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Identification and Distributions of Parasites on Developmental Stages of *Clarias gariepinus* Reared in Different Water Renewal Culture Systems

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Abstract

The intensification and commercialization of fish production often cause an imbalance in the water environment thereby exposing them to stress and biological pathogens – parasites, bacteria, fungi and viruses. Parasites are the primary causative agent of infections forming pathways for secondary infections whereas the knowledge about identification and distribution of parasites is vague to most farmers which prompted this study. The population size was 3% of functioning farms where five live fish were randomly collected from water renewal culture systems (Daily (DWR), Weekly (WWR) and Bi-weekly BWR)) for parasitological examination. Relevant keys were used for parasite identifications. Water parameters were measured for the community of parasites using standard methods. Descriptive statistics (percentages and mean) were used for analysis. The parasites observed across the culture systems in this study were categorized into three groups – protozoans *(Trichodina spp., Vorticella spp., Tetrahymena spp., Chilodonella spp., Ichthyobodo spp., Piscinoodinium spp.,* and *Ambiphyra* sp); helminths (*Dactylogyrus spp., Gyrodactylus spp.,* suspected *Salmonichus spp.,* and unidentified nematode *spp.,*) and crustacean (*Argulus* sp.). *Trichodina spp., Vorticella spp.,* and *Dactylogyrus spp.,* parasitized all developmental stages (fry, fingerlings, juveniles and adults) collected from DWR and WWR. *Trichodina spp.*was highly distributed on the skin (66%) and gills (84.5%) in BWR; *Vorticella spp.*on the skin (29.4%) and predominantly dominated the intestine (100%) in WWR; *Dactylogyrus spp.*was on the skin (2.5%) and gills (36.8%) in DWR. No *Vorticella spp.*and *Dactylogyrus spp.,* were recorded on gills and intestine respectively across the culture systems but nematode spp was predominantly found in the intestine. Therefore, the presence of parasites in all the culture systems and developmental stages indicates that neither a system nor developmental stage is exempted thereby more attention should be given to fish hygiene, especially with the awareness of different species of parasites in fish farms.

Key Words: distribution, *Clarias gariepinus,* predilection sites, parasites, water renewal management system

Introduction

Fish is an excellent protein source with essential micronutrients supporting growth and healthy living (Lilly *et al*., 2017 and Mohanty, 2015) thereby reducing the risks of malnutrition and non-communicable diseases (Elavarasan, 2018). Aquaculture is projected to be responsible for 60% of the food available for human consumption by 2030 (Elavarasan, 2018). Millions of people are involved in fish production which provides several benefits including employment, income generation and food security (Lehane, 2013) whereas many farmers had limited knowledge about the health management of fish coupled with the inability to identify and detect fish diseases occurrence (Walakira *et al*., 2014) which frequently leads to economic loss (Leung and Bates, 2013; Akoll and Mwanja, 2012). Fish parasites are ectoparasites and endoparasites, they infect all developmental stages of the fish. The parasitic diseases in fishes range from very serious pathogenic to virtually harmless ones and are mainly classified into protozoan and metazoan parasites (Jithendran, 2014).

*Trichodina spp.*belongs to a large assemblage of peritrichous ciliates of trichodinids and they are protozoan parasites of cultured and marine fish species globally (Lom and Dykova, 1992). Trichodinids are shaped with diameters reaching up to 100 µm depending on the species. They possess cilia around their shape for locomotory and feeding functions. Their body is strengthened by a rigid ring of interconnected discs known as a chitinoid lenticulalar ring food on their host skin and gills by spinning cilia motion which can cause injury to the host tissues.

*Vorticella spp.*is a sessile peritrich characterized by an inverted bell-shaped structure composed of a spherical shaped zooid ranging in size between 50 – 65 lm in diameter and scopula (Abdel – Baki *et al*., 2014). Members of vorticellid possess a single zooid associated with a retractile stalk and ribbon–shaped (Viljoeni and Van As, 1987).

