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Effects of Stocking Density on Digenean Trematode and Monogenean Infections in Nile Tilapia (*Oreochromis niloticus*, Linnaeus 1758) Reared in Cages in Uhanya Beach, Lake Victoria, Kenya

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Abstract

Cage cultured fish are susceptible to high disease incidence because of the high fish stocking densities as compared to other culture systems. This study was conducted from December 2017 to May 2018 with an objective of highlighting stocking density as a driver of monogenean and digenean trematode parasitism in Oreochromis niloticus in cages in Uhanya Beach, Lake Victoria, Kenya. The study adopted a systematic random sampling technique to sample an aggregate 600 fish during the rainy and dry seasons. Parasites isolated from the sampled fish were the monogenean Dactylogyrus and the digeneans; Tylodelphys, Clinostomum and Neascus. Dactylogyrus sp. was predominantly abundant in the fish from all the 10 cages. Mann-Whitney U-test revealed that mean intensity of Dactylogyrus sp. infestation considerably augmented with higher stocking density of fish ($p < 0.05$; $df = 298$). On the contrary, Clinostomum sp., Tylodelphys sp. and Neascus sp. exhibited significantly reduced infestation with increased stocking densities ($p < 0.0001$). All the individual fish showed allometric growth and the general fish condition as depicted by condition factor (Kn) was significantly better in cages with stocking densities of 2500 individuals/ Cage ($df = 298$; $p < 0.05$). The relationship between intensity of parasite infestation and fish condition factor was not also weak and significant in all the parasite species (Dactylogyrus sp.: $r^2 = 0.017$; Clinostomum sp.: $r^2 = 0.001$; Tylodelphys sp.: $r^2 = 0.008$; & Neascus sp.: $r^2 = 0.026$). The study recommends proper site selection for cages, water quality management and sourcing of fish seed from certified hatcheries to prevent the spread of parasitic diseases.

Keywords: Monogenea, Digenean Trematode, *Oreochromis niloticus*, Stocking Density, Cage Culture

INTRODUCTION

Tilapias are prime domesticated species for their good aquaculture attributes such as excellent growth rates even in low protein diets. It can also withstand wide ranges of environmental conditions, has little susceptibility to disease and is amenable to handling and captivity (Yadav, 2006). There are three taxonomic groups (genus) under which the tilapine fishes fall into, these are the Tilapia, *Oreochromis* and *Sarotherodon*. Species from the genus Tilapia lay their eggs on substrates, *Oreochromis* species are maternal brooders while *Sarotherodon* are

both maternal and parental brooders (Wegener, 2016). The natural range of tilapia is in Palestine, in the Nile and across to West Africa, and in Lakes Rudolf (Turkana) and Albert in East Africa (Lowe-McConnell, 1958). *Oreochromis niloticus* is native to Central and North Africa and the Middle East (Griffiths & Picker, 2011). The Nyanza Gulf suffers from Eutrophication by anthropological activities (Roegner *et al.,* 2020). Lake level fluctuations and an array of anthropogenic activities damage its ecological integrity by decreasing its area and the quality of goods and services it provides (Okotto-Okotto *et al.,* 2018). Pollutants can cause sub lethal physiological strain to fish thereby reducing its capacity to endure parasite attack potentially aggregating infection levels indirectly (Cable *et al.,* 2017). Fish and parasites live in equilibrium; this can be lost due to environmental turbulences such as the variations in water quality (Lafferty *et al.,* 2015) as well as poor management (Plumb, 2018) and high stocking densities (Mishra *et al.,* 2017). There has been a growing demand for fish and a significant reduction of fish catches in Lake Victoria (Njiru *et al.,* 2019). Therefore, cage culture is a promising undertaking towards increased production, employment creation and enhancement of economic wellbeing.

