PREVALENCE AND INTENSITY OF NEMATODE PARASITES OF *Poecilia reticulata* PETERS (1859) IN FOUR WASTEWATER DRAINS OF LAGOS STATE, NIGERIA

BY

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ABSTRACT

Poecilia reticulata (guppy) a common ornamental tropical fish is found in many wastewater drains in Nigeria. Guppies feed on copepods which are intermediate hosts of some nematode parasites of culturable fish species. The restriction on the importation of ornamental fishes into Nigeria has enhanced the demand for local species, usually sourced from the wild. There is dearth of information on the parasites of ornamental fishes in Nigeria. This study was aimed at determining prevalence and mean intensity of the nematode parasites of *P. reticulata* from four waste water drains in Lagos State.

Sampling was carried out monthly using a 2 mm mesh-sized scoop net along selected drains at Igi-Olugbin Street (A), Basil Ogamba Street (B), Ahmadu Bello Road (C) and Adenaike Alagbe Street (D) between March, 2004 and February, 2005. The selected drains were contiguous to human habitation and industrial activities but each in different local government areas of Lagos State. Sixty female and sixty male samples were randomly selected from each drain for dissection and microscopy. Nematodes observed were identified using standard identification guides. Prevalence was determined as percentage infection in guppies examined. Intensity was determined as total parasite count per host. They were calculated in relation to sex of guppies and drain location for sample collection. Measurements of wastewater temperature, Dissolved Oxygen (DO), pH and transparency were done according to APHA methods. Drain depth was determined using a calibrated pole. Data was analyzed using chi-square test, ANOVA and Pearson correlation coefficient.

Nematodes recovered were *Eustrongylides ignotus* from peritoneum, *Camallanus cotti* from duodenum and anus, *Capillaria pterophylli* from intestine and *Trichinella* species from muscle. Out of 4,320 fish hosts examined, *E. ignotus* was the most prevalent parasite (3.6 %) and *Trichinella* species the least prevalent (1.5 %). Prevalence did not differ significantly with sex and drain. Mean intensities were $0.3 \pm 0.3 - 5.0 \pm 1.8$: males, $0.3 \pm 0.3 - 4.9 \pm 1.8$: females and $1.3 \pm 1.3 - 7.0 \pm 3.0$: drains. Monthly mean intensity did not differ significantly with sex but differed significantly with drains ($p < 0.05$). Mean monthly physicochemical parameters for drains were: temperature, $25.0 \pm 1.1 - 26.0 \pm 1.1$ ^{o}C; DO, $7.8 \pm 2.1 - 8.4 \pm 1.8$ mg l^{-1}; pH, $6.9 \pm 0.5 - 7.3 \pm 0.4$; transparency, $3.5 \pm 0.8 - 23.0 \pm 3.6$ cm and drain depth, $9.6 \pm 2.3 - 14.8 \pm 3.2$ cm. There was no significant difference in mean monthly pH and DO across drains. However, mean monthly temperature, transparency and drain depth were significantly different across drain ($p < 0.05$). High correlation were observed at drain D between the prevalence of *Trichinella* species and wastewater DO ($r^2 = 0.8$), between prevalence of *C. pterophylli* and wastewater temperature ($r^2 = - 0.6$) and also between mean intensities of *C. pterophylli* and *Trichinella* species ($r^2 = 0.8$).

Prevalence and intensity of nematodes in *Poecilia reticulata* was low. However, the occurrence of parasites in the guppy requires appropriate treatment of fish before introduction into culture system.

Keywords: *Poecilia reticulata*, Nematodes, Wastewater drains, Lagos State.

Word Count: 497.

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CERTIFICATION

I certify that this work was carried out by Mr. M.M.A. AKINWALE in the Department of Zoology, University of Ibadan.

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CHAPTER ONE

INTRODUCTION

Ornamental fish is that species of fish kept alive in transparent homestead aquaria, ponds and outdoor waterfalls for their aesthetic values. These values range from their characteristic colour, shape, size, exudates and behaviour in water. In general terms, ornamental fish is incomplete without invertebrates such as mollusks and arthropods, corals, stones and live rocks having pores with encrusted colourful algae and sessile invertebrates for natural biofilteration of aquaria, aquatic plants, special enclosures and associated visual effects (Livengood and Chapman, 2007). There is no record encountered in literature indicating abhorrence of aquarium fish keeping as a hobby or trade by practitioners of any particular culture, religion or law in any human society (Sanders, 1990). Ornamental fish species have become ubiquitous all over the world and, therefore not surprisingly, many millions of ornamental aquaculture and ecotourism enthusiasts are easily found among children and young adults who made it at one time a hobby and at another a trade. As a result, colourful adaptations of ornamental fish, their habitat and associated organisms are the subject of some cartoon movies and space fiction reporting.

Ornamental fish industry (OFI) is currently valued at about 15billion United States Dollars (USD) according to Larkin (2003); Wabnitz *et al.* (2003); Pelicice and Agostinho (2005); Prang (2007); Wittington and Chong (2007) Moorehead and Zeng, (2010). Ornamental Fish industry grew from an annual USD 34million export business in the early 1950s (Conroy, 1975; Thurnberg, 1993 and Chapman *et al*., 1997) to a USD 282 million per annum export activity in 2006 (Livengood and Chapman, 2007). Considering sourcing, storage and conditioning of collected ornamental fish in the exporting countries where the industry has a ready poverty alleviating capacity; aquaria design and construction, specialized financing, retail and maintenance of resources in the destination countries estimations, as at 2006 indicated that global OFI was worth over USD 20 billion per annum (OFI, 2006).

Contemporary ornamental fish trade is considered low-risk because there is relatively more astringency attached to the trans-border transfer of ornamentals. Ornamental fish involved in live fish trade are mostly wrongly considered low-risk

articles because of their aesthetic value and the ease with which they have enjoyed trans-border transfers through the ages. As a matter of fact, a social school of thought considers the possession, popularity and the acceptance of ornamental aquaculture, an index of economic well-being of any human population (Sharma *et al*., 2007). Alarmingly more important, however, is the forensic link made by Hulme (2009) in the sequence between globalization and gradual loss of biodiversity. A connection was made linking improvement of socio-economic well-being of developing nations, concept and practice of globalization through free trade whose main driver is unfettered transportation and emigration which voluntarily breaks down physical and biological borders. Consequently, globalization liquefies natural biological isolations, redefining species and niches that culminate in invasion of alien species and, ultimately, bioconservation trouble for our planet, Earth. He tagged the trend as Trade, Transport and Trouble.

This means that if possession of ornamental fish is a ready reflection of social and economic status and means and, this would typify most human preferences under western civilization, it is no surprise that ornamental fishes have become ubiquitous. Moreso, that they are portable and adaptive to new climes in addition to being attractive, therapeutic and luxuriating to keep at home and at work place irrespective of personal ideology.

The most popular live-bearing freshwater aquarium fish in the world demanding relatively less specialized care to feed and reproduce is *P. reticulata* (Hargrove and Hargrove, 2006). It is therefore easier to keep even as it survives adversity when accidentally or intentionally discarded into wastewater drains. It retains the highest freshwater ornamental fish import value into the US, while Nigeria with Democratic Republic of Congo remain the highest African export sources of ornamental fish into the US (Chapman *et al*.,1997). It is called the millionfish because of its small size, high fecundity and aggressive colonization of otherwise inclement water conditions (Meffe and Snelson, 1989). This fish was intentionally introduced in some wastewater drains for mosquito control (Manna *et al*. 2008; Anogwih and Makanjuola, 2010; Lawal and Samuel, 2010) but later became ubiquitous due to its attractive colour displays, space adaptation and temperament in captivity. *P. reticulata* has the ability to reproduce between 2 and 50 fries, within 21 to 30 days gestation period and in hard-water and 25.5 - 27.8^{o}C temperature conditions (Welcomme, 1988). Meanwhile, the female is

ready to conceive immediately after delivery of fry with the fry swimming, eating and avoiding predators immediately after live-birth from their mothers (Froese and Pauly, 2007). Meanwhile, Chervinski (1984) working separately from Shikano and Fujio (1997a) and Shikano and Fujio (1997b) reported that *P. reticulata* is capable of colonizing water having over 150% salinity than normal freshwater. It therefore follows that for adequate conservation of aquatic wildlife, commercial aquaculture, proper management of avian intermediaries of fin-fish and shellfish diseases to take place, the current disease situation of *P. reticulata* in wastewater drains is critical to the larger Biosecurity of the Nigerian biome.

Obtaining data on the diseases of feral *P. reticulata* from their locations in Nigeria could also serve as a proper scientific prelude to an adequate risk identification, risk assessment and risk mitigation plan to local import substitution and enhancement of this ubiquitous and well-sought after ornamental fish species as export material. Meanwhile, a vision is incomplete when it considers positive scenarios at the exclusion of negative possibilities. This is why alluring consideration of the attractive possibilities of ornamental fish trade would fail reality test if it discountenances a repeat of Eldredge (2000) report that imported *P. reticulata* escaped into drains and streams only to later out-compete indigenous fish species that were trophically related to the invading fish species in Hawaiian streams. The result is that the niches of the indigenous species were later left as a gap unattended to. That was not all, parasites such as nematode *Camallanus cotti* and cestode *Bothriocephalus acheilognathi* that were hitherto not encountered in Hawaii streams became endemic because of the neo-tropical conditions of the streams there (Andrews *et al*., 1981; Font and Tate, 1994). In the light of the aforementioned case, a critical knowledge gap becomes apparent (Lindholm *et al*., 2005) because there is no record of nematode parasites of *P. reticulata* since their introduction into Nigeria and their eventual escape into wastewater drains and streams since the early 1970s (Welcomme, 1988 and Andrews, 1990).

Justification:

The per capita diversity, distribution and intractability of nematodes amongst the parasites of fish is not in doubt (Anderson and May, 1979; 1985; Anderson, 1988; 1996; Moravec and Nagasawa,1998; Subasinghe *et al*., 2001; Lodge and Shrader-Frechette, 2003; Corfield *et al*., 2008) hence the choice of nematode parasites of *P. reticulata* for this study. Already obvious in previous cases elsewhere is the fact that setting a risk assessment framework not based on verifiable data leads to precautionary measures that are unnecessary or lead to relaxation of rules that may mislead into

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allowing invasive species to tamper with a region's biodiversity (Corfield *et al.,* 2008). As a result and usually, only verifiable risk assessment form the basis for allowing a ban on importation of ornamental fish species from previously identified pathogen endemic areas by the WTO and OIE. Indeed, only this guarantees continuing security of ornamental fish export from an originating country. Unarguably, frequent risk assessments of standing parasite occurrence, load and distribution in wild and cultured ornamental fish stock is also the premium paid by developed economies for preventing difficult-to-verify origins of parasite outbreaks that culminate in brand damages impossible to subsequently indemnify. There is therefore a knowledge gap in the prevalence and intensity of parasites of *P. reticulata* obtained from wastewater drains in Lagos State where established populations of this fish exists (Anogwih and Makanjuola, 2010).

However, the need to fundamentally secure Nigeria's living aquatic borders from chance or deliberate infiltrations and aggressions of exotic pathogen invasions lead to the desirability of properly assessing the current status of the parasite and microbial loads of indigenous ornamental fish species and their major sister imports.

Aim:

The aim of this study is to determine the suitability of *P. reticulata* obtained from selected waste-water drains in Lagos, Nigeria that could satisfy requisite risk assessment parameters as disease-free ornamental fish export.

Objectives:

1. To determine types of nematode parasites of *P. reticulata*, their prevalence and intensity.
2. To investigate physicochemical parameters in relation to prevalence and intensity of nematode parasite of *P. reticulata*.

Hypotheses:
1.

Null Hypothesis H_o: there is no significant difference between the observed nematode parasite prevalence and the expected parasite prevalence in male and female samples of *P. reticulata*.

Alternate Hypothesis H_1: there is significant difference between the observed nematode parasite prevalence and expected parasite prevalence in male and female samples of *P. reticulata*.

2.

Null Hypothesis H_o: there is no significant difference in the nematode parasite prevalence in samples of *P. reticulata* obtained from the four drains.

Alternate Hypothesis H_1: there is significant difference in the nematode parasite prevalence in samples of *P. reticulata* obtained from the four drains.

3.

Null Hypothesis H_o: there is no significant difference between the observed nematode parasite intensity and the expected parasite intensity in male and female samples of *P. reticulata*.

Alternate Hypothesis H_1: there is significant difference between the observed nematode parasite intensity and expected parasite intensity in male and female samples of *P. reticulata*.

4.

Null Hypothesis H_o: there is no significant difference in the nematode parasite intensity in samples of *P. reticulata* obtained from the four drains.

Alternate Hypothesis H_1: there is significant difference in the nematode parasite intensity in samples of *P. reticulata* obtained from the four drains.

5.

Null Hypothesis H_o: there is no significant correlation between nematode parasite prevalence of *P. reticulata* and the means of monthly physicochemical parameters of wastewater obtained from the four drains.

Alternate Hypothesis H_1: there is significant correlation between nematode parasite prevalence of *P. reticulata* and the means of monthly physicochemical parameters of wastewater obtained from the four drains.

6.

Null Hypothesis H_o: there is no significant correlation between intensity of nematode parasites of *P. reticulata* and the means of monthly physicochemical parameters of wastewater obtained from the four drains.

Alternate Hypothesis H_1: there is significant correlation between intensity of nematode parasites of *P. reticulata* and the means of monthly physicochemical parameters of wastewater obtained from the four drains.

CHAPTER TWO

LITERATURE REVIEW

2.1. Habitat and morphology of *Poecilia reticulata*:

The fish *Poecilia reticulata* Peter (1859) is a member of the Phylum Chordata and belongs to the Class: Actinopterygii, the Order: Cyprinodontiformes, the Family: Poecilidae and the Genus: Poecilia. It is a tropical freshwater fish found occupying estuarine drains, streams and rivers. The common name it is oftentimes called is the guppy, a name credited after Reverend R.J. Lechmere Guppy, the first President of the Scientific Association of Trinidad. The guppy is also called topminnows or millions fish because it is prolific, very fecund and ubiquitous in the tropics (Meffe, 1989).

They are also found in weedy ditches and canals turbid enough to exclude other fish species in lowlands and high altitude locations (Arthington, 1989a). Courtenay and Meffe and Snelson (1989) reported a wide salinity tolerance for the guppy, it however survives in narrow temperature and water current ranges. *P. reticulata* is an omnivore that has been found to subsist on zooplanktons, insects, lower invertebrates and detritus. Apart from constituting a popular ornamental fish species, they are used as biological control agents against some aquatic insects that spread disease (Courtney and Moyle 1992; Courtney and Moyle 1996). In Lagos, Nigeria, just as in California, United States of America, *P. reticulata* is found in domestic and industrial waste water drains and channels (Arthington and Lloyd, 1989).

It is a very small fish of length ranging from 4.0 - 20cm. The entire body of the fish is elongate and moderately deep. The head is flattened; the entire body is covered with cycloid scales while the snout is short. The mouth is terminal, wide, oblique and protractible meant for picking, scraping, tearing and crushing food. There is a single dorsal fin with 6-19 soft rays; with their position relative to those of the anal fin. All fins have no spines (Rosen and Bailey, 1963). Anal fins have 9-soft rays with the third soft anal-fin rays unbranched in both sexes. The anal fins in males are modified into intromitent organ that is long, elongate and is called a gonopodium. This gonopodium is neither tubular nor scaled. There is also no sperm duct enclosed within it. Robin and Ray (1986) described *P. reticulata* as a fish whose pectoral fins have 9-16 soft rays, short, rounded and inserted on the high side of the body. Pelvic fins with 6 soft rays are

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sub-thoracic in the adult females but thoracic in the males. The lateral line is composed of a series of pits running end to end along the side of the body.

Sexual dimorphism is observed in *P. reticulata* with the males being smaller in size and shorter in length (2.5 - 3.5cm) than the females (4.0 - 6.0cm). While the males are more brightly coloured than the females, the latter bear the gravid spot anterior to the anal fin as a physical indication of bearing a young fish in the ovary. The gravid spot is however dorsal to the anal fin and at this stage the females have a prominently distended abdomen.

2.2. Ornamental fish trade in Nigeria:

Mbawuike and Ajado (2005) reported twenty four ornamental fish species excluding *Poecilia reticulata* known to be wildly sourced from the freshwaters of the Osun River terminating at the Ibiajegbende fish landing settlement in Epe area of Lagos State. Ornamental fish is also available from the wild in freshwater rivers of Lagos, Ondo, Ekiti, Ogun, Edo, Delta, Niger, Akwa Ibom, and Abia States (Areola, 2003). A list of thirty ornamental fish species constituting exports from Nigeria was drawn up by Areola (2003) who conservatively estimated regular and documented exports receipts from Nigeria, excluding revenue from local ornamental fish trade, to other countries to be in the region of USD 300, 000 per annum. This estimate is conservative because official ornamental fish cargo receipt alone from commercial airlines for export within the period of March and December, 2002 totaled over USD 411,000 (Areola, 2003). When these are contrasted with the USD 15 million gross annual continental ornamental fish exports receipts from Africa in the year 2000 (Mouton *et al*., 2001) of which not less than about a third is known to annually originate from Nigeria (Areola, 2003) it becomes clear that Nigeria's annual contribution to the sector is in multiples of million dollars. However, Nigeria exports as well as import ornamental fish.

The first record of introduction of *P. reticulata*, the guppy, into Nigeria occurred in 1971 (Welcomme, 1988) and it was ostensibly to help in the biological control of mosquitoes breeding in wastewater drains of Nigeria. It was considered an emergency in order to reduce the man-hours lost to malaria which heavily debilitate the post-colonial era workforce of Nigeria at a period of increasing human population drift to Lagos at that time. Mosquitoes were to be controlled in wastewater drains where mosquito eggs and larvae were expected to be fed on by guppies. Evidence is scarce to

confirm if guppies had any appreciable impact in eliminating mosquito spread in the wastewater drains. Indeed, experiments conducted by Manna *et al* (2008) on the effectiveness of *P. reticulata* in the biological control of mosquitoes in the wild questioned its success because other preys may have been available in the wastewater drains to distract the predator. *P. reticulata* was however not introduced into Nigeria and the wastewater drains of Lagos State as an ornamental fish for its aesthetic values but in biodefence against mosquito spread. *P. reticulata* has since then spread beyond the drains of Lagos State where it was originally released, though currently, those wastewater drains are some of the collection sources of *P. reticulata* for ornamental purposes in Nigeria.

2.3. Ornamental fish as potential source of exotic parasites:

While the danger of spread of diseases borne by indigenous ornamental fish species to aquatic wildlife and food fish is difficult to perceive as urgent, the translocation of the pathogens and transfer of diseases of exotic ornamental fish species constitutes clear and emerging danger to existing balance between indigenous fish hosts and their associated biome (Vitousek *et al.,* 1997; Kolar and Lodge 2001; Sakai *et al*., 2001; Dudgeon *et al.,* 2006). Collateral damage resulting from the ubiquity of ornamental fish everywhere culminates in the single, most unhindered translocation of exotic parasites across the globe (Courtenay 1990; Courtenay and Stauffer, 1990; Courtenay and Moyle, 1996; Fuller *et al.,* 1999; Canonico *et al.,* 2005). On record, ornamental fish constitutes the largest category of live animal and plant species moving across borders enjoying the most liberal application of quarantine and vetting regulations today (Corfield *et al.,* 2008). Consequently, as the most immediate purveyor of accidental invasion of alien species in new niches, ethical concerns raised by scientists, public sector administrators such as the International Union for Conservation of Nature, IUCN, (2001), Murray-Darling Basin Commission Native Fish Strategy (MDBC, 2006) and civil society about animal welfare abuses and zoonotic spread of exotic pathogens and parasites tends to find justifications.

For instance, *P. reticulata* which has found a universal spread, a small benthopelagic omnivore, was known to be a native of Trinidad and Tobago, Brazil, Guyana and Venezuela (Lindholm *et al.*, 2005 and Nico, 2010). As an invasive species it has been found to be hazardous and responsible for wiping out of native Cyprinids,

killifishes and damselflies by feeding on their eggs in their new climes of introduction as shown in Nevada and Wyoming, both in the United States (US). This information and those of other potential diseases of ornamental fish imports are made requisite for continuing trade relations by export destinations of Nigeria's ornamental fish in the developed economies of the world. Many international marketing, freighting and Animal Health regulators such as the World Trade Organization (WTO), International Air Transport Association (IATA) and Organisation International des Epizootes (OIE) also require this information for export certifications and brand enhancements. These requirements are to prevent potentially new infiltrations of their aquatic system by previously unidentified nematode parasites of *P. reticulata* and ornamental fish species into their holding, display and culture systems (United States Department of Agriculture: USDA / American Public Health Information Service: APHIS, 2008). Incidentally, Nigeria is a member of, and signatory to WTO, IATA and OIE treaties just as regular monitoring of existing and potential ornamental fish disease conditions are parts of Nigeria's relevant domestic laws such as Inland Fisheries Decree 108 of 1992 and Live Fish (Control of Importation) Act 209 of 1990.

Obtaining data on the prevalence and mean intensity of nematode parasites of feral *P. reticulata* from their locations in Nigeria could serve as a proper scientific prelude to an adequate risk identification, risk assessment and risk mitigation to local import substitution and enhancement. There is knowledge gap in the prevalence and mean intensity of nematode parasites of *P. reticulata* obtained from wastewater drains in Lagos State. Setting a risk assessment framework not based on verifiable data leads to precautionary measures that are unnecessary or lead to relaxation of rules that may mislead into allowing invasive species to tamper with a region's biodiversity (Corfield *et al.,* 2008). Usually, verifiable risk assessment is the basis for allowing for ban on importation of ornamental fish species from previously identified pathogen endemic areas by the WTO and OIE, and indeed, continuing security of ornamental fish export from an originating country (OIE, 2005).

2.4. Challenges of sourcing ornamental fish through aquaculture in developing countries:

The continuing desire and requirement by destination countries especially those with well organized and developed economies for ornamental fish originating from

aquaculture rather than from the wild, justifiable as it seems on account of ensuring the sustainability of biodiversity of the relevant fish species, raises its own challenges. One advantage is the immediate impetus it creates for human capacity building in the development and spread of appropriate technology in the originating countries before export. Unfortunately, the relevant technologies currently existing in the destination countries are in pre-generic state and are not commercially mature for adoption in the originating countries thereby widening the gulf between supply and demand ends of the market. Apart from the fact that the relevant technologies are not immediately available the intensive aquaculture method of raising fish and the attendant high stocking density it employs in the maximization of space is a ready recipe for encouraging disease conditions resulting from many stress factors governing intensive culture conditions (Akinwale and Ansa, 2004; Akinwale *et al.,* 2007).

2.5. Occurrence of *P. reticulata* in wastewater drains:

The fish *P. reticulata* Peters (1859) is very common in wastewater drains of many Lagos Streets (Anogwih and Makanjuola, 2010; Lawal and Samuel, 2010). The distribution often occurs in mature waste water drains already laden with debris emanating from residential and industrial activities surrounding the drains. Similar wastewater drain location had been described for *P. reticulata* in California in the United State of America (Courtney and Meffe, 1989; Courtney and Robbins, 1989; Courtney and Stauffer, 1990).

Meanwhile, different populations of guppies are known to have inter-racial isolation mechanisms (Baerends *et al*., 1955; Liley 1966) indicating intolerance of intraspecific interaction. It is important to mention that *P. reticulata* is known to be exotic to Nigeria and it is an ornamental aquaculture species reputed to have first been introduced into Nigeria around 1972 (Welcomme, 1988) though originally native to the Caribbean (Nico, 2010). It would by the definition of the IUCN (2001) constitute an alien and invasive species in Nigeria. Water is as important as the material it conveys from place to place hence the quality of water is often described by the radical presence of materials or organisms in it. For aquatic organisms and sundry biota of economic importance to man, water can therefore be considered not only as the fluid of life but also as the medium or basin of life (Raven and Johnson 1996). Meanwhile, just as water cycle is a dynamic continuum terrestrial water bodies also commune with one

another. To this extent, aquifer water which serves as source of well water and basins from where potable water are sourced are never isolated from floods and waste drains nor are they exclusive of industrial and domestic effluents (APHA 1998 and Acho-Chi 2001).