Monogeneans are parasitic flukes that mostly live on the skin and gills of the fish host. They are viviparous or oviparous displaying a high degree of host specificity. They possess specialized muscular posterior attachment organs (opisthaptor) associated with a variable array of sclerotized hooks, clamps, connecting bars or epidermal structures aiding attachment to fish host (Paladini *et al.* 2017). The penetration of opisthaptor caused damage to the host through the foraging action of the mouth thereby being regarded as a serious pathogen of culture fish (Ogawa 2002, Ernst *et al*. 2002, Grau *et al*., 2003). The most common representative of monogenean trematodes are *Dactylogyrus spp.*and *Gyrodactylus spp.Dactylogyrus spp.*is commonly found on the gill and thereby refers to as gill fluke. They are small in size and characterized by 2 – 4 eyespots, a pair of large anchor hooks an egg-laying parasite. They possess a pair of pigmented light receptors and two cephalic lobes at the anterior part responsible for the secretion of adhesive gland cells while the distal part has a muscular pharynx and a tubular confluent intestine. The haptor possesses pairs of hamuli and 14 marginal hooks. The eggs hatch into free-swimming larvae and transmit to a new host with ciliated movement aided by the water currents. Egg production and hatchability are temperature dependent (Paperna 1964, Bauer *et al.*, 1973). *Gyrodactylus spp.*are commonly referred to as "skin flukes" frequently found on the body surface of the fish including the fins and sometimes in the gills of cultured and marine fish (Bakke *et al*., 2007). They are small in size (0.3 – 1.0mm) and possibly visible to the naked eye. The adult *Gyrodactylus spp.*is viviparous and thereby carries a fully developed embryo with a pair of anchors, the replica of the adult which in turn carry another matured embryo and so on (Eissa, 2002). They are characterized by no eye spot, 16 marginal hooks, and a pair of anchors with both dorsal and ventral bars. They survive for a while after dislodging from their host but transmission is through fish-to-fish direct contact.

Ichthyobodo spp. is a parasitic flagellate with a direct life cycle causing heavy infestations on the skin and gills of the fish host

(Isaksen 2013). They are obligate ectoparasites, unable to survive, reproduce or multiply without an appropriate host (Becker 1977). *Ichthyobodo* spp exhibit two forms as the first recognized as pear-shaped is a parasitic feeding form often attached to fish skin and gills (Southgate, 1993) and the second form identified as kidney-shaped possessing two pairs of flagella is a non-feeding swimming form existing off the fish (Lom and Dykova 1992). The free-swimming form dislodged from the dead host and died between 30 – 60 minutes outside the host. *Ichthyobodo necatrix* is the most pathogenic causing costiasis.

Tetrahymena spp. are commonly distributed on the skin, eye socket, musculature, viscera, and spinal cord; masses of ciliates can be detected in copious amounts of mucus and between spaces in the damaged tissues (Lawhavinit *et al.*, 2002). They possess nucleated cilia positioned by basal bodies (Omori *et al*., 2020; Bottier, *et al.*, 2019). They possess different biological characteristics one of which is by undergoing encystment to survive harsh environments or change their oral morphology (from microstome bacterivore to macrostome carnivore) depending on food availability (in response to food availability) (Lynn and Doerdert, 2012).

*Chilodonella spp.*is a highly pathogenic holotrich ciliate. They are ectoparasites on the skin and gill of a wide range of freshwater fish globally. The parasite is around 80 µm in length and is characterized by a flattened, ovoid shape, covered by rows of cilia moving in a continuous gliding manner over the epithelial cells of the fish host. The infection caused a bluish-white coating mostly on the head region.

Piscinoodinium spp. is an important parasitic agent causing piscinoodiniasis or velvet disease in freshwater fish ((Noga and Levy, 2006) whose distribution is not host-specific (Martins *et al.*, 2001). The microscopic view revealed different shapes including pear-shaped, banana-shaped and mature rounded parasites of brownish color (Martins *et al*., 2001 and Foin, 2005).

Ambiphyra spp. is a sedentary barrel–shaped frequently found on the skin, fins or gills of cultured fish and characterized by the macronucleus (a long winding ribbon) and a wide scopula associated with an equatorial ciliary girdle which divided the body into the oral and basal region (Lom and Dykova, 1992). The length ranges between 60 – 80 lm while the width is 40 – 48 lm. The infundibulum was conspicuous while the peristomial disc was commonly convex in shape and surrounded by a conspicuous peritomial lip. There are many food vacuole but one contractile vacuole. The parasite attached to the host through the adhesive fibre associated with the scopula (Abdel-Baki, *et al.,* 2014).

