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Prevalence and intensity of ectoparasites in Nile tilapia hatcheries in Homa Bay County, Kenya

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Abstract
Nile tilapia is the most preferred fish species for rearing and human consumption in Africa. Despite being a prolific breeder, demand for tilapia seeds remains high, and hatcheries have been established to meet this demand. However, hatcheries face significant challenges with high costs associated with feed, electricity, fish diseases and parasitism. A study was conducted in two hatcheries in Homa Bay County, Kenya in March and April 2023, in a bid to establish the prevalence and intensity of ectoparasites on various age groups of Nile tilapia (*Oreochromis niloticus*) in these facilities. One hundred and forty samples were collected from the two hatcheries and examined *in-situ*. These included 20 scoops each for eggs, fry and larvae and, 40 pieces each for brood stock and fingerlings. The eggs, larvae and fry were examined wholly under the microscope, while for fingerlings and brooders, skin scrapings, fin and gill clips were examined. Overall ectoparasite prevalence was 66% (92/140) and was highest in brooders (80%; 32/40) and lowest in larvae (10%; 2/20). Five genera were recovered, with the monogenean, *Gyrodactylus* being found in all age groups at a prevalence of 35% (49/140). Others included the monogenean, *Dactylogyrus* (12%; 17/140), ectopropozoans, *Trichodina* (11%; 16/140) and *Epistylis* (4%; (6/140) and the fish louse, *Argulus* spp. (0.7%; 1/140). The prevalence of *Gyrodactylus* spp. and *Dactylogyrus* spp. was statistically significant (X^2=10, sig=0.039) and (X^2=20, sig<0.001) in the hatcheries, respectively. The ciliate, *Epistylis* spp. had the highest mean intensity of 30.8 relative to other ectoparasites and there was a significant difference between the two farms (X^2=4.4, sig=0.037). These findings indicate that ectoparasites are common in all age groups in hatcheries, and were most prevalent in brooders. Hatchery operators should implement strict biosecurity measures in order to avoid the spread of ectoparasites within the hatcheries and grower ponds.

Keywords: Epistylis, gyrodactylus, hatchery, lake victoria, monogenean, oreochromis niloticus

1. Introduction
Nile tilapia are prolific breeders which are able to breed in captivity and don't require to be induced by use of hormones or other methods. Spawning happens all year round in the tropics and in warm seasons in the subtropics [1]. Tilapia become sexually mature at the age of 6 months at a temperature of about 24 °C. Once the natural conditions are optimum, the males often change color to bright colors in order to attract females [2]. Mature females with ripe eggs identify the male, upon which the male makes a nest where the female lays eggs, they are fertilized by the male and mouth collected by the female for incubation [1]. Incubation for the eggs takes 1-2 weeks depending on temperature, until the yolk sac is fully absorbed, the fry is released to swim freely but can always escape predation by returning to the mouth of their mother [1].

The natural breeding of tilapia has its own challenges including inbreeding, overbreeding, lack of uniformity in fingerling sizes amongst others [3]. To help mitigate these challenges, commercial tilapia hatcheries have been set up. In these hatcheries, where many fingerlings are produced, the process is complex. Once brood stock has been selected, they are sexed and put in different holding places such as tanks, cages or hapa nets [4]. They are then provided with complete feeds until they are ready for spawning. Brood stock are selected and crossed at the ratio of 3 females to 1 male at a stocking density of 0.7Kg/M^2 for optimum results [5].

The brood stock are held in hapa nets that are erected in well prepared ponds. Mating occurs with the fish that are ready and the female incubates the eggs in its buccal cavity. The male now looks for other mates as the female continues to hold the eggs in the mouth where they are collected and incubated in hatching jars. It is in these hatching jars where right conditions of temperature, dissolved oxygen and flow rate are provided until the eggs hatch. Once the eggs hatch and are held in the hatchery as larvae in trays, basins or tanks until they exhaust the yolk sac and become fry[4].

The fry is now ready for feeding, where it is introduced to sex reversal feed, as producers often opt for production of only male fingerlings[5]. The fry can be held in tanks or hapa nets in ponds for 21-28 days being fed with sex reversal feed. After this period, depending on management levels, the fry is now a fingerling which is ready for stocking to cages, open ponds, dams or even lakes.

Fish ectoparasites attach on host external organs where they derive nutrients at the expense of the host, they cause injuries which can be a route for secondary infections. Fins, eyes, skin, mouth cavity and gills are some of the external organs that are mainly affected by ectoparasites. The parasites can result into mass mortalities of fish, reduced fertility, slow growth rate and loss of appeal to consumers when the lesions are visible [6, 7].