Neodermata is a group of platyhelminthes consisting of the Digenea, Aspidogastrea, Monogenea and Cestoda (Brusa *et al.,* 2020). Monogeneans, digeneans, leeches and certain groups of parasitic copepods are the most common parasites and can be extremely numerous in cultured fish leading to secondary bacterial and fungal infections or fish mortalities (Hutson *et al.,* 2018). The digenean trematodes are amongst the most common fish-borne parasitic diseases (Faruk, 2018). The digeneans have an intricate life cycle which encompasses developmental stages in one or more intermediate hosts and an adult stage in definitive hosts (Mesquita *et al.,* 2020). The backbone of digenean systematics is morphological examination of sexual adults from vertebrates (Bakhoum *et al.,* 2017). To spread to the vertebrate host, the cercariae either infiltrate directly through skin or mature into adults; this is species dependent. Nation *et al.,* 2020). The eggs laid by the adult worm pass to the environment and a miracidium may hatch and swim away or dependent on species, the egg may be ingested by the subsequent. The metacercariae crosses to a subsequent intermediate host, and awaits being swallowed or attaches to plants by metacercariae secrete, a resistant cyst wall where it awaits to be eaten (Halstead *et al.,* 2018). Larval stages of trematode flukes are common in fish, typically encysting as metacercariae within species specific sites (Stumbo *et al.,* 2012). It represents a phase where the potential to continue the life cycle and the ability to infect definitive hosts is retained and extended over a relatively long period (Tatonova & Besprozvannykh, 2019). Infectivity of metacercariae is passive, occurring only when the target host ingests the parasite and is at its highest at low temperatures (Zimmermann *et al.,* 2017).

There are two sub classes of monogeneans based on the nature of their haptor; the monopisthocotyleans (1 haptor as hooks for attachment) (Zhang *et al.,* 2018) and the polyopisthocotyleans (various parts to the clamped haptor. Polyopisthocotyleans are mostly found dwelling in gills and feed on blood (Weston, 2018) whereas Monopisthocotyleans could be present in gills, on skin and fins (Oroga, 2019). Monopisthocotyleans include the Genus *Gyrodactylus,* which has no eyespots and is viviparous (Zhang *et al.,* 2020). The *Dactylogyrus* is a genus of monopisthocotyleans monogeneans in the Dactylogyridae family, commonly known as gill flukes and is usually found on the gills of Cyprinid fishes (Zhang *et al.,* 2019). A large number of monogeneans are browsers, they move on the body surface feeding on the dermal mucus and gill debris (Petchimuthu *et al.,* 2018). They are equipped with a series of hooks for attachment during feeding (Garvey, 2020). Most monogenean species

are host and site specific (Benovics *et al.,* 2020) and require only a single host to complete its entire life cycle; some may even permanently remain attached to the host on a single site. Monogenean damages result to secondary bacterial and fungal infections (Doan *et al.,* 2020).

Aquaculture is increasingly being intensified resulting in higher stocking densities (Romano & Sinha, 2020). Rearing density and management of fish diseases are essential factors affecting aquaculture productivity and viability (Engle *et al.,* 2017). The crowding in cages promotes stress and allows disease causing organisms to spread rapidly (Mishra *et al.,* 2017). Nile tilapia stocked at high stocking densities experience a decrease in dissolved oxygen (Zaki *et al.,* 2020); this stresses fish resulting in decreased growth and survival making fish susceptible to parasitic infections. High stocking density reduces the growth and survival rates in fish culture (Paul *et al.,* 2016) whereas extremely high stocking densities of cage cultured tilapia reduces the sustainability and efficiency of the system (Monteiro *et al.,* 2016) through susceptibility to high disease incidences (Yarahmadi *et al.,* 2016). Monogenean transmission occurs primarily via direct contact therefore increased stocking density increases the contact between one fish and the other (Elsheikha & Patterson, 2013). The crowded nature of cages stimulates stress and permits the rapid spread of disease-causing organisms (Mishra *et al.,* 2017). The outbreak, persistence, and eradication of infectious diseases often depend on the density of hosts (Krkošek, 2017); therefore, chronic stress induced by high stocking densities in cage culture may have significant implications on fish immune-competence. This study highlights on the effects of stocking density on infection by monogenean and digenean trematode parasites.