Argulids are obligatory ectoparasites infringing severe damages to fisheries and aquaculture globally (Post 1987, Rushton-Mellor 1992). They are large parasites of a very distinctive oval shape with a flattened carapace measuring 6 - 6.5 mm (female) and 2-3 mm (male), visible to the naked eye (Cruz-Lacierda, 2001). They are characterized by compound eyes, a pair of large suckers, four pairs of branched thoracic swimming limbs, and a small unsegmented abdomen. Many species are identified in the water bodies with a loose corkscrew motion or a somersaulting action. They attach mostly to the skin with their strong suckers and inject cytolytic enzymes which made it easier to feed on host blood (Shimura and Inoue 1984). The completion of their life cycle is temperature dependent ranging between 30 – 100days (Shimura, 1981). The free swimming lice survive only for a few days while juveniles live for less than 48 hours (Kollatsch, 1959).

Materials and Methods

Study Area

The survey was carried out in Lagos state which lies between longitude 2^{0}41’20”E to 4^{0}21’10”E and latitude 6°22′10″N to 6^{0}42’15”N (Google Earth Satellite Imagery 2018). The collection of *Clarias gariepinus* was done randomly from the stratified Lagos agricultural zones (Lagos East, Lagos Far – East and Lagos West) and 3% of fish farms were randomly sampled from the lists of operating fish farms. The farms were purposively classified based on water usage management systems (WUMS) according to Okomoda *et al.,* (2016) as Daily Water Renewal (DWR), Weekly Water Renewal (WWR) and Bi-weekly Water Renewal (BWR). A structured questionnaire was used to obtain the source of water used for culture as borehole, well, stream, river and stagnant pond water.

Parasitological examinations

The fish were transported to the laboratory for parasitological examination. Wet smears from developmental stages (fry, fingerlings, juveniles and adults) were prepared from the skin, gills, intestine, trunk kidney, liver and blood. Hand lens was used to check the gross examination of ectoparasites on the epithelial cell before laboratory procedures as described by Tachia *et al*., (2010).

Ectoparasites Procedures

The skin was scraped with the cover slip from the head to the tail region to obtain the smear and placed on a clean slide (Noga, 2010) drop of distilled water was added using a dropper to avoid dryness when viewed under the microscope.

The gills of fry were obtained by removing the head portion and squashing between two clean slides while the gills of other stages were examined by removing the operculum case to expose the gill and a small quantum of gill arch was incised and placed on a clean slide for microscopic view filament and with scissor (Noga, 2010).

Endoparasites Procedures

The abdominal portion was slit opened from the anal opening towards the lower mandible to reveal the liver, intestine, trunk and kidney for endoparasites examination. The intestine was cut open and the content was removed carefully to expose the intestinal wall which was scraped with a coverslip and placed on a clean slide. A modified squash preparation was done for the liver and trunk kidney by squashing a small piece of tissue between two slides (Scott - Weber and Govett, 2009).

Microscopic Observation

The smears were observed for parasites using x10 and x40 objective compounds of the Olympus binocular microscope (Goselle *et al.,* 2008) fixed to a DCM 35E – 350pixel scope photo and connected to a computer device. The observed parasites were identified with the keys of freshwater fish parasites according to Pouder *et al., (*2005).

Statistical Analysis

Simple descriptive statistics including percentages, frequency and histograms were used for data analysis with the aid of excel 2016.