The ectoparasites spread very fast in crowded conditions, as it's always the case in many hatcheries where the stocking densities are high causing high mortalities [8]. In modern hatcheries the stocking density in hatching jars could be up to one kilogram of eggs which is approximately 100,000 eggs. These high densities may be associated with occurrence of ectoparasites like monogeneans which are highly prolific and can cause massive losses [9]. Common fish diseases in hatcheries and in early rearing systems are caused by protozoan ciliates, myxosporians, worms, opportunistic bacteria and fungi [10]. To date there is no report on fish parasites in hatcheries in the country. Thus, this study was undertaken to establish the prevalence and intensity of ectoparasites on various age groups of Nile tilapia in two commercial hatcheries in Homa Bay County, Kenya.

2. Materials and Methods

2.1 Ethical clearance

The Biosafety, Animal Use and Ethics Committee of the Faculty of Veterinary Medicine, University of Nairobi (UON), provided ethical clearance together with National Commission for Science, Technology and Innovations (NACOSTI) FVM BAUEC/2015/220) and respective fish hatcheries after the proposal was approved.

2.2 Study area

The investigation was undertaken in Homa Bay County which lies between latitudes 0°15' South and 0°52' South and between longitudes 34° East and 35° of East. The county sits along the shores of Lake Victoria within the broader region of South-Western Kenya and is rich in Nile tilapia aquaculture with many fish farms practicing both pond and cage culture. Two hatcheries within the county were purposively selected due to their expertise and experience in producing Nile Tilapia fingerlings, and their different production methods thus a representative of all other hatcheries in the county (Fig. 1).

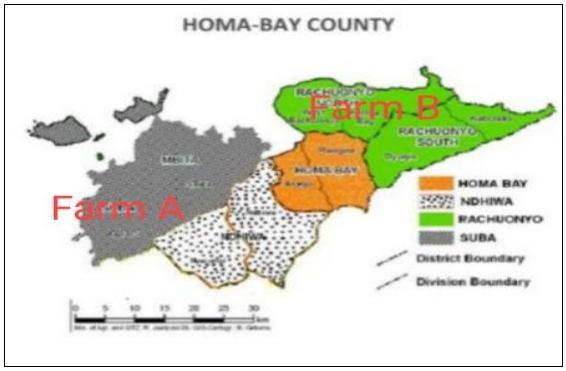


Fig 1: A map showing location of study farms in Homabay County, Kenya

2.3 Study design and sample size

Study samples were taken from two farms which had been purposively selected.

The sample size was calculated using the following formulae of Naing *et al.*[11] and Florio *et al.* [12]One hundred and forty samples were collected from farm A and B, each contributing 70 samples and these were divided into 5 age groups. Twenty broodstock fish and fingerlings were sampled, while for eggs, larvae and fry 10 scoops each using a spoon were sampled per farm. For every scoop of eggs and larvae approximately 60 eggs were placed on a slide and observations made, for fry approximately 5 fry were placed on a slide and observations made 5 times with different samples every time under a microscope.

2.4 Sample collection

Broodstock fish samples were captured using a net depending on the culture method employed by specific farms. In ponds where hapa nets were being used to keep the broodstock and fingerlings, the hapa nets were bagged using a bagging rod and samples taken out randomly, while in cage systems, a scoop net was used to capture the samples. The samples were transported in buckets which were aerated to ensure they get to the laboratory set up while still alive. The eggs were siphoned from the hatching jars while the fry and larvae were scooped from the rearing trays/basins using a scoop net.

2.5 Examination of parasites

Broodstock and fingerling were humanely killed by giving a single blow on the head using the blunt side of a strong knife. The skin was grossly examined for ectoparasites. Wet mounts of skin scrapings, gill and fins clips were collected on slides with saline and for parasites. The eggs, larvae and fry were placed on a slide per scoop and examined under a microscope at x4, x10 and x40. Ectoparasites recovered were characterized and identified using morphological features as described by Robert [13], Woo [14]and Mitiku *et al.*[15].

