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Original Research Article

Occurrence and effect of *Diplostomum* parasites in cultured *Oreochromis niloticus* (L.) and distribution in vector snails within Kisumu City, western Kenya

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ABSTRACT

Freshwater snails and larval trematode communities were studied in relation to diplostomiasis infection in fish. Out of 680 fish examined, 52.2% were positive for *Diplostomum* parasites. *Lymnea*, *Biomphalaria*, *Bulinus* and *Ceratophallus* snail species occurred, however *Diplostomum* larvae were only in *Biomphalaria* at a prevalence rate of 21.69%. There was no significant relationship between parasite abundance and fish condition factor in all the study sites, hence the wellbeing of the fish was not compromised by the parasites. Values of the regression co-efficient obtained for the length–body weight relationship in all the farms suggested isometric growth.

1. Introduction

The trematode genus *Diplostomum*, (family: Diplostomidae) represents a large group of parasites of economic importance worldwide due to their pathogenic metacercariae which parasitise the eyes of fish, in both natural and aquaculture systems (Chappell et al., 1994; Chappell, 1995). *Diplostomum* species parasites have a complex lifecycle. They mature in the small intestine of piscivorous birds and pass through snails as first intermediate host and fish as second intermediate host (Chappell et al., 1994). In fish, the parasites inhabit the lens, retina and aqueous humour of fish eyes as well as the brain, spinal cord and nasal spaces thereby resulting into substantial losses of wild and farmed fish.

Currently, the distribution of diplostomiasis infection in a given geographical area is determined largely by the presence or absence of metacercariae in fish eyes. This is evident from extensive studies regarding occurrence, pathology, taxonomy, lifecycle development and to a minor degree population biology of *Diplostomum* species that have been undertaken in the northern hemisphere (Field and Irwin, 1994; Chappell, 1995; Locke et al., 2011). Studies of diplostomiasis in fish farming within East Africa are few with reported findings by Kassaye et al. (2009), Fioravanti et al. (2009), Matolla (2009) and Mwita and Nkwengulila (2008). These laid emphasis on *Diplostomum* prevalence and intensity among the fish, occasionally trying to identify the intermediate snail hosts within the vicinities. Malacological surveys in Europe (Faltynkova, 2005; Faltynkova and Haas, 2006; Faltynkova et al., 2007; Voutilainen et al., 2009) have reported *Lymnaea stagnalis* as the sole vector snail of the parasite, however, reported studies on the same in Africa are scanty (Voutilainen et al., 2009) due to low sampling efforts extended to snail intermediate hosts. In addition, emphasis on vector snails as bioindicators of diplostomid communities in the parasite lifecycle has received little attention (Karvonen et al., 2004; Huspeni et al., 2005) compared to extensive

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studies that have focused on the parasite in fish. Larval trematodes found in snail hosts serve as one of the most promising bioindicators of the infection, because each infection in a snail host persists for the life of the snail unless the trematode is displaced by a superior competitor (Voutilainen et al., 2009). In addition, at temperatures above 20 °C, diplostomid cercariae production in snail vectors can range between 40 and 60,000 cercariae per day; implying that even at low numbers of infected snails, possibility of fish infection remains high (Karvonen et al., 2004). So far studies on *Diplostomum* spp. cercariae are rare as reported by Georgieva et al. (2012) whilst challenges faced in aquaculture as a consequence of the parasites. The major constraint is low sampling efforts attributed to pond snail intermediate hosts in tropical countries. Majority of temporary and spatial distribution studies on snail vectors have been limited to Schistosomatidae due to the significant socio-economic importance of the digenean family. Reports on Schistosomatidae have established that *Biomphalaria* and *Lymnea* species of snails play an important role in the transmission of schistosomiasis within the informal settlements of Kisumu City (Opisa et al., 2011). Kibos area, is among the informal settlements of Kisumu City, however, the area was not included in a pilot study conducted by Opisa et al. (2011) owing to financial constraints. Considering that Kisumu exhibit temperatures of between 22 °C and 32 °C which are optimum for snail development and reproduction, Kibos area which is located within Kisumu City share a similar microclimate environment as the studied informal settlements. Thus, lack of studies on the role of different snails in watershed environments in Kibos area predispose the community to major problems of health and fish farming development.