MATERIALS AND METHODS

Study Area

This study took place in Uhanya Beach, Yimbo in Siaya County from December 2017 to May 2018. Uhanya Beach is located in Usenge sub-location, Bondo Constituency, Siaya County in West Yimbo location with a total population of 21,931 (SCSP, 2019). The county lies between latitudes 0° 26’ and 0° 18’ N and longitudes 33° 58’ and 34° 33’ E (GOK, 2015). The Kenyan side of Lake Victoria has an overall of 3,696 fish cages, of which majority (n=3141; 85%) are situated in Siaya County (Aura *et al.,* 2018). The Uhanya Fish Cage Enterprise in Uhanya beach, Usigu ward was selected based on the number of cages (300). The cage farm lies between latitudes 0° 5.40’ to 0° 5.70’ south and longitudes 34° 4.62’ to 34° 4.86’ east (Figure1).

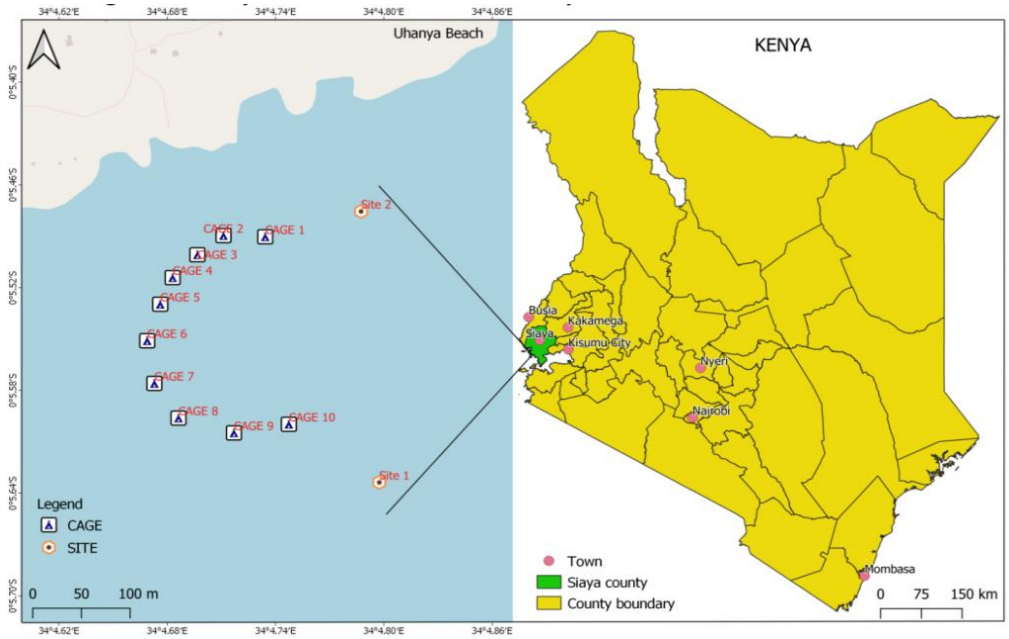

Figure 1: Position of Sampled Fish Cages in Uhanya Beach, Siaya County.

Study Design

A reconnaissance study of the cage site was conducted. With the help of the farm manager, identification of the operational cages, cage sizes, fish stocking densities and distances between the cages was established. The study focused on cages stocked with 2500 and 2000 monosex *O. niloticus* to determine the correlation of number of fish per cage on parasite prevalence and its effect on fish condition factor. The GPS coordinates of the farm was recorded and cages systematically selected to form sample cages for this study using a systematic random sampling technique.

Sampling Design

Systematic random sampling technique was used. The initial cage sampled was 300 metres off shore of the lake and the subsequent cages were selected after every 50 meters from the previous cage and at least 4 metres above the lake bottom. From a total of 246 fish cages, 124 cages measured 2 x 2 x 3 metres of which 64 had a stocking density of 2,500 and the remaining 60 had a stocking density of 2,000 monosex O. niloticus. Out of these, 10 cages were sampled. Five of these cages were stocked at a density of 2,000 monosex *O. niloticus* fingerlings whereas the other 5 were stocked at a density of 2,500 fish and reared for eight months. To achieve randomization, a scoop net was used to randomly pick 30 fish from each study cage, stocked at a density of 167 fish/m^3 and 208 fish/m^3, respectively. The fish were carried in buckets and labelled according to name, location and stocking density of cage then taken to a field laboratory for parasite isolation, observation and analysis. A total of 600 fish were collected from 10 cages, 300 fish collected from December 2017 to May 2018 to establish the influence of stocking density on digenean trematode and monogenean parasitism.