Results

The parasites observed across the culture systems in this study were categorized into three groups – protozoans *(Trichodina spp., Vorticella spp., Tetrahymena spp.Chilodonella spp., Ichthyobodo spp., Piscinoodinium spp.,* and *Ambiphyra spp.*); helminths (*Dactylogyrus spp., Gyrodactylus spp.,* suspected *Salmonichus spp.,* and unidentified nematode sp.) and crustacean (*Argulus* sp.) (Plate 1). *Trichodina* sp, *Vorticella spp.*and *Dactylogyrus spp.*parasitized all developmental stages (fry, fingerlings, juveniles and adults) collected and examined from the daily water renewal system (DWR) and weekly water renewal system (WWR) except the fry that was unparasitized with *Vorticella spp.*in WWR, nevertheless, *Trichodina spp.* and *Dactylogyrus spp.* parasitized the fingerlings and juveniles in bi-weekly water renewal system (BWR) (Table 1). However, *Gyrodactylus spp.* parasitized all developmental stages in DWR except fry, only juveniles in WWR and adults in BWR. Other parasites like *Tetrahymena spp., Chilodonella spp., Ambiphyra spp., Piscinoodinium spp.* and *Ichthyobodo spp.* predominantly parasitized the fingerlings in DWR except for *Chilodonella spp.* that was found on juveniles. Suspected *Salmonichus spp.* predominantly parasitized the fry reared in the DWR system. In WWR, *Tetrahymena spp.* and *Argulus spp.* parasitized the fingerlings while in BWR, *Chilodonella spp.* parasitized the fingerlings and juveniles as well as *Ichthyobodo spp.*found only on adult fish. No parasite was recovered from the liver, trunk kidney and blood.

*Trichodina spp.*was harvested on the skin, gills and intestine of *Clarias gariepinus* examined in all the culture systems except for the intestine in bi-weekly water renewal systems. *Vorticella spp.*was recovered on the skin and intestine in both daily water renewal (DWR) and weekly water renewal (WWR). *Dactylogyrus spp.* (gill fluke) and *Gyrodactylus spp* .(skin fluke) were both found on the gills and skin of *C. gariepinus* reared in DWR but on gills and skin respectively in WWR and BWR (Table 2). Apart from *Piscinoodinium spp.* that was found on the skin and gills, *Argulus spp.* on the gills and unidentified nematode *spp.* found in the intestine; all other parasites such as *Tetrahymena spp., Chilodonella spp., Ambiphyra spp., Ichthyobodo spp.* and suspected *Salmonichus spp.* were found on the skin of fish reared in respective culture systems. The percentage distribution of parasites on predilection sites across the culture systems was shown in Figure 1. *Trichodina spp.* was highly distributed on the skin (66%) and gills (84.5%) in BWR followed by 59.7% and 67.6% in WWR respectively. *Vorticella spp.* was highly distributed on the skin (29.4%) and predominantly dominated the intestine (100%) in WWR. No *Vorticella spp.* was found on the gills. The percentage distribution of *Dactylogyrus spp.*was highest on the skin (2.5%) and gills (36.8%) in DWR. No *Dactylogyrus spp.* was recorded in the intestine across the culture systems. Other parasites were found on the skin (34%) and intestine (100%). The parasites found in the intestine were predominantly nematode sp.

Discussion

Occurrence of parasite at the developmental stage across the culture systems

Trichodina spp., Vorticella sp, *Dactylogyrus spp.* and *Gyrodactylus spp.* were found to parasitize all developmental stages (fry, fingerlings, juveniles and adults) at different periods across the culture system while *Tetrahymena spp.* may be associated with fingerlings due to their observation only on this stage in DWR and WWR but they were reported to be none host-specific (Martins, *et al*., 2001) likewise *Chilodonella spp.*.found on fingerlings and juveniles both in DWR and BWR. Other parasites (*Ambiphyra* sp, *Piscinoodinium spp., Ichthyobodo spp., Argulus spp.* and nematode *spp.*) were found on one of the developmental stages in a particular culture system showing no possible specificity for a particular stage of fish across the culture systems. More ectoparasites were found than endoparasites supporting the report of (Tiya *et al.,* 2019) who also recorded *Trichodina spp, Tetrahymena spp., Chilodinella* sp, *Dactylogyrus spp.,* and *Argulus spp.,* on Tilapia *spp.* similar to parasites identified in this study. The presence of parasites was noticed most in WWR compared to DWR and BWR which may be due to the period of staleness of culture water before renewal unlike daily water renewal (DWR) system bi-weekly water renewal (BWR) which were predominantly spring/stream fed earthen ponds.