Comprehensive studies on the effect of *Diplostomum* spp. on the growth of fish in privately managed farms in Kisumu City is equally lacking due to inadequacy in provision of extension services and training to farmers. Evidence of decline in the average size of fish (100–180 g) from 300 g has been reported previously in Kibos area over the last 3–5 years (Otieno, 2010). In addition, current small sizes of fish available in Kisumu City fish market clearly suggest that fish farming in the area is faced by major challenges. A report by Shitote et al. (2011) examined the challenges facing fish farming development in western Kenya and established that farmers do not know/understand diseases affecting fish and it is, therefore, difficult for them to identify whether diseases have a negative impact on their fishing endeavours. Monitoring the growth of farmed fish is necessary for accurate prediction of production levels in fish farms. In addition, information on the length and weight measures of fish is important in elucidating the health status of broodstock and juveniles which determine the amount of seed to be produced for stocking of ponds and quality of fingerlings to be used in stocking of fish farms.

It is therefore imperative to identify snail intermediate hosts for *Diplostomum* parasites in Kisumu, which will aid in elucidating the associations between biotic ecology (vector snails) with parasitology data from the fish and their environment.

The overall aim of this study was to determine the occurrence of *Diplostomum* parasites in fish and distribution of diplostomid trematodes in pond snails within fish farms in Kisumu City. In addition, the environmental and physicochemical factors that may influence *Diplostomum* development and snail distribution were determined. This study further investigated the effect of *Diplostomum* parasites on the length–weight relationships of farmed tilapia in Kisumu City.

2. Materials and methods

2.1. Study area

The study was conducted in Kibos area within Kisumu City, Kenya. Kibos is located between latitudes –0.07001′ N/0°4′0.0012″ S and longitudes 34.81092′ E/34°49′0.0012″ E. This area is largely endowed with black cotton soil which is very sticky, with pH values ranging between 6.55 and 6.85, base saturation percentages of 95.3 and cation exchange capacity estimated at 72.5 cmol/kg (Bowman and Seim, 1995). These characteristics enhance aquaculture through low soil permeability, relatively high availability of nutrients, relatively low amounts of acidity, and high base saturation percentages, which result in minimal lime requirements. Three farms were selected for the study based on their location near a major fry production centre in Kibos, which serve as the main supplier of fingerlings in the western region of the country. Selected farms were distantly situated (>30 km) to ensure spatial distribution of parasites affecting fish.

2.2. Fish sample size

The species was Nile tilapia, *Oreochromis niloticus* L.1758; the sample size used was estimated according to the formula by Daniel (1999).

2.3. Study design

A cross-sectional study design was adopted in three fish farms, namely a hatchery centre (Farm 1) and two privately owned fish farms (Farm II and Farm III) in Kibos area. Three fish ponds were selected per fish farm using a computer random number generator technique to increase chances of establishing diversity of *Diplostomum* parasites among the fish.

2.4. Fish sampling procedure and transportation

Sixty four fish were sampled per pond for every three fish ponds in a farm. Sampling of fish was conducted between 0900 hours and 1100 hours which coincided with fish feeding.

Sampling was conducted after every three weeks for a period of three months (December 2011–February 2012). A seine net of 1.5 m diameter and 6 mm mesh was used for sampling. Sampled fish were then transported in an iced cool box at 8 °C to the Department of Zoology laboratory, Maseno University, for analysis.

2.5. Sampling of snail population and transportation

A minimum of 100 snails were randomly collected from each farm using a scoop or by hand collection from the ponds. Snail sample size calculation was estimated according to Huspeni et al. (2005). Sampling was performed between 0830 h and 1030 h. This was following the hypotheses that most snails during morning hours are dormant and begin shedding of cercariae at 1100 hours coinciding with the presence of the next host (Combes et al., 1994). At each collection time, snails from each site were appropriately labelled and transported in separate perforated plastic containers to the Department of Zoology laboratory, Maseno University for analysis.

2.6. Physicochemical characteristics of the pond water

Water pH and temperature were measured directly in the field using Hanna HI 9828 hand held multiparameter metre.

2.7. Examination of fish specimens for Diplostomum parasites

Fish eyes were dissected and then examined for metacercariae with a stereoscopic microscope using procedures as described by Yamaguti (1971) and Gibson (1996). The metacercariae extracted from each eye were counted as separate lots, and placed in a petri dish containing saline solution before storing in 95% ethanol.

2.8. Examination of fish for length–weight relationship

Specimens were mopped on filter paper to remove excess water from their body surfaces. Total and standard lengths were then measured using a ruler in centimetres. The total length was measured as the distance from snout to the tip of the caudal fin while the standard length was measured as the distance from the snout to the caudal peduncle. The body weight was taken using a tabletop weighing balance to the nearest 0.1 g. Fish samples were recorded according to their collection sites as F1, F2 and F3.