This is the same reason accidental fin and shellfish escapees reach wastewater drains when water is dislodged from earthen ponds or more intensive aquaculture and fish seed propagation systems (de Graff *et al*., 1995; Akinwale *et al*., 2004). As a result, exchange or translocation of parasite fauna and biocoenose between aquaculture water impoundments and wastewater drains are not only made probable (Noble and Noble 1971) but become a practical and present danger in developing nations like Nigeria where waste water disposal systems are primordial (Boyd 1990; Genthe and Seager, 1996; Forch and Bremann, 1998; Lacorchak *et al*., 1998 and Obi *et al*., 2002). The parasite ecology of fish communities in wastewater drains should therefore be of interest to aquaculture and fish parasite evolution studies (de Graff *et al*., 1995). Beyond this however, the potential of aquaculture in further enhancing the intensity of newly established fish parasite fauna through massive stocking densities hitherto unavailable in waste-water drain fish ecosystems transcends the level of scenario to that of a nudging reality (Cole *et al*., 1999). The abatement of such an unpleasant prospect should therefore begin with the assessment of the existing parasite fauna of the major fish in waste water drains and the existing type of association or potentially evolving parasitemia amongst this major biota of the drains.

2.6. Bases for host-parasite relationships:

Relationship between organisms and their environment in every community are governed by easily yielding rules and dynamics. Rules of co-habitation, toleration, contention and subversion are established, re-assessed and redrawn to suit changing realities in resultant evolutionary directions that are in the main, adaptive rather than refractive, with no permanent interest constantly served (Noble and Noble, 1971).

Again, as previously reviewed by Pigliucci and Murren (2003), is it possible that we have missed the small shifts in parasite evolution because evolutionary Scientists pay attention only to the impact of the big strokes on the even bigger canvas of evolutionary portraits?

Warren (1971) defined a parasite as an organism which lives in or on another organism (the host) and which depends on the host for its food, has a higher reproductive potential than the host, and is suspected of harming the host when present in large numbers. Parasites are therefore generally very spontaneous in reproduction while timing the host's life cycle for the best transmission mode so that not only is the parasite's interest enhanced between generations of its hosts, the parasite adapts more quickly to the changes in the host's environment while inducing it to behave mostly in the parasite's best interest.

Often, large healthy fishes effectively sustain greater parasite populations before showing clinical signs of infection than smaller bodied fish like *P. reticulata* in the same aquatic environment. However, because the skin and gill are the initial landing sites for most parasites on fish hosts, parasites of small bodied fish species like *P. reticulata* that can adopt susceptible larger bodied fish species in aquaria and in aquaculture tend to be more aggressive and successful in establishing themselves on such new hosts to the detriment of the affected fish culture systems.

2.7. Previously known nematode parasites of *Poecilia reticulata*.

Previously published records of nematode parasites of *P. reticulata* include those of adult samples of *C. cotti* recovered from *P. reticulata* (Kennedy *et al*. 1987; McMinn 1990; Rigby *et al*. 1997; Moravec *et al*. 1999; Levsen, 2001, Kim *et al*. 2002 and Font 2003). Similarly, *Capillaria philippinensis* had also been obtained from *P. reticulata* by Ko (1995) while Thilakaratne *et al*. (2003) recovered adult *Capillaria spp*. from *P. reticulata* surveyed amongst ornamental fishes in Sri Lanka. Only larval samples of *Aochotheca philippinensis* were obtained from *P. reticulata* in the Phillipines by Arthur and Lumanlan-Mayo (1997).

CHAPTER THREE

MATERIALS AND METHOD

3.1. Study Area

Lagos state is one of the most densely populated of all the states in Nigeria while Lagos city is reputed as one of the most rapidly growing cities of the world. It lies in the south western part of Nigeria and it consists of many metropolitan, urban and rural areas. It is bordered in the north by Ogun state and the Bight of Benin part of Gulf of Guinea in the South. The Niger Delta is to the East of Lagos State but it is bordered to the West by Benin Republic. Lagos is currently the largest city in Nigeria with the highest concentration of sea ports and airports. However, Lagos is a city consisting of several islands separated by creeks but surrounded by many beaches which protect them from the damaging inundations of the Atlantic Ocean which lie to the South of these Islands stretching over a 100 km to the east and west. Lagos Island is south west of the Lagos Lagoon while Victoria Island lies to the South east. They are linked by the Bonny Camp Bridge. Lagos is found between Latitude (lat.) 6^0 .35'N and 6.583^0 N to Longitude (long.) 3^0 .45'E and 3.75^0 E.

Lagos retains a humid tropical savannah climate with coastal sand banks, discontinuing patches of swamps, mangroves, heavy rain forests and secondary growths. Fadama III (2011) estimates that the vegetation of Lagos State consists of brackishwater swamps (10%), freshwater swamps (30%), rain forests (15%), regenerative growths (15%) and savannah bush fallows (25%). The brackishwater swamp, found in Badagry, Ojo, Ibeju-Lekki, Epe and Ikorodu, is mainly characterized by two mangrove plants, *Rhizophora mangle* (the red mangrove) and *Rhizophora racemosa* (the black mangrove) plants with zonation consisting of nearshore having *R. mangle*, followed by *R. racemos*a inland and further by *Avicennia africana*, *Laguncularia racemosa*. Where the coast is lined by regosol soil, the palms of coconut, *Prodococcu wateri* and *Ancistrophylum opacum* dominate the vegetation of the immediate shore (Fadama III, 2011). Aquatic macrophytes such as *Eichhlornia crassipes* that bloom seasonally and others that remain in water perennially are found in the Ogun, Isheri Rivers as well as in the Lagos lagoon.

In the case of freshwater swamps into which rivers Yewa, Ogba, Isaku, Opamu, Ogun, Solodo, Berre, Owa, Aye, Owo and Oshun drain, wetlands resulting from the sporadic deluge of freshwater floods from these rivers sustain such vegetations as Kolanut, Oil palm, raffia and bamboo trees. Meanwhile, most local government areas of Lagos state have a bit of these freshwater swamps as the Lagos lagoon ramifies the viscera of Lagos State.

The typical rainforests of Lagos State found in areas such as Igbokuta, Ijede, Agura and Agbede in Ikorodu LGA; Igbodu and Oko-Afo, Agbowa – Ikosi areas of Epe LGA are characterized by deciduous plants like *Afromosia laxiflora, Burkea africana, Daniella oliveri and Laoberlinia doka* while the low and secondary growth forest areas found in Ibowon, Agbowa-Ejirin, Itoikin in Epe LGA, Igbogbo in Ikorodu LGA have plants like *Alchornea cordifolia, Gmelina arborea and Gliricidia sepium.*

Typical savannah of low shrubs, herbs and grasses spread over about 25% of Lagos terrestrial space are characterized by *Oryza sativa, Discorea esculenta, Zea mays* and *Chromolaena odoratum* (Fadama III, 2011).

Fadama III (2011) also reported that the geologic structure of Lagos State as mainly sedimentary but of the tertiary and quarternary state while the soil consists of ferrosol (18%), regosol (33%), with the hydromorphic and organic soil constituting remaining 49% of the soils of Lagos State. Ferrosols require drainages; regosols are useful for coconut plantations, while the remaining alluvial and clayey soil requires fertilization and other conservation measures to enhance forestry, and agro-allied industry potential of the State.

Lagos has a high rainfall profile marked by two heavy rain seasons initially between May and July and then between September and October. Building Nigeria's response to Climate change Project (2012) reported monthly rainfall means ranging between 1567.2mm - 1750mm of every year. However, heavy floods result from the heavy rainfall peaks due to poor drainage of soil type characteristic of coastal lowlands found in Lagos. These two rain maxima often experience an interregnum of a few weeks called the August break spanning between August and September (monthly mean rainfall - 75mm) of the year while remaining rainfall tappers spasmodically to as little as monthly mean of 1.5mm in early January. Highest monthly atmospheric temperature maxima of 30°C occur between November and December and also between February and March of each year. Thereafter, rains are replaced by disruptive

harmattan wind from the Sahara Desert that actually begins to gradually visit the Lagos area from late December till February of every year (BNRCC, 2012). The highest monthly atmospheric mean temperature of $27^{o}C$ was usually attained in March while the lowest ($25^{o}C$) was recorded in July (Fadama III, 2011).

Lagos is currently home to many tribes of Nigerian origin but the original settlers were the Aworis and the Eguns while other parts of Lagos state especially to its northeast were inhabited by the fringe Ijebus as the first settlers.

Though a cosmopolitan city located in the coastal region of southwestern Nigeria, the growth of freshwater aquaculture as a result of increasing demand for fish and fish products has made aquaculture to be of great economic importance to its citizens (Fakoya *et al*., 2004).

Commensurate demand for ground water has therefore also increased pressure on potable water (Grabow, 1996; Genthe and Seager, 1996; Lehlossa and Muyima, 2000) in the face of failing public portals for domestic and industrial use (Forch and Bremann, 1998). This consequentially limits the quality of water employed in aquaculture throughout Lagos and expands the cost of obtaining water which is one of the main factors of production in aquaculture (Boyd 1995).

3.2. Study location and design:

This study was carried out in Lagos State, Nigeria between March, 2004 and February, 2005. Waste water drains on four different streets in four different local government areas of Lagos State were chosen for this study based on the human population density and the adjacent industrial activity surrounding the selected portal drains serving as the habitat of *Poecilia reticulata.* The streets were Igi-Olugbin (Fig. 3.1), Basil Ogamba (Fig. 3.2), Ahmadu Bello (Fig. 3.3) and Adenaike Alagbe (Fig. 3.4) streets. Each street was coded as: Igi-Olugbin (A); Basil Ogamba (B); Ahmadu Bello (C) and Adenaike Alagbe (D) for labeling of samples, tabulation and graphical illustration of nematode parasite occurrence as well as for calculating parasite prevalence and intensity. A stratified random sampling design was adopted with ten sampling stations identified along the length of each drain on the four selected streets. The global positioning system (GPS) co-ordinates of the sampling sites were acquired

while the sampling stations were spaced in such a way as to cover as many *P. reticulata* schools swimming in open wastewater in each of the drains.

Sampling stations were identified on each street and spread along the length of waste water drains where *P. reticulata* were available while noting the descriptive particulars of the sampling areas, street landmarks, the Longitude and Latitude co-ordinates of sampling location, and the characteristics of the adjoining structures in terms of anthropogenic activities impacting on the wastewater drains.

Thereafter, the mercury-glass thermometer, pH and Oxyguard meter for measuring temperature, pH and DO were inserted separately in wastewater to measure the corresponding physicochemistry.

3.3. Field Study

3.3.1. Preliminary observations:

Visual identification and a pilot study of the sector and part of the wastewater drain where populations of *P. reticulata* were collected were conducted for each of the selected stations. This informed the decision as to the best approach in obtaining both fish and water samples for direct *in situ* measurement of wastewater physicochemical parameters.

Fish samples collected from these drains were examined externally for significant indices of sexual dimorphism. Fish host sex was determined using features outlined by Rosen and Bailey (1963), Houde (1997), Froese and Pauly (2007. The Global positioning system (GPS) co-ordinates of the drain were obtained using a handheld digital meter (Garmin - etrex brand). Sampling sites comprising of many stations for collection of both fish and water samples were spread along the length of wastewater drains while noting the descriptive particulars of the sampling areas, street landmarks, the Longitude and Latitude co-ordinates of sampling location, and the characteristics of the adjoining structures in terms of anthropogenic activities impacting on the abutting wastewater drains.

3.3.2. Fish sample collection and transportation:

On sampling day, *P. reticulata* were obtained from the waste water drains with a 2mm mesh size scoop net from March 2004 to February, 2005.

Figure 3.1.Section of Somolu LGA Showing sampling stations (dots) at Igi-Olugbin Street (ArcGIS mapping software by ESRI, 2011).
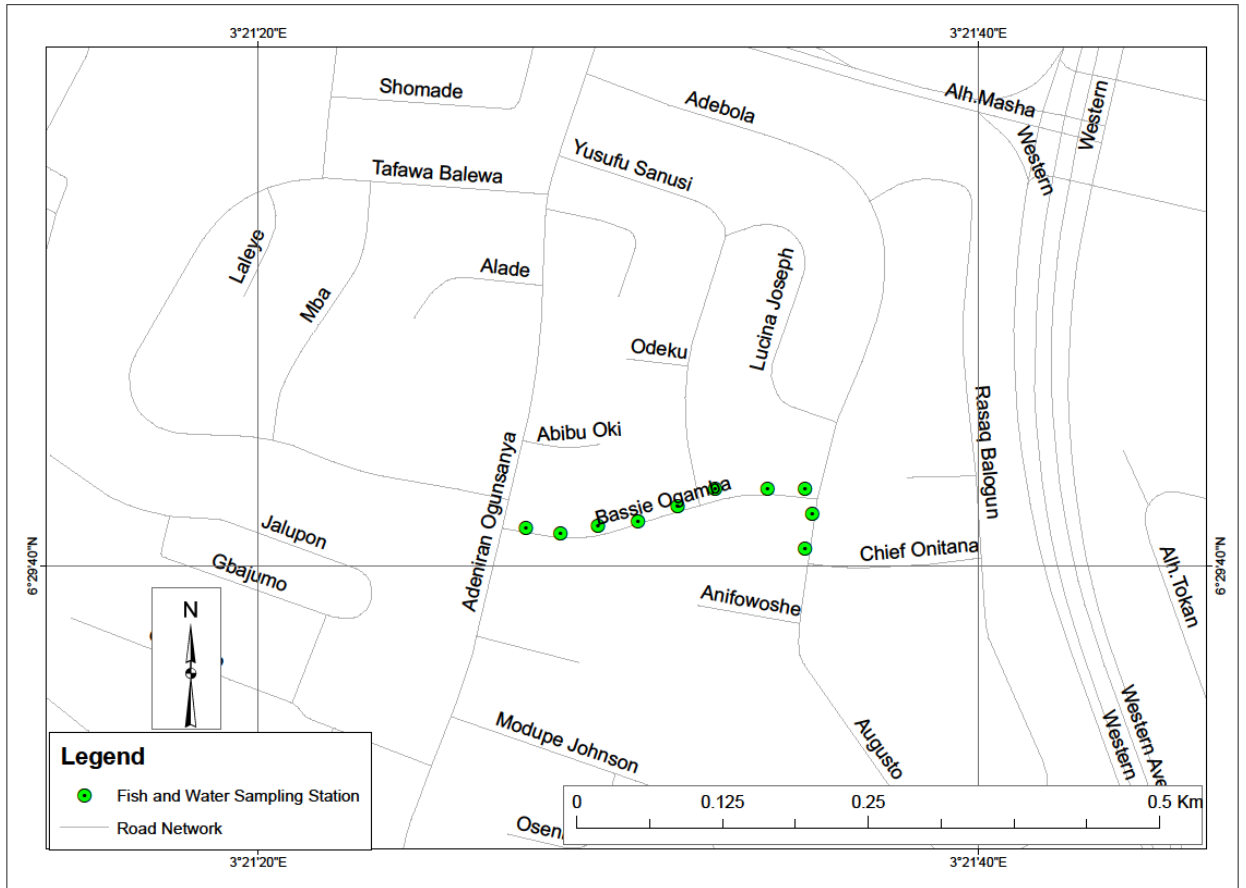
Figure 3.2.Section of Surulere LGA Showing sampling stations (dots) on Basil Ogamba Street (ArcGIS mapping software by ESRI, 2011).

Figure 3.3.Section of Eti-Osa LGA Showing sampling stations (dots) on Ahmadu
Bello Road (ArcGIS mapping software by ESRI, 2011).
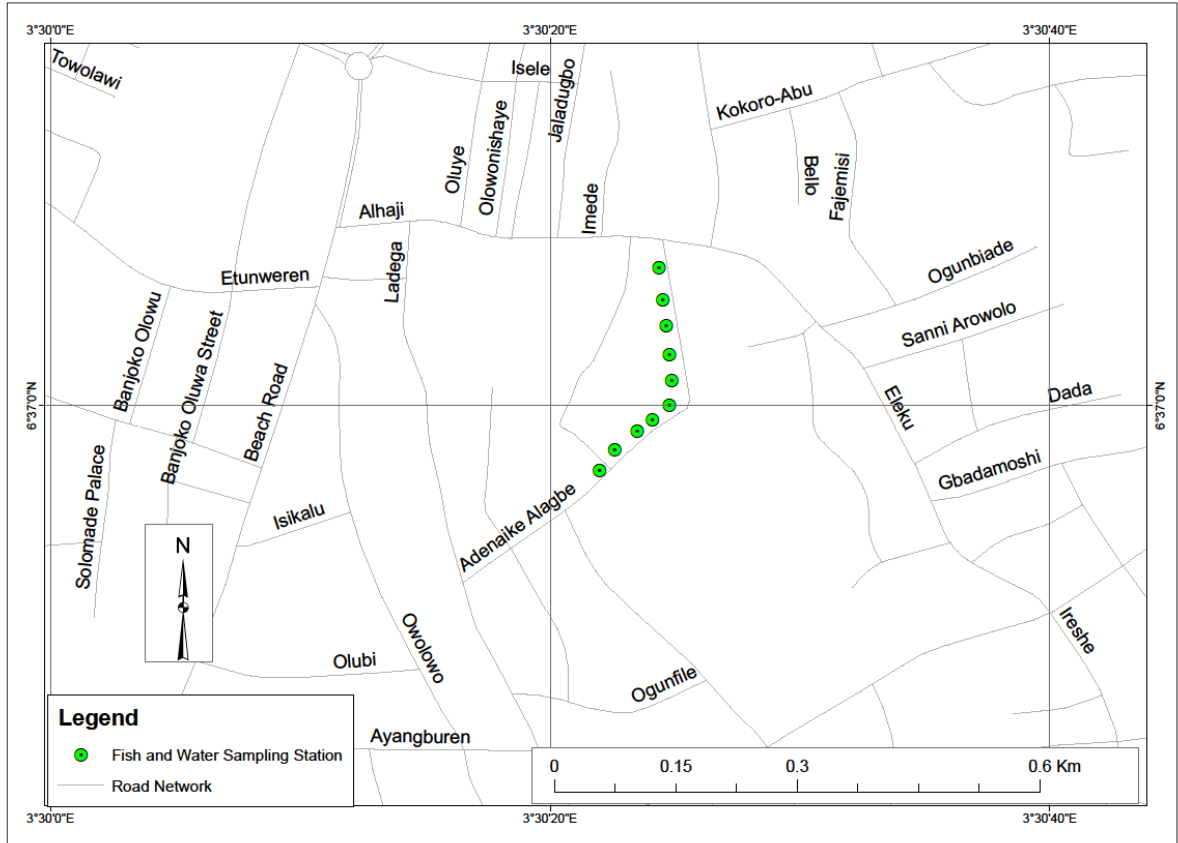
Figure 3.4.Section of Ikorodu LGA Showing sampling stations (dots) on Adenaike Alagbe Street (ArcGIS mapping software by ESRI, 2011).

Collection of *P. reticulata* samples was between 10.00am and 12.00noon, transported to the laboratory in a plastic bucket containing about fifteen liters of wastewater from the particular drain where collection occurred.

3.3.3. Water sample collection:

A one liter glass beaker was used to obtain water samples from the more rigorously mixed portions of the wastewater that often coincide with the higher collection of *P. reticulata* schools in the drains. This is because the sampling strategy is to capture the main physicochemical parameters associated with the major region of wastewater where fish hosts mostly inhabit which were in line with the objective of these measurements. Water samples collected are analyzed for wastewater physicochemistry.

3.4. Laboratory investigation

Two hundred *P. reticulata* samples were collected from each drain on the field. They were sorted by sex into sixty males and sixty females in the laboratory through direct visual inspection for immediate dissection under a dissecting microscope. Nematodes encountered in the gills, muscle tissues, viscera and Gastrointestinal Tract (GIT) of *P. reticulata* were extracted from the organs.

Those collected from waste water drains were examined for the species and number of nematode parasites in relation to the fish host sex, drain location of fish host before correlating them to the physicochemical parameters of the wastewater in the drain. The relationship of the occurrence of a particular nematode parasite and its number in each sex of the fish host obtained were later pooled for each of the wastewater drain locations.

3.4.1. Sample sorting and identification:

Sex determination followed the methods and criteria used by Haskins and Haskins (1951), Rosen and Bailey (1963), Houde (1997), Alexander and Breden (2004), Froese and Pauly (2007) in separating male *P. reticulata* from the female. Range of colour patterns on fish host samples were observed and applied in the preliminary determination of their sex. The colour patterns along the trunk region posterior through the operculum of fish all the way to the anterior region of the caudal

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peduncle were noted. The structure of the anal fin of fish specimens were also observed if modified into an intromitent organ called the gonopodium for copulation in *P. reticulata* males. Guppy mark that distinguishes gravid female *P. reticulata* from immature females and males were also searched for to assist in sex determination.

3.4.2. Parasite recovery:

Fish dissection was carried out using standard methods described by Southgate (1994). Saline was dispensed (0.9% sodium chloride solution) into a set of three small watch-glasses. Before decapitation of fish sample, the opercula flap of each sample was raised with a pair of forceps to expose the gill arch, the opercula flap was cut with a small pair of scissors, while gill filaments were also cut with the same pair of scissors. The gills were then removed from the gill chamber, separated into two parts. One part was dropped into one of the watch glasses described above with the other dry gill part dropped on a microscope slide. A drop of saline solution was pipetted on the dry gill part mounted on the slide for microscopy. Thereafter, the wet gill sample in saline solution of the first watch glass was then transferred into a Perspex sample bottle. This bottle containing gill was shaken vigorously for about ten minutes intermittently in this saline solution to free any available parasite lodged within the filaments. Fluid resulting from this gill immersion was then pipetted onto another glass slide for microscopy of nematodes present. Subsequently, the gill itself was directly lifted onto a different glass slide crushed between slides before microscopic examination of nematode parasite lodged within its lamella.

A fin of the fish sample was severed with a pair of scissors and placed in another watch glass containing saline solution before transfer into another Perspex bottle for dislodgement of nematode parasites for wet mount microscopy as done in the case of the gills above. A dry fin is also mounted on a glass slide before dabbing with saline as described for the gills above. Scales lying posterior to the operculum of fish samples were shaved off with a scalpel to give access for cutting a flap of abdominal muscle tissue in order to reach the internal organs of fish sample under a dissecting microscope since *P. reticulata* is a particularly small fish with relatively small organs. The third watch glass was retained for any organ requiring re-examination in saline fluid.

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The kidney, heart, gonads, liver, peritoneum, muscle tissue, alimentary canal and the anus of *P. reticulata* were very small. However, each organ excepting the peritoneum and anus was placed in a Petri dish before a wet-mount was made for each of the organs. Each organ sample was punctured, ripped open with the help of a pair of forceps and scalpel, dabbed with saline solution in the Petri-dish in which it was retained before mounting under the binocular microscope to examine tissue for nematode parasite. Nematodes sighted in petri dish are removed with a needle and placed on glass slide for further microscopic examination. Depending on the length of stay of fish sample on bench, a squash-sample of a whole organ was carried out by placing organs between two glass slides and squashed to visualize nematode parasites to stave rapid deterioration of fish samples.

3.4.3. Parasite count, preservation and identification:

Each of the parasites were included as tally entries on tables drawn with a column each titled with the name of each identified nematode with rows indicating organ where parasite was lodged. They were counted and entered as tallies into tables separately for each sex and pooled for drain locations.

The identification of nematode parasite larvae and adult followed thorough and full microscopic examination according to standard identification keys by Yamaguti (1961), Wilmott and Chabaud (1974). Where photomicrographs taken with the aid of mounted digital camera were adjudged of less quality and too fragmented to represent whole microscope mounts, Microsoft windows 7 Paint graphics software was used to draw parasite features.

3.4.4. Water quality analysis:

The major physicochemical parameters measured for each of the sampling sites on the selected streets and road were water temperature (ºC), the pH, the dissolved Oxygen (mg/L), the Secchi disc-extinction and water transparency (cm) and the varying drain depth (cm) which indirectly measured the volume of water at each sampling station to the periodic accumulation of debris and therefore substrate in each of these drains. Temperature of wastewater was measured with a glass-mercury bulb *in situ* by dipping thermometer underneath water surface for 3 minutes before taking thermometer readings. The pH of water was measured with a pH/Conductivity meter model ARH-1

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CE (Manufactured by Myron L Company, Carlsbad, California, USA) while dissolved Oxygen (DO) was measured by the use of electronic probe equipment (Oxyguard). Secchi disc was used to measure water transparency with an improvised white plastic plate, weighted with a small stone tied up in the middle of the plate with a five-meter length plastic-twine chord. Drain depth was measured with a calibrated pole. Physicochemical parameters taken monthly and all data were recorded in a table.