Occurrence of parasites on predilection sites of developmental stages across the culture systems

Ectoparasites were recovered on the skin and gills cultured *C. gariepinus.* on developmental stages reared across the culture systems. The results on the skin agreed with Tachia *et al.*, (2010) and Afolabi *et al*., (2020) which may be due to direct contact of the skin with the culture environment or continuous flow of water over the skin. Parasites were found on the gills as also reported by Omeji *et al.* (2011) which may likely be due to being the core center of filtering food substances with the aid of a gill raker possibly trapping some parasites (Somerville, 1984).

Trichodina spp. was found in fingerlings intestine in DWR apart from the conventional sites on the skin and gills which could be ingested though was reported to be endoparasites in the intestine, kidney and urinary bladder of fish (Lom and Dyková, 1992). The *Vorticella spp.* found on the skin was reported to be a free-living parasite on the skin, fins and gills of many fishes (Abdel-Baki *et al.*, 2014; Dash *et al*., 2015). They become facultative under stressed or fouled environmental situations (El – Tantawy and El – Sherbiny, 2010). Dash *et al*., (2015) reported the presence of *Vorticella spp.* on the gill contrary to the findings of this study. However, the presence of *Vorticella spp.* noticed in the intestine may be an occasional occurrence as the parasite is considered to be a free-living using its host as a living substrate to conveniently gain access to the source of food like organic debris and water-borne bacteria thereby adapted specifically to attaching to the surface of fish hosts (Scheubel, 1973). *Dactylogyrus spp.*, and *Gyrodactylus spp.* were recovered from both the skin and the gills supporting (Afolabi *et al*., 2020) and also agreed with the report got on the skin and gills of catfish (Wooten, 1974) and *Dactylogyrus spp.* on Tilapia gills (Donald *et al*., 2017). Monogenean trematodes are frequently found on the gills, skin, and fins of fish, though the adult stage may be site specific unlike the larvae stages. *Gyrodactylus spp.*are generally found on the skin and fins of fish but invaded gills at high infestation (Ergens *et al*., 1988). The monogeneans were not recorded in other organs or tissue apart from skin and gill contrary to their infections in the nasal cavities and urinary system (Takemoto *et al*., 2004) where they feed on host blood and tissues (Lupchinski Jr. *et al.*, 2006). *Chilodonella spp.* are free-living but parasitizes the skin, gills and fins of freshwater fish (Pádua *et al*., 2013a) which supports their observations on the skin in this study. It partially agreed with the observation of *Chilodonella hexasticha* on skin and gills in all developmental stages of tench (*Tinca tinca*) (Svobodova and Kolarova, 2004). The nematode was identified in the intestine conforming to the nematode (*Camallanus spp.*) found in the intestines of *Labeo rohita* (Bhuiyan *et al.,* 2007) though the species observed was unidentified. Some authors reported nematodes in the swim bladder, gonads, liver, gills, eyes and skin.

Relevance of parasitic infection in the present status of fish farming in Lagos

The aquatic environment is endowed with parasites thereby making natural or cultured organisms victims of parasitic infections (Marcogliese, 2005) agreed with Baldwin *et al.,* (1967) who reported that susceptible relationships frequently occurred between different fish species and parasite infection. However, the importance of parasites on fish health is commonly neglected unless affected by the fish species of interest or causing severe economic loss since no environment is pristine or pathogenic free especially parasites (Iwanowicz, 2011). Therefore many farms across Nigeria are not exempted from parasitic infections (Inyang – Etoh and George, 2018; Eyo *et al*., 2015; Adeogun *et al*., 2014; Bichi and Dawaki, 2010) likewise Lagos State (Dimelu *et al*., 2018; Okere and Adeyemo, 2014). Most farmers in Lagos State attributed mortality to poor management practices especially fouled water quality (Dimelu *et al*., 2018; Okere and Adeyemo, 2014) whereas less concern is given to parasitic infections compared to secondary infections due to its virulence thereby making it difficult for farmers to affirm the impact of parasites on fish production probably due to low or lack of knowledge of fish parasites. However, the effects of parasitic infection on farmed fish would be a threat if farmers can identify the contributions of parasites to mortality rate instead of general opinion on environmental factors which would help the immediate response to remediate further occurrence.