2.9. Confirmation of snail infection

Snails were rinsed and placed individually in 24-well culture plates (Corning Glass Works, Corning, NY, USA) containing 1 ml of filtered de-chlorinated water for a period of 24 h. The snails were exposed to a 15-h light/9-h dark lighting regimen for shedding cercariae (Steinauer et al., 2008). After 24 h, plate wells were examined with a dissecting microscope for the presence of cercariae shed by the snails. Identification of cercariae was made according to their morphology and behaviour, as strigeoid cercariae form a distinctive right-angle resting position and the furcae spread apart at an angle of 180° (Niewiadomska, 1986). Isolated cercariae were picked and stored in vials containing 95% ethanol and stored at 4 °C.

3. Data analysis

3.1. Analysis of parasite occurrence among three fish farms

Prevalence (%) of *Diplostomum* parasites was estimated as the ratio between the number of infected fish and the number of examined fish expressed in percentages. The mean intensity (M.I.) was determined as the ratio between the total number of parasites in a sample and the number of infected fish in a sample. The mean abundance (M.A.) was determined as the ratio between the total number of parasites in a sample and the total number of fish examined (infected + uninfected).

One-way ANOVA was used to test for differences in abundance of parasites among the farms. To determine the possible correlation between the parasite number and host standard length, Pearson's linear correlation "*r*," was used. Kruskall–Wallis *H* test was used to test for differences in parasite number among the host length classes. Comparison for prevalence of infection between the snail populations among the farms was performed using Fisher's exact test. Associations between snail abundance and physicochemical variables were determined using spearman correlations. Results from various statistical tests were considered significant at $p \leq 0.05$ using SPSS v. 17.00 (USA) software packages.

3.2. Analysis of Fulton's condition factor

The length measurements were converted into length frequencies with constant class intervals of 5 cm. The mean standard lengths and weights of the classes were used for data analysis, the format accepted by FISAT (Gayando and Pauly, 1997). The Fulton condition factor (*K*) of the experimental fish was compiled from values of the growth exponent according to Froese (2006):

$$K = \frac{100W}{L^b}$$

where *K* is the Fulton's condition factor; *W* the mean weight of all specimens in a given length class (g); *L* the mean length of the respective length class (cm) and *b* is the regression coefficient. *K* values obtained were compared with the standard $K = 1.0$ by Student's *t*-test. The independent-samples *t*-test was used to test for differences in the *K* values between parasitised and un-parasitised hosts. To determine the relationships between condition factor and the number of parasites, Pearson product moment correlation coefficient was used.

3.3. Analysis of the length–weight data

Relationship between weight (*W*) and length (*L*) of fish was expressed by equation: $W = aL^b$ (Froese, 2006). Where *W* is the mean weight of all specimens in a given length class (g); *L* the mean length of the respective length class (cm); *a* the exponent describing the rate of change of weight with length (=the intercept of the regression line on the *Y* axis); *b* is the slope of the regression line (also referred to as the allometric coefficient). The "*a*" and "*b*" values were obtained from a linear regression of the length and weight of fish. The degree of adjustment of the model studied was assessed by the correlation coefficient (*r*). Student's *t*-test was applied to verify whether the declivity of regression (constant "*b*") presented a significant difference of 3.0. In all cases a statistic significance of 5% was adopted.

Table 1
Summary of the distribution and prevalence of trematodes in pond snails collected from three fish farms in Kibos area.

Site	Type of snail species	Number of snails collected	Number of snails infected with:			
			Mammalian cercariae	Xiphidiocercariae	Amphistomes	Strigeoid cercariae
Fish farm I	*Biomphalaria* spp.	288	0	0	0	30 (10.42%)
	Lymnea spp.	180	0	0	0	0
	Ceratophallus	0	0	0	0	0
	Bulinus spp.	6	0	0	0	0
Fish farm II	*Biomphalaria* spp.	270	0	18 (6.67%)	24 (8.89%)	15 (5.56%)
	Bulinus spp.	156	0	0	1 (0.64%)	0
	Lymnea spp.	12	0	0	0	0
Fish farm III	*Biomphalaria* spp.	420	0	0	12 (2.86%)	24 (5.71%)
	Lymnea spp.	3	0	0	0	0
	Bulinus spp.	24	0	0	0	0