2.6 Data analysis

The raw data collected was entered into Microsoft excel data sheet and analyzed using R statistical software. Descriptive statistics, percentages and 95% confidence intervals were used to summarize the proportion of infected fish. Statistical significance level was set at $P < 0.05$. Quantifying of parasites was done using prevalence and intensity as shown in the following equations described by Margolis *et al.* [16] and Bush *et al.* [17].

$$\text{Prevalence} = \frac{\text{Number of samples infested with parasite(s)}}{\text{Total number of samples}} \times 100$$

$$\text{Mean Intensity} = \frac{\text{Specific parasite total count}}{\text{Number of fish infested with that parasite}}$$

3. Results

3.1 Prevalence of ectoparasites

Overall prevalence was 66% (92/140) and brood stock had the highest prevalence at 80% (32/40) while larvae had the lowest at 10% (2/20). There was significantly high prevalence of monogenean genera *Gyrodactylus* (fingerlings = 45%) and *Dactylogyrus* in the two hatcheries while that of *Trichodina*, *Epistylis* and *Argulus* species was low (Table 1).

Table 1: Prevalence of ectoparasite genera recovered from Nile tilapia in two hatcheries in Homa Bay County

Genera	Eggs, N = 20 (%)	Larvae, N = 20 (%)	Fry, N = 20 (%)	Fingerling, N = 40 (%)	Brood stock, N = 40 (%)	chi-statistic	p-value	Significance
Gyrodactylus	7 (35)	1 (5.0)	7 (35)	18 (45)	16 (40)	10	0.039	*sig.
Dactylogyrus	1 (5.0)	0 (0)	0 (0)	3 (7.5)	12 (30)	20	<0.001	sig.
Trichodina	0 (0)	0 (0)	4 (20)	7 (18)	5 (13)	8.1	0.087	**ns
Epistylis	0 (0)	0 (0)	0 (0)	2 (5.0)	4 (10)	5.9	0.2	ns
Argulus	0 (0)	0 (0)	0 (0)	0 (0)	1 (2.5)	2.5	0.6	ns

*Sig. = Significant; **ns = Not significant

The two hatcheries showed different prevalence levels of ectoparasites recovered with Farm A having significant prevalence of *Gyrodactylus* spp. and *Trichodina* spp. (Table 2). Farm B had significant prevalence levels of *Gyrodactylus* spp., *Trichodina* spp. and *Dactylogyrus* spp. (Table 3). Four ectoparasites were recovered from each farm with *Epistylis* spp. recovered from Farm A while, *Argulus* sp. was recovered from Farm B.

Table 2: Prevalence of ectoparasite genera recovered from Nile tilapia in farm A in Homa Bay County

Genera	Eggs, N=10 (%)	Larvae, N=10 (%)	Fry, N=10 (%)	Fingerlings, N= 20 (%)	Brood stock, N= 20 (%)	chi- statistic	p- Value	Significance
Gyrodactylus	6 (60)	1 (10)	1 (10)	4 (20)	8 (40)	10	0.037	*sig.
Dactylogyrus	1 (10)	0 (0)	0 (0)	2 (10)	6 (30)	8.4	0.077	**ns
Trichodina	0 (0)	0 (0)	0 (0)	7 (35)	5 (25)	12	0.021	sig.
Epistylis	0 (0)	0 (0)	0 (0)	2 (10)	4 (20)	6.2	0.2	ns
Argulus	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)			

*Sig. = Significant; **ns = Not significant

Table 3: Prevalence of ectoparasite genera recovered from Nile tilapia in farm B in Homa Bay County

Genera	Eggs, N = 10 (%)	Larvae, N = 10 (%)	Fry, N = 10 (%)	Fingerling, N = 20 (%)	Brood stock, N = 20 (%)	chi-statistic	p-value	Significance
Gyrodactylus	1 (10)	0 (0)	6 (60)	14 (70)	8 (40)	19	<0.001	*sig.
Dactylogyrus	0 (0)	0 (0)	0 (0)	1 (5.0)	6 (30)	13	0.012	sig.
Trichodina	0 (0)	0 (0)	4 (40)	0 (0)	0 (0)	25	<0.001	sig.
Epistylis	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)			
Argulus	0 (0)	0 (0)	0 (0)	0 (0)	1 (5.0)	2.5	0.6	**ns

*Sig. = Significant; **ns = Not significant

Farm prevalence difference was only significant for *Epistylis* spp. which was only recovered in farm A (8.6%) as shown in Table 4.

Table 4: Comparative prevalence between the two study farms in Homa Bay County

Genera	Farm A, N = 70 (%)	Farm B, N = 70 (%)	chi-statistic	p-value	Significance
Gyrodactylus	20 (29)	29 (41)	2.0	0.2	**ns
Dactylogyrus	9 (13)	7 (10)	0.07	0.8	ns
Trichodina	12 (17)	4 (5.7)	3.5	0.063	ns
Epistylis	6 (8.6)	0 (0)	4.4	0.037	*sig.
Argulus	0 (0)	1 (1.4)	0.00	>0.9	ns

*Sig. = Significant; **ns = Not significant

3.2 Mean intensity of ectoparasites

The mean intensity of ectoparasites found on eggs, larvae and fry are as shown in Table 5.