Drains have concrete floors which do not substantially change except for erosion of the surfaces and the periodic deposit of debris from floods during rains and those backfills from monthly residential area sanitation exercises conducted throughout Lagos State. Drain depth, measured with a calibrated metal pole, was the difference between first impact on debris and ultimate drain floor.

3.5. Data Analyses
3.5.1. Determination of monthly frequency of occurrence:

The number of months in which each nematode parasite species was recovered from *P. reticulata* was recorded as the monthly frequency of occurrence for that species. These were derived from sex and drain-related nematode parasite prevalence tables.

3.5.2. Determination of prevalence, intensity, mean intensity and relative monthly percentage composition:

Prevalence, expressed as a percentage, is the number of individuals of a host species infected with a particular parasite species divided by number of hosts examined. Intensity, expressed as a numerical range, is the number of individuals of a particular parasite species (determined directly or indirectly) in each infected host. However, mean intensity is the mean number of individuals of a particular parasite species per infected host in a sample (Margolis *et al*, 1982).

Prevalence was determined for nematode parasites recovered from each sex of *P. reticulata* examined per month before determining prevalence of nematode parasites recovered for all *P. reticulata* pooled for drain location on each of the selected Streets irrespective of sex of fish host.

Intensity was determined for nematode parasites recovered from each sex of *P. reticulata* examined per month before determining intensity of nematode parasites

recovered for all *P. reticulata* pooled for drain location on each of the selected Streets irrespective of sex of fish host.

Therefore, where

α = number of individuals of a host species infected with a particular parasite species;

β = total number of host samples examined;

μ = number of individuals of a particular parasite species and

π = number of infected host samples

Ω = total number of individuals of all parasite species found in infected host sample then:

(i) prevalence was calculated as = α / β x 100

(ii) intensity was calculated as = μ / π .

(iii) Relative monthly percentage composition = μ / Ω x 100

Mean intensity was calculated as mean of aggregated intensity of parasite species categories ± standard deviation.

3.5.3. Statistical analyses:

Microsoft Excel (Office 2007) and SPSS 15 computer software packages were used for statistical analyses.

The Chi square analyses were used to determine if differences in treatment means were due to sampling chance in sex-based parasite prevalence and sex-based parasite intensity after basic descriptive statistics for each group of data. Two-way Analysis of Variance (2-Way ANOVA) was used to determine drain-based parasite prevalence drain-based parasite intensity variations.

3.5.4. Correlation co-efficient:

Correlation Coefficient (r^2) of the array of prevalence and intensity data of nematode parasite in *P. reticulata* to each of the array of physicochemical parameters of temperature, pH, DO, transparency and drain depth were determined.

CHAPTER FOUR

RESULTS AND OBSERVATIONS

4.1. Preliminary Observations

4.1.1. Sampling location:

Human habitation and anthropogenic influence of the contents of wastewater were evident in the contents and nature of sewer in the drains. Effluents from bathrooms, kitchen wastes, bin wastes and materials swept into drains from the tarred road are some of the constituents of wastewater and the mix in the drain inhabiting *P. reticulata*.

Activities from places such as fuel retail centers, carpentry sheds and other commercial concerns that inadvertently release domestic and industrial wastes into wastewater drains influence the chemical status of water that may impact on *P. reticulata* and its parasites.

The low walls of the concrete wastewater drains on Basil Ogamba was vastly eroded in more than half of its length with many occasional predators such as the White Egret, *Ardea alba*, visiting regularly to prey on *P. reticulata* (Appendix 1). The concrete wastewater drains on Igi-Olugbin Street, Ahmadu Bello Road and Alake Adenaike Street were mostly even, though with structural failure of the concrete walls especially around the culverts intersecting adjoining roads and failed plumbing lines. Heavy floods observed in June, 2004 in all wastewater drains, brought the heavy collection of debris. Overgrowth of aquatic macrophytes resulting in occlusion of the drains ultimately leading to loss of habitat for such wastewater fauna as *P. reticulata* was observed (Appendix 2). The result was that no fish sample was available in the drains at the sampling locations in July, 2004 from the selected Streets.

During the dry season, between November, 2004 and January, 2005, the drains were mostly dry and muddy in other places. The sector of drain where fish and water samples were then obtained was restricted to only shorter wastewater sanctuaries especially near entry-points of the drains closer to residences demanding greater sampling effort in all the drains.

4.1.2. Fish host features and behaviour:

Many schools of *P. reticulata* were observed swimming in open wastewaters though some of them often hide under aquatic macrophytes as sanctuary from avian and sundry predators. Stalks of aquatic macrophytes and the flat and long fronded leaf of macrophytes provide shade and sanctuary from aerial predators.

Male *P. reticulata* observed in this study were more brightly coloured than their female counterparts. Colour patterns observed on male fish samples ranged from orange, pink, red and black variegations of the regions posterior to the operculum of fish. These run through the trunk all the way to the anterior region of the caudal peduncle. Male *P. reticulata* were relatively smaller, slender and shorter in size while female *P. reticulata* samples were longer, broader and rotund in the posterior part of their abdomen (Plate 4.1).

Gravid female *P. reticulata* bore guppy marks which have darkened spots around the posterior third of the abdomen indicating the position of the head and eye buds of the developing embryo. Heavy deposition of chromophores were observed on the cranium of male *P. reticulata* juveniles under microscopic examination (Plate 4.2) while their female counterparts had heavier melanin deposits rather than chromophores on the anterior cranial region (Plate 4.3). The gonopodium, a modified intromitent organ for transferring sperm into female *P. reticulata*, was supported by anal fin numbers 3, 4 and 5 as shown on Plate 4.4. The 4th and 5th anal fins are not as acutely arched upwards as found in the other Poeciliids such as *Gambusia spp*. found in the wastewater drains and streams where they sometimes co-exist with *P. reticulata.* This arching and the structure of the gonopodium as well as its distal end are distinguishing features for these fish species.

Caudal region colour dimorphism observed in the males was not seen in the females. Lateral view of the caudal peduncle of a male *P. reticulata* shows chromophores responsible for the multicolor pattern on male fish ramifying blood vessels and dermis of fish (Plate 4.5) which are greatly reduced in the case of their female counterparts (Plate 4.6).
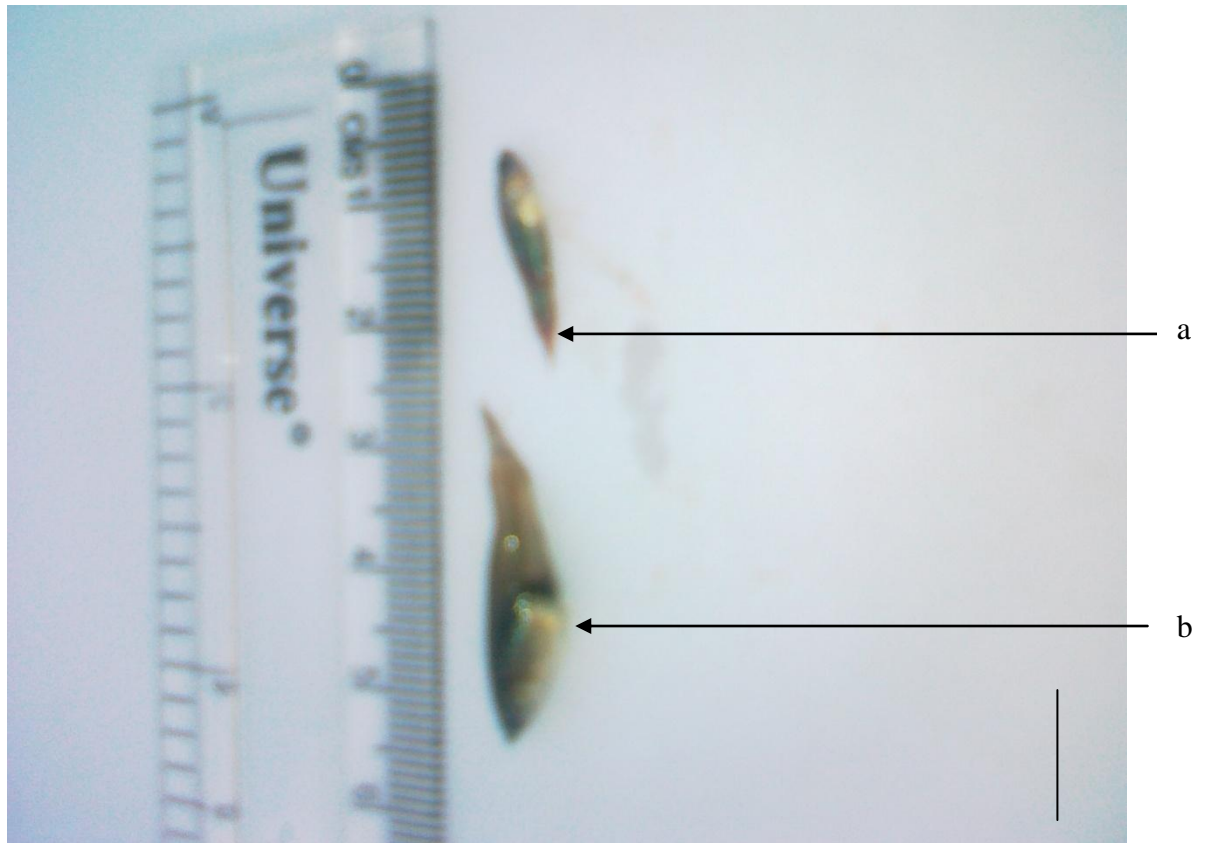
Plate 4.1. Lateral and comparative views of *Poecilia reticulata* (a) male with slender but multicolored caudal and abdominal regions and (b) female with broader abdominal and caudal regions. Bar (1cm).
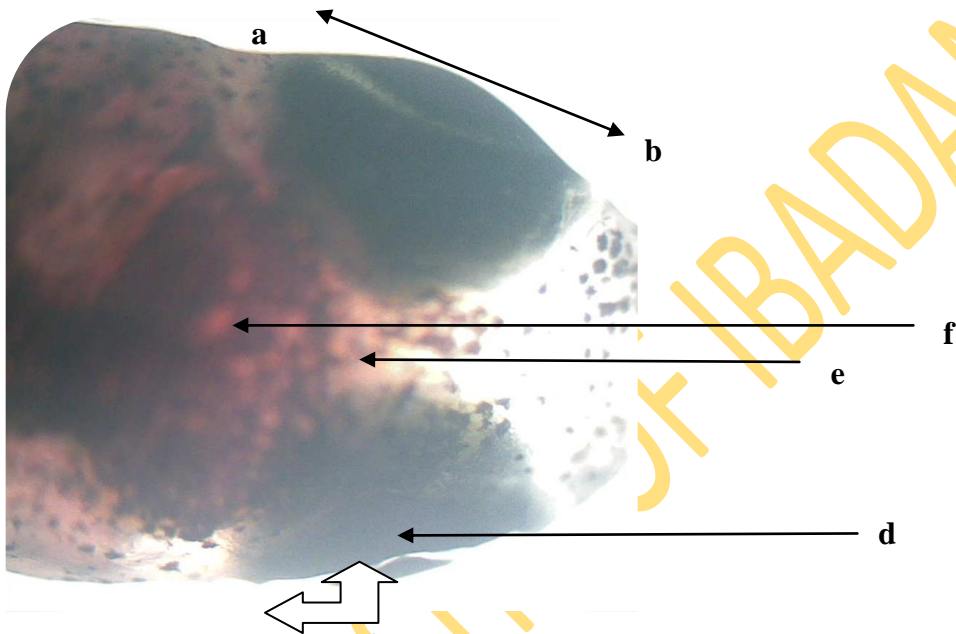
Plate 4.2. Dorsal view of cranium of male *P. reticulata* juvenile with horizontal eye view (a - b); and right angled eye view. Chromophore deposits (e and f). (Mag. x 64.)
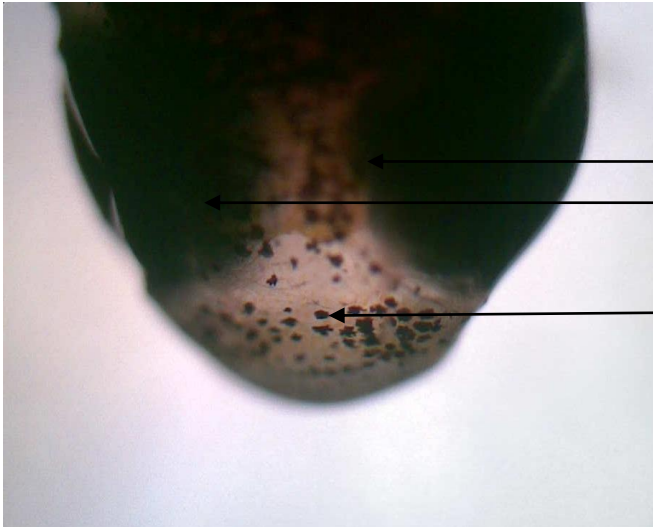
Plate 4.3. Dorsal view of cranium of female *P. reticulata* juvenile with heavier melanin deposits (a and b) and superior, oblique and sub-terminal mouth (c). (Mag. x 64.)
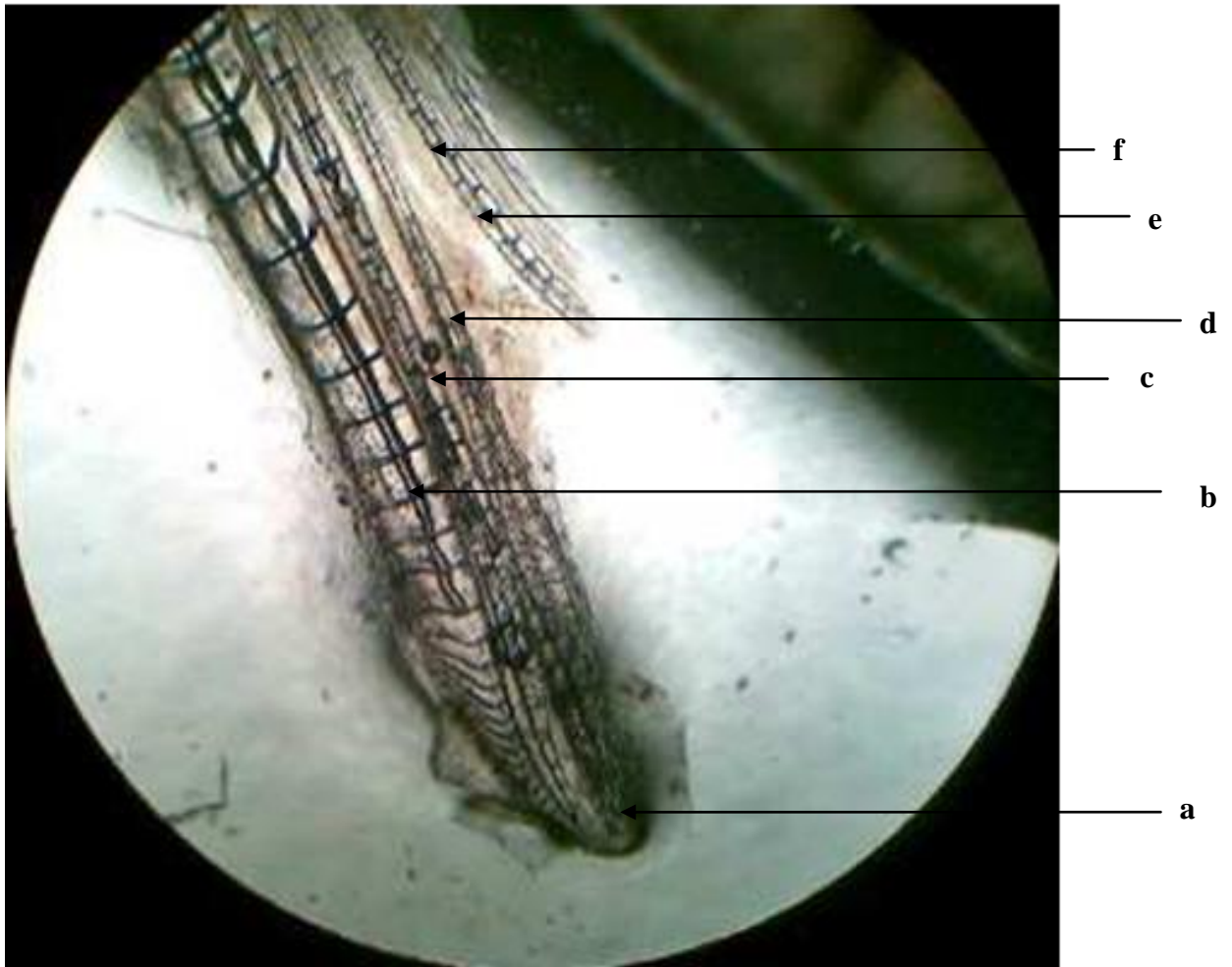
Plate 4.4. A ventro-lateral view of the gonopodium of male *P. reticulata* showing (a) fleshy palp of gonopodium; (b) main gonopodium made of 1st anal fin; (c) 2nd anal fin part of gonopodium; (d) 3rd anal fin; (e) 4th anal fin and (f) 5th anal fin.(Mag. x 160).
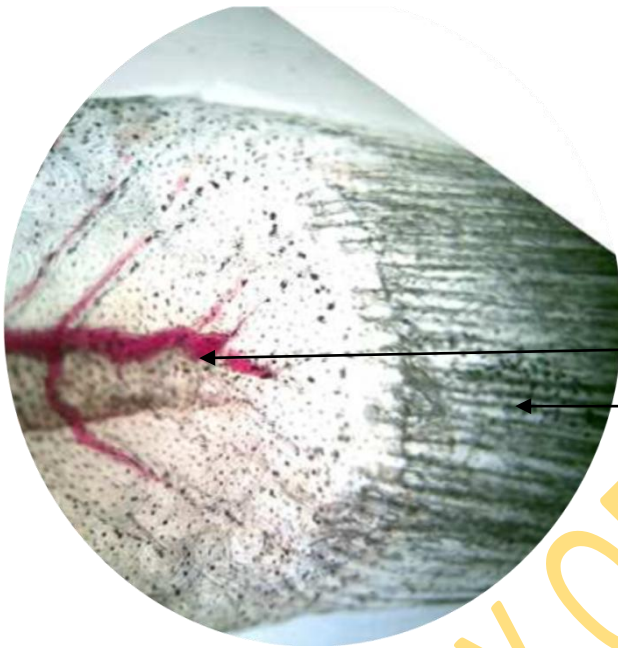
Plate 4.5. Lateral view of the caudal region showing chromophores deposits ramifying blood vessels and dermis of a male *P. reticulata* (a) peduncle and (b) fin (Mag. x 160).
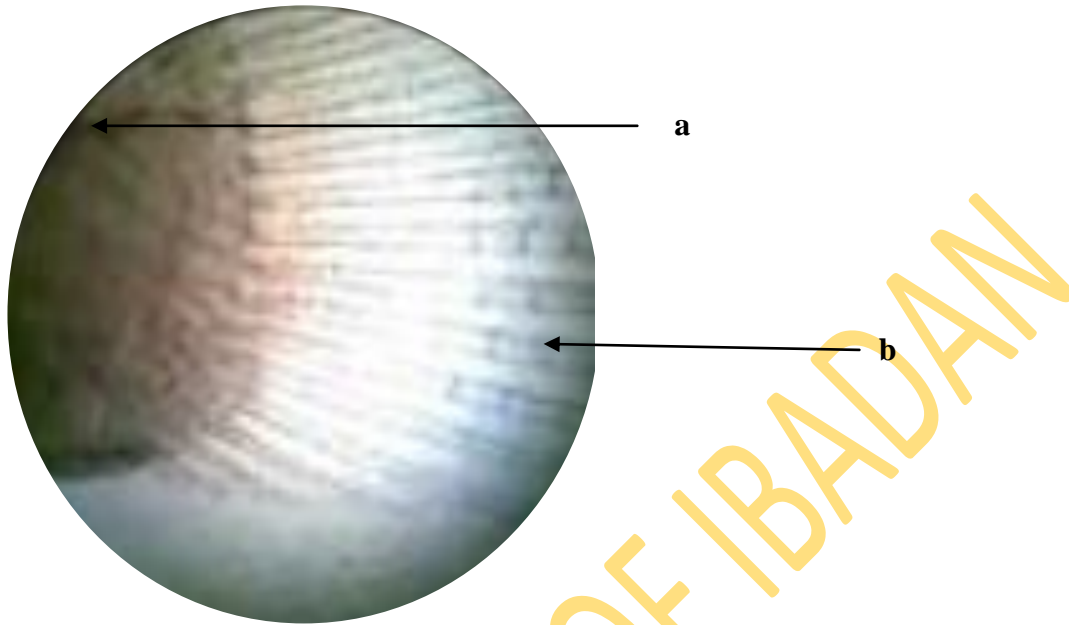
Plate 4.6.   Lateral view of the caudal region showing (a) dark pigmentation on peduncle and (b) fin of a female *P. reticulata* (Mag. x 160).

4.2. Parasite types encountered

Four nematode parasite species from four different nematode families were observed in *P. reticulata* samples examined. These were *Eustrongylides ignotus* Jagerskold 1909*, Camallanus cotti* Fujita 1927*, Capillaria pterophylli* Heinze 1933 and *Trichinella* species Railliet 1895. The parasite, *E. ignotus*, belongs to the family Eustrongylidae while *C. cotti* belongs to the family Camallanidae. The nematode, *C. pterophylli*, is a member of the family Capillaridae while *Trichinella spp.* was the only member of the family Trichinellidae encountered in this study.

The nematode *E. ignotus* was obtained from the peritoneum of infected *P. reticulata*. They were not encyted though the occurrence and infection of *P. reticulata* by *E. ignotus* caused external inflamation of the abdominal cavity of the host fish. The third and fourth stage larvae of *E. ignotus* encountered were found ramifying the peritoneum of *P. reticulata.* This nematode was usually bright red but atimes transluscent pink to faded-brown in colour with transversely striated and thick cuticle. They were thread-like long bodied worms tapering to the anterior region with two rings of cephalic and labial pappilae (Fig. 4.1). Each of the six labial papillae in the first inner ring of papillae was more pointed while those of the outer ring of papillae were not as sharp at their tips. The anterior region of this nematode terminates in a protractile labium. The rounded posterior of a male *E.ignotus* larva is spatulate (Fig. 4.2) while that of the female is ovoid (Fig. 4.3) in shape. There were caudal papillae at the posterior region of both male (Fig. 4.2) and female larvae (Fig. 4.3) of *E. ignotus*.

There were coiled larvae of *Trichinella* spp. lodged in the muscle fibers of *P. reticulata*. These were found below darkened spots and lessions on the skin of the fish host. Some larval forms were found in the intestine of *P. reticulata*. The encysted larval forms in the muscle tissues of *P. reticulata* (Fig. 4.4) were often coiled unto themselves. Those lodged within muscle of *P. reticulata* presented with a dirty white but transparent colour upon dissection of infected *P. reticulata* host in contrasts with the dark pigmentation on the dermis corresponding to the lesion of encystment. This nematode possessed stichosomes on the ventrolateral sides of the oesophagus at the anterior half region of the nematode (Fig. 4.4). This worm tapers sharply at the anterior region terminating into a stylet but broadens widely and evenly to almost the tip of the caudal region when stretched and relaxed.