4. Results

4.1. Snail species, distribution, abundance and infection in snails

A total of 1359 snails were collected from the three sites. Out of these, 124 (9.12%) snails were infected with trematodes; they represented only two species of the freshwater snail community. The general prevalence of natural infection in host snails was, 0.64% (1) in *Bulinus* spp. and 12.58% (123) in *Biomphalaria* spp. (Table 1). The larval trematode community was depauperate and composed of 3 types of cercariae, (based on cercarial morphology), namely: xiphidiocercaria type I, amphistomes and strigeoid cercariae. No individual snail was infected with more than one digenetic cercariae. The most prevalent cercariae were strigeoid cercariae (21.69%) (genus *Diplostomum*) recovered from snails of *Biomphalaria* spp. No infection for mammalian cercariae was observed among the snails. Mean snail abundance varied significantly across the 3 farms in the different monthly sampling periods ($F_{2,\ 72} = 4.918$, $p = 0.0001$), while there was a marginal difference in snail abundance among different sites for the assayed period ($F_{2,\ 72} = 4.93$, $p = 0.506$).

4.2. Environmental factors

The most common vegetation in close proximity to the fish farms was grass, trees, papyrus and sugarcane plantations. Qualitative data indicated that the vegetation cover was associated with snail abundance in the farms. Birds namely; cormorants, kingfisher, egrets and eagles were observed visiting the farms as well as the papyrus (Plates 1–3)

4.3. Physicochemical parameters of water

The mean values of water temperature ranged between 26 and 30 °C. This significantly influenced the overall snail

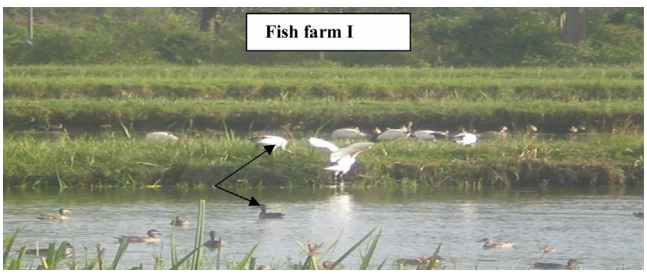

Plate 1. Ducks, egrets and cormorants in a pond in Fish farm I.

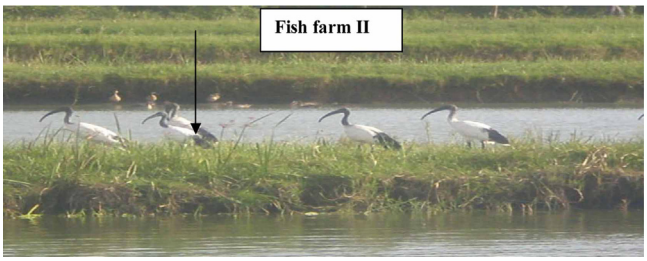

Plate 2. Sacred ibis in Fish farm II.

Plate 3. Cormorants in Fish farm III.

abundance ($F_{2,\ 900} = 21.23$, $p < 0.01$). There was a positive association between water temperature and overall snail abundance ($r = 0.8$, $p = 0.01$). The pH levels of water did not vary greatly at the sites. The mean pH of water from fish farm I was 6.62 ± 1.1 (range = 5.2–6.2), fish farm II was 5.34 ± 1.2 (range = 5.1–5.38) and fish farm III was 5.15 ± 0.31 (range = 5.1–5.3). pH was positively associated with snail abundance from all the sites ($r = 0.733$, $p < 0.001$).

4.4. Prevalence, mean abundance and mean intensity of *Diplostomum* parasites from three fish farms in Kibos area

A total of 680 fish were collected from the three fish farms in Kibos area. General prevalence of infection in the area was 52.21% with 355 fish infected. Farm I which is also a fry production centre had a prevalence of 47.42% ($n = 272$) with a mean intensity ranging between 2 and 15 parasites; farm II had a prevalence of 43.23% ($n = 192$) with a mean intensity ranging between 0 and 8 parasites and farm III had a prevalence of 66.2% ($n = 216$) with a mean intensity ranging between 2 and 10 parasites. There was a statistically significant effect of the fish farms on the number of *Diplostomum* parasites (ANOVA $p < 0.0001$). Comparison of the farms using Tukey's post hoc test indicated that at least one of the farms was different from another ($p < 0.000$) hence the difference in parasite intensity among the farms.