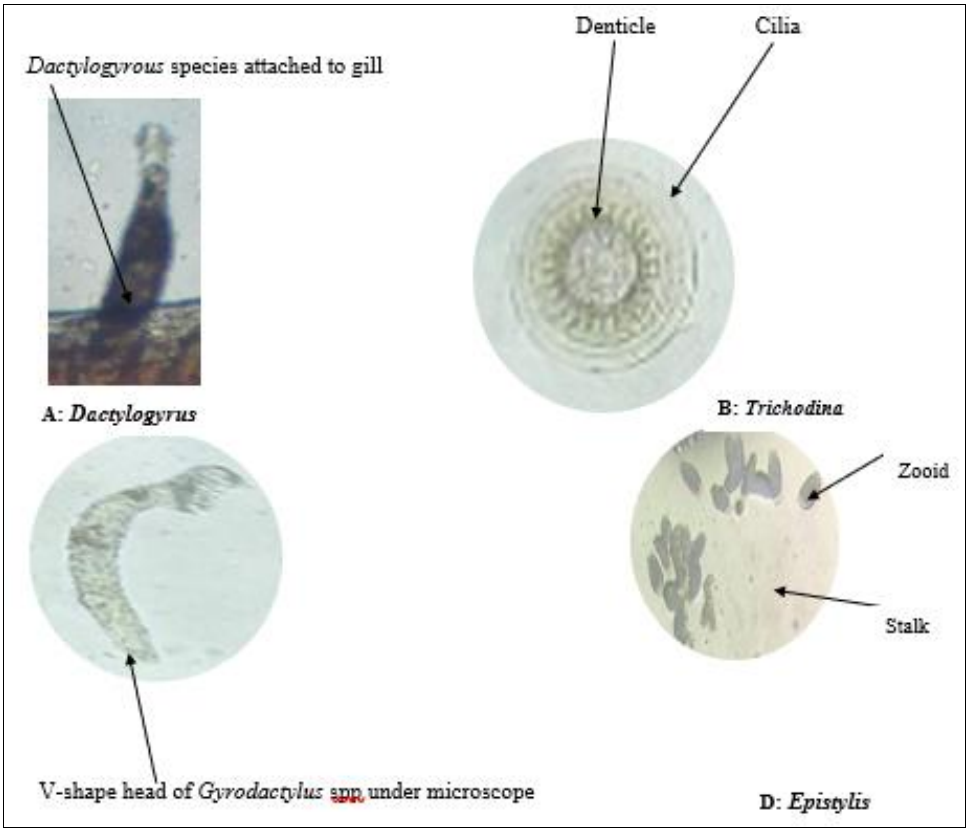
Table 5: Mean intensity of ectoparasites on Nile tilapia eggs, larvae and fry from study farms in Homa Bay County

Age group	Genera	Number of samples	Number of infected samples	Number of ectoparasites	Mean intensity
Eggs	*Gyrodactylus*	20	7	12	1.7
	Dactylogyrus	20	1	8	8
Larvae	*Dactylogyrus*	20	2	2	1
Fry	*Gyrodactylus*	20	7	70	10
	Trichodina	20	4	9	2.3

The highest mean intensity in fingerlings and brood stock was recorded by *Trichodina* (6.6) and, *Epistylis* (38.3), respectively (Table 6). Figure 2 shows some genera recovered on Nile tilapia from study hatcheries in Homa Bay County.

Table 6: Mean intensity of ectoparasites on Nile tilapia fingerlings and Brood stock from study farms in Homa Bay County

Age group	Genera	Number of samples	Number of infected samples	Number of ectoparasites	Mean intensity
Fingerlings	*Gyrodactylus*	40	18	70	3.9
	Dactylogyrus	40	3	3	1
	Trichodina	40	7	46	6.6
	Epistylis	40	2	2	1
Brood stock	*Gyrodactylus*	40	16	119	7.4
	Dactylogyrus	40	13	38	2.9
	Trichodina	40	5	25	5
	Epistylis	40	4	153	38.3
	Argulus	40	1	1	1



. **Fig 2:** Ectoparasite genera observed from various Nile tilapia age groups in the two farms in Homa Bay County

4. Discussion

Five different genera of ectoparasites were recovered from five different age groups of Nile tilapia studied. The prevalence and intensity varied across different age groups with only the monogenean, *Gyrodactylus* spp. found in all age groups from the eggs to the brood stock. *Gyrodactylus* spp. had a fusiform-like body shape with a V-shaped head and a pair of anchor rods with marginal hooks for attaching to the skin, and no eyespots as described by others [18 19]. *Gyrodactylus* spp. attacks cause fading colors, scale loss, secretion of excessive secretion of mucus and eventually leading to death of fish [20].