Isolation and Identification of Parasites from Fish Samples

Fish samples were examined for both the internal and external parasites through observation and microscopy. Fish were killed by dislocating the cervical vertebrae as described in Matvienko *et al.* (2015) prior to parasitological examinations. Gross examination of the skin, fin and gills was done using a magnifying lens of a

magnification of ×10 to determine the presence of fish ectoparasites. A cover slip was used to make skin and gill scrapings and thereafter a wet mount prepared on a microscope slide and observed under a dissecting microscope at a magnification of ×100. For the endoparasites, the samples were dissected and the eyes, kidney, intestine, swim bladder and body cavity were inspected. Parasites isolated were categorized as either monogeneans or digenean trematodes. Monogenean and digenean parasites were identified based on their morphological features and with the aid of standard identification keys and illustrative picture guides (Pouder *et al.,* 2011; Scholz & Choudhury, 2014). Parasite counts were recorded.

Water Quality Parameters

Water physico-chemical variables were measured *in situ* throughout the sampling period with the aid of a YSI multi-probe water quality meter, model number (556 MPS, Yellow Springs Instruments, Ohio, USA) at each cage and the two off-cage sites. These parameters were; water temperature, dissolved oxygen concentration (DO), pH, Total Dissolved Solids (TDS) and salinity. These physico-chemical variables were measured at depths of 1, 2 and 3 m levels below the surface.

Determination of Parasitic Indices

The following parasitic indices were determined according to Bush *et al.* (1997) and calculated as follows;

a). Relative abundance of parasites (RA)

$$RA = \frac{ij}{Ij} * 100$$

Where: ij = No of individuals of parasite species *i* in host fish *j*

IJ = No of individuals of all parasite species in host fish *j*

b). Mean intensity of parasitic infestation (MI)

$$MI = \frac{i}{Fi}$$

Where: *i* = overall number of individuals of parasite species *i*

Fi = total number of host fish infested

(c). Prevalence of parasitic attack (PA)

$$PA = \frac{fi}{Fe} * 100$$

Where: *fi* = total number of host fish infested by parasite species *i*

Fe = total number of host fish examined

Data Analysis

All data sets were first examined for parametry (normality) and outliers were excluded from the datasets before being subjected to univariate statistics both descriptive and inferential using SPSS (version 16) and advanced tools of MS Office Excel (2016). Mann-Whitney U-test was used to test for significant differences in water quality parameters between the two stocking densities (167 fish/m^3 and 208 fish/m^3) of monosex *Oreochromis niloticus*, respectively. Kruskall-Wallis test was then used to examine the relationship between significant water quality parameters (Temperature, DO, and TDS) and mean intensity of parasitic attack (*Dactylogyrus sp*., *Clinostomum sp*., *Tylodelphys sp*. and *Neascus sp*.).

RESULTS

Identification of Monogenean and Digenean Parasite species infecting *Oreochromis niloticus* reared in cages in Uhanya Beach, Lake Victoria, Kenya

The monogenean parasites identified were of the genera *Dactylogyrus* while the digeneans were of the genera *Tylodelphys*, *Clinostomum* and *Neascus*. The *Dactylogyrus sp.* parasite was found attached on gills. They had eye spots and a series of hooks on the anterior end. *Tylodelphys sp.* was found attached in the vitreous fluid of the eyes. The *Clinostomum sp.* was found encysted in the buccal. *Neascus* sp. larvae were encysted on the skin where it formed black spots as a result of pigment

mobilization on the skin of *O. niloticus* (**Plate 1**).