4.5. Condition factor of *Oreochromis niloticus* sampled in three fish farms in Kibos area

4.5.1. Comparison of condition factor between parasitised fish and non-parasitised fish

t-Test revealed no significant difference in the condition factor values (K) for parasitised fish ($M = 1.787 \pm 0.04793$) and non-parasitised fish ($M = 1.970 \pm 0.08437$; $t\ (4) = 1.886$, $p = 0.1324$) in fish farm I. Similarly, results obtained from fish farm II and fish farm III did not reveal any significant differences in the condition factor values between parasitised and non-parasitised fish ($t\ (5) = 0.09920$, $p = 0.9248$; $t\ (7) = 0.1428$, $p = 0.8905$) respectively. Hence, there was no significant difference in the fish condition factor (K) between parasitised and non-parasitised fish in all the study sites ($p = 0.253$).

4.5.2. Relationship between the number of parasites and the fish's condition factor

There was no significant relationship between number of parasites and fish condition factor in all the study sites (Pearson correlation; $p = 0.516$, $p = 0.565$, $p = 0.357$ respectively). This meant that the condition factor of the fish did not decrease with increase in the number of *Diplostomum* sp. metacercariae in the eyes of the fish.

4.6. Length–weight relationships

There was a strong significant relationship between length and weight of fish (p values < 0.001) and a high degree of positive correlation between total length and total weight of all individuals (r^2 0.90–0.99). The estimated values of the exponent b ranged from 2.63 to 3.18. Parasitised fish in farm II and III had 'b' values < 3 ($p < 0.05$); implying a negative allometric growth whereas non-parasitised fish in farm I and III showed positive allometric growth ($b > 3$; $p < 0.05$). In summary, the exponent b values were around the hypothetical value '3' showing isometric growth for the farmed fish.

5. Discussion

5.1. Prevalence, abundance and intensity of *Diplostomum* parasites from three fish farms in Kibos area

Occurrence of *Diplostomum* infection in the farms was directly associated to the presence of a high abundance of *Biomphalaria* spp. snails shedding cercariae of *Diplostomum* parasites (Table 1). *Biomphalaria* snails live on water plants and mud that is rich in decaying organic matter (Karvonen et al., 2005). Open earthen ponds are common in tropical countries and are preferred by most fish farmers due to their affordability in construction and increased profitability as reported by Jacobi (2013). However open fish ponds accumulate nutrients, mud, clay and silt at the bottom of the pond which is stirred up from the pond bottom or comes into the pond with rainwater. This accumulation facilitates development of different biotic life such as insects and snails. *Biomphalaria* snails in this study were collected at the bottom of the ponds as well as near the shores of the ponds. These snails commonly occur in water that is moderately polluted with organic matter, such as faeces and urine (Karvonen et al., 2005). Faecal matter along the shores of the ponds in this study was mainly contributed by birds that frequently visited the pond areas as shown in Plates 1–3. The presence of snails and birds in the environment showed that colonisation of parasites with a complex life cycle was strongly favoured. Similarly, a study conducted by Fioravanti et al. (2009) in

Kenyan earth pond-based farms reported 1–4 metacercariae in farmed *Oreochromis niloticus* with a prevalence of 40.7%. Results from these findings therefore concur with findings by Fioravanti et al. (2009) that indicate earthen ponds provide a conducive environment that play a significant role in proliferation of parasites. In addition, the fact that these were open ponds, there is a high possibility that snails found their way into the ponds as a result of surface run offs from the vegetation cover found in close proximity to the farms. According to Bertman (1980), the highest prevalence of trematode infection in snail populations took place in small, shallow and overgrown still waters containing many aquatic organisms rather than in flowing waters like rivers and streams. As shown from the qualitative data in this study (Plates 1–3), vegetation such as papyrus reeds, sugarcane plantations and grass dominated the nearby areas of the fish ponds. This is suggestive that the vegetation acted as reservoir for snails and other aquatic organisms. As suggested by Karvonen et al. (2005), plants serve as substrates for feeding and oviposition of snails as well as providing protection from high water velocities and predators such as fish and birds. The vegetation also seemed to offer a conducive environment for eggs emerging from motile vertebrate hosts (i.e. birds) to hatch and meet with miracidium emerging from the snails. Therefore, findings from this study concur with the suggestion by Hechinger and Lafferty (2005) that snails serve as important intermediate hosts in the completion of *Diplostomum* life cycle.

