



## Prevalence of fish parasites in Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*) and physicochemical characteristics of pond water in Arusha and Morogoro, Tanzania

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### Abstract

Fish parasites can cause significant production and economic losses in aquaculture. This study examined 130 small-scale fish ponds in the Arusha and Morogoro regions of Tanzania between December 2019 and February 2020 to determine pond water physicochemical parameters and the prevalence of parasites in farmed Nile tilapia (*Oreochromis niloticus*) and African catfish (*Clarias gariepinus*). Most ponds sampled were small ( $20 \leq 300 \text{ m}^2$ ) and majorly of concrete. The physicochemical water parameters measured varied between sites and regions. The overall length and weight of fish sampled ranged from 6 to 23 cm (mean  $14.4 \pm 5.7$  cm) and 30 to 412 g (mean  $227 \pm 124$  g). The most and least prevalent parasites were *Acanthocephalus* sp. (49.2 – 50.7%) and leeches (4.6 – 7.6%). The prevalence of *Diplostomum* sp. (36.9 – 38.4%), *Dactylogyrus* sp. (32.3 – 47.6%), *Gyrodactylus* sp. (36.9 – 47.6%), *Contracaecum* sp. (41.5 – 49.2%), and *Trichodina* sp. (41.5 – 44.6%) were higher than reported in other parts of East Africa. We recommend awareness of fish health and proper farm management practices to minimize parasitic infection and improve aquaculture in Tanzania.

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## Introduction

Freshwater fish farming using small-sized earthen or concrete ponds is the most commonly practiced form of aquaculture system in Tanzania, both for domestic consumption and international trade (MLFD, 2015). It's estimated that more than 20,000 freshwater fish ponds are scattered across Mainland Tanzania, with Ruvuma, Iringa, Mbeya, and Kilimanjaro regions each having more than 1000 fish ponds (MLFD, 2015; Rukanda and Sigurgeirsson, 2018). The distribution of fish ponds is determined by several factors, including the availability of water, the suitability of land for fish farming, and economic potential in fish farming (URT, 2005). Nile tilapia production is especially preferred for its superior growth characteristics, followed by African catfish (de Graaf, 2004).

Inland aquaculture in Tanzania is dominated by small-scale fish farmers (average size of 150 m<sup>2</sup>) (Wetengere, 2010), which hugely contribute to poverty alleviation in the country (MLFD, 2015). Arusha and Morogoro regions are found in the northern and eastern regions of Tanzania, respectively, and are increasingly becoming urbanized. Fish farmers in both regions obtain their water supply for their aquaculture activities from dams, rivers, streams, and boreholes. Food safety and nutrition security in aquaculture-dependent communities can be threatened by several factors including aquatic parasitic infections (Quiazon, 2015). Helminthic worms such as cestodes, nematodes, and trematodes are the predominant parasitic groups reported to significantly affect farmed fish in the East African region, causing mortalities, low production, and economic losses in fish (Walakira *et al.*, 2014; Mavuti *et al.*, 2017). Their physiological effects on fish include muscle degeneration, liver dysfunction, cardiac, endocrine, and reproductive disruptions, castration as well as interference with spawning and growth rates (Sindermann, 1987). In pond fish farming, some of the predisposing factors of fish to parasitic infestations include high stocking densities, poor water quality management, lack of biosecurity measures, poor nutrition, anthropogenic transfer of

stocks, and age (Mdegela *et al.*, 2011). Consequently, the absence of biosecurity strategies at all levels poses a huge risk in many aquaculture facilities in the country. There are very limited parasite studies that have been conducted in fish ponds in Tanzania. It is for these reasons that research on aquatic parasites concerning water and pond management systems and potential food safety in Tanzania becomes critical. The objective of the present study was to examine the current prevalence of fish parasites in Nile Tilapia and African catfish reared under the small-scale fish farming system in the Arusha and Morogoro regions of Tanzania to understand the extent of parasitic infections and help prevent potential disease outbreak that may lead to economic losses to the farmers.

## Materials and methods

### Study area

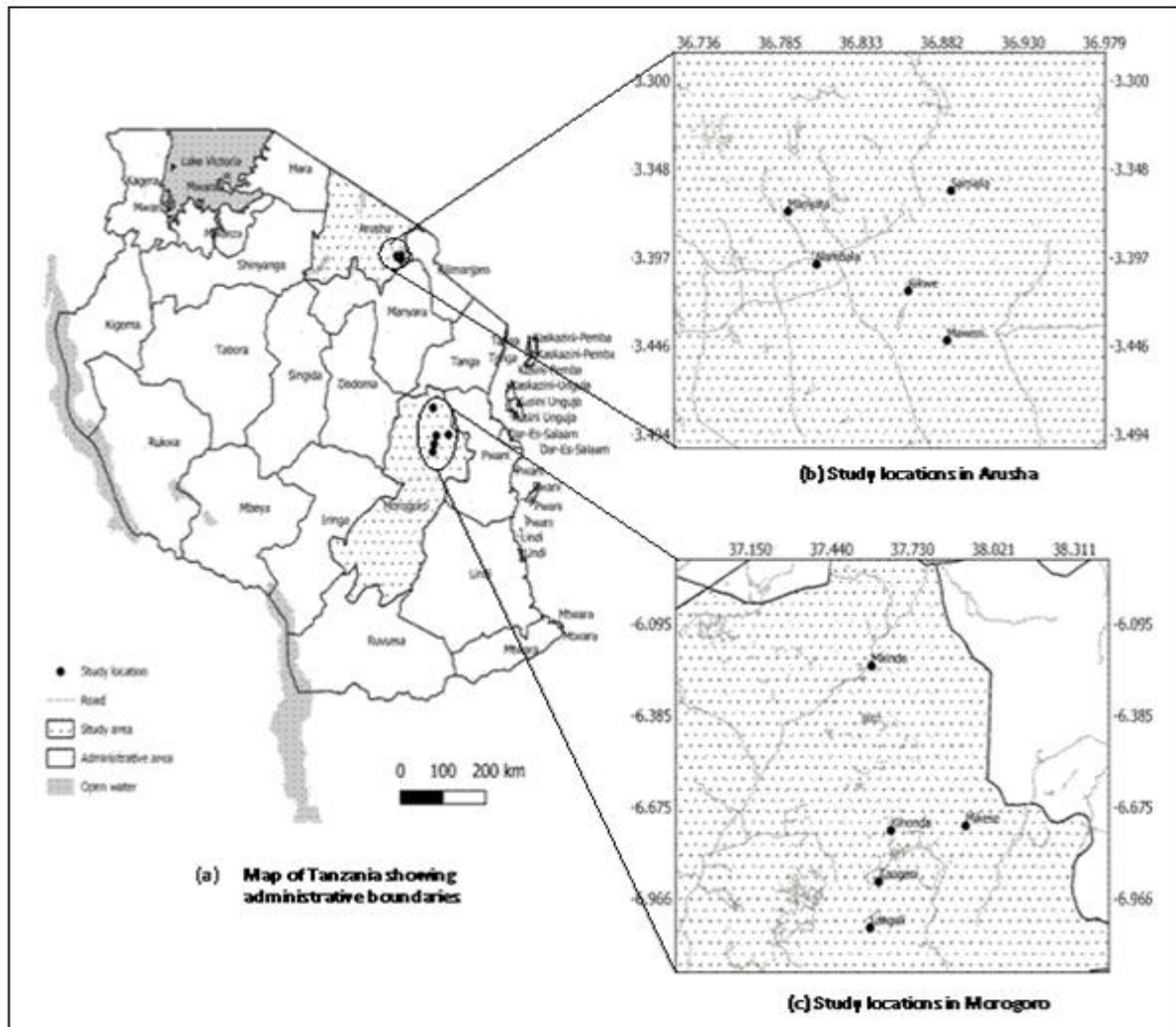
This study was carried out between December 2019 and February 2020 in fish ponds located in 10 sites within Arusha (3.3869° S, 36.6830° E) and Morogoro (6.8278° S, 37.6591° E), which lie in the northern and eastern regions of Tanzania, respectively (Figure 1).