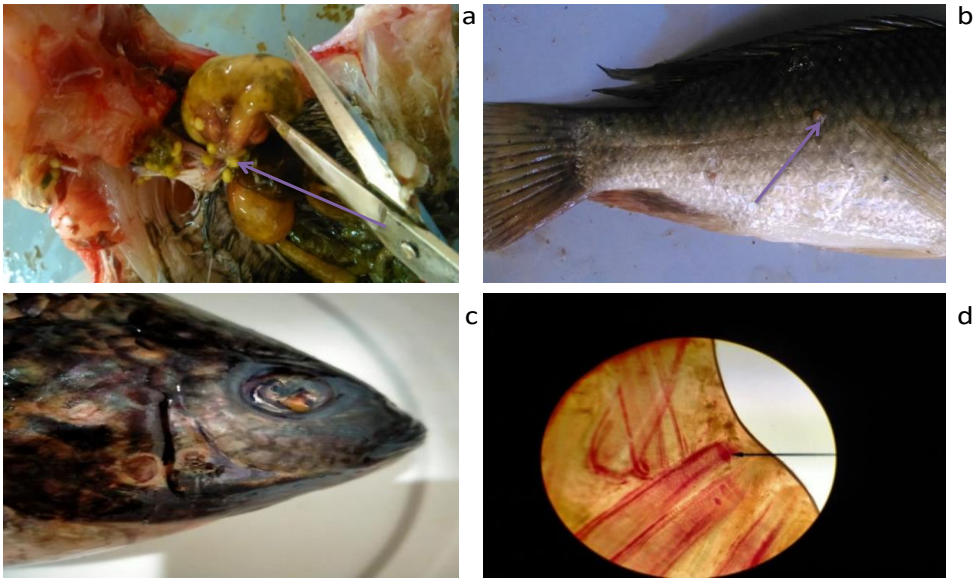

Plate 1*:* (a) *Metacercariae* of *Clinostomum sp.* encysted in the buccal cavity, (b) *Metacercariae of Neascus sp.* encysted on the skin, (c) Total blindness, and (d) monogenean Dactylogyrus on the gill filament of cage cultured monosex *Oreochromis niloticus* in Uhanya Beach, Lake Victoria (Photograph taken in 2018 by Julie Bwoga).

Mean Intensity and Prevalence of infestation of digenean trematodes and monogeans in *Oreochromis niloticus* at different stocking densities

Student's t-test revealed that mean intensity of *Dactylogyrus sp.* infestation significantly increased with increased fish stocking density ($t = -1.94$: $p < 0.05$; $df = 298$). On the contrary, *Clinostomum sp., Tylodelphys sp. and Neascus sp.* exhibited significantly reduced infestation with increased stocking densities ($t = 5.18$; $df = 298$; $p < 0.0001$; $t = 1.83$: $p < 0.05$; $df = 298$ and $t = 5.10$: $p < 0.0001$; $df = 298$, respectively) (Figures 2 & 3).