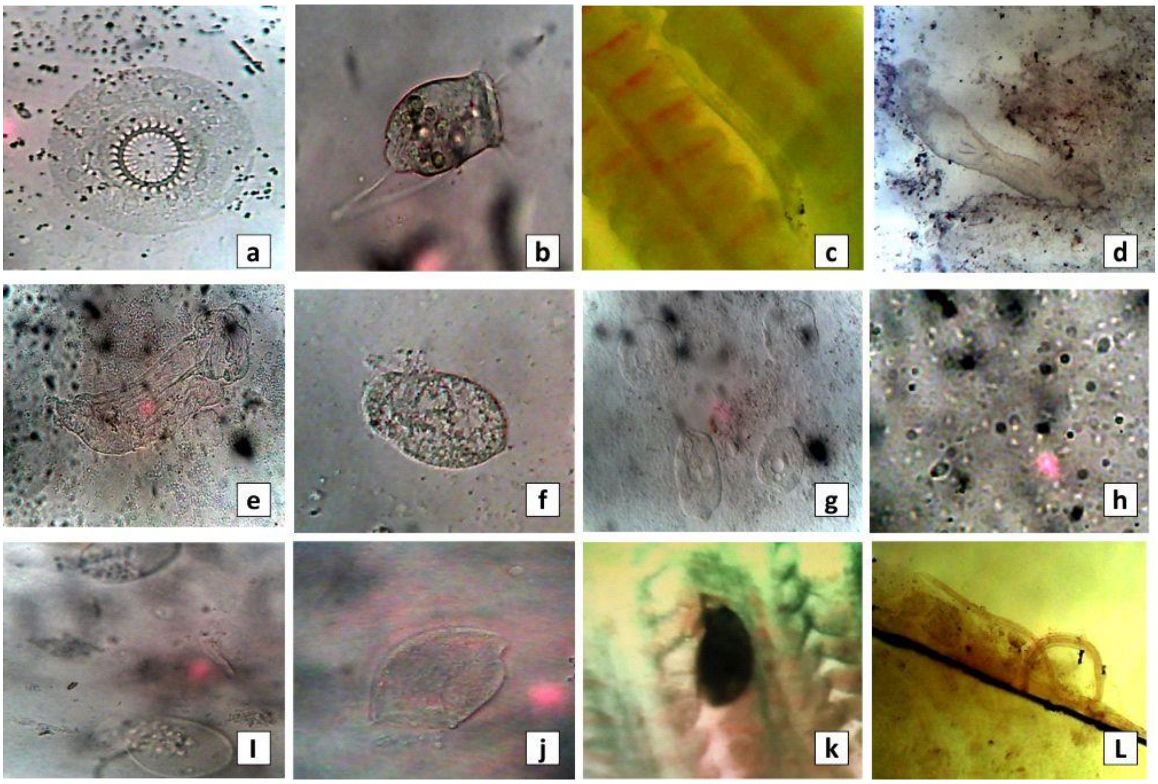

Plate 1: Microscopic view of (a) *Trichodina spp.*x 400 (b) *Vorticella spp.*x 100 (c) *Dactylogyrus spp.*x 100 (d) *Gyrodactylus spp.*x 400 (next generation shown by arrow) (e) Suspected *Salmonichus spp.*x 100 (f) *Tetrahymena spp.*x 100 (g) *Chilodonella spp.*x 100 (h) *Ichthyobodo spp.*x 100 (i) *Piscinoodinium spp.*x 100 (j) *Ambiphyra spp.*x 100 (k) *Argulus spp.*x 100 (l) Nematode *spp.*

Table 1: Occurrence of parasites on different developmental stages across the culture systems