The sites sampled in the Arusha region were Kikwe, Nambala, Maweni, Manyata, and Samalia; while those in the Morogoro region were Kihonda, Langali, Mikese, Mkindo, and Tangeni, and each site had 13 ponds. Fish samples were collected from ponds owned by select fish farmers willing to participate in the study. One hundred fish ponds rearing Nile tilapia and thirty with African catfish were sampled for this study. Sites studied in the Arusha region had 26 earthen and 39 concrete ponds; while those in the Morogoro region had 17 earthen and 48 concrete ponds. The mean annual precipitation in Arusha and Morogoro is 1100 mm (43.0 inches) and 889 mm (35.0 inches), respectively.

The mean maximum temperature ranges for Arusha and Morogoro are between 20 – 28 °C; while their mean minimum temperature ranges are between 8 – 13 °C. Both direct observation and structured questionnaires were used to gather information from

the fish farmers about the stocking density, fish feeding, feed type, pond type, water source, and frequency of changing water. Pond sizes were recorded using a tape measure and physicochemical

water quality was assessed before fish sample collection. For ease of data analysis, fish ponds were arbitrarily classified into three size ranges, that is,  $20 \leq 100 \text{ m}^2$ ,  $101 \leq 200 \text{ m}^2$ , and  $>200 \text{ m}^2$ .



**Fig. 1.** Map showing (a) Tanzania including its administrative boundaries; study locations in (b) Arusha and (c) Morogoro.

#### *Collection of fish samples and freshwater samples*

Fishing was done using  $1 \times 1 \text{ m}$  seine net following the method by Mdegela *et al.*, 2011. Fish were randomly captured from each of the 130 fish ponds studied in both Arusha and Morogoro regions and then transferred to separate bucket containers filled with respective pond water and aerated using a battery-powered air pump to keep the fish alive. Water samples for physicochemical analyses were collected aseptically from the pond's water surface (maximum 1 feet depth) using sterile cap bottles and then transported to the laboratory in an ice-packed

container. All samples were transported to the Microbiology Laboratory at the Department of Microbiology, Parasitology, and Biotechnology in the College of Veterinary Medicine and Biomedical Sciences, Sokoine University of Agriculture (SUA), Morogoro.

#### *Parasitological examination and identification*

The weight and standard length of each fish were measured and recorded. Live fish were stunned with a single blow to the back of the head and pithed to separate the central nervous system from the spinal

cord. Gross examination of the skin was done for ectoparasites. Wet mounts of the skin scrapings and gill filaments were collected on slides with saline and examined under the microscope for ectoparasites. The eyes were removed and contents were expressed on a slide and examined for eye flukes. Post mortem of the fish was performed as described by Noga (Noga, 1996). For endoparasite identification, the dissection plate was used to lay every fish on its correct side with the mid-region coordinated towards the dissector.

The body cavity was opened into two cuts by using a pair of scissors. A dark shaded plate was used to put the removed gastrointestinal tract (GIT). The GIT was then opened and the internal content was removed. Around 100 ml of distilled water was added, delicately shaken to clean out the digestive organs, and analyzed for parasites utilizing laboratory lamps with the aid of a magnifying lens. Any parasite found was carefully transferred using plastic forceps into bottles containing 15 mL of 70% alcohol and stored for identification.

Stainless steel sieves (W.S. Tyler Incorp. Mentor, OH, USA) with approximately 212  $\mu\text{m}$  pore size were used to filter the remaining contents. After filtration, the blend was transferred to a Petri dish for additional assessment of parasites under a stereo microscope following the method of Mdegela *et al.*, 2011. Briefly, the filtrates were centrifuged at 425.6G force for five minutes using a centrifuge machine (Sigma, USA). After discarding the supernatants, a drop of the collected sediments was placed on a microscope glass slide followed by two drops of saline, carefully smeared, and covered using a glass coverslip. A light microscope (10 $\times$  and 40 $\times$  magnification) was used to examine the content for the presence of worm eggs, adult parasites, and coccidian oocyst. Additionally, wet smears of the intestinal mucosa were prepared by sampling the contents with a glass slide at multiple areas and the parasites observed identified using standard fish parasite identification keys as described by Paperna (Paperna *et al.*, 1996). A stereo microscope (20 $\times$  amplification) with a side lamp was

used to observe any parasites present in the samples previously stored in 70% alcohol and smeared on a magnifying instrument glass slide with a drop of lactophenol before placing a coverslip.

#### *Analysis of water quality in pond using physicochemical parameters*

Physicochemical water quality parameter analysis (e.g. nitrate, ammonia, alkalinity and hardness, turbidity, and BOD) were done using Water test kits (Tetra GmbH, Germany) and according to the manufacturer's instructions. Water Ph, DO and the temperature was measured using a portable handheld multi-parameter probe (Eco pond supply, USA).

#### *Statistical analysis*

Simple descriptive statistics were used to give summaries such as the mean and standard deviation of weight and length of fish and other physicochemical parameters. Analysis of variance (ANOVA) was used to test the study hypotheses. R software was used to statistically analyze the prevalence of fish parasite infestation in the two regions.

## **Results**

### *Pond type, size, location, and management practices*

Concrete ponds were the most common in Arusha (60%) and Morogoro (73.8%), established mainly in the rural and peri-urban areas.

