms (Ina-Salwany et al., 2019; Alom et al., 2019).

3.3 Parasite infestation versus fish body size

In terms of fish size, fish length of 20-24 cm had the most parasite prevalence. Generally, the number of parasites increased with the increase in fish length. The length group of fish > 24 cm had the highest mean

intensity of parasite, followed by the length group 20-24 cm, with values of 2.5 and 1.64, respectively (Figure 3).

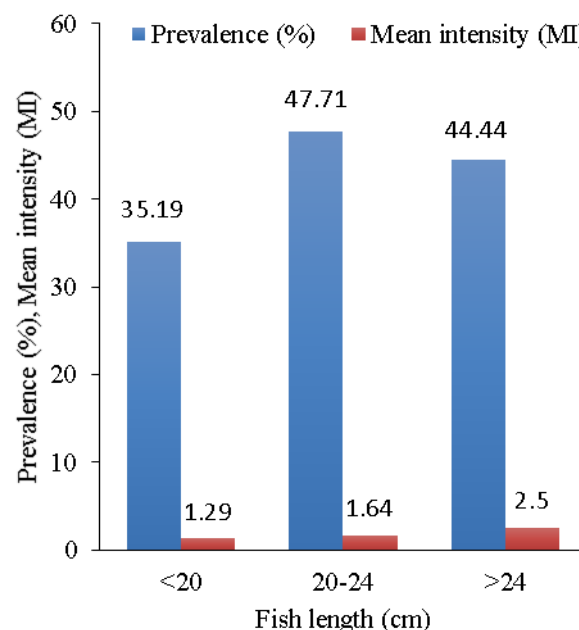


Figure 3: Prevalence (%) and mean intensity of *A. africanus* based on total length of *O. niloticus*

In terms of body weight, samples with weights g \geq 300 (g) were most infested, with a prevalence of 72.73%, followed by fish weighing between 251-300 (g) and 201-250 (g) (Figure 4). The highest mean intensity of *A. africanus* was found in weight group \leq 100 (g), and, generally, it showed decreasing pattern as the weight increased.

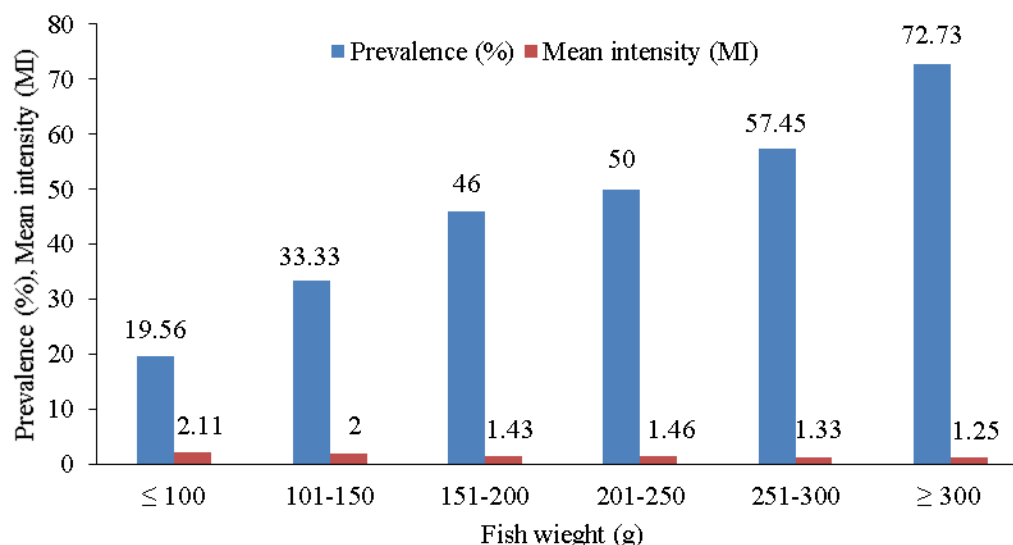


Figure 4: Prevalence (%) and mean intensity of *Argulus africanus* based on weight of *O. niloticus*