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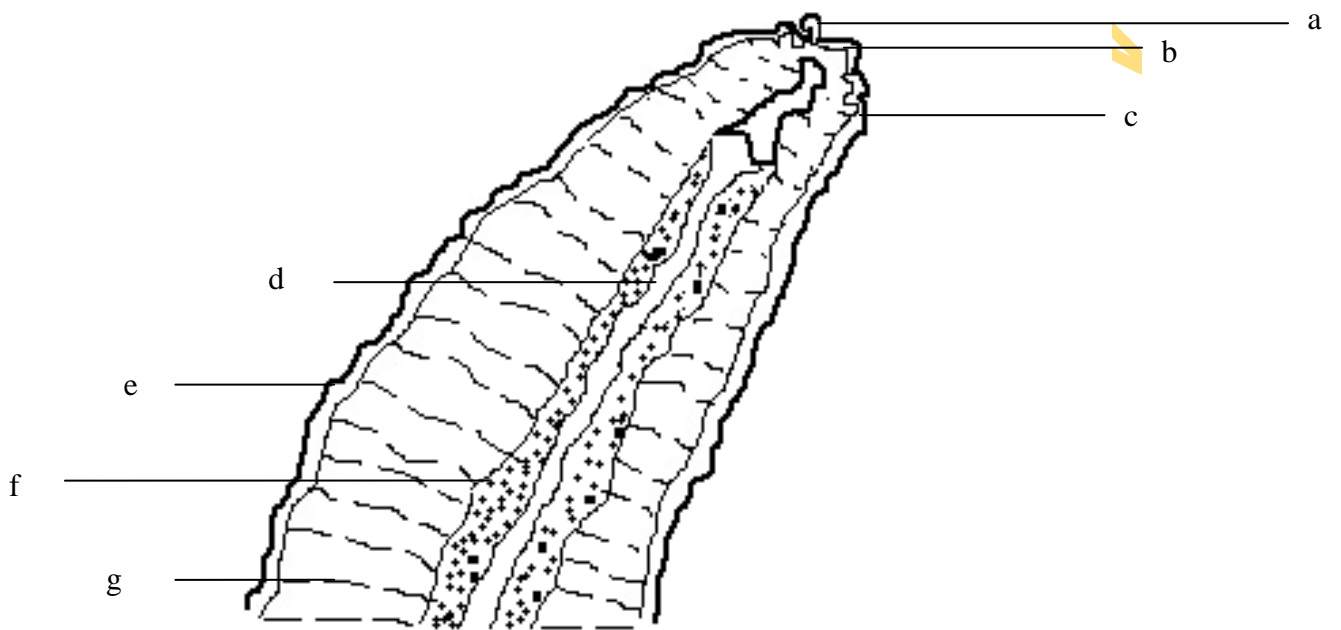
Figure 4.1. A drawing of lateral section of the anterior region of *Eustrongylides ignotus* fourth-stage larvae obtained from *P. reticulata* peritoneum showing (a) one of the inner ring of labial papillae, (b) cephalic tip and (c) one of the outer ring of labial papillae (d) long oesophagus (e) inner cuticular layer (f) glandular oesophagus and (g) transverse striations (Mag. x 640).
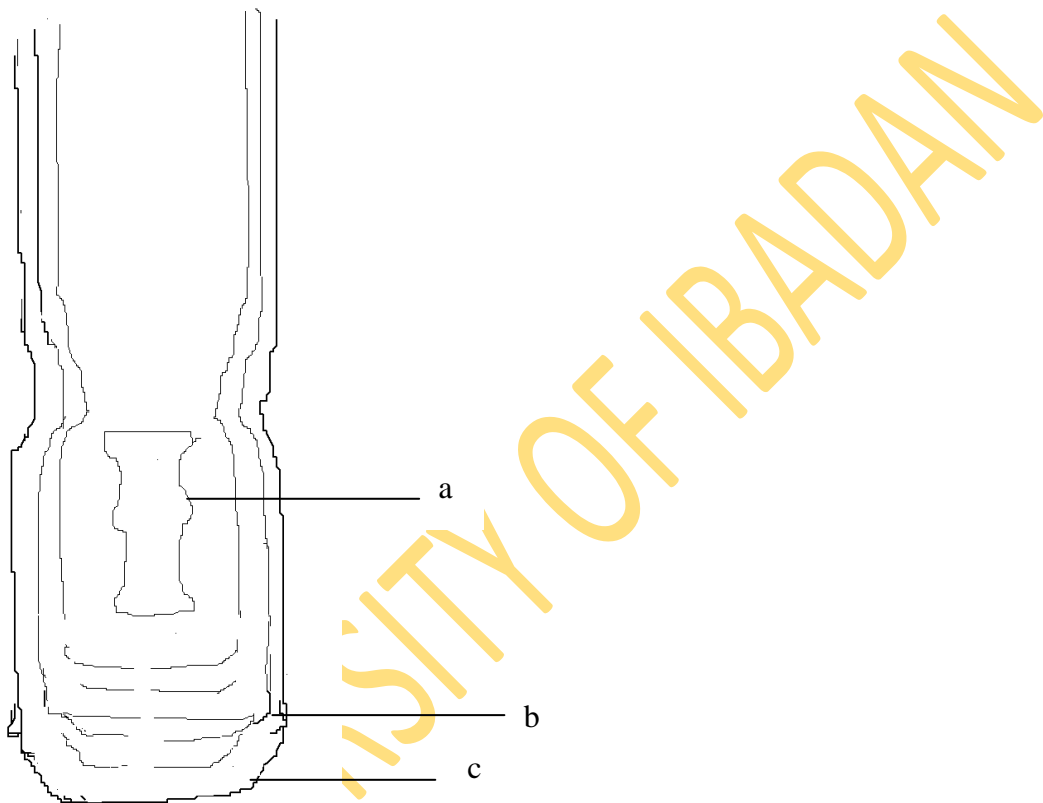
Figure 4.2. A drawing of dorsal section of the posterior end of male *Eustrongylides ignotus* fourth-stage larvae showing (a) cuticular folds (b) caudal papillae and (c) outer cuticle (Mag. x 640).
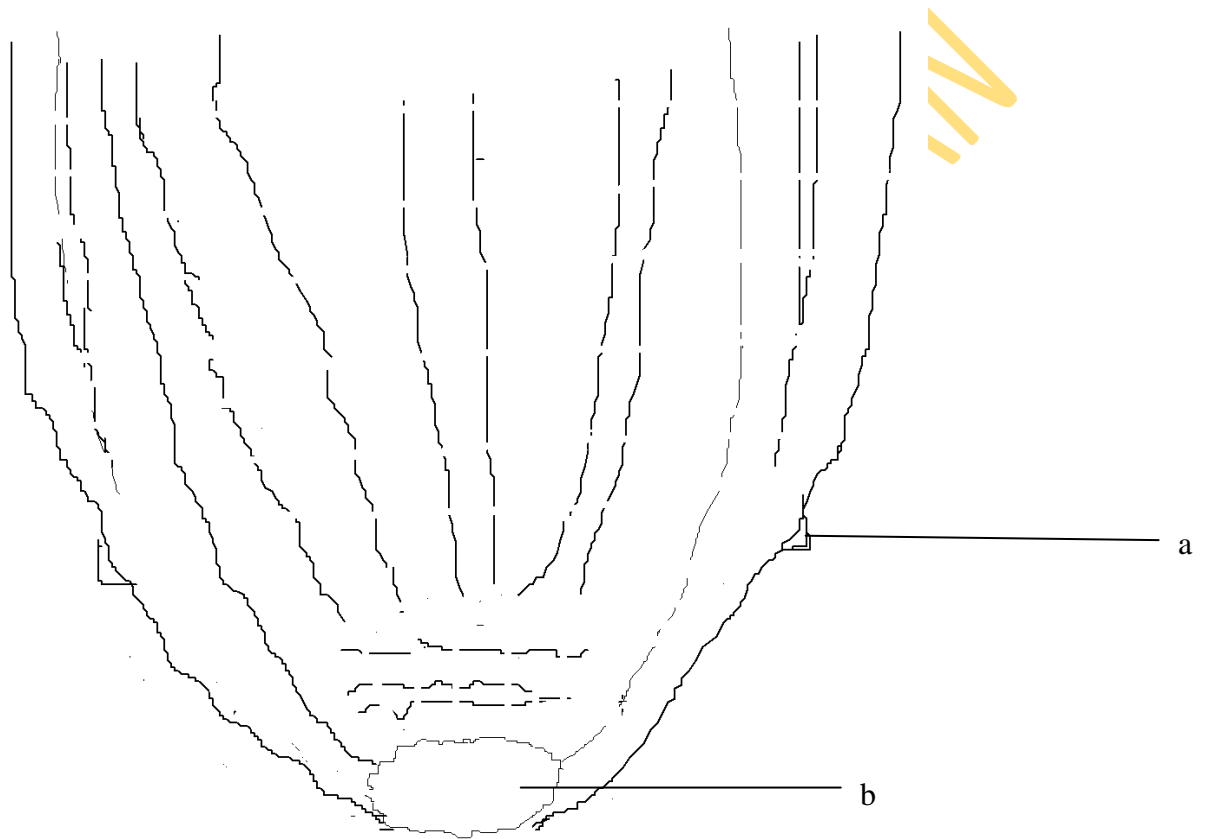
Figure 4.3. A drawing of dorsal section of posterior end of female *Eustrongylides ignotus* fourth-stage larvae showing (a) caudal papilla and (b) terminal anus (Mag. x 640).
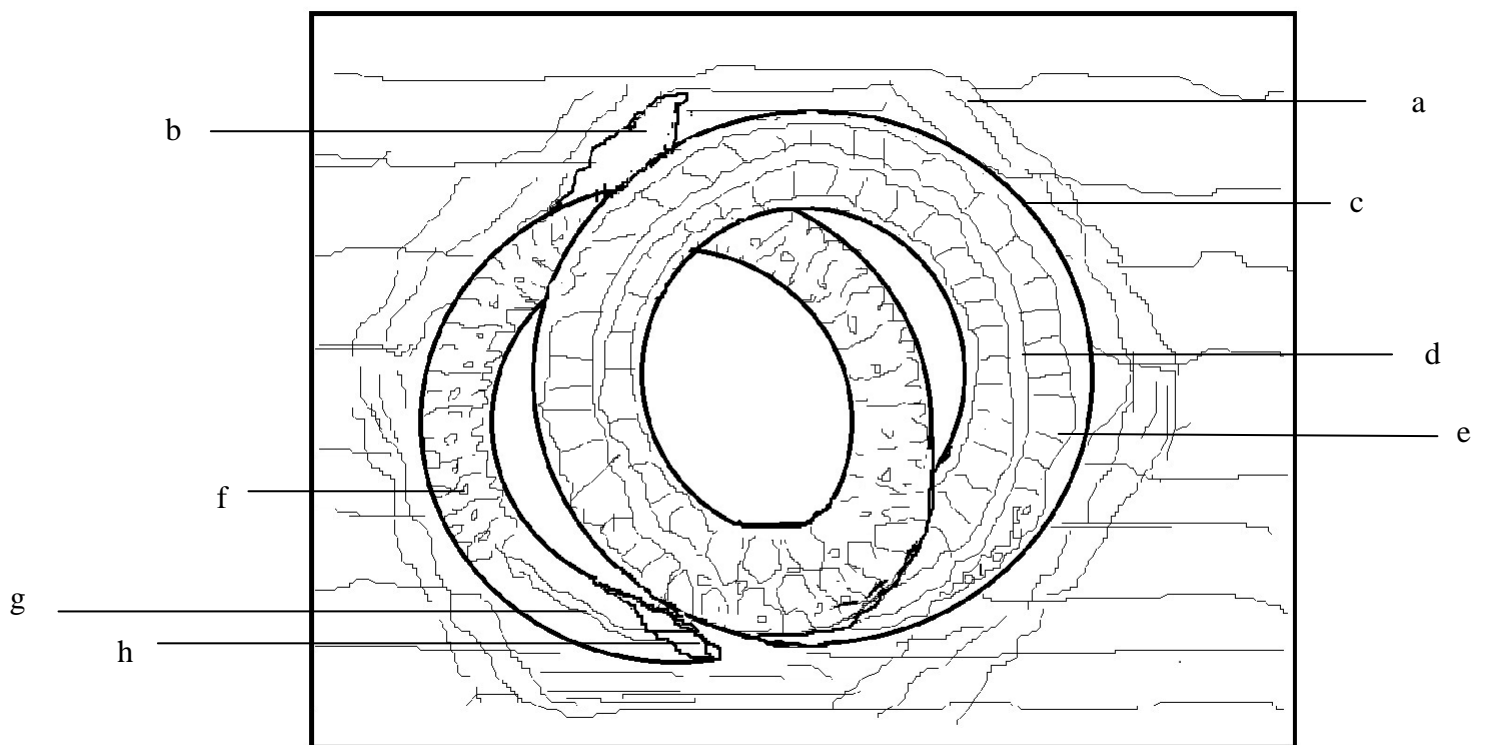
Figure 4.4. A drawing of lateral view of *Trichinella spp.* obtained from *P. reticulata* muscle tissues showing (a) debris of host tissue, (b) posterior end, (c) cuticle, (d) intestine, (e) , (f) stichosome, (g) oesophagus and (h) stylet at anterior end of worm (Mag. x 64).

The nematode parasite *C. pterophylli* belongs to the Class Adenophorea, subclass Enoplia, Order Trichurida, Family Capillaridae and Genus Capillaria. Adult *C. pterophylli* samples were observed in the infected *P. reticulata* dissected. This parasite is filliform in shape, transparent in colour and considerably long within the intestine of infected *P. reticulata* samples dissected. At the anterior end, the mouth is recrudescent without clearly distinct cephalic papillae (Fig. 4.5). There was a single line of slender stichosomes, each with a large centrally placed nucleus (Fig. 4.6). The male adult *C. pterophylli* has a distal end terminating in a prominent set of twin lobes around which are a pair of circumcloacal papilla on the ventral side (Fig. 4.7). Each of these two lobes also bears a papilla each. However, the male genital opening, the cloaca, houses a sheated spicule where that of the female terminates in a vulvar with a partially raised upper lip through which mature embryo are extruded from the nematodes's uterus (Fig. 4.8). The maturing gelatinized string of embryos is set in a single file along the uterus. Some extruded embryos are found separately outside adult worms in the intestine of some infected *P. reticulata*. The female *C. pterophylli* are usually longer and broader than their male counterparts. Their eggs are oval shaped with gentle striations on the apices and lateral wall of the mature embryo but less so in the middle (Fig. 4.8). This worm has a direct life cycle in *P. reticulata*.

The general taxonomic description of *C. cotti* is Phylum: Nematoda, Order: Spirurida, SubOrder: Camallaninae, Superfamily: Camallanoidae, Family: Camallanida, and Genus: Camallanus. Adult *C. cotti* were observed lodged in the duodenum and anus of infected *P. reticulata* hosts. This fusiform nematode, *C. cotti*, (Plate 4.7) has a cephalic region that is somewhat dorsovetrally flattened with a buccal capsule that gives the anterior end of this nematode a golden brownish colour that contrasts with the transparent but reddish colour of the remaining parts of the whole worm. There is a frontally placed slit-like mouth opening from left to right at the anterior-most end of the parasite. On top and below this slit-like mouth is a pair of cephalic papillae. So also is one laterally placed trident structure indentured on both sides of the head with their triad prong end pointing anteriorly but their single arms pointing posteriorly. On the caudal extremity of this nematode, the male worm often maintain a dorsoventrally curved orientation with the cloaca located in the deeper cup of the folded distal part (Plate 4.8). The cloaca is surrounded by circumcloacal papillae and a triad of papillae at the distalmost end of the tail tip.
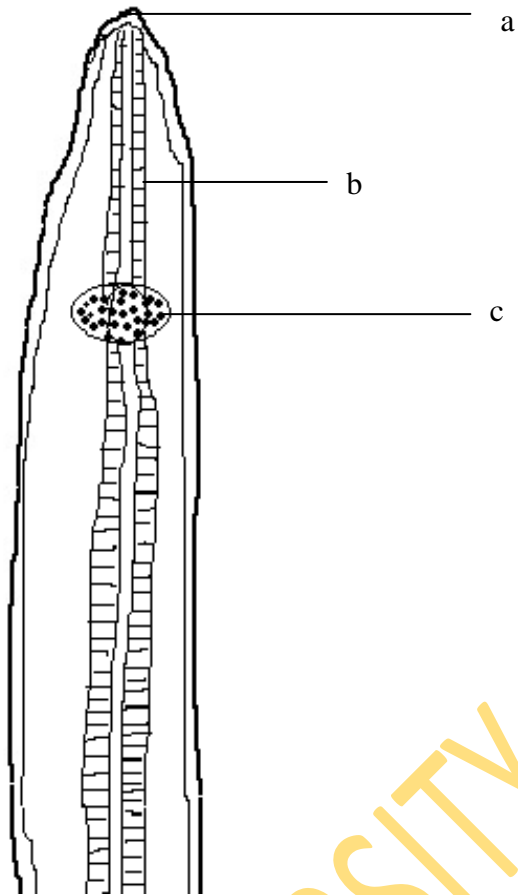
Figure 4.5. A drawing of lateral view of the anterior region of *Capillaria pterophylli* obtained from *P. reticulata* showing (a) indistinct mouth at anterior region (b) oesophagus and (c) nerve ring (Mag. x 64).
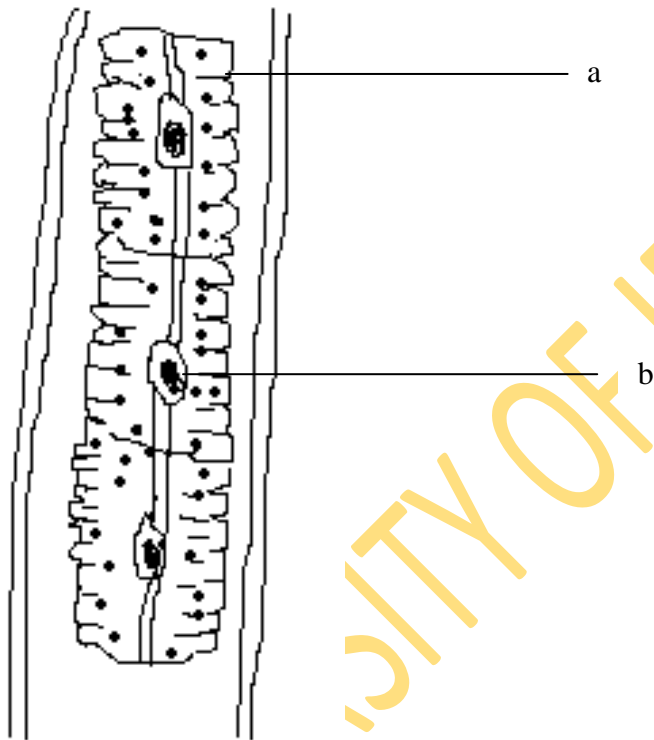
Figure 4.6. A drawing of lateral view of a midsection of *Capillaria pterophylli* obtained from *P. reticulata* showing (a) slender stichosome and (b) conspicuous nucleus of stichocyte (Mag. x 64).
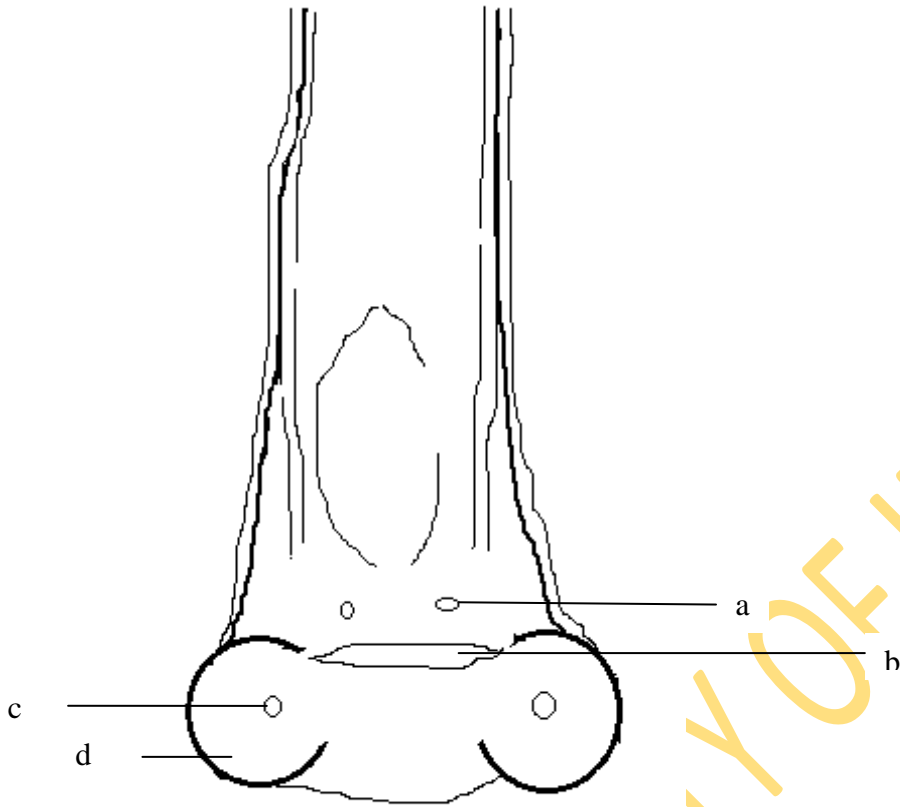
Figure 4.7. Drawing of ventral view of the distal end of *Capillaria pterophylli* obtained from *Poecilia reticulata* showing (a) circumclocal papilla (b) cloaca (c) papillae of ventral lobe and (d) ventral lobe (Mag. x 64).
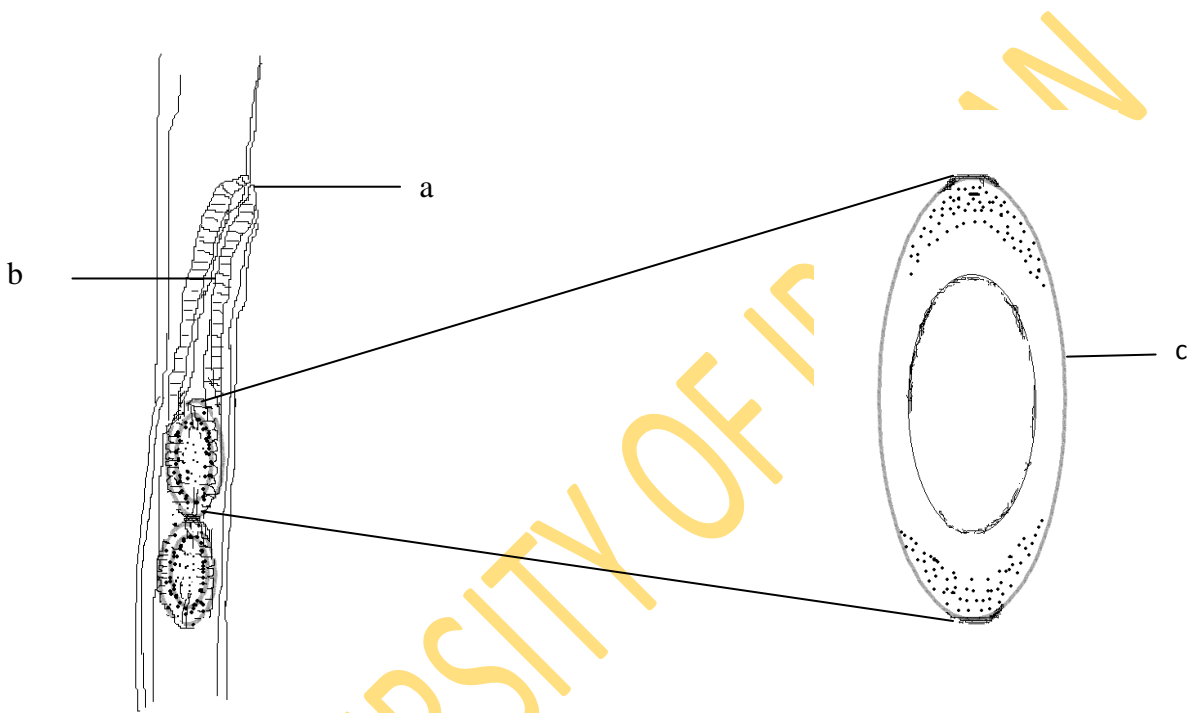
Figure 4.8. Lateral view of the gonadal section of a gravid female *Capillaria pterophylli* obtained from *Poecilia reticulata* showing (a) vulva (b) uterus and (c) mature egg ready for extrusion (Mag. x 64).