The number of fish ponds ranging from 20 to 200 m<sup>2</sup> in size in the Arusha and Morogoro regions was 87.7 and 83.1%, respectively (Table 1), with water sourced from boreholes, small rivers, streams, and tap water. Arusha and Morogoro had 72.3 and 70.8% of farmers, respectively, who stocked less than 1000 fingerlings in their ponds at the beginning of the production cycle. These farmers relied majorly on on-farm made feed formulations and to a lesser extent commercial feeds. Household food waste was rarely used in feeding the fish (Table 1). Pond water cleanliness was a major concern to fish health in both regions. Only four farmers reported changing pond water weekly. Unfortunately, the rest of the farmers took longer

periods, even months, to change their pond water throughout the production cycle. Considering the observed overstocking in some ponds (>2000 fingerlings). Disease infections were prevalent in both regions regardless of pond type, suggesting the

importance of maintaining proper aquaculture management practices. 75.4% and 76.9% of fish farmers in the Arusha and Morogoro regions, respectively, reported dealing with disease outbreaks in their ponds in the past.

**Table 1.** Type, size, location, and management of fish pond.

Region		Arusha					Morogoro				
Site (village)		Kikwe	Nambala	Maweni	Manyata	Samalia	Kihonda	Langali	Mikese	Mkindo	Tangeni
Factor		n for each site = 13					n for each site = 13				
Pond type	Concrete	3(23.0)	9 (69.2)	10 (76.9)	10 (76.9)	7 (53.8)	10 (76.9)	8 (61.5)	7 (53.8)	11 (84.6)	12 (92.3)
	Earth pond	10 (76.9)	4 (30.7)	3 (23.0)	3 (23.0)	6 (46.1)	3 (23.0)	5 (38.4)	6 (46.1)	2 (15.3)	1 (7.6)
Pond size (m <sup>2</sup> )	20–100	7 (53.8)	7 (53.8)	9 (69.2)	7 (53.8)	8 (61.5)	9 (69.2)	6 (46.1)	8 (61.5)	6 (46.1)	9 (69.2)
	101≤ 200	4 (30.7)	3 (23.0)	3 (23.0)	5 (38.4)	3 (23.0)	2 (15.3)	6 (46.1)	3 (23.0)	4 (30.4)	1 (7.6)
	>200 ≤ 300	2 (15.3)	3 (23.0)	1 (7.6)	1 (7.6)	2 (15.3)	2 (15.3)	1 (7.6)	2 (15.3)	3 (23.0)	3 (23.0)
The initial number of fish	<100	0 (0)	0 (0)	1 (7.6)	0 (0)	1 (7.6)	0 (0)	0 (0)	1 (7.6)	1 (7.6)	1 (7.6)
	101–1000	8 (61.5)	8 (61.5)	10 (76.9)	8 (61.5)	11 (84.6)	7 (53.8)	10 (76.9)	9 (69.2)	8 (61.5)	9 (69.2)
	1001–2000	3 (23.0)	4 (30.7)	1 (7.6)	2 (15.3)	2 (15.3)	3 (23.0)	2 (15.3)	4 (30.7)	4 (30.7)	3 (23.0)
	>2000	2 (15.3)	1 (7.6)	2 (15.3)	3 (23.0)	0 (0)	3 (23.0)	0 (0)	0 (0)	1 (7.6)	1 (7.6)
Feeding type	Commercial	0 (0)	3 (23.0)	4 (30.7)	0 (0)	3 (23.0)	4 (30.7)	4 (30.7)	1 (7.6)	0 (0)	0 (0)
	On-farm made	13 (100)	10 (76.9)	9 (69.2)	12 (92.3)	10 (76.9)	8 (61.5)	9 (69.2)	9 (69.2)	13 (100)	13 (100)
	Food scrap	0 (0)	0 (0)	0 (0)	1 (7.6)	0 (0)	1 (7.6)	0 (0)	3 (23.0)	0 (0)	0 (0)
Frequency of water change	Weekly	1 (7.6)	0 (0)	1 (7.6)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (15.3)	0 (0)
	Monthly	3 (23.0)	1 (7.6)	1 (7.6)	1 (7.6)	3 (23.0)	6 (46.1)	6 (46.1)	5 (38.5)	5 (38.4)	0 (0)
	Twice	2 (15.3)	10 (76.9)	7 (53.8)	8 (61.5)	6 (46.1)	2 (15.3)	2 (15.3)	8 (61.5)	0 (0)	0 (0)
	Once	7 (53.8)	2 (15.3)	5 (38.5)	4 (30.7)	4 (30.7)	5 (38.4)	5 (38.4)	8 (61.5)	6 (46.1)	13 (100)
Disease occurrence	Yes	13 (100)	8 (61.5)	10 (76.9)	11 (84.6)	7 (53.8)	9 (69.2)	12 (92.3)	10 (76.9)	13 (100)	6 (46.1)
	No	0 (0)	5 (38.4)	3 (23.0)	2 (15.3)	6 (46.1)	4 (30.7)	1 (7.6)	3 (23.0)	0 (0)	7 (53.8)

Data collected through questionnaires and direct observation during sample collection.

Legend: *n* is the number of ponds. The numbers in parentheses are percentages (%).

#### Physicochemical analysis of pond waters

The mean water temperature in Arusha and Morogoro regions was  $25.9 \pm 0.2$  °C and  $27.1 \pm 0.2$  °C, respectively (Table 2). The mean pH of pond water sampled in Arusha and Morogoro regions were  $9.8 \pm 1.4$  and  $7.2 \pm 0.8$ , respectively, and were highest in all sites studied in Arusha ( $8.6 \pm 0.9$  to  $11.2 \pm 0.8$ ) compared to the latter ( $6.4 \pm 0.1$  to  $8.3 \pm 0.3$ ) (Table 2). The mean alkalinity of the pond water sampled from all sites studied in Arusha and Morogoro regions was  $48.6 \pm 22.3$  mg/L and  $44.6 \pm 14.6$  mg/L, respectively, and were highest in Nambala ( $79.8 \pm 11.1$  mg/L) and Maweni ( $59.1 \pm 15.0$  mg/L) in Arusha region as well as Mikese ( $55.5 \pm 8.2$  mg/L) and Langali ( $53.6 \pm 15.0$  mg/L) in Morogoro region (Table 2). The mean nitrate levels reported in pond water in Arusha and Morogoro regions were  $8.4 \pm 5.2$  mg/L and  $4.9 \pm 2.4$  mg/L, respectively, and were highest in

Samalia ( $13.4 \pm 2.8$  mg/L), Manyata ( $12.8 \pm 2.4$  mg/L) and Maweni ( $10.7 \pm 1.8$  mg/L) in Arusha followed by Tangeni ( $8.9 \pm 2.1$  mg/L) and Langali ( $4.8 \pm 0.9$  mg/L) in Morogoro region (Table 2). The mean ammonia levels in pond water samples studied from Arusha and Morogoro regions were  $1.3 \pm 0.9$  mg/L and  $1.2 \pm 0.9$  mg/L, respectively, and were highest in Samalia ( $1.9 \pm 1.0$  mg/L) and Nambala ( $1.6 \pm 0.9$  mg/L) in Arusha as well as Mikese ( $1.6 \pm 1.0$  mg/L) in Morogoro region (Table 2).