Physicochemical parameters of water served a significant role in snail population densities and transmission rates. According to Levitz et al. (2012), temperature and pH play a significant role in snail and parasite development. This study demonstrated that these physicochemical parameters of water appeared to be the key determinant of increased trematode prevalence among the snails. The pH of water in all the farms (range 5.1–6.6) was lower than the recommended range of pH for cultured fish (range 6.8–8.7), while the association with snail abundance was ($r = 0.733$, $p < 0.001$) positive. Although tilapia can survive in pH ranging from 5 to 10, they do best in a pH range of 6 to 9. Reduced pH in the water was likely to be related to sugarcane plantation found within the vicinity of the farms. Most of the research done in sugarcane plantations, have pointed reduction of soil pH to the use of nitrogenous fertilizers which reduce soil pH during ammonification and nitrification processes (Oliver, 2004). Equally, water temperature appeared to be a key determinant of snail abundance. The positive association between snail abundance and water temperature observed in our study ($r = 0.3$, $p = 0.01$) suggested optimal reproduction in the snail. This is in agreement with observations that demonstrated *Biomphalaria pfeifferi* grew and survived better at 25 °C than at 19 °C (Sturrock, 1966).

The present study was conducted between December and February; these months were dry and hot with recorded temperature range of the pond waters being 26–32 °C. Although there was a high prevalence of *Diplostomum* infection in fish, prevalence of trematodes in the snails seemed low with the highest prevalence (10.42%) observed in snails collected from LBFPC. The overall high temperatures recorded (26–32 °C) might have contributed to temperature stress and ultraviolet radiation that affected trematode survival and ability of miracidia to infect snails. This is typical of infection by digenean larvae (Esch and Fernandez, 1993) as high prevalence of trematode parasitism in snail population render a remarkable proportion of snails infertile due to the host-castrating effect of trematodes. As a result, the host density is reduced (Puurtinen et al., 2004). Snail prevalence of less than 10% of *Diplostomum* infections have similarly been reported in lymnaeid snails in both Finland (Väyrynen et al., 2000; Faltynkova et al., 2007) and other European countries (Faltynkova, 2005; Faltynkova and Haas, 2006).

This study therefore indicated that very low proportion of snails tend to shed cercariae that translate to high levels of diplostomiasis infection in the farms. This is because, infection prevalence between 1% and 10% in snails is considered sufficient to generate 100% infection rates in fish (Stables and Chappell, 1986). In contrast to findings from this study, Nkwengulila and Kigadye (2005) reported higher infection prevalence levels of digenean trematodes in snails during the dry season compared to other seasons. Other workers (Väyrynen et al., 2000; Karvonen et al., 2006) have reported seasonal variation of prevalence of digeneans in snails attributing high prevalence during dry seasons to be contributed by reduced water volume in conjunction with increased density of snail hosts and intensified use of the habitat by definitive hosts. This study was conducted only during the dry season with no comparisons to the other seasons.

5.2. Effect of Diplostomum parasites on length–weight relationship of farmed Oreochromis niloticus

The length–weight relationship (LWR) showed that parasitisation did not reduce the growth of infected fish. Curtis (1981) and Marcogliese et al. (2001) also observed no significant effect of *Diplostomum* parasitism on the condition of fish despite the high number of parasite intensity observed ranging between 1 and 248 parasites per host. In contrast, massive fish kills due to *D. compactum* metacercariae infections in the eyes of cultivated tilapias have been documented in Malpaso and La Angostura reservoirs in the state of Chiapas, Mexico (Chappell, 1995) with total blindness reported where metacercariae counts exceeded 40 individuals per eye, depending on fish size (Evans et al., 1976). However, a number of other factors (e.g. sex, seasons, environmental conditions, stress, food supply (quantity and quality), spawning conditions and other factors, such as, age, gonad maturity and stomach fullness) also affect the growth condition of fish. The fish specimens in this study were in good condition; similarly, the weight increased logarithmically with an increase in length which is desirable quality in fish for a fish farm.

6. Conclusion

In summary, our findings indicate that *Diplostomum* parasites are present in fish ponds within Kisumu City and its transmission patterns are closely related to the abundance and distribution of *Biomphalaria* snails. Even

though the metacercariae counts in the eyes of the studied fish were not sufficiently high to affect survival, preventive measures such as vegetation control and removal of snail populations are needed to prevent any possible diplostomiasis outbreaks in the culture systems of the City of Kisumu.

Conflict of interest

None declared.

Financial disclosure

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