Plate 4.7. Lateral view of an adult *Camallanus cotti* obtained from *P. reticulata*
with (a) buccal capsule; (b) position of trident; (c) oesophageal junction and (d)
position of genital pore
(Mag. x 40).
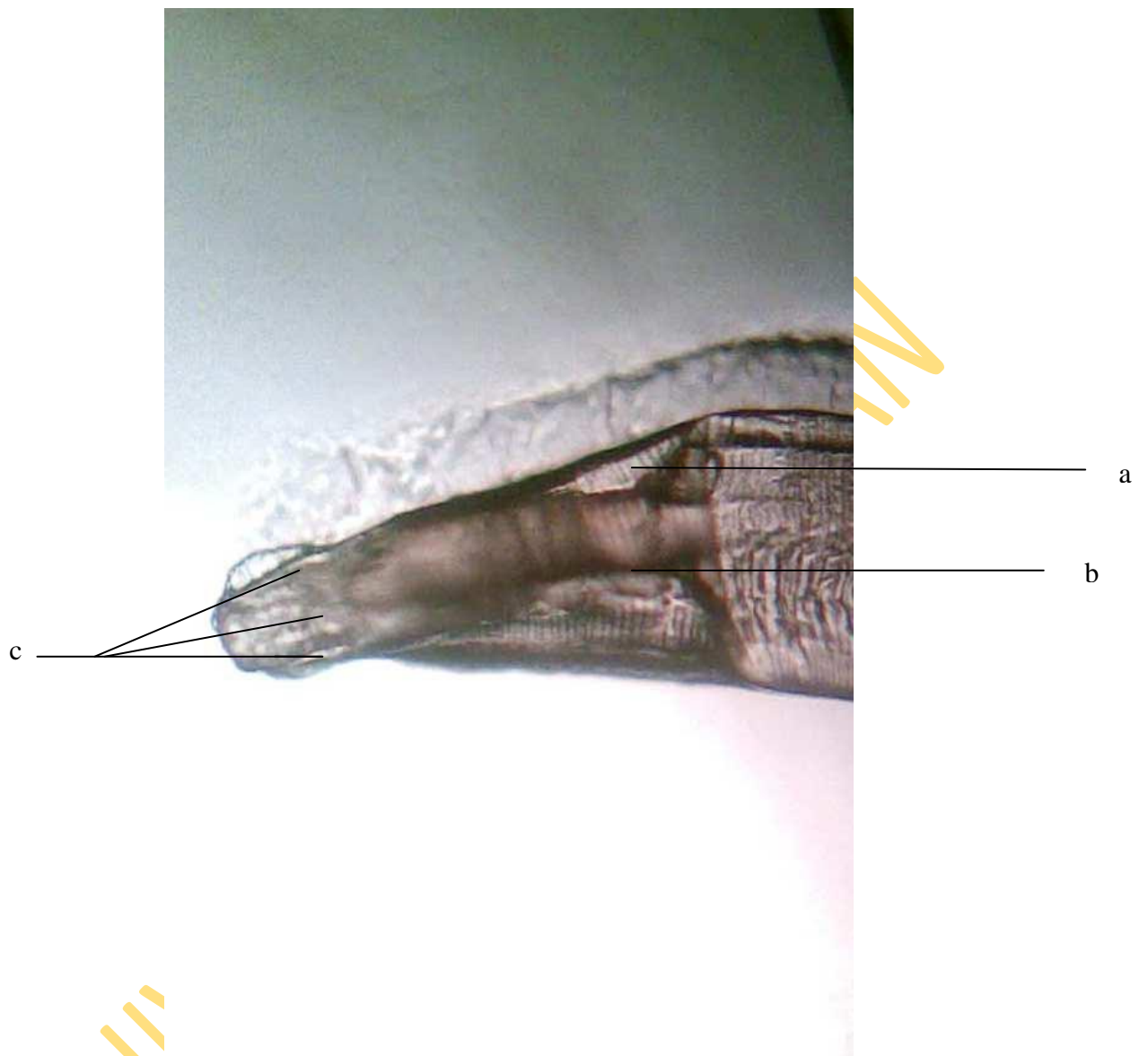
Plate 4.8. Ventral view of the posterior region of a male *Camallanus cotti* showing (a) circumcloacal papillae (b) cloaca and (c) triad of distal papillae at the posterior tip of the worm (Mag. x 640).

4.3. Monthly Frequency of Occurrence

4.3.1. Overall Percentage Frequency of Occurrence Patterns:

Out of the total of 4320 *P. reticulata* samples examined a total of 519 (12.0%) were infected with at least one, or a combination of, nematode parasite. *E. ignotus* had the highest overall percentage frequency of occurrence (PFO) of 30.0% with 156 *P. reticulata* infections while *Trichinella spp.* had the lowest overall percentage of 13.0% with 65 *P. reticulata* infections (Table 4.1).

4.3.2. Frequency of Occurrence Pattern in Relation to Host Sex

The frequency of occurrence of each nematode parasite of *P. reticulata* is derived from the sex-related prevalence table (Table 4.2) according to the percentage of the number of months in which each parasite occurred in either sex of fish host over the entire period of this study.

E. ignotus: The frequency of occurrence of *E. ignotus* in male *P. reticulata* was seven months which constituted 28.0% in percentage frequency of Occurrence while *E. ignotus* occurred in eight months in female *P. reticulata* also constituted 28.0% in PFO (Table 4.2).

Trichinella spp: This occurred in four months in male *P. reticulata* at 16.0% PFO but five months in female *P. reticulata* at 18.0% PFO (Table 4.2).

C. pterophylli: It occurred in seven months of the study with a 28.0% PFO in male *P. reticulata* but for eight months with 29.0% PFO in female *P. reticulata*.

C. cotti: It occurred in seven months in both sexes with a 28.0% PFO in male *P. reticulata* but a 25.0% PFO in female *P. reticulata*. A student t-test revealed no significant differences in the PFOs of the male and female *P. reticulata* (Table 4.2).

4.4. Prevalence Pattern

4.4.1. Prevalence by Parasite type:

Out of the 4320 samples of *P. reticulata* examined 156 (3.6%) of them were infected by *E. ignotus*, 152 (3.5%) were infected by *C. pterophylli*, 146 (3.4%) were infected by *C. cotti* while only 65 (1.5%) of the *P. reticulata* samples were infected by *Trichinella spp.*

Table 4.1. Number of infected male and female *Poecilia reticulata*

Month	Nematode	Male fish Street Igi-Olugbin	Basil Ogamba	Ahmadu Bello	Adenaike Alagbe	Female fish Street Igi-Olugbin	Basil Ogamba	Ahmadu Bello	Adenaike Alagbe
March, 2004	*Eustrongylides ignotus*	1.0	0.0	1.0	2.0	6.0	0.0	6.0	0.0
	Trichinella spp.	0.0	0.0	0.0	0.0	0.0	0.0	4.0	0.0
	Capillaria pterophylli	0.0	0.0	7.0	3.0	0.0	0.0	8.0	6.0
	Camallanus cotti	0.0	0.0	4.0	0.0	0.0	0.0	0.0	0.0
April, 2004	*Eustrongylides ignotus*	0.0	0.0	0.0	0.0	6.0	0.0	0.0	0.0
	Trichinella spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Capillaria pterophylli	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Camallanus cotti	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
May, 2004	*Eustrongylides ignotus*	3.0	2.0	1.0	9.0	11.0	0.0	6.0	3.0
	Trichinella spp.	3.0	0.0	2.0	0.0	3.0	0.0	1.0	2.0
	Capillaria pterophylli	1.0	0.0	0.0	1.0	5.0	1.0	3.0	0.0
	Camallanus cotti	0.0	0.0	0.0	1.0	2.0	0.0	0.0	2.0
June, 2004	*Eustrongylides ignotus*	0.0	0.0	1.0	12.0	0.0	0.0	2.0	8.0
	Trichinella spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Capillaria pterophylli	9.0	5.0	1.0	0.0	2.0	3.0	0.0	0.0
	Camallanus cotti	0.0	0.0	0.0	0.0	0.0	0.0	0.0	2.0
July, 2004	*Eustrongylides ignotus*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Trichinella spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Capillaria pterophylli	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Camallanus cotti	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
August, 2004	*Eustrongylides ignotus*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Trichinella spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Capillaria pterophylli	0.0	4.0	3.0	0.0	0.0	2.0	0.0	2.0
	Camallanus cotti	5.0	0.0	0.0	11.0	5.0	0.0	0.0	2.0
September, 2004	*Eustrongylides ignotus*	3.0	0.0	0.0	6.0	3.0	1.0	2.0	2.0
	Trichinella spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Capillaria pterophylli	5.0	0.0	0.0	4.0	5.0	0.0	0.0	1.0
	Camallanus cotti	13.0	1.0	11.0	7.0	15.0	1.0	3.0	15.0
October, 2004	*Eustrongylides ignotus*	3.0	5.0	0.0	3.0	1.0	0.0	0.0	8.0
	Trichinella spp.	0.0	6.0	5.0	2.0	0.0	0.0	5.0	3.0

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	Capillaria pterophylli	0.0	0.0	0.0	0.0	6.0	0.0	0.0	1.0
	Camallanus cotti	1.0	3.0	1.0	2.0	2.0	5.0	0.0	6.0
November, 2004	*Eustrongylides ignotus*	0.0	0.0	0.0	1.0	0.0	0.0	0.0	1.0
	Trichinella spp.	1.0	3.0	0.0	0.0	7.0	2.0	0.0	0.0
	Capillaria pterophylli	1.0	0.0	3.0	0.0	1.0	0.0	0.0	0.0
	Camallanus cotti	0.0	0.0	0.0	2.0	0.0	0.0	0.0	6.0
December, 2004	*Eustrongylides ignotus*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Trichinella spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Capillaria pterophylli	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Camallanus cotti	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
January, 2005	*Eustrongylides ignotus*	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Trichinella spp.	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Capillaria pterophylli	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	Camallanus cotti	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
February, 2005	*Eustrongylides ignotus*	7.0	6.0	8.0	1.0	3.0	2.0	8.0	2.0
	Trichinella spp.	4.0	0.0	0.0	5.0	2.0	0.0	0.0	5.0
	Capillaria pterophylli	7.0	5.0	8.0	1.0	6.0	17.0	13.0	2.0
	Camallanus cotti	0.0	4.0	0.0	0.0	2.0	6.0	2.0	4.0

Table 4.2. Monthly prevalence of sex-related nematode parasites of *Poecilia reticulata*

		Nematode parasite			
Month	Sex of fish host	*Euustrongylides ignotus*	*Trichinella spp.*	*Capillaria pterophylli*	*Camallanus cotti*
March, 2004	Male	1.7	0	4.2	1.7
	Female	5	1.7	5.8	0
April, 2004	Male	0	0	0	0
	Female	2.5	0	0	0
May, 2004	Male	6.3	2.1	0.8	0.4
	Female	8.3	2.5	3.8	1.7
June, 2004	Male	5.4	0	6.3	0
	Female	4.2	0	2.1	0.8
July, 2004	Male	0	0	0	0
	Female	0	0	0	0
August, 2004	Male	0	0	2.9	6.7
	Female	0	0	1.7	2.9
September, 2004	Male	3.8	0	3.8	13.3
	Female	3.3	0	2.5	14.2
October, 2004	Male	4.6	5.4	0	2.9
	Female	3.8	3.3	2.9	5.4
November, 2004	Male	0.4	1.7	1.7	0.8
	Female	0.4	3.8	0.4	2.5
December, 2004	Male	0	0	0	0
	Female	0	0	0	0
January, 2005	Male	0	0	0	0
	Female	0	0	0	0
February, 2005	Male	9.2	3.8	8.8	1.7
	Female	6.3	2.9	15.83	5.8

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4.4.2 Monthly prevalence by Host Sex:

In this study, the differences observed in the monthly sex- related nematode parasite prevalence throughout the study were not statistically significant. It is though noteworthy, that *C. pterophylli* had the highest monthly prevalence (15.8%) observed in female *P. reticulata* collected in February, 2005 as against the prevalence of 8.8% in male *P. reticulata* (Table 4.2).

E. ignotus: The highest monthly prevalence observed in male *P. reticulata* (9.2%) was observed in February, 2005 while the highest prevalence (8.3%) in female samples of *P. reticulata* was also obtained in May, 2004 (Table 4.2).

Trichinella spp.: For *Trichinella spp.*, the highest monthly prevalence (5.4%) in male *P. reticulata* was observed in October, 2004 while that for female *P. reticulata* (3.8%) was observed in November, 2004 (Table 4.2).

C. pterophylli: The highest monthly nematode parasite prevalence 15.8% in the entire study was *C. pterophylli* observed in female *P. reticulata* in February, 2005. The highest *C. pterophylli* monthly prevalence in male *P. reticulata* was 8.8% also observed in the February, 2005 samples (Table 4.2).

C. cotti: The highest monthly prevalence of nematode parasites in male and female *P. reticulata* occurred in September, 2004 and they were relatively comparable with each other unlike in the previous cases already discussed. The highest prevalence was 13.3% in male *P. reticulata* and 14.2% in female *P. reticulata* (Table 4.2).

4.4.3. Drain-related nematode parasite prevalence in *P. reticulata*:

There were no statistically significant differences in the monthly drain-related prevalenc'e in the nematode parasites of *P. reticulata* obtained from drains of the four selected streets.

Igi-Olugbin Street: *C. cotti* had the highest drain related nematode parasite prevalence of 23.3% amongst all the nematodes observed in *P. reticulata* samples obtained and examined throughout the study and it was observed in samples obtained from Igi-Olugbin Street examined in September, 2004 (Table 4.3).

The highest prevalence of *E. ignotus* was 11.7% in May, 2004 while that for *Trichinella spp.* was 6.7% in November, 2004. The highest drain-related prevalence of *C. pterophylli* was 10.8% in February, 2005 (Table 4.3).

Table 4.3 Monthly prevalence of drain-related nematode parasites of *Poecilia reticulata* obtained from each sampling location irrespective of sex of sample

Month	Nematode parasite	Igi-Olugbin	Basil Ogamba	Ahmadu Bello	Adenaike Alagbe
March, 2004	*Eustrongylides ignotus*	5.8	0.0	5.8	1.7
	Trichinella spp.	0.0	0.0	3.3	0.0
	Capillaria pterophilli	0.0	0.0	12.5	7.5
	Camallanus cotti	0.0	0.0	3.3	0.0
April, 2004	*Eustrongylides ignotus*	5.0	0.0	0.0	0.0
	Trichinella spp.	0.0	0.0	0.0	0.0
	Capillaria pterophilli	0.0	0.0	0.0	0.0
	Camallanus cotti	0.0	0.0	0.0	0.0
May, 2004	*Eustrongylides ignotus*	11.7	1.7	5.8	10.0
	Trichinella spp.	5.0	0.0	2.5	1.7
	Capillaria pterophilli	5.0	0.8	2.5	0.8
	Camallanus cotti	1.7	0.0	0.0	2.5
June, 2004	*Eustrongylides ignotus*	0.0	0.0	2.5	16.7
	Trichinella spp.	0.0	0.0	0.0	0.0
	Capillaria pterophilli	9.2	5.0	0.8	0.0
	Camallanus cotti	0.0	0.0	0.0	1.7
July, 2004	*Eustrongylides ignotus*	0.0	0.0	0.0	0.0
	Trichinella spp.	0.0	0.0	0.0	0.0
	Capillaria pterophilli	0.0	0.0	0.0	0.0
	Camallanus cotti	0.0	0.0	0.0	0.0
August, 2004	*Eustrongylides ignotus*	0.0	0.0	0.0	0.0
	Trichinella spp.	0.0	0.0	0.0	0.0
	Capillaria pterophilli	0.0	5.0	2.5	1.7
	Camallanus cotti	8.3	0.0	0.0	10.8
September, 2004	*Eustrongylides ignotus*	5.0	0.8	1.7	6.7
	Trichinella spp.	0.0	0.0	0.0	0.0
	Capillaria pterophilli	8.3	0.0	0.0	4.2
	Camallanus cotti	23.3	1.7	11.7	18.3
October, 2004	*Eustrongylides ignotus*	3.3	4.2	0.0	9.2
	Trichinella spp.	0.0	5.0	8.3	4.2
	Capillaria pterophilli	5.0	0.0	0.0	0.8
	Camallanus cotti	2.5	6.7	0.8	6.7
November, 2004	*Eustrongylides ignotus*	0.0	0.0	0.0	1.7
	Trichinella spp.	6.7	4.2	0.0	0.0

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	Capillaria pterophilli	1.7	0.0	2.5	0.0
	Camallanus cotti	0.0	0.0	0.0	6.7
December, 2004	*Eustrongylides ignotus*	0.0	0.0	0.0	0.0
	Trichinella spp.	0.0	0.0	0.0	0.0
	Capillaria pterophilli	0.0	0.0	0.0	0.0
	Camallanus cotti	0.0	0.0	0.0	0.0
January, 2005	*Eustrongylides ignotus*	0.0	0.0	0.0	0.0
	Trichinella spp.	0.0	0.0	0.0	0.0
	Capillaria pterophilli	0.0	0.0	0.0	0.0
	Camallanus cotti	0.0	0.0	0.0	0.0
February, 2005	*Eustrongylides ignotus*	8.3	6.7	13.3	2.5
	Trichinella spp.	5.0	0.0	0.0	8.3
	Capillaria pterophilli	10.8	18.3	17.5	2.5
	Camallanus cotti	1.7	8.3	1.7	3.3

Basil Ogamba Street: The highest drain-related prevalence of *E. ignotus* was observed in February, 2005 at 6.7%, that of *Trichinella spp.* was at 5.0% in October, 2004 and November, 2004. The highest drain related prevalence (18.3%) of *C. pterophylli* was observed in February, 2005 while the highest drain related prevalence of *C. cotti* at 6.7% was observed in October, 2004 (Table 4.3).

Ahmadu Bello Road: The most prevalent nematode found in the drain related *P. reticulata* samples obtained from Ahmadu Bello Road was *C. pterophylli* at 17.5% and this was followed by *E. ignotus* at 13.3% also in February, 2005. However, the highest prevalent *Trichinella spp.* (8.3%) was observed in October, 2004, the most prevalent *C. pterophylli* (12.5%) was observed in March, 2004 while the most prevalent *C. cotti* at Ahmadu Bello Road (11.7%) was observed in September, 2004 (Table 4.3).

Adenaike Alagbe Street : *C. cotti* was the most prevalent nematode 18.3% found in *P. reticulata* obtained from this Street and this was during the month of September, 2004 with the most prevalent *E. ignotus* (16.7%) observed in *P. reticulata* obtained from Adenaike Alagbe Street in June, 2004 (Table 4.3).

4.4.4. Sex-related nematode parasite intensity in *P. reticulata*:

Total counts for each of the nematode parasites observed in *P. reticulata* dissected during this study were recorded (Table 4.4). Parasite intensity was calculated according to the sex of *P. reticulata* host from which they were observed (Table 4.5). However, after parasite mean intensity data was subjected to ANOVA no statistically significant difference was observed. A range of low mean intensity value of 0.3 ± 0.3 (mean intensity $\pm$ standard deviation) to the highest mean intensity at 5.0 ± 1.8 in male *P. reticulata* samples were observed. In the case of female *P. reticulata* samples, the range was from as low as 0.3 ± 0.3 nematode parasite mean intensity to as high as 4.9 ± 1.8.

The highest topical monthly intensity in male *P. reticulata* samples was 9.0 and that was *C. cotti* in May, 2004 which was followed by the monthly intensity 5.5 for *C. pterophylli* in the same month (Table 4.5).

E. ignotus: The highest sex-related *E. ignotus* intensity in male *P. reticulata* observed in the study was 3.0 in March, 2004 (Fig. 4.5) and that in female *P. reticulata* was 3.9 in September, 2004 (Table 4.5).

Trichinella spp.: The highest sex-related intensity was 2.3 and it was observed in *P. reticulata* female in March and May, 2004 (Table 4.5).

Table 4.4. Total count of nematode parasites in *Poecilia reticulata* obtained from the wastewater drains of four selected streets in Lagos State

	Street Name	Igi-Olugbin	Igi-Olugbin	Basil Ogamba	Basil Ogamba	Ahmadu Bello	Ahmadu Bello	Adenaike Alagbe	Adenaike Alagbe
	Sex	Male	Female	Male	Female	Male	Female	Male	Female
March, 2004	*Eustrongylides ignotus*	1	6	0	0	4	9	7	0
	Trichinella spp.	0	0	0	0	0	9	0	0
	Capillaria pterophylli	0	0	0	0	11	9	11	11
	Camallanus cotti	0	0	0	0	8	0	0	0
April, 2004	*Eustrongylides ignotus*	0	11	0	0	0	0	0	0
	Trichinella spp.	0	0	0	0	0	0	0	0
	Capillaria pterophylli	0	0	0	0	0	0	0	0
	Camallanus cotti	0	0	0	0	0	0	0	0
May, 2004	*Eustrongylides ignotus*	6	13	5	0	7	9	9	6
	Trichinella spp.	9	4	0	0	2	5	0	5
	Capillaria pterophylli	6	5	0	5	0	5	5	0
	Camallanus cotti	0	7	0	0	0	0	9	3
June, 2004	*Eustrongylides ignotus*	0	0	0	0	6	8	26	5
	Trichinella spp.	0	0	0	0	0	0	0	0
	Capillaria pterophylli	16	5	12	7	1	0	0	0
	Camallanus cotti	0	0	0	0	0	0	0	7
July, 2004	*Eustrongylides ignotus*	0	0	0	0	0	0	0	0
	Trichinella spp.	0	0	0	0	0	0	0	0
	Capillaria pterophylli	0	0	0	0	0	0	0	0
	Camallanus cotti	0	0	0	0	0	0	0	0
August, 2004	*Eustrongylides ignotus*	0	0	0	0	0	0	0	0
	Trichinella spp.	0	0	0	0	0	0	0	0
	Capillaria pterophylli	0	0	11	6	8	0	0	3
	Camallanus cotti	10	11	0	0	0	0	11	9
September, 2004	*Eustrongylides ignotus*	9	6	0	10	0	6	8	9
	Trichinella spp.	0	0	0	0	0	0	0	0
	Capillaria pterophylli	5	5	0	0	0	0	8	6
	Camallanus cotti	20	28	8	6	13	3	9	24
October, 2004	*Eustrongylides ignotus*	6	6	5	0	0	0	9	9

	Trichinella spp.	0	0	9	0	4	8	5	8
	Capillaria pterophylli	0	13	0	0	0	0	0	4
	Camallanus cotti	10	8	8	5	5	0	5	9
November, 2004	*Eustrongylides ignotus*	0	0	0	0	0	0	1	1
	Trichinella spp.	3	12	5	6	0	0	0	0
	Capillaria pterophylli	4	3	0	0	8	0	0	0
	Camallanus cotti	0	0	0	0	0	0	2	6
December, 2004	*Eustrongylides ignotus*	0	0	0	0	0	0	0	0
	Trichinella spp.	0	0	0	0	0	0	0	0
	Capillaria pterophylli	0	0	0	0	0	0	0	0
	Camallanus cotti	0	0	0	0	0	0	0	0
January, 2005	*Eustrongylides ignotus*	0	0	0	0	0	0	0	0
	Trichinella spp.	0	0	0	0	0	0	0	0
	Capillaria pterophylli	0	0	0	0	0	0	0	0
	Camallanus cotti	0	0	0	0	0	0	0	0
February, 2005	*Eustrongylides ignotus*	9	9	6	2	8	8	1	2
	Trichinella spp.	4	5	0	0	0	0	5	5
	Capillaria pterophylli	8	10	5	24	13	13	1	2
	Camallanus cotti	0	5	6	8	0	2	0	4

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Table 4.5. Monthly sex-related mean intensity of nematode parasites of *Poecilia reticulata*

Month	Sex	Male	Female
March, 2004	*Eustrongylides ignotus*	3.0	1.3
	Trichinella spp.	0.0	2.3
	Capillaria pterophylli	2.2	1.4
	Camallanus cotti	2.0	0.0
April, 2004	*Eustrongylides ignotus*	0.0	1.8
	Trichinella spp.	0.0	0.0
	Capillaria pterophylli	0.0	0.0
	Camallanus cotti	0.0	0.0
May, 2004	*Eustrongylides ignotus*	1.8	1.4
	Trichinella spp.	2.2	2.3
	Capillaria pterophylli	5.5	1.7
	Camallanus cotti	9.0	2.5
June, 2004	*Eustrongylides ignotus*	2.5	1.3
	Trichinella spp.	0.0	0.0
	Capillaria pterophylli	1.9	2.4
	Camallanus cotti	0.0	3.5
July, 2004	*Eustrongylides ignotus*	0.0	0.0
	Trichinella spp.	0.0	0.0
	Capillaria pterophylli	0.0	0.0
	Camallanus cotti	0.0	0.0
August, 2004	*Eustrongylides ignotus*	0.0	0.0
	Trichinella spp.	0.0	0.0
	Capillaria pterophylli	2.7	2.3
	Camallanus cotti	1.3	2.9
September, 2004	*Eustrongylides ignotus*	1.9	3.9
	Trichinella spp.	0.0	0.0
	Capillaria pterophylli	1.4	1.8
	Camallanus cotti	1.6	1.8
October, 2004	*Eustrongylides ignotus*	1.8	1.7
	Trichinella spp.	1.4	2.0
	Capillaria pterophylli	0.0	2.4
	Camallanus cotti	4.0	1.7
November, 2004	*Eustrongylides ignotus*	1.0	1.0
	Trichinella spp.	2.0	2.0
	Capillaria pterophylli	3.0	3.0
	Camallanus cotti	1.0	1.0
December,	*Eustrongylides ignotus*	0.0	0.0

2004

	Trichinella spp.	0.0	0.0
	Capillaria pterophylli	0.0	0.0
	Camallanus cotti	0.0	0.0
January, 2005	*Eustrongylides ignotus*	0.0	0.0
	Trichinella spp.	0.0	0.0
	Capillaria pterophylli	0.0	0.0
	Camallanus cotti	0.0	0.0
February, 2005	*Eustrongylides ignotus*	1.1	1.4
	Trichinella spp.	1.0	1.4
	Capillaria pterophylli	1.3	1.3
	Camallanus cotti	1.5	1.4

C. pterophylli: In the case of female *P. reticulata*, the most intense infection was 5.5 observed in May, 2004 (Table 4.5).