The mean DO report in Arusha and Morogoro regions were  $4.0 \pm 1.3$  mg/L and  $6.0 \pm 1.93$  mg/L, respectively (Table 2). We found none of the fish ponds had aeration. Sites with ponds that had DO levels above 5 mg/L were Mkindo ( $9.1 \pm 4.1$  mg/L), Mikese ( $6.1 \pm 2.7$  mg/L), Langali ( $5.9 \pm 2.3$  mg/L), and Tangeni ( $5.3 \pm 2.3$  mg/L), all located in Morogoro region (Table 2).

**Table 2.** Mean physicochemical water parameters in fish ponds in sites within Arusha and Morogoro regions.

Region	Arusha (n = 65)					Overall Arusha mean	Morogoro (n = 65)					Overall Morogoro mean	
	Village (site)	Kikwe	Nambala	Maweni	Manyata		Samalia	Kihonda	Langali	Mikese	Mkindo		Tangeni
	n for each site = 13						n for each site = 13						
Water quality parameter	Temperature (°C)	25.6±0.1	26.0±0.1	25.9±0.1	25.8±0.1	26.0±0.1	25.9±0.2	27.2±0.4	26.9±0.1	27.0±0.1	27.2±0.2	27.0±0.1	27.1±0.2
	pH	9.2±0.8	8.6±0.9	11.2±0.8	9.9±1.6	10.3±1.3	9.8±1.4	6.4±0.1	6.9±0.1	8.3±0.3	7.5±0.7	6.8±0.4	7.2±0.8
	Nitrate (mg/L)	3.2±0.9	2.1±0.7	10.7±1.8	12.8±2.4	13.4±2.8	8.4±5.2	3.9±0.8	4.8±0.9	3.7±0.7	3.1±0.7	8.9±2.1	4.9 ± 2.4
	Ammonia (mg/L)	1.2±0.7	1.6±0.9	1.0±0.7	1.0±0.6	1.9±1.0	1.3±0.9	1.0±0.5	1.3±0.8	1.6±1.0	1.0±0.8	1.0±1.1	1.2±0.9
	DO (mg/L)	3.7±1.1	4.3±1.8	4.3±1.5	3.6±0.8	4.2±1.1	4.0±1.3	3.8±1.4	5.9±2.3	6.1±2.7	9.1±4.1	5.3±2.3	6.0±3.1
	BOD (mg/L)	12.7±2.7	16.8±5.5	19.3±5.4	22.4±4.3	25.6±4.4	19.4±6.3	34.2±4.3	29.4±5.0	32.6±5.2	29.8±4.4	23.1±4.8	29.8±6.0
	Alkalinity (mg/L CaCO <sub>3</sub> )	35.8±8.7	79.8±11.1	59.1±15.0	45.7±9.1	22.4±7.3	48.6±22.3	36.2±7.0	53.6±15.0	55.5±8.2	48.7±11.2	29.2±10.3	44.6±14.6
	Turbidity (NTU)	8.9±4.3	15.8±5.5	4.6±3.3	3.2±1.5	15.8±8.5	9.7±7.4	19.8±6.4	25.7±5.7	12.3±3.9	17.8±6.4	25.6±9.8	20.2±8.3
	Hardness (mg/L CaCO <sub>3</sub> )	125±26.1	260±77.9	140±32.7	78.0±23.5	60.0±22.7	133±81.6	200±33.0	195±46.8	220±41.7	189±73.3	300±57.3	221±65.3
	Fish	Fish length (cm)	15.7±5.5	17.5±5.8	15.4±6.0	13.7±6.1	13.1±4.8	15.1±5.7	12.5±4.4	13.5±6.0	14.1±6.7	14.8±5.5	13.2±5.5
Fish weight (g)		267.1±97	277.0±133	243.5±131	199.4±145	212.2±135	239.8±129	218.7±116	216.4±119	206.8±135	217.0±118	211.4±122	214.1±119

Values are mean ± standard deviation

Legend:

n is the number of ponds.

DO = Dissolved Oxygen

BOD = Biological Oxygen Demand

mg/L = concentration expressed in milligrams per liter.

NTU = Nephelometric Turbidity Units, a turbidimeter (nephelometer) measurement of light intensity as a beam of light passes through a water sample at 90 degrees.

cm = centimeter

g = grams.

On the other hand, Biological Oxygen Demand (BOD) indicates biodegradable organic content in the pond water. The recommended BOD level in pond water is 20 mg/L (Boyd, 2003). In this study, we found that the mean BOD levels in Arusha and Morogoro regions were  $19.4 \pm 6.3$  mg/L and  $29.8 \pm 6.0$  mg/L, respectively (Table 2). All sites studied in the Morogoro region had BOD levels above the recommended 20 mg/L concentration.

Turbidity measurement was taken to determine water clarity or discoloration. In this study, turbidity varied considerably from site to site and ranged from  $3.2 \pm 1.5$  to  $15.8 \pm 5.5$  NTU and  $12.3 \pm 3.9$  to  $25.7 \pm 9.8$  NTU in sites sampled in Arusha and Morogoro region, respectively (Table 2). These levels are ideal for tilapia and catfish farming. Ponds with clear waters were mainly found in Manyata ( $3.2 \pm 1.5$  NTU) followed by Maweni ( $4.6 \pm 3.3$  NTU), both in the Arusha region (Table 2).

Finally, water hardness was measured since it indicates calcium concentration in pond water. In our study, water hardness varied significantly from site to site and ranged from 60 – 260 mg/L and 189 – 300 mg/L in Arusha and Morogoro region, respectively (Table 2). The highest and lowest hardness values were found in Tangeni ( $300 \pm 57.3$  mg/L) in Morogoro and Samalia ( $60 \pm 22.7$  mg/L) in the Arusha region.

#### *Prevalence of parasites in Nile tilapia and African catfish in Arusha and Morogoro*

The standard length of fish captured in this study varied because ponds were at different production cycles and ranged from 8 cm to 23 cm (mean  $15.1 \pm 5.7$  cm) and 6 cm to 22 cm (mean  $13.6 \pm 5.6$  cm) in Arusha and Morogoro regions, respectively; while their weights in those two regions ranged from 46 g to 412 g (mean =  $239.8 \pm 129$ g) and 30 g to 395 g (mean =  $214.1 \pm 119$ g), respectively (Table 2).