Findings from this study verify the results of Oniye *et al.* (2004), which demonstrated a positive correlation between host size and parasite prevalence. Generally, bigger fish have a greater parasitic burden than their smaller counterparts (Alom *et al.*, 2019). Larger host organisms offer a larger surface area for parasite settlement (Webb, 2008). Fish species consisting mainly of larger individuals showed the greatest prevalence of *A. foliaceus* infestation; in fact, the infested fish were typically heavier than the uninfected ones (Walker *et al.*, 2008). There is link between the size of fish bodies and the load of *A. africanus*, a potentially hazardous factor for commercial fish hatchery development. Thus, a severe infestation caused by *Argulus* sp. necessitates the implementation of effective, user-friendly, and low-maintenance protective measures as soon as possible.

3.4 Prevalence and intensity of infestation in relation to fish sex

The overall prevalence and intensity in male and female *O. niloticus* were assessed to observe the influence of *A. africanus* and presented in Table 2. The result shows that the prevalence of male and female fish to *A. africanus* was almost the same. Intensity of parasite on sex of fish was 1.69 and 1.37 in male and female *O. niloticus*, respectively.

Understanding host-parasite relationships is significantly influenced by a key biotic factor, especially, the host's sex. Physiological, biological, and behavioural disparities between males and females (Hawley & Ezenwa, 2022) are thought to contribute to a persistent yet moderate sexual bias in infection rates, potentially resulting in host sex effects on parasitism levels. The current findings agree with those of Hayatbakhsh *et al.* (2014), who found a marginal difference in infection rates between males and females. Males may be more susceptible to parasites due to higher testosterone levels, which can weaken their immune system.

4. Conclusion and Recommendations

The incidence and infestation of *Argulus africanus* on *O. niloticus* of Hawassa Lake are higher during the dry season and lower during the wet season. The life cycle stages of *Argulus* sp., including egg development, hatching, molting, and growth, are all affected by temperature. High recruitment of juveniles into the population occurred with increasing temperature, thereby boosting infestation levels. A large number of *Argulus* species are attached to the gills of *O. niloticus*, due to the presence of soft tissues within the gills and gill cavity, which can be easily penetrated by the pre-oral spine of *Argulus* to access food. The larvae of *Argulus* sp. typically find the gills a safe environment in which to reside. The attachment site preference of *Argulus* sp. on the host body is significant for feeding and survival, and the presence of gills as a primary filter-feeding organ and gas exchange site may contribute to the increased number of ectoparasites found in Lake Hawassa. Fish of greater size tend to have a higher incidence of *Argulus* sp. compared to smaller counterparts.

The stakeholders, including farmers, government bodies, fishery institutions, are highly suggested to play their role in managing the release of organic fertilizers from farmland, and municipal and domestic effluent into the lake, as these pollutants compromise water quality and facilitate the proliferation of parasites. Establishing a pollution-reducing buffer zone by cultivating trees in areas where agricultural and urban wastewater discharge is prevalent. Moreover, providing short training and raising awareness among fishermen and fish traders to prevent the disposal of fish waste in the lake, which contains high levels of protein and is broken down by microbes, resulting in dissolved oxygen levels being depleted for aquatic organisms and causing stress to the fish population, is also recommended.

Table 2: Prevalence and mean intensity of *A. africanus* based on sex of *O. niloticus* (n=270)

Sex	No. of fish		Prevalence (%)	No. of <i>A. africanus</i>	Mean Intensity	Mean Abundance
	Examined	Infected				
Male	155	67	43.23	113	1.69	0.73
Female	115	48	41.74	66	1.37	0.57

$$\chi^2 = 11.6; p < 0.05; df = 1$$

Additional research is required to identify effective methods of minimizing parasitic infestation in capture and culture fisheries, ultimately improving fish yields.

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