C. cotti: The most intense *C. cotti* infection in female *P. reticulata* was 3.5 observed in June, 2004 samples (Table 4.5).

4.4.5. Drain-related nematode parasite intensity in *P. reticulata*:

The drain-related intensity of nematode parasites in *P. reticulata* samples aggregated from the different drains without distinguishing their sexes were subjected to ANOVA. It yielded ranges from a low mean intensity of 1.3 ± 1.3 to as high as 7.0 ± 3.0.

Monthly mean intensity differed significantly with drains ($p < 0.05$). *C. cotti* had the highest drain related monthly intensity of 10.5 in the entire study and it was observed in Basil Ogamba Street in September, 2004 (Table 4.6). This was followed by *C. cotti* in October, 2004 at Igi-Olugbin Street and *E. ignotus* in May, 2004 at Basil Ogamba Street at intensity of 6.0 each (Table 4.6)..

In May 2004 and February, 2005, *E. ignotus*, *Trichinella spp.*, *C. pterophylli* and *C. cotti* were all found to have occurred in the *P. reticulata* obtained with not less than intensity of 1 from both Igi-Olugbin and Adenaike Alagbe Streets (Table 4.6).

4.5. Relative percentage parasite composition of infection

4.5.1. Sex-related relative monthly percentage composition (rmpc) of nematode parasite:

The sex-sensitive relative monthly percentage composition of nematode parasite (rmpc) illustrates gender-linked success of a nematode in dominance over other nematode parasites of *P. reticulata* at a given time. Sex-related prevalence of each nematode parasite of *P. reticulata* was related to each other in percentages for this purpose (Table 4.2). The relative monthly percentage parasite composition (rmpc) of *E. ignotus* and *C. pterophylli* were high in male and female *P. reticulata* samples obtained in March and June, 2004 on one hand, while a combination of *C. pterophylli* and *C. cotti* constituted the higher rmpc in August and September, 2004 on the other (Fig. 4.1).

In August, 2004, a combination of *C. pterophylli* and *C. cotti* were the only two nematode parasites of male *P. reticulata*.

Table 4.6. Monthly drain-related mean intensity of nematode parasites of *Poecilia reticulata* on drain locations irrespective of sex of Host

Month	Nematode	Igi-Olugbin Street	Basil Ogamba Street	Ahmadu Bello Road	Adenaike Alagbe Street
March, 2004	*Eustrongylides ignotus*	1.0	0.0	1.9	3.5
	Trichinella spp.	0.0	0.0	2.3	0.0
	Capillaria pterophylli	0.0	0.0	1.3	2.4
	Camallanus cotti	0.0	0.0	2.0	0.0
April, 2004	*Eustrongylides ignotus*	1.8	0.0	0.0	0.0
	Trichinella spp.	0.0	0.0	0.0	0.0
	Capillaria pterophylli	0.0	0.0	0.0	0.0
	Camallanus cotti	0.0	0.0	0.0	0.0
May, 2004	*Eustrongylides ignotus*	1.4	6.0	2.3	1.3
	Trichinella spp.	2.2	0.0	2.3	2.5
	Capillaria pterophylli	1.8	0.0	1.7	5.0
	Camallanus cotti	3.5	0.0	0.0	4.0
June, 2004	*Eustrongylides ignotus*	0.0	0.0	4.7	1.6
	Trichinella spp.	0.0	0.0	0.0	0.0
	Capillaria pterophylli	1.9	1.6	1.0	0.0
	Camallanus cotti	0.0	0.0	0.0	3.5
July, 2004	*Eustrongylides ignotus*	0.0	0.0	0.0	0.0
	Trichinella spp.	0.0	0.0	0.0	0.0
	Capillaria pterophylli	0.0	0.0	0.0	0.0
	Camallanus cotti	0.0	0.0	0.0	0.0
August, 2004	*Eustrongylides ignotus*	0.0	0.0	0.0	0.0
	Trichinella spp.	0.0	0.0	0.0	0.0
	Capillaria pterophylli	0.0	3.2	2.7	1.5
	Camallanus cotti	2.1	0.0	0.0	1.5
September, 2004	*Eustrongylides ignotus*	2.5	0.0	3.0	2.1
	Trichinella spp.	0.0	0.0	0.0	0.0
	Capillaria pterophylli	1.0	0.0	0.0	2.8
	Camallanus cotti	1.7	10.5	1.1	1.5
October, 2004	*Eustrongylides ignotus*	3.0	1.0	0.0	1.6
	Trichinella spp.	0.0	2.2	1.2	2.6

	Capillaria pterophylli	2.2	0.0	0.0	4.0
	Camallanus cotti	6.0	1.6	5.0	1.8
November, 2004	*Eustrongylides ignotus*	0.0	0.0	0.0	1.0
	Trichinella spp.	1.9	1.0	0.0	0.0
	Capillaria pterophylli	3.5	0.0	2.7	0.0
	Camallanus cotti	0.0	0.0	0.0	1.0
December, 2004	*Eustrongylides ignotus*	0.0	0.0	0.0	0.0
	Trichinella spp.	0.0	0.0	0.0	0.0
	Capillaria pterophylli	0.0	0.0	0.0	0.0
	Camallanus cotti	0.0	0.0	0.0	0.0
January, 2005	*Eustrongylides ignotus*	0.0	0.0	0.0	0.0
	Trichinella spp.	0.0	0.0	0.0	0.0
	Capillaria pterophylli	0.0	0.0	0.0	0.0
	Camallanus cotti	0.0	0.0	0.0	0.0
February, 2005	*Eustrongylides ignotus*	1.8	1.8	1.0	1.0
	Trichinella spp.	1.5	0.0	0.0	1.0
	Capillaria pterophylli	1.4	0.8	1.2	1.0
	Camallanus cotti	2.5	0.6	1.0	1.0

C. pterophylli had rmpc of 30.2% while *C. cotti* dominated with an rmpc of 68.8% in male *P. reticulata.* In the case of female *P. reticulata*, *C. pterophylli* had rmpc of 37.0% while *C. cotti* dominated with an rmpc of 70.0% in the same month of August, 2004 (Fig. 4.9).

The parasite *E. ignotus* had rmpc of 100% in April, 2004; 50.9% in May, 2004 and 59.2% in June 2004 in female *P. reticulata* while the rmpc of *E. ignotus* in male *P. reticulata* were 65.6% in May, 2004; 50.0% in June, 2004 and 39.2% in February, 2005 (Fig. 4.9).

For the female samples of *P. reticulata*, *Trichinella spp.* had the following rmpc: 41.9% in October, 2004 and 37.0% in November, 2004 in contrast with the rmpc of 22.2% in October, 2004 and 53.5% in November, 2004 observed in male *P. reticulata* (Fig. 4.9).

The highest rmpc for *C. pterophylli* in male *P. reticulata* samples were 55.3% observed in March, 2004 followed by 50.0% in June, 2004; 30.2% in August, 2004; 37.0% in November, 2004 and 39.2% in February, 2005 though in female *P. reticulata* it was 31.7% in March, 2004; 23.3% in May, 2004; 29.6% in June, 2004; 37.0% in August, 2004; 40.9% in November, 2004 and 51.3% in February, 2005 (Fig. 4.9).

The nematode parasite *C. cotti* was observed in *P. reticulata* with the highest rmpc maxima of 71.0% in female *P. reticulata* samples obtained in September, 2004 but 69.8% in male *P. reticulata* dissected in August, 2004. It was similarly observed to have had rmpc of 63.0% in female *P. reticulata* samples obtained in August, 2004 and 63.75% in male *P. reticulata* samples obtained in September, 2004 (Fig. 4.9).

4.5.2. Drain-related relative monthly percentage composition of nematode parasite prevalence:

The nematode parasites *E. ignotus*, *C. pterophylli* and *C. cotti* constituted major parasites recovered in their hosts in August and September, 2004 as well as in February, 2005 (Fig. 4.10). *E. ignotus* had 100% rmpc in *P. reticulata* obtained from Igi-Olugbin Street in March and April, 2004 while *Trichinella spp.* had 100% rmpc in *P. reticulata* obtained from Basil Ogamba Street in November, 2004 without prejudice to the sex of the fish hosts (Fig. 4.10).
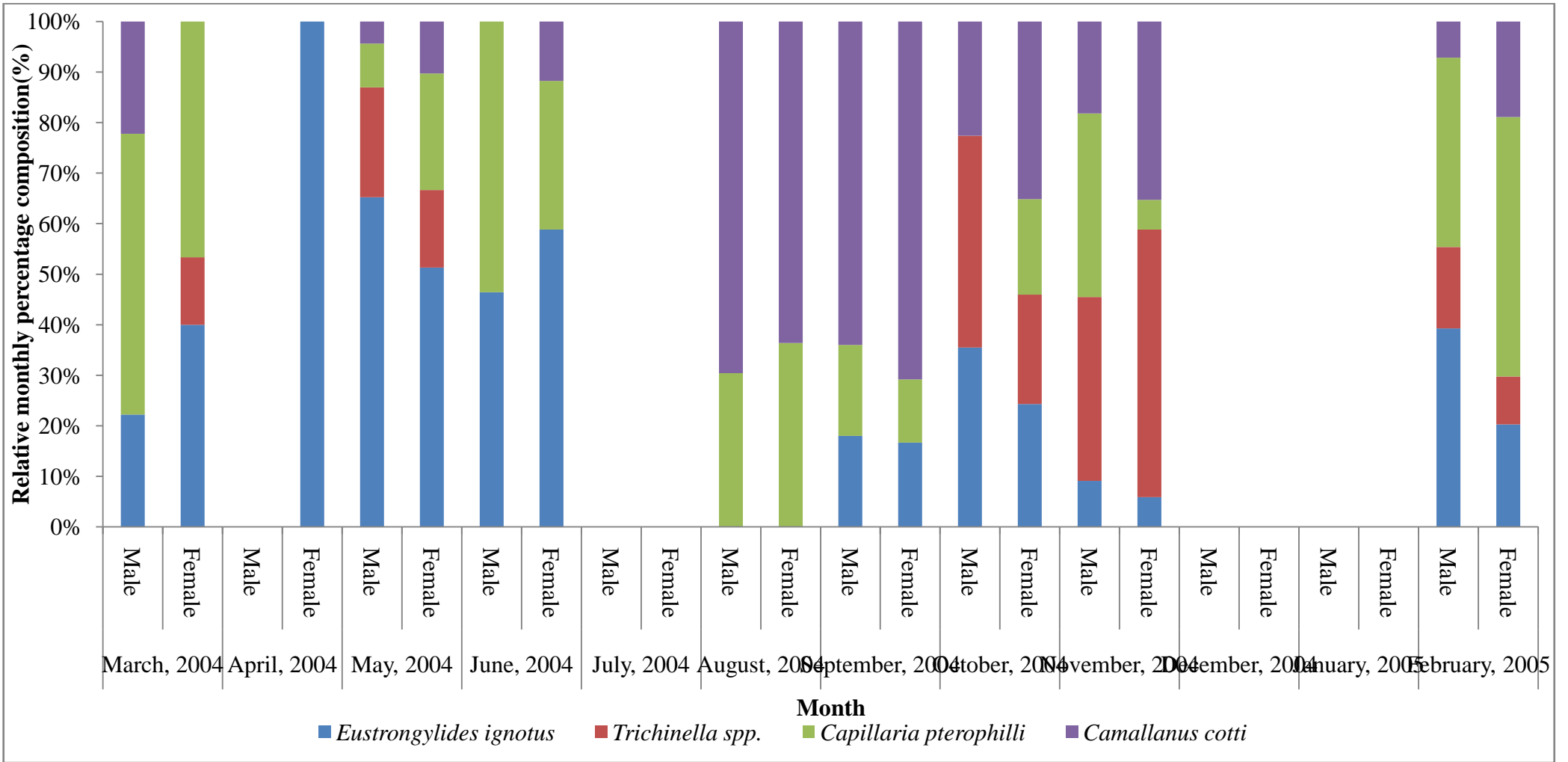
Figure 4.9.Sex-related Relative monthly percentage composition (rmpc) of nematode parasite prevalence in *P. reticulata* examined.
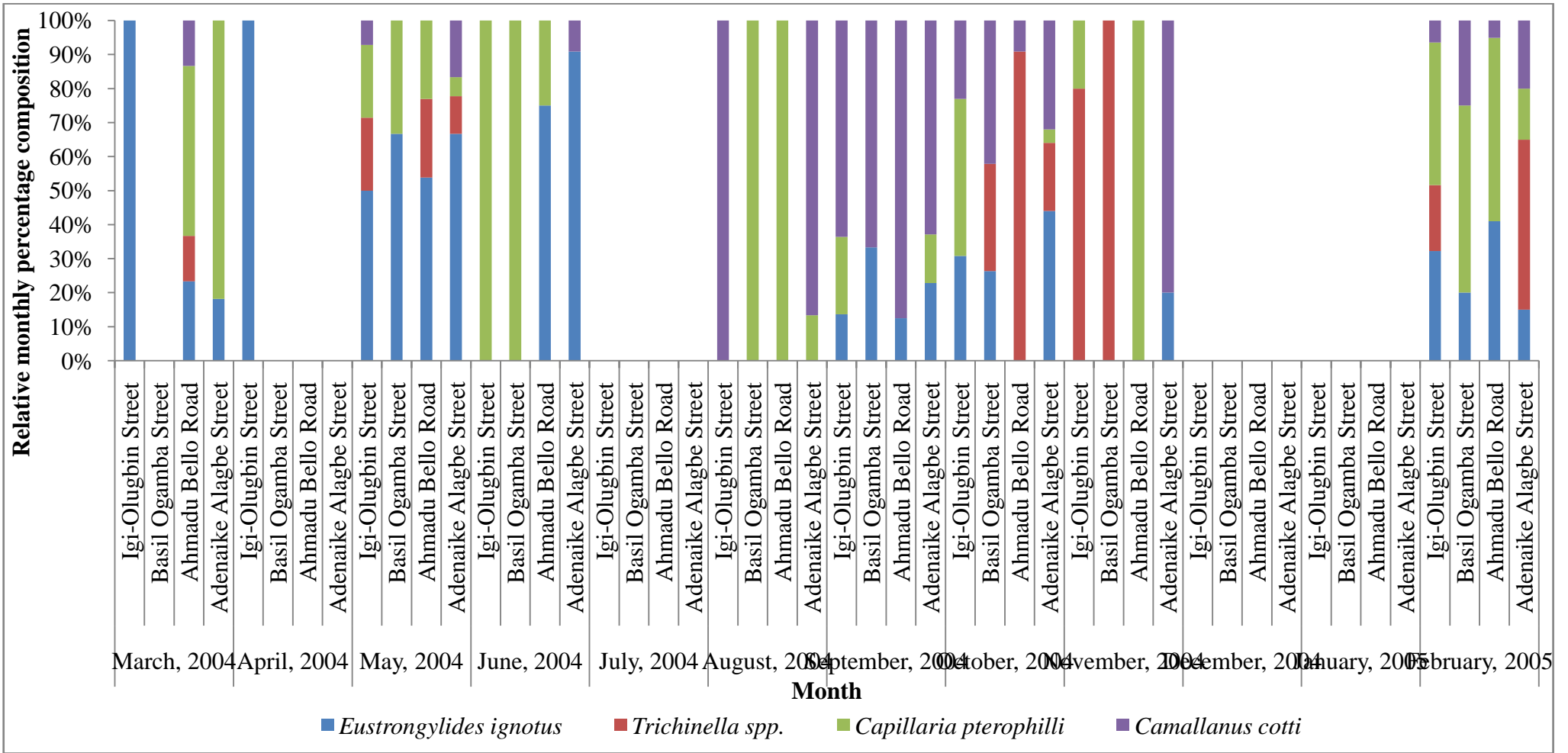
Figure 4.10. Drain-related Relative monthly percentage composition (rmpc) of nematode parasite prevalence in *P. reticulata* examined.

Similarly, only *C. cotti* was prevalent in all the *P. reticulata* samples obtained from Igi-Olugbin Street in August, 2004. The exclusive prevalence of 100% rmpc for *C. pterophylli* occurred at Basil Ogamba Street and Ahmadu Bello Road in August, 2004 (Fig. 4.10).

4.6. Mean physicochemical parameters

There was no significant difference in the mean monthly pH and DO of wastewater obtained from all the four selected Streets in Lagos State. However, there were significant differences in the wastewater temperature, transparency and drain depth during the course of this study ($p < 0.05$). Mean monthly physicochemical parameters of wastewater are on Table 4.7.

4.6.1. Mean monthly physicochemical parameters

Temperature: The highest mean monthly wastewater temperature was observed in July, 2004 at 26.75 ± 0.79 ºC while the lowest mean monthly temperature of 23.69 ± 0.80 ºC was observed in January, 2005.

pH: Though, the lowest mean monthly pH of 6.85 ± 0.92 was obtained in May, 2004 the highest mean monthly pH of 7.66 ± 0.77 was observed in August, 2004.

DO: The high mean monthly DO of 10.33 ± 1.52 mgl^{-1} was observed in October, 2004 while the lowest mean monthly DO of 6.43 ± 1.18 mgl^{-1} was observed in March, 2004.

Transparency: However, the lowest mean monthly transparency of 12.10 ± 7.46 cm was observed in July, 2004 when the highest mean monthly transparency of 14.93 ± 11.85 cm was observed in January, 2005.

Drain Depth: Though the highest mean monthly drain depth of 13.33 ± 2.34 cm was observed October, 2004, the lowest mean monthly drain depth of 8.55 ± 4.78 cm was observed in March, 2004.

4.6.2. Mean physicochemical parameters in drains.

There were significant differences in the temperature, transparency and drain depth of wastewater samples collected from each of the selected drains ($p < 0.05$). However, no significant differences were observed in the DO and pH recorded from the wastewater samples from the drain.

Table 4.7. Means of Monthly Physicochemical Parameters Measurement in the Selected Drains Throughout the Experiment

Streets	Igi-Olugbin Street					Basil Ogamba Street					Ahmadu Bello Road					Adenaike Alagbe Street				
Sampling Months	Temperature (°C)	pH	Dissolved Oxygen (mg/l)	Transparency (cm)	Drain Depth (cm)	Temperature (°C)	pH	Dissolved Oxygen (mg/l)	Transparency (cm)	Drain Depth (cm)	Temperature (°C)	pH	Dissolved Oxygen (mg/l)	Transparency (cm)	Drain Depth (cm)	Temperature (°C)	pH	Dissolved Oxygen (mg/l)	Transparency (cm)	Drain Depth (cm)
March, 2004	26.5	7.2	8	25	6	24.5	7	6	3	5.2	26.5	6.5	6.5	15.2	15.6	25.5	7	5.2	8.2	7.5
April, 2004	25.0	7.0	7.6	24.6	7.4	26.3	6.9	7.5	4.5	13.4	26.8	7.4	6.3	15.2	18.8	25.0	6.8	8.2	8.2	8.5
May, 2004	25.5	6.2	5.0	22.5	8.4	25.5	7.9	7.5	3.0	6.5	25.0	7.4	7.0	17.9	20.1	26.5	6.0	8.3	8.2	9.5
June, 2004	25.0	6.2	8.2	21.8	11.4	27.5	8.1	8.1	4.0	11.0	25.0	8.2	8.1	14.8	18.8	26.8	6.0	6.2	8.0	11.6
July, 2004	25.8	7.8	6.0	20.8	7.4	26.5	7.7	8.4	4.0	13.2	27.5	6.2	8.0	15.5	16.7	27.3	7.0	6.2	8.2	9.3
August, 2004	25.5	8.6	8.1	20.3	6.8	27.0	7.4	8.2	2.5	16.8	25.8	8.0	8.1	24.7	15.3	27.5	6.8	5.0	8.3	10.3
September, 2004	25.8	8.6	8.8	20.1	12.8	26.3	7.1	9.7	2.5	16.9	26.3	7.0	10.8	22.4	13.9	26.5	8.0	6.3	7.9	9.2
October, 2004	25.8	8.3	10.8	19.7	11.5	27.0	7.6	8.1	3.0	16.6	25.0	7.4	10.9	18.4	13.6	27.3	7.4	11.6	8.1	11.7
November, 2004	24.5	7.0	10.2	19.8	11.8	25.3	7.1	9.9	4.5	16.6	24.3	7.8	9.2	21.2	11.3	25.3	7.0	10.6	8.3	11.2
December, 2004	24.0	6.8	11.0	22.4	10.6	24.3	7.1	10.1	4.5	16.0	24.0	7.1	7.3	19.9	11.3	25.3	7.1	8.0	8.7	11.2
January, 2005	22.5	6.7	9.5	28.5	10.5	24.0	7.1	7.1	2.5	16.0	24.3	7.2	8.3	16.6	11.5	24.0	7.0	7.9	8.7	11.2
February, 2005	24.3	7.4	8.1	30.8	10.6	23.0	7.1	6.1	3.5	17.0	24.0	7.1	8.8	16.5	11.5	24.8	7.0	9.9	9.0	11.0

Temperature: The highest mean temperature of 25.96 ± 1.14ºC was observed at Adenaike Alagbe Street while the lowest, 25.00 ± 1.06 ºC was observed at Igi-Olugbin Street.

pH: The highest mean pH of 7.32 ± 0.38 was observed at Basil Ogamba Street, with the lowest observed 6.90 ± 0.54 observed at Adenaike Alagbe Street.

DO: The highest mean DO of 8.43 ± 1.79 mgl^{-1} observed at Igi-Olugbin Street contrasts with that of the mean temperature while the lowest mean DO was obtained at 7.75 ± 2.11mgl^{-1}was observed from Adenaike Alagbe Street.

Drain Depth: Meanwhile, the highest mean drain depth of 14.84 ± 3.21cm was observed at Ahmadu Bello Road with the lowest mean drain depth of 9.60 ± 2.27cm at Igi-Olugbin Street.

4.7. Correlation Co-efficient of physicochemical parameters with prevalence and intensity

4.7.1. Correlation Co-efficient of physicochemical parameters with nematode parasite prevalence in *P. reticulata*:

The monthly prevalence of *C. pterophylli* positively correlated at 0.6 with the drain depth of Igi-Olugbin Street while *C. cotti* correlated at 0.7 with pH (Appendix 3). The monthly prevalence of *C. pterophylli* moderately but positively correlated with the temperature at 0.5 and pH of wastewater at 0.6 in Basil Ogamba Street. However, monthly prevalence of *E. ignotus* highly correlated at 0.9 with those of *Trichinella spp.* and *C. cotti* each (Appendix 4).

At Ahmadu Bello Road, monthly prevalence of *C. pterophylli* correlated at 0.7 with that of *E. ignotus* (Appendix 5).

At Adenaike Alagbe, wastewater transparency and monthly prevalence of *C. pterophylli* negatively correlated at -0.6 with that of the wastewater temperature while *E. ignotus* correlated at 0.6 with drain depth though *Trichinella spp.* highly correlated with DO at 0.8 (Appendix 6).

4.7.2. Correlation Co-efficient of physicochemical parameters with nematode parasite intensity in *P. reticulata*:

The intensity of *C. pterophylli* correlated at 0.6 each with Drain depth and the intensity of *Trichinella spp.* while it correlated with the intensity of *E. ignotus* at 0.7 at Igi-Olugbin Street (Appendix 7).