Fish farmers in both regions mostly preferred Nile tilapia production. 73.1% and 70.8% of farms in the Arusha and Morogoro region, reared Nile tilapia (Table 3). Overall, we recovered 7 parasite species in both Nile tilapia and African catfish samples studied in both regions. The thorny headed worm *Acanthocephala* sp. was the most prevalent (Arusha (49.2%); Morogoro (50.7%)); while leeches were the least (Arusha (4.6%); Morogoro (7.6%)) occurring parasites in both Nile tilapia and African catfish samples studied (Table 3). Under light microscopy, *Acanthocephala* sp. is small, bilaterally symmetrical

worms with a retractable spined proboscis. Leeches had body segmentation, with an anterior and a rear sucker differentiating them from common free-living annelids. The overall prevalence of digenean trematode *Diplostomum* sp. (eye flukes), recovered from the vitreous humour of the eyes of fish, were found as 36.9% and 38.4% in Arusha and Morogoro regions, respectively, especially in African catfish samples from Arusha (72.7%). *Diplostomum* sp. had a cup-shaped front structure with suckers, with immature gonads contained in a cylindrical hind body.

**Table 3.** The occurrence of parasites on Nile tilapia and African catfish in Arusha and Morogoro.

Parasites	Parasite occurrence, n (%)							
	Arusha			$\chi^2$	Morogoro			$\chi^2$
	Nile tilapia (n = 54)	African catfish (n = 11)	Total (n = 65)		Nile tilapia (n = 46)	African catfish (n = 19)	Total (n = 65)	
<b>Endoparasites</b>								
<i>Acanthocephala</i> sp.	26 (48.1)	6 (54.5)	32 (49.2)	0.301 <sup>ns</sup>	25 (54.3)	8 (42.1)	33 (50.7)	0.631 <sup>ns</sup>
<i>Diplostomum</i> sp.	16 (29.0)	8 (72.7)	24 (36.9)	2.306*	22 (47.8)	3 (15.7)	25 (38.4)	1.426*
<i>Contracaecum</i> sp.	26 (48.1)	6 (54.5)	32 (49.2)	0.985 <sup>ns</sup>	17 (36.9)	10 (52.6)	27 (41.8)	1.011 <sup>ns</sup>
<b>Ectoparasites</b>								
<i>Trichodina</i> sp.	25 (46.2)	4 (36.3)	29 (44.6)	0.253 <sup>ns</sup>	19 (19.5)	8 (42.1)	27 (41.5)	1.73*
<i>Dactylogyrus</i> sp.	26 (48.1)	5 (45.4)	31 (47.6)	0.868 <sup>ns</sup>	16 (34.7)	5 (26.3)	21 (32.3)	0.611 <sup>ns</sup>
<i>Gyrodactylus</i> sp.	20 (37.0)	4 (36.0)	24 (36.9)	0.023 <sup>ns</sup>	23 (50.0)	8 (42.1)	31 (47.6)	0.913 <sup>ns</sup>
<i>Leeches</i>	3 (5.5)	0 (0)	3 (4.6)	0.836 <sup>ns</sup>	4 (8.6)	1 (5.2)	5 (7.6)	0.920 <sup>ns</sup>

Legend:

*n* is the number of fish samples

The numbers in parentheses are the percentages (%) of detected bacteria from two different fish species.

Statistically significant at \**P* < 0.05; ns = not significant.

The overall prevalence of nematode *Contracaecum* sp., recovered from the intestines of fish, were found as 49.2% and 41.8% in Arusha and Morogoro regions, respectively; again, with the highest occurrence in African catfish samples (Table 3). Visible to the naked eye, these worms are round, with a solid cuticle. The overall prevalence of Ciliophora *Trichodina* sp., a protozoan recovered from the skins of fish, was 44.6% and 41.5% in Arusha and Morogoro regions, respectively. Under light microscopy, *Trichodina* sp. had hooked ring-like denticles, appeared circular when observed dorsally, with a cup-shaped structure. Finally, we also recovered monogenean trematodes *Dactylogyrus* sp. and *Gyrodactylus* sp. from the gills and skin of fish. The overall prevalence

of *Dactylogyrus* sp. in Arusha and Morogoro regions was 47.6% and 32.3%; while the occurrence for *Gyrodactylus* sp. in the fish was 36.9% and 47.6% in Arusha and Morogoro regions (Table 3).

Under light microscopy, *Dactylogyrus* sp. contained a scalloped head with anteriorly eye spots while *Gyrodactylus* sp. had a V-shaped head, an opisthaptor at the back end, and no eyespots. Statistically, there was no significant difference (*P* < 0.05) in the prevalence of the parasites recovered on both Nile tilapia and African catfish in the sites studied, except for *Diplostomum* sp. in the Arusha region and *Trichodina* sp. and *Diplostomum* sp. in the Morogoro region (Table 3).

*Prevalence of fish parasites infesting farmed Nile tilapia and African catfish in select sites within Arusha and Morogoro*

The endoparasites recovered in the fish studied were *Acanthocephala* sp., *Diplostomum* sp., and *Contracecum* sp. The highest occurrence of *Acanthocephala* sp. in the Arusha and Morogoro region was found in Nambala (76.9%) and Tangeni (76.9%). From our results, this parasite least occurred in Samalia (23.0%) in Arusha region. Statistically, there was a significant ( $P < 0.05$ ) difference in the occurrence of *Acanthocephala* sp. between the individual sites sampled in both regions (Table 4). The highest occurrence of *Diplostomum* sp. in the Arusha region was found in ponds within Kikwe (53.8%) and Maweni (53.8%); while in the Morogoro region, this parasite mostly occurred in Tangeni

(53.8%) and Langali (46.1%). There was a significant ( $P < 0.05$ ) difference in the occurrence of this parasite across the ponds studied in Arusha (Table 4).

The highest occurrence of *Contracecum* sp. in the Arusha region were found in Maweni (76.9%), followed by Kikwe (69.2%); while in the Morogoro region, the most occurrence happened in Tangeni (76.9%), followed by Langali (38.4%), and Mikese (38.4%). There was a significant ( $P < 0.01$ ) difference in the occurrence of *Contracecum* sp. across the ponds sampled in the Arusha region, which we didn't find in sites from the Morogoro region (Table 4). Overall, the highest occurrences of endoparasites in this study were found in Kikwe, Nambala, and Maweni in Arusha region as well as Tangeni in the Morogoro region.