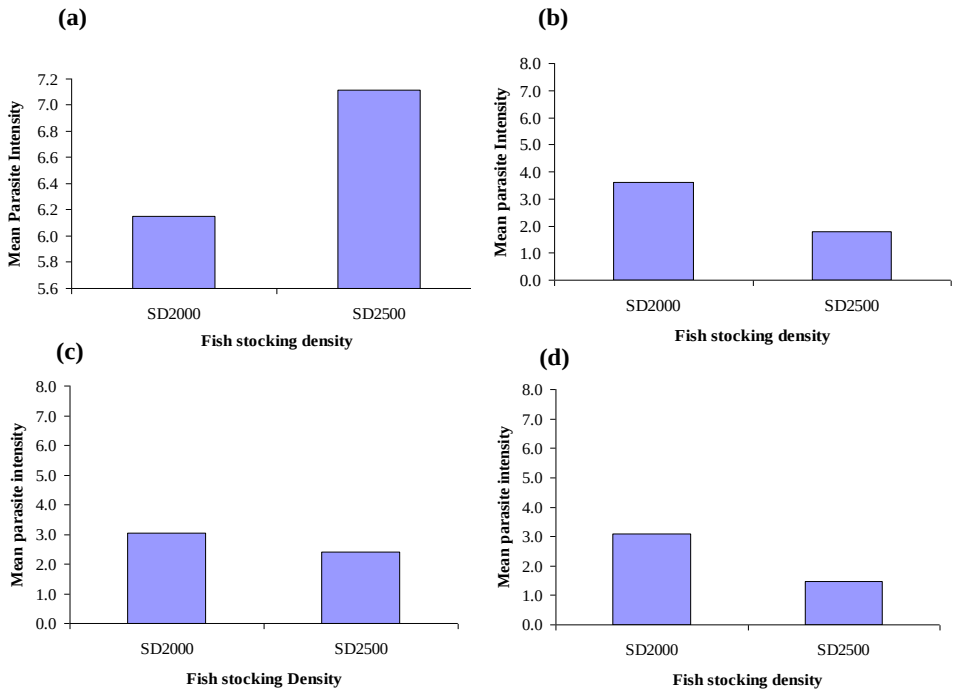

Figure 2: Mean (± SE) intensity of infestation by fish parasite: (a) *Dactylogyrus sp*.; (b) *Clinostomum sp*.; (c) *Tylodelphys sp*.; and (d) *Neascus sp*. across different stocking densities in fish cages in Uhanya Beach of Lake Victoria.

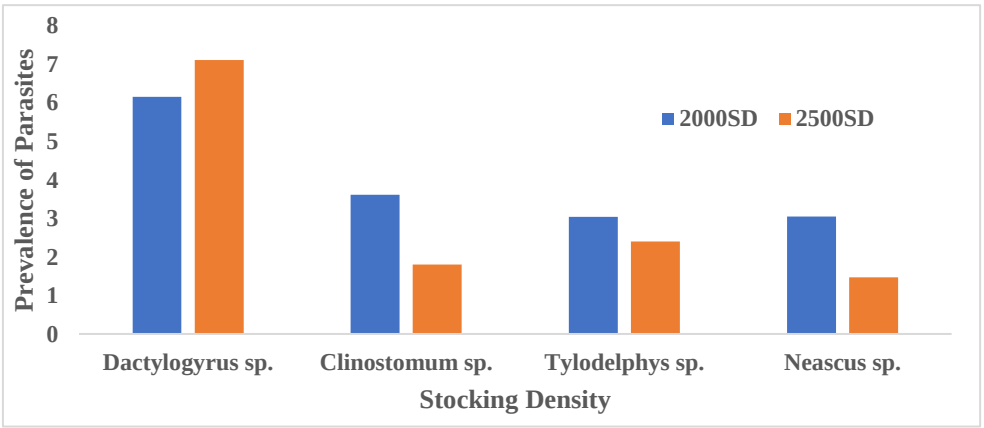

Figure 3: Prevalence of parasites (a) *Dactylogyrus sp*.; (b) *Clinostomum sp*.; (c) *Tylodelphys sp*.; and (d) *Neascus sp*. across different stocking densities in fish cages in Uhanya Beach of Lake Victoria.

Relative abundance of parasites

Dactylogyrus sp. was predominantly abundant in the fish from all the 10 cages (**Table 1)** shows multiple comparison of the parasite composition by relative abundance from the 10 cages.

Table 1: Post hoc Multiple Comparison (Bonfenori) of relative abundance between parasites (1. *Dactylogyrus sp.*, 2. *Clinostomum sp.*, 3. *Tylodelphys sp.*, 4. *Neascus sp.*)

					95% Confidence Interval	
(I)Parasites	**(J)Parasites**	**Mean Difference (I-J)**	**Std. Error**	**Sig.**	**Lower Bound**	**Upper Bound**
	2	42.647	1.028	0.000**	39.93	45.36
1	3	43.284	1.028	0.000**	40.57	46.00
	4	46.818	1.028	0.000**	44.10	49.54
	1	-42.647	1.028	0.000**	-45.36	-39.93
2	3	0.637	1.028	1.000^{ns}	-2.08	3.35
	4	4.171	1.028	0.000**	1.45	6.89
	1	-43.284	1.028	0.000**	-46.00	-40.57
3	2	-0.637	1.028	1.000^{ns}	-3.35	2.08
	4	3.534	1.028	0.004*	0.82	6.25
	1	-46.818	1.028	0.000**	-49.54	-44.10
4	2	-4.171	1.028	0.000**	-6.89	-1.45
	3	-3.534	1.028	0.004*	-6.25	-0.82