The correlation coefficient between the temperature and pH of wastewater in Basil Ogamba Street was moderately at 0.6 (Appendix 8) while there was no correlation between the nematode mean intensity and physicochemical parameters of wastewater.

In the case of Ahmadu Bello Road, temperature correlated with Drain Depth at 0.6 with no correlation between mean intensity of any of the nematode parasites obtained from *P. reticulata* (Appendix 9).

At Adenaike Alagbe Street, the intensity of *C. cotti* negatively correlated with pH at -0.6 while *C. pterophylli* correlated with *Trichinella spp.* at 0.8 (Appendix 10).

CHAPTER FIVE
DISCUSSION

Nematode parasite prevalence in *P. reticulata* obtained from the four selected Streets of Lagos State was neither sex-dependent nor drain-dependent. The nematodes *E. ignotus*, *Trichinella spp.*, *C. pterophylli* and *C. cotti* encountered in this study are not currently amongst established DNOs and OSDs within the definition of the OIE. Therefore, evidence from this study suggests that *P. reticulata* from Igi-Olugbin, Basil Ogamba, Adenaike Alagbe Streets and Ahmadu Bello Road would safely qualify as restriction-proof commercial ornamental fish for culture and domestic display as well as for export since only viral fish infections presently constitute DNOs by OIE definitions.

Although, according to the OIE fifth Strategic Action Plan (OIE, 2009), the identification, prevention and management control of epizootics and zoonoses helps mankind throughout time, the overall prevalence of 3.6%, 3.5% and 3.4% for *E. ignotus*, *C. pterophylli* and *C. cotti* in *P. reticulata* probably reflects the susceptibility of *P. reticulata* samples to nematode parasite transmission through feeding habit preferences, life cycle dynamics and environmental synchrony between host and parasite types (Anderson and May, 1979; Anderson and May, 1985 and Anderson, 1988).

The above levels of overall prevalence do not infer inherent carriage of these nematode parasites when compared to the relatively higher prevalence of some of the same nematode parasites observed in *P. reticulata* or other Poeciliids obtained from homestead aquaria (Kim *et al.*, 2002) and natural drains from other regions of the world. In addition, the prevalence of these four nematode species do not reflect an intractable association between the *P. reticulata* obtained from the selected Streets of Lagos and the nematodes encountered in this study.

Though *C. pterophylli* had the highest sex-related prevalence of 15.8% prevalence in female *P. reticulata* samples obtained in February, 2005 (Table 4.2), it was *C. cotti* that had the highest sex-related nematode prevalence of 13.3% in male *P. reticulata* in September, 2004 (Table 4.2). Despite the fact that there was no statistical significance in sex-related differences in nematode prevalence in *P. reticulata*, the pertinent issue is, could one fail to notice that *C. cotti* also held the reputation as the

99nematode with the highest drain-related prevalence at 23.3% observed in *P. reticulata* obtained in September, 2004 from wastewater drains at Igi-Olugbin Street (Table 4.3 which was by record the all-time maxima in this study? One explanation for the persistence and prominence of *C. cotti* as a very prevalent nematode parasite of *P. reticulata* in this wastewater drains could be its adaptation to hibernation in the absence of a suitable intermediate host and its by-pass of the latter in re-infection of new hosts once wastewater renewal occurs between rain seasons.

Certainly, *E. ignotus* had the highest sex-related monthly intensity of 3.9 in female *P. reticulata* observed in September, 2004 (Table. 4.5). However, as regards mean intensity of nematode parasites in male *P. reticulata*, *C. cotti* still had the highest sex-related monthly intensity of 9.0 observed in May, 2004 (Table. 4.5) and the highest drain related monthly intensity of 10.5 in the entire study which was observed at Basil Ogamba Street in September, 2004 (Table. 4.6). If it had been entirely co-incidental that *C. cotti* which had the highest sex-related prevalence in male *P. reticulata* and also had the highest drain-related prevalence in *P. reticulata*, the reputation of this nematode as the parasite with the highest sex-related and drain-related monthly intensity in *P. reticulata* should draw appreciable attention to it.

All this notwithstanding, the highest monthly prevalence of *C. cotti* in *P. reticulata* obtained from both sex-related and drain-related segment of this study compares markedly far less than the 51% prevalence of *C. cotti* reported by Moravec and Justine (2006) in the gobiid, *Awouis guamensis,* obtained from the La Foa river of New Caledonia and the 71% of *C. cotti* in *P. reticulata* obtained from export ready stock in South Korea and Indonesia by Kim *et al*., 2002. Notably though, the monthly mean intensity of 10.5 in *P. reticulata* of this present study is more than that of 5 reported in the gobiid by Moravec and Justine (2006).

On record, the first reported case of *C. cotti* infection of any fish host was in 1927 by Fujita, the next known report of this nematode infecting an ornamental fish species, such as *P. reticulata*, in Korea and Indonesia was by Kim *et al.* (2002). The current study would represent the first known investigation of the prevalence and intensity of nematode parasites of feral *P. reticulata* in Nigeria. In addition, although the prevalence study of *C. cotti* in *P. reticulata* by Kim *et al*., 2002 was not sex-related as in the current instance, the sample size of *P. reticulata* hosts in Kim *et al*., 2002 may have been too small to reflect a proper demographic illustration of *C. cotti* prevalence

in *P. reticulata* feral populations. Meanwhile, *C. cotti* is now gaining attention as a cosmopolitan nematode parasite of ornamental fish species with the uncommon advantage of monoxeny that facilitates dormancy in the face of intermediate host bottleneck by directly infecting other susceptible fish hosts within immediate reach (Levsen and Jakobsen, 2002).

Except for the study at export-ready holding farms, where Thilakaratne *et al*. (2003) found a 0.7% prevalence of *Capillaria spp.* in *P. reticulata* in their study of parasitic infections in freshwater ornamental fishes in Sri Lanka, no other published record of the prevalence or mean intensity of *C. pterophylli* in *P. reticulata* had been encountered. The low prevalence in Thilakaratne *et al*. (2003) could be as a result of screening and removal of moribund samples of *P. reticulata* and the extra effort made to preclude diseased samples from the quarantine farm unlike in the case of this study where feral stocks were examined for nematode parasites.

Relative percentage monthly composition (rmpc) reflects the domination of one nematode parasite encounters in *P. reticulata* in this study by another. Therefore, instances where a particular nematode is found to have exclusively retained a 100% rmpc could infer a suitable host physiological or wastewater physicochemical milieu enhancing that given nematode parasite establishment as reflected in prevalence or endemy as measured in intensity. As a result, one should not fail to note such instances of a 100% in drain-related rmpc analyses of this study. Only in one instance was a sex-related 100% rmpc observed. And this was in the case of *E. ignotus* in *P. reticulata* females in April, 2004 (Fig. 4.1).

There were however many cases of drain-related 100%rmpc in this study such as that of *E. ignotus* in March and April, 2004 at Igi-Olugbin Street; *Trichinella spp.* in November, 2004 at Basil Ogamba Street; *C. pterophylli* in June at Igi-Olugbin and Basil Ogamba Street as well as in November, 2004 at Ahmadu Bello Road and *C. cotti* in August, 2004 at Igi-Olugbin Street (Fig. 4.2). Other notable rmpc were *E. ignotus* at 92%rmpc in June, 2004at Adenaike Alagbe Street, *Trichinella spp.* at 92% in October, 2004 at Ahmadu Bello Road and November, 2004 at Igi-Olugbin Street while *C. pterophylli* had 82%rmpc in March, 2004 (Fig. 4.2). This notwithstanding, conscious avoidance of collecting female *P. reticulata* from the wastewater drains in April when they were found to have *E. ignotus* of 100% rmpc and male *P. reticulata* in May with *E. ignotus* of 65% rmpc is advised. Collection of *P. reticulata* from the wastewater

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drains may also be unprofitable in August and September because those in this study indicated a 73% and 69% rmpc for *C. cotti* in female and male *P. reticulata*, respectively.

High rmpc may symptomize possible intervention of new intermediate or paratenic hosts for the concerned parasites out-competing other nematodes in the identified wastewater drains. It may also be as a result and effect of particular anthropogenic activities and habits of people living around those areas that impact the abutting wastewater drains. All these notwithstanding, it would be naïve not to take notice of the fact that the rudiments of sexual characters of the adult *E.ignotus* which should clearly be completed in their final hosts, piscivorous wading birds, had started to form in the fourth stage larvae of *E. ignotus* (Fig. 4.8 and Fig. 4.9) even when *P. reticulata* is not the final host.

Apart from the twin effect of globalization and climate change, rapid unplanned urbanization resulting in increasing population growth at Igi-Olugbin and Basil Ogamba Streets within the more densely populated sectors of Lagos State (National Population Commission of Nigeria, 2006 and Curry, 2009) lead to poor sanitation and uncontrolled roaming of household pets such as cats, dogs and occasional visits of rodents, poultry and ducklings to these drains. The carrions of these animals were often encountered on some occasions particularly in wastewater drains of these Streets during fish sample collection. While the existence of Cyclopod copepods in the drains would not seem strange, the possibility of *P. reticulata* feasting on these dead avian, feline pets and rodents could lead to transfer of encysted *Trichinella spp.* across previously known species barriers to *P. reticulata*.

There was no significant difference in mean monthly pH and DO across all the four drains sampled in this study. And this is not surprising since photic adaptations of wastewater phytoplanktons that modify pH and DO through diurnal photosynthesis is known to occur in the daytime when water physicochemical parameters in this study were measured. However, foraging activities of *P. reticulata*, egg-laying mosquitoes, their larvae and occasional visitors to the wastewater in drains may continually influence daily temperature, transparency and drain depth and ultimately their mean monthly values that were found to have been significantly different across drains. The high correlation observed at Adenaike Alagbe drains between the prevalence of *Trichinella* species and wastewater DO, between prevalence of *C. pterophylli* and

wastewater temperature and also between the mean intensities of *C. pterophylli* and *Trichinella spp.* in *P. reticulata* may be related to ones daily encounter with animal ritual sacrifices at some of the walkways near sectors of the wastewater drains that may have lead to recrudescent slouch of infected animal carrion into wastewater during episodic rains. Indeed, two different shrines for African traditional worship exist at the ends of Adenaike Alagbe Street in Ikorodu LGA of Lagos State.

On the other hand, it is interesting to note that while the highest mean wastewater DO of 8.43 ± 1.79mgL^{-1} and transparency of 23.02 ± 3.60 cm were observed at Igi-Olugbin Street wastewater that same Street had the lowest mean temperature of 25.00 ± 1.06 ºC and drain depth of 9.60 ± 2.27cm. Although, wastewater temperature and transparency moderately correlated negatively at -0.5 (Appendix 3) to buttress the highest mean transparency and lowest mean temperature at Igi-Olugbin Street above, the moderate negative correlation between *E. ignotus* and DO at -0.5 coefficient finds relevant support from the observations of Coyner *et al*. (2003). They indicated that anthropogenically altered wastewater sites in Florida, USA that were comparable to those of Igi-Olugbin Street in Lagos State, Nigeria where monthly environmental sanitation exercises occur, are characterized by higher mean densities of piscifaunal and oligochaete hosts of *E. ignotus* accompanied by decreased surface water DO but increased total phosphorus, nitrogen, chlorophyll-a, emergent vegetation and grasses. So also is the observation in this study that prevalence of *E. ignotus* in *P. reticulata* was not sex-related comparable to the conclusions of Coyner *et al.* (2003) that the ratio of infected male and female *Gambusia holbrooki*, another wastewater piscifaunal, by *E. ignotus* did not differ significantly.

At Basil Ogamba Street, where the highest mean wastewater pH at 7.32 ± 0.38 and the lowest mean wastewater transparency at 3.46 ± 0.81cm was observed , the prevalence of *C. cotti* and *Trichinella spp.* were found to be very highly correlated at 1.0 though the prevalence of *E. ignotus* correlated with those of *Trichinella spp.* and *C. pterophylli* at 0.9. Similarly, *C. pterophylli* was also found to have moderately correlated with temperature at 0.6 and with pH at 0.5 (Appendix 4) indicating that the prevalence of this nematode somehow optimized in its *P. reticulata* host with increasing wastewater temperature and pH. However, the near perfect inter-nematode parasite prevalence correlated at high mean pH and very low mean wastewater

transparency at Basil Ogamba Street suggests a wastewater nematode faunal utopia where fair-play access to nutrient, intermediate hosts and final hosts equilibrium exists.

The moderately high correlation between the prevalence of *C. pterophylli* and *E. ignotus* at 0.7 on Ahmadu Bello Road where the highest mean drain depth at 14.84 ± 3.21cm (Appendix 5) was recorded may reflect the confluence between nematode parasite availability and the benthophagous habit of *P. reticulata* in highly debriated wastewater drains where contact between benthophagous hosts and nematode parasites intermediate hosts such as Oligochaetes are far better assured. This interplay of mean temperature, transparency and drain depth is also reflected in the moderate correlation between prevalence of *C. pterophylli* and mean transparency at 0.5 on one hand and the correlation of prevalence of *E. ignotus* and mean drain depth at 0.6 at Adenaike Alagbe Street where the highest mean temperature at 25.94 ± 1.14 °C but the lowest mean pH at 6.90 ± 0.54 and DO at 7.75 ± 2.11 mgl-1 were observed (Appendix 6).

Even if wastewater mean pH and DO were found to have not been significantly different in the whole study, the negative correlations between mean transparency and mean temperature at -0.6 on Adenaike Alagbe Street in one instance, and prevalence of *C. pterophylli* and mean temperature at -0.6 (Appendix 6) on the other, makes the coupling effect of water chemistry on the transmission of *C. pterophylli* within wastewater piscifauna become obvious. This would mean that Trichurid nematodes such as *C. pterophylli* reputed to be capable of transmitting infection from one aquatic vertebrate host to another by side-stepping the need for intermediate hosts (Moravec *et al*., 1987) could survive the impact of climate change more resiliently than other nematodes. The anomalous property of water that does not accommodate too wide mean temperature ranges and turbidity or low transparency of flooded wastewater that are often readily resolved into eutrophication during succeeding high nutrient load that follow floods may not deter the transmission of *C. pterophylli* during outfalls of natural disasters.

In the correlation of nematode parasite mean intensity with physicochemical parameters, the mean intensities of *C. pterophylli* correlated with that of *Trichinella spp.* at 0.7 just as the mean intensity of *C. cotti* correlated with that of *E. ignotus* also at 0.7 on Igi-Olugbin Street (Appendix 7). It is safe to infer that similar water quality with direct or indirect regards to water chemistry predispose *P. reticulata* to enhanced intensification of infection by all these four nematodes found at Igi-Olugbin Street. It

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was also observed that the mean intensities of *C. pterophylli* correlated with that of *Trichinella spp.* at 0.8 on Adenaike Alagbe Street (Appendix 10) though the mean intensity of *C. cotti* correlated with that of *Trichinella spp.* and that of *C. pterophylli* at 0.5 each, respectively on *P. reticulata* obtained from Adenaike Alagbe Street. It is noteworthy, however, that a general pattern of moderate correlation between drain depth and DO was observed at 0.6 on Igi-Olugbin Street (Appendix 7), at 0.5 on Basil Ogamba Street (Appendix 8) and Adenaike Alagbe Street (Appendix 10). The only negative correlation with regards to nematode parasite mean intensity between drain depth and DO was observed at -0.5 on Ahmadu Bello Road (Appendix 9).

Nonetheless, drain-dependence in nematode parasite mean intensity correlating with wastewater chemistry in this study indicates the possibility of other underlying water quality factors apart from host sex that could have predisposed obtained fish samples to spontaneity in nematode parasite visibility and multiplication at the particular months of heightened intensity of *E. ignotus*, *C. cotti* and *C. pterophylli*. As a result, it is necessary to underline the fact that since mean temperature, transparency and drain depth were found to be statistically significant in this study, the months of March, 2004 when the lowest mean drain depth was observed at 8.55 ± 4.78cm; July, 2004 when the highest mean temperature of 26.75 ± 0.79 ºC but the lowest transparency of 12.10 ± 7.46cm was observed; and January, 2005 when the lowest mean temperature at 23.69 ± 0.80 ºC but the highest mean transparency was observed at 14.93 ± 11.85cm deserve epizootiological notice and enquiry. It would not be too presumptive to therefore flag the months of January, March and July as indicative of critical nematode-intense *P. reticulata* collection months particularly at Ahmadu Bello Road and Adenaike Alagbe Street where substantial correlations amongst wastewater mean temperature, transparency and drain depth have been noted.

It is noteworthy that the focus of previously reported nematode parasite prevalence and intensity in ornamental fish species had been limited to the end-point of the OFI value chain (Ponpornpisit *et al*., 2005). For this reason, only prevalence of most nematode parasites in homestead aquaria, retail points and directed aquaculture systems of ornamental fish in developed economies of the world have enjoyed sustained studies. With the growing exceptions of newly directed attention on OFI in neo-tropical and sub-tropical regions of the World such as Trinidad and Tobago, the Amazon region (Chao, 1992; 1993; Chao and Prang, 1997; Chao, 2001), New

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Zealand, Australia where conservation concerns of Reef damage due to deplorable harvest of ornamental fish from the wild occur (Chan and Sadovy, 2000; Claydon, 2005) and, the often well reported Asian continent's experience in aquaria business (Chou *et al*., 2002), nematode parasite impact on the OFI in Africa has been clearly and largely underreported.

P. reticulata was unofficially introduced into Lagos wastewater drains as a biological control measure against mosquitoes breeding in such drains by European officials of the colonial government of Nigeria as the earliest records in the 1970s shows (Welcomme, 1988). Malaria was the major scourge of European colonial life in the West Africa and Asia of twentieth century (Manna *et al*., 2008). Even if official sanction of the introduction of this fish into Nigeria as pet may not officially exist, just as they are wont elsewhere, it is all the same necessary to draw a new risk map for invasive alien fish species in Nigeria. One may disagree with any of the above mentioned routes as the means of introduction of *P. reticulata* into Nigeria. What is not in doubt is the ready availability of *P. reticulata* in many wastewater drains of Lagos State (Anogwih and Makanjuola, 2010; Lawal and Samuel, 2010). *P. reticulata* still retains its elegant colour and aggressive nature whether in drains or aquarium tanks qualifying it as an ornamental fish species though the standing nematode parasite fauna may have immigrated with the host in the original translocation into Lagos wastewaters. Other studies have shown that many alien invasions follow the pattern of transportation, escape or intentional introduction before full aggressive establishment and spread to exert propagule pressure on an ecosystem (Courtenay and Stauffer, 1990; Courtenay and Moyle, 1996; Dugan, *et al*., 2006).

Although body and fin colour, caudal fin shapes and size of *P. reticulata* account for contemporary grading and sorting of samples at the downstream of the OFI, disease susceptibility, carriage at swimming and other features that help in export product qualification in the OFI are health related (Zion *et al*., 2008). For this reason, it is difficult to ignore all the fine points predisposing to potential disease state as well as identify measures for ensuring upstream product quality assurance as in the case of *P. reticulata* that is in universal demand (Courtenay, 1999).

However, drain-dependence in nematode parasite mean intensity in this study may indicate that another underlying factor apart from host sex could be predisposing obtained fish samples to spontaneity in nematode parasite visibility and multiplication

at the particular months of heightened intensity of *E. ignotus*, *C. cotti* and *C. pterophylli*. Shed *E. ignotus* eggs are ingested by primary benthophagous hosts such as Chironomids and Oligochaetes (Frederick *et al*., 1996) that are eaten by several intermediate hosts such as *P. reticulata* and other Poeciliids while larger piscivorous fishes serving as paratenic hosts facilitate their development to third stage larva. Fourth stage larvae are ingested by Ciconiiforms such as White Egrets, final hosts of *E. ignotus*, when they prey on these fishes where *E. ignotus* finally develops into mature adults (Measures, 1987; 1988; Spalding *et al*., 1993; Frederick and McGhee, 1994). It would appear that the observation of these water wading birds within the drains in Lagos completed the infection cycle of *E. ignotus* in *P. reticulata* in this study.

Frederick *et al*., 1996 noticed a decline in *E. ignotus* prevalence from 39% observed in *G. affinis*' in the Northern Florida quartz-like drains (Brown *et al*., 1990) to 10% prevalence in the *G. affinis* samples obtained from the thick peats of Southern parts of the same Florida peninsular. His explanation that altered soil conditions in the Southern parts may make sinking *E. ignotus* eggs inaccessible to Oligochaetes that are intermediate hosts to, and foragers of, *E. ignotus* eggs becomes plausible. Constant disturbance of soil types through recurrent excavations lead to the exposure of clay, sandy and lime overlays to bring parasite eggs to the water/soil interphase that had been otherwise lost to intermediate hosts to the surface for ingestion by bacteriophylic and detritivorous Oligochaetes. The 1.5% prevalence of *Trichinella spp.* represents the first such record in *P. reticulata*. Though the prevalence is low, further studies in confirming the exact species of Trichinella cyst in the muscle of *P. reticulata* would be necessary (Bruschi and Murrell, 2002). It may have originated from the close nocturnal interaction of house rodent carriers such as domestic rats, *Rattus rattus*, domestic cats and dogs that frequent wastewater drains at night that drink and defecate into these drains. It is normal to expect that since terrestrial and aquatic scavengers feed on carcasses of dead organic living matter, the known persistence of *Trichinella spp.* larvae in carcasses (Kim, 1983) would ensure transmission of Trichinosis in man and other animals once the cyclozoonotic loop of carnivore and scavenger is complete.

Similarly, omnivorous fish species such as *Poecilia reticulata* and *Clarias gariepinus* that scavenge on dead organic matter and carcasses or cannibalize moribund individuals of their species of other fish species have the potential of becoming paratenic hosts to infective *Trichinella spiralis* larvae. AdelNabih *et al* (2003) reported

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experimental infection of *C. gariepinus* with *T. spiralis* larva which though failed to encyst in the muscles of infected catfishes, yet the larvae persisted in the intestine of these catfish and when the latter were fed to albino rats 48 hours after experimental infection of fish, viable *T. spiralis* larvae were found in the rats. Indicative biochemical response with regards to aspartate aminotransferase and alanine aminotransferase changes associated with liver and muscle tissue damages following Trichinosis have been noted in the experimental infection of catfishes. This gives credence to *C. gariepinus* as a potential paratenic hosts to *Trichinella spp*. and this may explain the infection of *P. reticulata* by *Trichinella spp*. What is however further surprising in the case of *Trichinella spp*. is the susceptibility and successful experimental infection of both carnivorous and herbivorous animals pigs, monkeys, sheep, cattle, horse young chickens, pigeons, magpies and rooks (Cram, 1941), horses (Adel *et al*., 2003) and rabbits (Yacoub *et al*., 1993) by this parasite. Campbell (1983) and MacLeane *et al*.(1989) reported that marine mammals and herbivores have been experimentally infected with *Trichinella spp*. while Asatrian *et al*. (2001) were also able to experimentally infect reptiles *Lacerta agilis* and *Agama caucasica* with *Trichinella spp*.

There is converging consensus that wastewater in altered sites are generally characterized by hypereutrophication leading to very low DO in the sub $2mgl^{-1}$ limits, high soil oxygen demand (SOD) due to the resulting microbial action of chemotrophic and anaerobic microbes, respiration of benthic invertebrates (Culp *et al*., 1983; Wetzel, 1983); increases in total carbon and chlorophyll a (Brenner *et al*., 1990); detritivous that speed up decomposition of retiring biota such as photosynthetic and chemotrophic algae leading to high biomass input (Gale and Reddy, 1994); increases in total nitrogen (TN) and total phosphorus (TP) as shown by the works of Huber *et al*.,1982 and Nordlie, 1990. In particular relevance to the results of this study, Chironomids and Oligochaetes rely on constant substrate particle heterogeneity (Block *et al*., 1982; Culp *et al*., 1983 and Waters, 1995) leading to temporary nutrient renewal to the point where only such species tolerant of anorexic wastewater-sheds survive. When this occurs, Poeciliids such as *P. reticulata* usually survive these dramatic DO sinks by subsurface breathing of atmospheric oxygen (Meffe and Snelson, 1989)

If all these were to be considered with the additional fact that infected Ciconiiform birds' prefer foraging in recently disturbed watersheds devoid of emergent macrophytes then the enhanced prospect of complete *E. ignotus* infection cycle through

regular wastewater disturbance by excavation during monthly sanitation exercises in Lagos State becomes clearer. Where these birds defecate in wastewater drains but ingest newly exposed infected livebearer fish species such as *G. affinis* and *P. reticulata*, hitherto hidden in temporary sanctuaries, by recurrent monthly excavations, then multiple infections of both oligochaete and intermediate host Poeciliid fish occur culminating in increased *E. ignotus* intensity as against consistent level of prevalence that may not be affected by fish host re-infection. Results of the studies by Coyner *et al.* (2003) support this assertion. This may also explain the statistically significant difference in drain based mean intensity of *E. ignotus* in *P. reticulata* observed in the current study.