**Table 4.** Prevalence of endoparasites and ectoparasites in farmed fish in study sites within Arusha and Morogoro.

Bacteria species	Parasite occurrence, n (%)													
	Arusha (n for each site = 13)							Morogoro (n for each site= 13)						
	Kikwe	Nambala	Maweni	Manyata	Samalia	Total	$\chi^2$	Kihonda	Langali	Mikese	Mkindo	Tangeni	Total	$\chi^2$
<b>Endoparasites</b>														
<i>Acanthocephala</i> sp.	8 (61.5)	10 (76.9)	5 (38.4)	6 (46.1)	3 (23.0)	32 (49.2)	9.824*	7 (53.8)	4 (30.7)	7 (53.8)	5 (38.4)	10 (76.9)	33 (50.7)	15.758*
<i>Diplostomum</i> sp.	7 (53.8)	5 (38.4)	7 (53.8)	1 (7.6)	4 (30.7)	24 (36.9)	4.727*	5 (38.4)	6 (46.1)	3 (23.0)	4 (30.7)	7 (53.8)	25 (38.4)	8.254??
<i>Contracecum</i> sp.	9 (69.2)	5 (38.4)	10 (76.9)	4 (30.7)	4 (30.7)	32 (49.2)	12.123**	4 (30.7)	5 (38.4)	5 (38.4)	3 (23.0)	10 (76.9)	27 (41.5)	4.701 <sup>ns</sup>
<b>Ectoparasites</b>														
<i>Trichodina</i> sp.	10 (76.9)	4 (30.7)	6 (46.1)	4 (30.7)	5 (38.4)	29 (44.6)	15.255*	7 (53.8)	5 (38.4)	4 (30.7)	3 (23.0)	8 (61.5)	27 (41.5)	8.098 <sup>ns</sup>
<i>Dactylogyrus</i> sp.	8 (61.5)	5 (38.4)	9 (69.2)	6 (46.1)	3 (23.0)	31 (47.6)	7.58 <sup>ns</sup>	3 (23.0)	4 (30.7)	2 (15.3)	6 (46.1)	6 (46.1)	21 (32.3)	6.500 <sup>ns</sup>
<i>Gyrodactylus</i> sp.	5 (38.4)	3 (23.0)	11 (84.6)	3 (23.0)	2 (15.3)	24 (36.9)	5.591 <sup>ns</sup>	6 (46.1)	5 (38.4)	6 (46.1)	4 (30.7)	10 (76.9)	31 (47.6)	6.243 <sup>ns</sup>
<i>Leeches</i>	0 (0)	2 (15.3)	0 (0)	0 (0)	1 (7.6)	3 (4.6)	3.259 <sup>ns</sup>	0 (0)	0 (0)	0 (0)	4 (30.7)	1 (7.6)	5 (7.6)	6.429*

Legend: n is the number of fish samples

Numbers in parentheses are the percentages of detected bacteria from two different fish species

Statistically significant at \* $P < 0.05$ , \*\* $P < 0.01$ ; ns = not significant.

The four ectoparasites recovered in this study were *Trichodina* sp., *Dactylogyrus* sp., *Gyrodactylus* sp., and leeches. The highest occurrence of *Trichodina* sp. in Arusha was found in Kikwe (76.9%) followed by Maweni (46.1%); while in the Morogoro region, this parasite mostly occurred in Tangeni (61.5%), followed by Kihonda (53.8%). Prevalence of *Trichodina* sp. significantly ( $P < 0.05$ ) differed across sites studied in the Arusha region, which wasn't the case in the Morogoro region (Table 4). Furthermore, the results

show that there was higher *Dactylogyrus* sp. infection in the Arusha region compared to Morogoro, with the highest occurrence in ponds within Maweni (69.2%). Furthermore, we found that the occurrence of *Gyrodactylus* sp., the other monogenean trematode, was higher in Maweni (84.6%) in the Arusha region and Tangeni (76.9%) in the Morogoro region, with no significant difference in their prevalence across all the sites studied. Lastly, leeches rarely occurred in the fish samples, and none



existed on fish sampled from ponds within Kikwe, Maweni, Manyata, Kihonda, Langali, and Mikese (Table 4). Overall, the highest occurrences of ectoparasites found in fish sampled in this study were from ponds within Kikwe and Maweni in the Arusha region and Tangeni in the Morogoro region.

### Discussion

Aquaculture in Arusha and Morogoro regions is entirely informal; the farmers surveyed mainly reared fish for supplementary household income and home consumption. A majority of the fish farmers we interviewed had limited or lack accurate and critical information on proper aquaculture production. Most of the 130 fish farmers used farm-made feeds consisting of grain crops to minimize production costs. Since a majority of the ponds were  $\leq 200\text{m}^2$ , their initial stocking capacities were mostly less than 1000 fingerlings. We found 75.4% and 76.9% of farms in the Arusha and Morogoro region, respectively, had disease occurrences, indicating the importance of frequency of water change, pond cleanliness, and good aquaculture practices despite the scale of operation.

Aquaculture productivity is dependent on a wide range of factors. Successful management of aquaculture systems requires an understanding of water quality parameters, which is determined by abiotic factors such as temperature, pH, nitrate, ammonia, dissolved oxygen (DO), biological oxygen demand (BOD), alkalinity, turbidity, and hardness, among others (Bhatnagar and Devi, 2009).

The ideal water temperature is considered optimum for feeding, spawning, good health, and growth of Nile tilapia and African catfish is about 25 to 27 °C (Kausar and Salim, 2006). The mean temperature previously reported in 13 fish ponds located in urban and rural areas of the Morogoro region was  $26 \pm 3.1$  °C (Mdegela *et al.*, 2011). Other researchers gave a range of between 20 and 35 °C as ideal for tilapia culture (Ngugi *et al.*, 2007). These previous studies are consistent with our current findings. Different types of fish tolerate different pH levels. The ideal pH

for tilapia culture ranges from 6.0 to 9.0 (DeWalle *et al.*, 1995). Fish and other aquatic vertebrates have an average blood pH of 7.4. Therefore, ponds with pH levels close to fish blood pH would be ideal, the majority of which were found in the Morogoro region. H below 5.0 or above 10 may stress fish and cause heavy mortality (Ekubo and Abowei, 2010). pH values higher than 10.0 were reported in ponds within Samalia and Maweni in the Arusha region. From our results, some fish farmers in Arusha and Morogoro must begin monitoring the pH by recording weekly readings to provide an excellent indication of any developing problem. Previously, Mdegela *et al.*, 2011 reported mean pH from 13 fish ponds located in urban and rural areas of Morogoro as  $6.8 \pm 0.8$ , which was within the recommended standard range for Nile tilapia and African catfish production.