Parasites	Daily Water Renewal				Weekly Water Renewal				Bi - Weekly Water Renewal			
	Fry	Fingerlings	Juveniles	Adults	Fry	Fingerlings	Juveniles	Adults	Fry	Fingerlings	Juveniles	Adults
Trichodina sp	+	+	+	+	+	+	+	+	-	+	+	-
***Vorticella* sp	+	+	+	+	-	+	+	+	-	-	-	-
Dactylogyrus sp	+	+	+	+	+	+	+	+	-	+	+	-
Gyrodactylus sp	-	+	+	+	-	-	+	-	-	-	-	+
Tetrahymena sp	-	+	-	-	-	+	-	-	-	-	-	-
Chilodonella sp	-	+	+	-	-	-	-	-	-	+	+	-

Ambiphyra sp	-	+	-	-	-	-	-	-	-	-	-	-
Piscinoodinium sp	-	+	-	-	-	-	-	-	-	-	-	-
Ichthyobodo sp	-	+	-	-	-	-	-	-	-	-	-	+
*Suspected *Salmonichus* sp	+	-	-	-	-	-	-	-	-	-	-	-
Argulus sp	-	-	-	-	-	+	-	-	-	-	-	-
Unidentified Nematode sp	-	-	-	-	-	-	-	-	-	-	+	-

** New parasites found in cultured *Clarias gariepinus* * Suspected new parasite

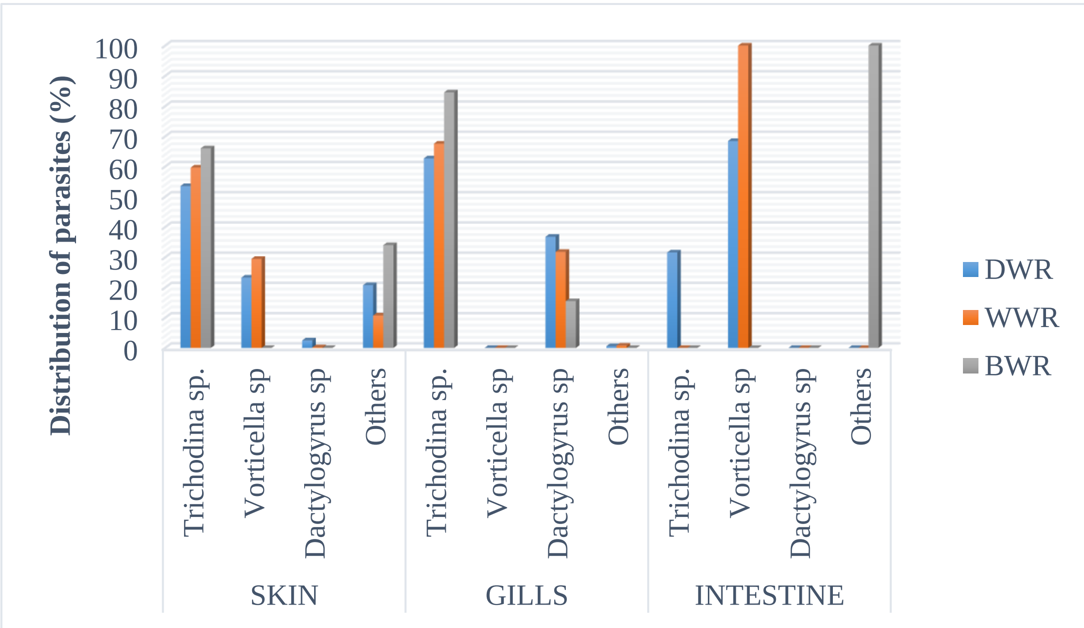

Figure 1: Percentage distributions of parasites on predilection sites of *C. gariepinus* across *the* culture systems in Lagos State

Conclusion

Almost all the parasites identified in this study were protozoan indicating that they have a direct life cycle which influenced the ease of propagation within *C. gariepinus* in culture systems especially *Trichodina spp.*with a vast distribution pattern. The new entrant observed (*Vorticella* sp) shows the possibility of diverse parasitic fauna yet to be discovered in the aquaculture industry in Nigeria. However, the least prevalence of endoparasites (nematode spp) indicated that few or no intermediate hosts (snails, copepods and fish-eaten birds) cohabit directly or indirectly with the cultured fish. This information can be a guide and improve the confidence of farmers to easily relate with the laboratory reports of fish experts to improve management procedures.

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