DISCUSSION

The study shows a link between stocking density of fish in cages and parasitism. *Dactylogyrus sp.* was predominantly abundant in the fish from all the 10 cages, this concurs with the study by Otachi *et al.* (2014) in which the monogenean parasite species were found to be the dominant taxa infesting two cichlid fishes in Lake Naivasha, Rift Valley, Kenya. Modi & Prasanna (2018) also reported *Dactylogyrus fotedari* as the most prevalent monogenean infesting gill arches of freshwater fish in India. The escalated intensity of infestation with increased stocking density of *Dactylogyrus sp*. witnessed in this study, agrees with the study by Garcia *et al.* (2013) where it was established that use of the lowermost initial stocking density reduces the risk of disease outbreak and therefore no need for therapeutic disease control. In their study, the death rate and incidences of disease and deformity reduced with decreased stocking density. In the same study, the dissolved oxygen level inside the cages increased with decreased stocking density. This study expresses a case of a co-infection by both monogenea and digeneans where a single fish is infected by both parasite groups. Abdel *et al.* (2020) also described co-infections commonly arising when two or multiple different pathogens infect the same host leading to parasitic outbreaks amongst other negative impacts. On the contrary, the reduced mean intensity of *Clinostomum* sp., *Tylodelphys sp*. and *Neascus sp.* with increased stocking densities may perhaps be explained by the dilution effect caused by linear escalation in contact with high host density. In cage reared tilapia in Bangladesh, the prevalence of *Tylodelphys sp.* identified was incomparable to the gill monogenean *Gyrodactylus* (Doulah *et al.*, 2019), this conforms to the findings of this study. This can be explained by reduction in encounter as has been recorded in trematodes (Keesing *et al.*, 2006). These astounding effects could be a function of the elevated density and restricted mobility of these parasites, and their overall low probability of effectively infecting a host. Eye flukes are famous for causing detrimental effects through inducing cataract, once inside a fish's eye; it can cause partial blindness and several behavioural changes to the intermediate host (Flink *et al.*, 2017). Some fish species can absorb the transmittable stages of parasites regardless of its ability to infect the fish consequently reducing encounters between the most competent hosts and parasites (Civitello *et al.*, 2013).

This study agrees with that of Zaki *et al.*, (2020) where it was observed that Nile tilapia stocked at high stocking densities

experienced a decrease in dissolved oxygen which in turn stresses fish, leading to decreased growth and survival thus making fish susceptible to parasitic infections. This study identified fish suffering from partial and total blindness and tissue injuries in both stocking densities. This concurs with findings by Calabrese *et al.* (2017) where increasing fish stocking density negatively affected feed utilization (FCR) and the external welfare of fish leading to fin damage and formation of eye cataracts (Calabrese *et al.,* 2017). Mean intensity of *Dactylogyrus sp*. infestation significantly increased with increased fish stocking density. This can be attributed to the transmission of monogeneans from fish to fish which occurs primarily via direct contact, where increased stocking density increases the contact between one fish and the other (Elsheikha & Patterson, 2013). Therefore, in this regards, extremely high stocking densities of cage cultured tilapia reduce the sustainability and efficiency of the cage culture system (Monteiro *et al.,* 2016) in the long run. Andree *et al.* (2013) validates that exposing fish to stress agents such as water temperature, low DO and high stocking densities has a substantial upsurge in the pathogenicity of the invading pathogens.

CONCLUSION AND RECOMMENDATIONS

The study identified the monogenean *Dactylogyrus* as the predominant parasite affecting tilapia in cages in Uhanya Beach, Lake Victoria. The study further established that stocking fish at the rate of 2,500 individuals leads to a decline in parasite numbers in individual hosts as a result of dilution effect in host density for trematodes parasites. However, for the monogenean group, an escalation in rearing density led to an upsurge in parasite attack because the parasite is transmitted by direct contact and with an increase in fish numbers in a cage, the contact chance is increased. The study recommends the use of 2,500 stocking density for maximum profits if water quality is good and feeding is done as required of in intensive aquaculture.

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