Naturally, water is saturated with dissolved oxygen (DO) in equilibrium with air but fluctuates considerably depending on the prevailing temperature of the water (Meck, 2000; Eze and Ogbaran, 2010). Decreased DO in the pond may lead to poor feeding of fish, starvation, reduced growth, and fish mortality (Bhatnagar *et al.*, 2000). DO level  $> 5$  mg/L is essential for good pond productivity (Bhatnagar *et al.*, 2000; Singh, 2010). Though sensitivity to low levels of DO is species-specific, most fish species are distressed when DO falls to 2 – 4 mg/L, leading to detrimental effects on growth and feed utilization, while mortality usually occurs at concentrations less than 2 mg/L (Bhatnagar *et al.*, 2000). From our results, 80% of the sites studied in the Morogoro region had mean DO levels ranging from  $5.3 \pm 2.3$  mg/L to  $9.1 \pm 4.1$  mg/L, which are excellent for pond life. On the other hand, average DO levels in ponds studied in Arusha ranged from  $3.6 \pm 0.8$  mg/L to  $4.3 \pm 1.8$  mg/L, and would most likely be lower during the night (Boyd, 2003). This is particularly concerning in constrained environments considering a concentration of  $>4$  mg/L DO is recommended for optimum pond life (Ntengwe and Edema, 2008). Reports indicate that African catfish can tolerate 20 to 30 mg/L CO<sub>2</sub> in pond water if DO concentration is above 5 mg/L (Wanja *et al.*, 2020).

Since aquatic plants use CO<sub>2</sub> for photosynthesis, water quality guidelines established by Bhatnagar and Devi provide a framework that small-scale fish farmers in Arusha and Morogoro may use to control amounts of aquatic weeds and phytoplankton in their ponds to manage DO levels (Bhatnagar and Devi, 2013). We must note, however, that minor fluctuations in the DO content in pond water are a natural occurrence and that fish have developed adaptive mechanisms to cope with these changes.

The Biological Oxygen Demand (BOD), which is a measure of biodegradable organic matter in the ponds, has an inverse relationship with DO. High BOD levels in pond water might be harmful to aquatic life and can arise from the unconsumed feed, fish waste, as well as surface runoff and soil erosion caused by rainfall (Odokuma and Okpokwasili, 1993; Mukherjee *et al.*, 2016). The recommended BOD level in pond water is 20 mg/L (Boyd, 2003). In this study, BOD values for Arusha were consistently lower than for Morogoro sites, suggesting a geographic influence on water sources and organic matter composition. The highest BOD was found in Kihonda in the Morogoro region, suggesting higher organic matter in these ponds than the rest. Such a load of organic matter upon degradation can mineralize to release sufficient nutrients for phytoplankton and other aquatic plants to thrive.

Water alkalinity and hardness levels were measured because they can have profound effects on pond productivity. Generally, the total alkalinity of > 20 mg/L CaCO<sub>3</sub> is necessary for good pond productivity. Lower alkalinity reduces the buffering capacity of water (Eze and Ogbaran, 2010). From our results, alkalinity values measured in both regions ranged between 20 – 80 mg/L and varied from site to site. Our results concur with Mdegela *et al* report who found mean alkalinity values in fish ponds located in the Morogoro region as 78.7 ± 34.1 mg/L (Mdegela *et al.*, 2011).

Hardness level in the range of 100 to 250 mg/L is ideal for aquaculture; a value of 250 mg/L hardness

matches the calcium concentration of fish blood (Wanja *et al.*, 2020). In this study, the only sites that had water hardness levels lower than 100 mg/L were Manyata and Samalia, both in Arusha region. All sites in the Morogoro region had water hardness in the range of  $\geq 189 \leq 300$  mg/L, and it would be reasonably safe to assume that these hardness levels reflect sufficient calcium concentrations for fish. Previously, researchers reported average water hardness of 76.6 ± 24.1 mg/L from 13 fish ponds located in Morogoro urban and rural areas, suggesting possible differences in characteristics of soil and bedrock where ponds surveyed in this study were located (Mdegela *et al.*, 2011).

Nitrogen is usually present in fish ponds as ammonia or nitrate. Ideally, the ammonia concentration in pond water should be zero. The minimum acceptable ammonia level suitable for pond fishery is < 0.2 mg/L (Bhatnagar and Singh, 2010). According to the Bureau of Fisheries and Aquatic Resources (BFAR), ammonia levels of between 0.2 – 0.5 mg/L are optimum for tilapia growth (Makori *et al.*, 2017). In this study, the ammonia levels in Arusha and Morogoro regions ranged from 1.0 ± 0.7 mg/L to 1.9 ± 1.0 mg/L and 1.0 ± 0.8 mg/L to 1.6 ± 1.0 mg/L. Fortunately, the alkalinity levels in all sites studied were ≤ 80 mg/L; this is desirable since the less toxic ionized form of ammonia (ammonium, NH<sub>4</sub><sup>+</sup>) is more prevalent in low alkaline waters (Handy and Poxton, 1993). Previously, other researchers reported ammonia levels of 1.0 ppm in fish ponds located in Morogoro urban and rural areas, which concurs with our findings in Kihonda, Mkindo, and Tangeni sites (Mdegela *et al.*, 2011). Reports indicate that an ammonia concentration of >0.6 mg/L in pond water can cause mortality in fish (Wanja *et al.*, 2020). Sadly, all ponds surveyed lacked aeration, yet several farmers failed to replace pond water regularly throughout the production cycle.

The favorable range of nitrate in the aquaculture pond is 0.1 to 4.0 ppm (Bhatnagar and Devi, 2013). The presence of nitrate in ponds could be from the fish feed and surface water runoff. Excessively higher

nitrate concentrations in pond water are indicative of pollution. Several researchers reported extremely low nitrate concentrations (0.20 ppm) in water samples from multiple ponds within the Morogoro region, which included Mkindo where we found levels in the range of  $3.1 \pm 0.7$  mg/L (Mdegela *et al.*, 2011). Though nitrate is very important to aquatic plants, its concentrations in the pond water should be controlled to avoid eutrophication. This can be effectively achieved through routine water changes and utilization by plant algae (Meck, 2000).

Turbidity measurements were taken to determine water clarity or discoloration levels. Reports indicate turbidity levels between 30 – 35 NTU are favorable for tilapia growth and the lower the better for freshwater fish (Ojwala *et al.*, 2018). In this study, turbidity values varied considerably from site to site, but were all less than 35 NTU, therefore are ideal for fish health. Though turbidity is usually only an aesthetic problem (such as muddy water), high turbidity can hinder sunlight penetration into the pond, affecting pond life. Zooplankton blooms, surface runoffs, disturbance of sediments by fish, and using rivers as a water source can all affect pond water discoloration.

Overall, substandard fish environment and poor pond management were observed in multiple fish ponds surveyed in the current study, especially in farms with earthen ponds which often provide a favorable environment for benthic microinvertebrates to proliferate (Kirjusina and Vismanis, 2007).