Trichodina spp. had a round shape with a size and body parts such as radial pins, membranes, an adoral zone and measures between 50 - 90 µm [21]. This ectoprotozoan cause damage by feeding on mucus and detritus covering the surface of the gills and skin of fish causing irritation to the epithelial layer of cells. This can result in hyperplasia (proliferation) of the epithelial cells, clubbing of the gill filaments and even fusion of the gill filaments [22]. This affects the ability of the gills to maintain optimal respiratory and excretory activities, and the ability of the skin to maintain proper homeostatic osmoregulatory properties. Massive infestations of these parasites on fish can also directly result in superficial to deep ulcerative skin lesions which then allow for secondary bacterial and fungal infections to develop at the affected site [23].

Epistylis spp. had a fusiform shape and its length can reach 2 mm and a width of 400 um. It has 2 pairs of eyespots on the anterior end and the mouth is located near to the anterior end of the body. At the posterior end of the body is an engaging tool consisting of two pairs of large hooks (anchors) surrounded by 14 smaller hooks called opisthaptor [21]. *Epistylis* spp. apparently secretes an enzyme that dissolves fish scales or spines and produces pit-like inflamed lesions. Bacterial infections often occur secondarily to the *Epistylis* spp. Infestation [24].

Dactylogyrus spp. was mainly recovered from the gills of the fingerlings and brood stock. Adult worms are up to 0.2 - 2 mm with two pairs of eye spots on the anterior end. They

have a sucker located near to their anterior end. At the posterior end of the body, there is a sticking device which consists of 2 large hooks surrounded by 14 smaller hooks called opisthaptor [21]. Fish infected by *Dactylogyrus* spp. show significant behaviour changes like partial suffocation, lethargic swimming behavior, pale gills, haemorrhage and inflammation at point of parasite attachment [25].

The brood stock had the most parasites as they have lived longer and might have accumulated parasites in their growth period. The constant handling of broodstock during egg collection, hand sexing stresses the fish and thus predisposes them to ectoparasites.

Eggs were mainly affected by the skin (*Gyrodactylus* spp.) and gill (*Dactylogyrus* spp.) monogenean ectoparasites which were mainly found in the culturing water or embedded in the dirt films along the base or bottom of the hatching jars.

The larvae had significantly lower infestation of ectoparasites relative to other groups and this was due to reduced stocking densities. High stocking densities lead to overcrowding which favour the spread of monogeneans [26]. The holding trays/basins used to rear the larvae had no dirt films and were stocked at 100g of larvae. Only two *Gyrodactylus* spp. were found attached on the tail of two different larvae in one of the sampled farms.

Fry were infested by *Gyrodactylus* spp. found attached on the tail fin, head and the skin as was observed in Kafreilsheick hatcheries in Egypt [27] where they cause tail erosion and mortalities. In this study, fry exhibited eroded tails, restless swimming due to irritation caused by these parasites and individual fry had more than one parasite.

Brood stock which were actively used for egg production had the greatest number of various parasites (361) representing a prevalence of 80%. The brood stock examined were reared in hapas and were handled weekly during egg collection, where the females are held by hand and their mouths opened to spit out the eggs into a scoop net. The constant handling stresses the fish leading to loss of mucus and scales which predisposes fish attack by ectoparasites. Since mucus acts as the barrier between the fish body and its surrounding [28].

5. Conclusions

- From this study, ectoparasites were found on all age groups of Nile tilapia sampled.
- Monogeneans were found across all age groups including eggs, larvae, fry, fingerlings and brooders.
- Culturing practices such as handling, culturing facility stocking densities, biosecurity measures affected the prevalence and intensity of ectoparasites.
- Hatchery owners should always employ the best management practices to minimize ectoparasitic infestations.
- Routine (biweekly) monitoring of ectoparasites using microscopy.

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7. Conflict of interest

The authors declare that they have no competing interests that could have influenced the conduct of this study or the interpretation of its results.

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