Monogeneans are flatworms (Platyhelminthes), are host- and site-specific, ectoparasitic and have special posteriorly positioned organs for attachment onto their host's skin or gills (Iyaji and Eyo, 2008). Monogenean trematodes such as *Dactylogyrus* sp. and *Gyrodactylus* sp. can proliferate rapidly especially in ponds under high stocking densities as their life cycle require only one host, thus poses a great threat to fish cultures (Mansell *et al.*, 2005). In this study, we recovered *Dactylogyrus* sp. and *Gyrodactylus* sp. from the gills of Nile tilapia and

African catfish samples from both Arusha and Morogoro regions, and their prevalence varied by region. Multiple reports exist on the prevalence of monogenean infection in East Africa. For instance, in Kenya, *Dactylogyrus* sp. was reported in farmed tilapia at a prevalence of 48.1% in Nyeri County and 3.5% in Kiambu County, which affected the health and quality of fish (Maina, 2017; Mavuti, 2017). Thus, fish farmers in Tanzania need to be informed that if left uncontrolled, monogenean infestation could lead to serious high morbidity and mortality and thus economic losses.

In our study, *Acanthocephalus* sp. had the highest prevalence among all parasites recovered, which concurred with studies conducted by Ashmawy *et al.*, 2018. *Acanthocephalus* sp. are mostly endoparasitic with at least one intermediate host in their life cycle. We recovered *Acanthocephalus* sp. from the gastrointestinal tract of both Nile tilapia and African catfish and found a significant ( $P < 0.05$ ) difference in the prevalence of this parasite between all sites studied in both regions. Comparatively, Mavuti *et al.*, 2017 isolated *Acanthocephalus* sp. from tilapia reared in earthen ponds at a lower prevalence of 0.8% in Tetu, Nyeri County, Kenya (Mavuti, 2017). Florio *et al.* also found the prevalence of *Acanthosentis* sp. in Kenya (7.1%) and Uganda (13%) in all the fisheries systems (wild and caged) (Florio *et al.*, 2009). Additionally, Mwita and Lamtane reported the prevalence of *Acanthocephalus* sp. as high as 47.9% in unconfined environments such as Lakes Uba and Ruwe in Tanzania (Mwita and Lamtane, 2014). The disparity between our results and these other reports can be ascribed to the differences in feeding habits or the availability of intermediate hosts such as crustaceans. Earthen ponds with overgrown vegetation are the most at risk of encouraging *Acanthocephalus* sp. infestation because intermediate hosts such as amphipods, isopods, copepods, or ostracods thrive in such environments (Eyo *et al.*, 2013). In our survey, some farmers admitted sourcing fingerlings from fellow farmers whose quality is unknown. This could lead to the transmission of parasites such as *Acanthocephalus* sp. between farms.

Furthermore, since most farmers produced fish on a subsistence level, several admitted to practicing partial harvesting and left some fish to continue inbreeding in the ponds.

Digenean trematodes such as *Diplostomum* metacercariae require multiple hosts (e.g. snails, fish, and piscivorous birds) to propagate and can be found externally or internally in the fish organs (Thon *et al.*, 2019). *Diplostomum* sp. In this study, we recovered *Diplostomum* sp. from the vitreous humour of the eyes of fish and found that it infested significantly high numbers of African catfish in Arusha (72.7%) than in the Morogoro region (15.7%). We found a significant ( $P < 0.05$ ) difference in the overall prevalence of *Diplostomum* sp. in both Nile tilapia and African catfish in the Arusha and Morogoro regions. A similar influence of ecological zone on parasite infestation was also reported in Kenya where the prevalence of *Diplostomum* sp. in farmed Nile tilapia was found as 54.3% in Tana River and 1.9% in Nyeri County (Mathenge, 2010; Mavuti, 2017). Earthen ponds are good environments for digenean helminths because snails, crustacean copepods, and leeches can be vectors/intermediate hosts. Eliminating intermediate hosts through good aquaculture management can substantially minimize its prevalence in aquaculture systems.

In this study, we recovered the larvae of *Contracaecum* sp. from the abdominal cavity of Nile tilapia and African catfish samples from both Arusha and Morogoro regions. In both regions, the occurrence of *Contracaecum* sp. was higher in African catfish than Nile tilapia. This may be related to diet and water sources. Both fish species feed on all intermediate hosts including detritus, benthic invertebrates like arthropods, mollusks, mud, and other small fish, potentially accumulating the parasite larvae (Mathenge, 2010; Mavuti, 2017). This presents a huge challenge to farmers using earthen ponds because chance proximity of infected mollusks has implications on *Contracaecum* sp. prevalence. Therefore, the aquaculture sector must focus on the control of nematodes to safeguard public health and

prevent production losses.

Protozoans in fish are highly pathogenic. Ciliophora, especially trichodinids, is characterized by cilia for locomotion, round shape when seen dorsally, and a ring with hook-like denticles (Mavuti, 2017). In this study, we recovered *Trichodina* sp. from the skin of Nile tilapia and African catfish samples. Compared to our results, Mavuti *et al* reported *Trichodina* sp. occurred in both farmed Nile tilapia and African catfish at a much lower overall prevalence of 1.4% in Nyeri County, Kenya (Mavuti, 2017). All infested fish were from earthen ponds, which are highly prone to siltation and vegetation cover that often support parasitism. Overstocking and poor pond management can exacerbate protozoan infections in fish. Other reports from the East Africa region showed a high prevalence of ciliates in various aquaculture systems (Akoll *et al.*, 2012). Therefore, for profitable and sustainable aquaculture in Tanzania, awareness of fish health and good farm management practices should be encouraged. Leeches are differentiated from monogenean parasites by the presence of body segmentation. Leeches suck blood from the soft tissues and exposed organs, which can potentially kill the fish (Mavuti, 2017). In this study, we observed leeches in gills, nostrils, and the anus of both Nile tilapia and African catfish samples. The use of rivers as a source of water could be a possibility of leeches being introduced into farmed fish reared exclusively in concrete ponds. Comparatively, reported leeches in the gills of farmed Nile tilapia and African catfish with an overall prevalence of 2.7% in Nyeri Central sub-county, Kenya. Additionally, in Nigeria, Iyaji and Eyo (2008) reported leeches on the mouths of silver catfish with an overall prevalence of 19% (Iyaji *et al.*, 2009; Mavuti, 2017).

### Conclusion

Based on our observation and results, farmers need to be informed on how key water quality parameters, pond management, feed quality, stocking density, pond type, source of water, and frequency of water change, among others, affect pond productivity, parasite infestation, disease outbreaks, fish quality,

and human health. Our results highlight a need to strengthen extension services to the small-scale and subsistence fish farmers in Tanzania. We recommend that farmers establish small cooperative fish farmer groups within the Aquaculture Association of Tanzania (AAT) to improve on-farm knowledge transfer. Due to the high prevalence of several parasites in these two most economically important fish species in Tanzania, we also recommend proper pond management practices, awareness of fish health, and fish disease prevention at the community level to minimize parasitic infection and improve aquaculture in Tanzania. Finally, strengthening collaborative research on aquaculture between government research institutions and academia should be encouraged for better fish health management in Tanzania. Of course, still more studies are required to explore the magnitude of the parasitic infestation in fish under aquaculture farming in other regions of the country.

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