Perhaps the most important factors determining and enhancing infection cycle of *P. reticulata* by *E. ignotus* in wastewater drains, in agreement with the conclusion of previous studies by Frederick and McGhee (1994), Brenner et al (1990) and Coyner *et al*. (2003) on similar drains in Florida, are the interplay in constant release of domestic sewage in wastewater reaching drains on Lagos Streets. High nutrient refluxes lead to high primary productivity at varying levels of eutrophication. In order to ease waste-drain traffic flow, recurrent excavation of wastewater substrate and drain soil leads to substrate renewal and soil particle heterogeneity during monthly public sanitation exercises in Lagos State.

In effect, physicochemical conditions in the wastewater drains play a significant role in determining transitional eutrophication in the drains (Snieszko, 1974; Akinwale and Ansa, 2004) through environmental alteration that promote *E. ignotus* transmission (Coyner *et al.*, 2003). This spontaneity usually coincides with the loss of refugia to infected *P. reticulata* that are thereafter exposed to predation by piscivorous fish and Ciconiiform birds. It is not very clear to what extent these monthly wastewater substrate alteration helps in promoting the transmission of *Trichinella spp.*, *C. pterophylli* and *C. cotti* in *P. reticulata*.

Here however, unlike the report of Loftus and Eklund (1994) that the highest prevalence of *E. ignotus* in *G. affinis* was observed in the dry season in Florida, the highest monthly prevalence of *E. ignotus* in *P. reticulata* in this study was observed in May, 2004 during rain season in Lagos. Loftus and Kushlan (1987) also found out that the highest predation of piscivorous fish on Poeciliids occur in the Dry season in

Florida. There is no comparative study on predation of *P. reticulata* by piscivores in Nigerian in lotic and lentic waters or in wastewater drains.

Nigeria is a developing economy with a growing need for expanding foreign exchange revenue base and profile. The needed growth is matched by the yawning gap in balance of trade between Nigeria and many of her major trading partners. Although, balance in trade is not only a desirable imperative but one of the conventional means of measuring the state of health and relative attractiveness of any national economy, yet an unbalanced economy is symptomatic of a poorly regulated and an insecure economy (Ploeg *et al*., 2009). Nigeria's dependence on petroleum-based foreign exchange earnings as singular major revenue source is an example of such a mono-cultural economy with inherent defects. Therefore, a gradual diversification of Nigeria's foreign exchange gross-earning portfolio such as can be enhanced through relatively low-risk and high-yield commerce engendered by the current state of international ornamental fish trade is an unavoidable option. An option that would eventually further facilitate greater employment opportunities, new skills acquisition, technology transfer along the entire value chain of local ornamental fish trade, industrialization, ecotourism and other social collateral effects as experienced elsewhere.

However, the need to fundamentally secure Nigeria's living aquatic borders from chance or deliberate infiltrations and aggressions of exotic pathogen invasions leads to the desirability of properly assessing the current state of parasite and microbial load of indigenous ornamental fish species and their major sister imports.

The choice of *P. reticulata* for this study out of as many as forty ornamental fish species listed as commonly available for export in Nigeria by Areola (2004) was informed by its ubiquity in Lagos wastewater drains, its exotic nature and therefore its potential for invasion and propagule pressure on existing ichthyofauna of wastewater and deliberate aquaculture of contiguous aquifer. The invasion of new biota and watersheds in Nigeria by *P. reticulata* and contemporary nematode fauna may as a result lead to new problems hence the need for this study. *P. reticulata* also retains the reputation of being the most widely sold freshwater ornamental fish in the world. This emphasizes the point that it is difficult to do a risk assessment of obtaining export grade of *P. reticulata* product without an examination of the objectives of export substitution in the absence of conscious reminder of the amount of foreign exchange earning

potential of *P. reticulata* that Nigeria could gain from developing a local export substitute to the various forms of *P. reticulata* modified for the market.

It would be necessary to evaluate what Nigerian OFI clients expend in the import of *P. reticulata* as ornamental fish today, what is infrastructure to put in place to satisfy quality assurance and also what is needed to further conclude study on other non-nematode parasites of *P. reticulata*.

So far none of the parasites found in this study are amongst the pathogens included in OIE list of identifiable diseases (OIE, 2003 and 2005). The fish diseases listed under the OIE lists are Epizootic hematopoietic necrosis (EHN),Infectious hematopoietic necrosis (IHN), Spring viraemia of carp (SVC),Viral hemorrhagic septicemia (VHS), Infectious pancreatic necrosis (IPN),Infectious salmon anemia (ISA), Epizootic ulcerative syndrome(EUS), Bacterial kidney disease (BKD) and Gyrodactylosis (caused only by *Gyrodactylus salari*s), Red sea bream iridoviral disease and Koi herpesvirus disease (KHV). Of all these, only three (VHS, EUS and KHV) have been shown to affect ornamental fish species all over the world while only the last, KHV can infect *P. reticulata*. Luckily for Nigerian ornamental fish mongers and exporters, KVH can only survive in its host only below 17°C or above 25°C temperatures. Wastewater drain and aquaria temperatures in Nigeria are rarely outside this range thereby enhancing the export potential of *P. reticulata* from these drains.

It is already clear that none of the four nematodes can be said to be indigenous to Nigerian clime. If they are all exotic to Nigerian aquatic biota, then they must have been originally introduced with the same or other exotic parasites in the far or recent past through accidental or unethical disposal from aquaria settings. Further enlargement of this study would do well to note that transmission of autogenic against allogenic nematode parasites have comparative but contrasting niche, environmental, transmission and trophic strategies (Dobson, 1986; Dobson, 1988 and Esch *et al*., 1990) for propagation hence the differences in their prevalence and intensity in *P. reticulata*. Proper risk abatement measures against the retention of these nematode parasites in export substitutes should therefore discriminate between these in its sourcing, quarantine and transportation focus. Other studies of this magnitude for the prevalence and mean intensity of protozoan and trematode parasites of *P. reticulata* deserve consideration as well.

Finally, the role of genetic facilitation and subversion of nematode parasitism by the *P. reticulata* host in the suitability of wastewater derived samples for export market substitution would not be comprehensive enough without a proper re-evaluation of possible speciation taking place in the current feral stock in Lagos drains as described by Webb *et al*. (2007) for *Trichogaster trichopterus* in Australian waters. It is suggested that DNA barcoding method of evaluating the evolutionary history of the *Poecilia* genus (Steinke *et al*., 2009) be done to ascertain how many species and strains of *Poecilia* are currently existing in these wastewater drains (Dawes, 1991). This is beside same level of taxonomic scrutiny necessary for the attendant fish disease causing pathogens observed in the ornamental fish hosts within the Nigerian biome since their current economic importance need proper validation. Spontaneous and dynamic changes may be already occurring in our freshwater and marine aquatic environments un-noticed as would be indicated by previous works by Whitfield (1979), Wallace (1961), Toft (1986), Barratt and Medley (1990), William and Jones (1994), Rigby *et al*., 1997 and Thomas *et al*. 2005 .

There had been this special, albeit, unusual situation, where the rigor applied to the control of imported plant and animal cargo across borders are not applied to live ornamental fish species (Freyhof and Korte 2005) ostensibly because of their fragility and the need to reduce the impact of stress on them in order not to cause mortality en-route their destination. Consequently, the trans-border transportation of ornamental fishes occurs at times in large volume in most parts of the world and is done by virtually anyone with little hindrance because they are somehow considered as harmless pets.

Realization of the potential risks of pathogen transfer through ornamental fish imports (Langdon 1988; Read, 1990; Rowland and Ingram 1991; Dove *et al*. 1997; Dove 1998; 2000; Dove and Ernst 1998; Dove and Fletcher 2000) only became critical relatively recently in comparison with those of other animal imports. In Nigeria, the import of ornamental fish is now regulated under the Live fish (Control of importation) Decree 209 of 1990 (See Volume XI of laws of the Federation of Nigeria, 2000). In New Zealand, it was previously considered under the Biosecurity Act of 1993 but now modified in the Hazardous Substances and New Organisms Act of 1996. There, only exotic ornamental fish species of tropical origin that are known not to be able to survive the temperate condition of New Zealand have been further allowed on the permitted list

for imports. As a result, ornamental fish species of temperate origin such as the Goldfish (*Carassius auratus*) and carp (*Cyprinus spp.*) are not on the permitted list of imported ornamental fish into New Zealand. Even then, the presence of geothermal streams and artificial warm water bodies in some northern parts of New Zealand, in addition to continuing climatic changes induced by gradual global warming ultimately mimic tropical conditions. These geothermal stream have been confirmed by the New Zealand Freshwater Fish Database (2005) to now sustain local populations of four major tropical species of ornamental fish species such as the guppy (*P. reticulata)*, the sailfin mollies (*Poecilia latipinna),*the swordtail fish (*Xiphophorus helleri)*, and the caudo (*Phallocerus caudimaculatus*). These species were previously considered safe in New Zealand because of the thought that their characteristic pathogens would hardly survive in the temperate condition of the country not surprisingly as experienced by the studies and reviews of Poulin (2002); Pigliucci and Murren (2003). By extension, global warming realities would demand that Nigeria develops a more comprehensive risk map and mitigating measures that can officially screen for potential pathogens of both tropical and temperate origin in ornamental fish imports. Such ornamental imports may inadvertently adapt to Nigeria's climate and establish themselves in local fish populations because of thinning climatic barriers (May, 1988 and Dobson, 1990).

Furthermore, ongoing global climate changes could already be impacting on the local stock of *P. reticulata* existing in wastewater drains thereby amplifying possible endemicity of nematode parasites or establishment of exotic ones on this ornamental fish species (May and Anderson, 1979; Dobson, 1988; Pigliucci, 2001; Buckling and Rainey, 2002; Poeser, 2011). In the event that these occur, aggressive infusion or spontaneous invasion of Nigeria's wastewater biosphere (Esch *et al*., 1975; Fuller *et al*., 1999 and Dunn, 2005) possibly occasioning loss of germplasm may result (Courtenay and Meffe, 1989; Gozlan *et al*., 2010). Obtaining empirical data on the parasites of *P. reticulata* is therefore critical to its prospects in the Nigerian segment of the OFI (Dobson, 1986; Courtenay and Stauffer, 1990 and Courtenay and Moyle, 1996).

Symptom of diseases in ornamental fish kept in aquaria are easily treated at that macrocosmic level however making the fish pet survive and continue as a carrier of the germ (Arthington, 1989a; Courtenay and Robbins, 1989). When sanitation of aquarium tanks or unintended escape of ornamental fish take place, accidental translocations to

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wastewater drains often occur thereby making native fish species in the drains that are susceptible to the parasites become new hosts (Arthington and Lloyd, 1989). Oftentimes, these new hosts are food fish species (deGraaf *et al*., 1995) with potential for transferring these parasites to humans (Arthington, 1989b; Moyle and Li, 1994).

Though many freshwater ornamental fish species number amongst the export list from Nigeria destined for the US, EU and South East Asia (Mbawuike and Pepple, 2003; Mbawuike *et al*., 2004; Mbawuike and Ajado, 2005) the potential for the more economically rewarding marine species of ornamental fish species are wide because of the many deep water wrecked abandoned vessels in Nigeria's Exclusive Economic Zone (EEZ). Coral reefs that result from some of these ship wrecks have for years constituted refugia for the many colonizing invading poriferans and ichthyofauna that constitute highly financially rewarding gains in the ornamental fish market (Larkin and Degner, 2001; Larkin *et al*. 2003) elsewhere in the US. Same potential exists in deep waters of Nigeria's EEZ. Perhaps, the major factor militating against a repeat of the wanton destruction of these deep water habitats for exploitation of deep water marine ornamental fish species is the absence of deep water divers in Nigeria unlike in the Southeastern coast of Africa in the Seychelle islands and Mauritania.

The taxonomy of *Capillaria spp.* has clearly been under constant review while the true status of parasitic finds in food-fish and ornamental fish species are also being validated by molecular biology methods (Moravec and Gut, 1982; Paperna, 1996). Another case in point is the occurrence of *Trichinella spp.* in *P. reticulata* in this study that suggest a host-parasite species barrier conquest because *Trichinella spp.* are nematode parasites of feline mammals and not previously encountered in fish samples (Murrell *et al*., 2000; Bruschi and Murrell, 2002). This is just as Moravec *et al*. 1990 in their first record of *Raphidascaris* (*Sprentascaris*) *hypostomi* occurrence in Brazil questioned the taxonomic status of the host genus *Sprentascaris*. Moravec *et al*. (1998a) similarly made new observations of *Mexiconema cichlasomae* (Nematoda: Dracunculoidea) from fishes of Mexico. Furthermore, Moravec *et al*, 1998b found fish as paratenic host of *Serpinema trispinosum* while the report by Gonzalez and Hamann (2007) explained the new role of the amphibian, *Lysapsus limellum* Cope, 1862 (Amphibia: Hylidae) as new paratenic host in the life cycle of *Serpinema trispinosum* (Nematoda: Camallanidae).

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All these reports were initially controversial. Further validation of these startling identifications of fish parasites, unusual fish and other taxa as paratenic hosts through molecular biology techniques would be necessary to settle grey areas generated by their discovery (Bondad-Reantaso *et al*., 2005; Bolton and Graham, 2006). It would not be surprising if current helminthic parasite fauna of freshwater ornamental fish species already over-reach the limits set by the checklist of Khalil and Polling (1997). On-going genetic characterization of parasites species using DNA barcodes seem to hold contemporary sway in parasitology and Nigerian Scientists would need to play active roles. This is necessary because legal disputes of the future on control of natural aquatic resources, determination of assets and the burden of its liability, in the face of panoply of natural disasters, may very well be based on the possession of intellectual property accessions to buttress claims to natural assets of regional scope. Indeed, the world's living aquatic resources have moved far from the works of Linnaeus (1758) while the *P. reticulata* of Peters (1859) is actively under review.

Import and export records from Custom authorities and FAO in virtually all points of origin and destination of ornamental fish are currently, scientifically unreliable as regards true origin and defendable taxa of these organisms. The impeccability of DNA barcodes that employs the use of a short stretch (685 bp) of the mitochondrial DNA of all organisms to evolutionarily profile and identify organisms is clear (Steinke *et al*., 2009). It is the newest approach to trans-border traffic control of pets. Practically and relatively less invasive than other methods, with the collection of hairs, blood sample and fish skin snipes immediate confirmation of the true cluster of origin of an ornamental fish can be generated and transmitted to and from globally accessible internet servers to obviate for some of the expensive and tortuous certification mechanisms put in place by UNCTAD, CITES and OIE that stifles true free trade amongst nations.

Meanwhile, there are more marine ornamental fish species that draw greater profit per unit catch and sale in the export than the freshwater species (Chan and Sadovy, 1998 and 2000) even when greater care as enunciated by (Chao, 1992; 1993; Chao and Prang, 1997 and Chao, 2001) has to be ensured to conserve our natural marine and coastal reefs from irresponsible fishing methods. To source, warehouse, trade and retain ornamental fish assets, adoption of DNA barcoding methods for profiling therefore seem unavoidable.

The Nigerian state may be in possession of assets whose currency and validity has since long ago changed. We have to be actively involved in all relevant and current research, in a global knowledge economy, where possession of natural resources are validated by only intellectual property development of such resources for economic access. It is poetic that such nations of the world lacking in natural resources with a surfeit of natural disasters (temperate regions, inclement weather conditions, earthquakes, Tsunamis, Hurricanes forest fires and landslides) but that attach great policy and implementation premium to human capital development such as Japan, Malaysia, EU, China and the US are far ahead of those endowed with natural resources (such as Nigeria and Democratic Republic of Congo) in the productive harness of natural resources.

CHAPTER SIX
CONCLUSIONS

Prevalence of *E. ignotus*, *Trichinella spp. C. pterophylli* and *C. cotti* in *P. reticulata* obtained from Igi-Olugbin, Basil Ogamba, Ahmadu Bello and Adenaike Alagbe Streets of Lagos State is neither sex-related nor drain-related though monthly mean intensity differed significantly with drains. Therefore, the collection of *P. reticulata* from the above Streets for exploring their export potential is safe as regards intolerable nematode infections and intensity.

Access of terrestrial household pets and animals such as stray cats, dogs, poultry, ducks and wading water birds to wastewater drains where collections of *P. reticulata* is to be made should be limited if not totally avoided. This is because the observed nematode parasites and others such as non-nematode helminthes and protozoan parasites need these occasional visitors to wastewater drains to complete their cycle. Similarly, the monthly sanitation exercise in Lagos State that entails, amongst other activities, the clearing of drains by removal of debris and soil substrates onto the main road turn out to be counter-productive to the effort in stemming nematode parasite cycle completions. This so because the excavated debris are in many cases not evacuated away from the point of deposition near the drains with subsequent rains washing the sewage debris and nutrients back into the drains thereby increasing eutrophication of wastewater. These high nutrient load, sewage debris and flood water renewal expose regular refugia of *P. reticulata* making them revealed preys to avian and quadruped predators. The attraction of wading birds and the release of their fecal matter into wastewater particularly enhance recurrent *E. ignotus* egg loading of the wastewater.

It is also advised that animal carrion be properly disposed away from the drains. An alternative means of preventing all these is the use of well covered drains inaccessible to occasional visitors. Monitoring of ornamental fish aquaculture and trade through regular review of risk assessment and mitigation plans should be directed at socio-economic gains and biological conservation of these species. Since, the proper genetic characterization of these organism are critical to possession and access, it is recommended that the use of DNA barcoding method of identification be adopted for both ornamental fish and their parasite fauna to stave off inhibitory litigation cost that

may result from unfair trade practices injurious to Nigeria's economy in the near future. This would also be important in determining dynamic level of speciation expected to be under serious yield to climate change, the origin, wild or domestic nature of fish samples and their parasites, forensic confirmation of source of harvest, route of import and method of obtaining them which would ultimately reduce licensing and certification cost for Nigerian traders and exporters.

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APPENDIX 1

White Egrets (a), *Ardea alba*, occasional visitors to a failed section of drain on Basil Ogamba Street.

APPENDIX 2

An occluding wastewater drain on Ahmadu Bello Road in June, 2004 with (a) gradual
loss of habitat to wastewater fauna including *P. reticulata* and (b) fully blocked section.

Correlation co-efficient of Nematode parasite prevalence in *P. reticulata* obtained from Igi-Olugbin Street in relation to the means of monthly physicochemical parameters of wastewater in drains.

	Temperature (OC)	PH	Dissolved Oxygen (mg/L)	Transparency (cm)	Drain Depth (cm)	Eustrongylides ignotus	Trichinella spp.	Capillaria pterophylli	Camallanus cotti
Temperature (OC)	1.0								
Ph	0.4	1.0							
Dissolved Oxygen(mg/L)	-0.4	0.2	1.0						
Transparency (cm)	-0.5	-0.3	-0.1	1.0					
Drain Depth (cm)	-0.4	0.0	0.6	-0.1	1.0				
E. ignotus	0.5	-0.1	-0.5	0.0	-0.2	1.0			
Trichinella spp.	-0.2	-0.4	0.1	-0.3	0.2	0.2	1.0		
C. pterophylli	0.1	-0.1	0.0	-0.3	*0.6**	0.2	-0.1	1.0	
C. cotti	0.2	*0.7**	0.0	-0.2	0.4	0.1	-0.3	0.4	1.0

Correlation co-efficient of Nematode parasite prevalence in *P. reticulata* obtained from Basil Ogamba Street in relation to the means of monthly physicochemical parameters of wastewater in drains.

	Temperature (OC)	PH	Dissolved Oxygen (mg/L)	Transparency (cm)	Drain Depth (cm)	*Eustrongylides ignotus*	*Trichinella spp.*	*Capillaria pterophylli*	*Camallanus cotti*
Temperature (OC)	1.0								
pH	0.6	1.0							
Dissolved Oxygen (mg/L)	0.3	0.0	1.0						
Transparency (cm)	0.0	-0.1	0.3	1.0					
Drain Depth (cm)	0.0	-0.3	0.5	0.0	1.0				
E. ignotus	0.3	0.4	0.0	-0.3	0.0	1.0			
Trichinella spp.	0.3	0.2	0.0	-0.2	0.2	**0.9**	1.0		
C. pterophylli	0.6	0.5	0.0	-0.1	-0.1	-0.2	-0.1	1.0	
C. cotti	0.4	0.2	0.1	-0.3	0.3	**0.9**	**1.0**	-0.2	1.0

Correlation co-efficient of Nematode parasite prevalence in *P. reticulata* obtained from Ahmadu Bello Road in relation to the means of monthly physicochemical parameters of wastewater in drains.

	Temperature (OC)	pH	Dissolved Oxygen (mg/L)	Transparency (cm)	Drain Depth (cm)	Eustrongylides ignotus	Trichinella spp.	Capillaria pterophylli	Camallanus cotti
Temperature (OC)	1.0								
pH	-0.5	1.0							
Dissolved Oxygen (mg/L)	-0.2	0.1	1.0						
Transparency (cm)	-0.2	0.3	0.4	1.0					
Drain Depth (cm)	0.6	0.1	-0.5	-0.4	1.0				
E. ignotus	0.2	-0.1	-0.4	-0.3	0.5	1.0			
Trichinella spp.	0.0	-0.1	0.3	-0.1	0.1	0.2	1.0		
C. pterophylli	0.3	-0.3	-0.4	-0.2	0.2	**0.7**	0.3	1.0	
C. cotti	0.3	-0.3	0.5	0.3	-0.1	0.2	0.0	0.1	1.0

Correlation co-efficient of Nematode parasite prevalence in *P. reticulata* obtained from Adenaike Alagbe Street in relation to the means of monthly physicochemical parameters of wastewater in drains.

	Temperature (OC)	PH	Dissolved Oxygen (mg/L)	Transparency (cm)	Drain Depth (cm)	Eustrongylides ignotus	Trichinella spp.	Capillaria pterophylli	Camallanus cotti
Temperature (OC)	1.0								
pH	-0.1	1.0							
Dissolved Oxygen (mg/L)	-0.3	0.1	1.0						
Transparency (cm)	-0.6	0.0	0.3	1.0					
Drain Depth (cm)	0.0	-0.1	0.5	0.3	1.0				
E. ignotus	-0.1	-0.3	0.2	-0.1	*0.6*	1.0			
Trichinella spp.	-0.2	0.1	**0.8**	0.4	0.4	0.0	1.0		
C. pterophylli	-0.6	0.3	0.1	0.5	0.3	0.3	-0.1	1.0	
C. cotti	0.3	0.6	-0.1	-0.2	0.1	0.0	-0.1	0.0	1.0

Correlation of Mean intensity of nematode parasites in *P. reticulata* obtained from Igi-Olugbin Street in relation to means of monthly physicochemical parameters of wastewater in drains.

	Temperature (OC)	PH	(mg/L)	Transparency (cm)	Drain Depth (cm)	ignotus	Trichinella spp.	Capillaria pterophylli	Camallanus cotti
Temperature (OC)	1.0								
pH	0.4	1.0							
Dissolved Oxygen (mg/L)	-0.4	0.2	1.0						
Transparency (cm)	-0.5	-0.3	-0.1	1.0					
Drain Depth (cm)	-0.4	0.0	0.6	-0.1	1.0				
E. ignotus	0.4	0.4	0.0	0.0	0.2	1.0			
Trichinella spp	-0.1	-0.4	-0.3	0.1	0.1	0.0	1.0		
C. pterophylli	0.0	-0.2	0.2	-0.3	**0.6**	0.2	**0.7**	1.0	
C. cotti	0.3	0.4	0.0	-0.2	0.2	**0.7**	0.2	0.4	1.0

APPENDIX 8

Correlation of Mean intensity of nematode parasites in *P. reticulata* obtained from Basil Ogamba Street in relation to means of monthly physicochemical parameters of wastewater in drains.

	Temperature (OC)	PH	Dissolved Oxygen (mg/L)	Transparency (cm)	Drain Depth (cm)	Eustrongylides ignotus	Trichinella spp.	Capillaria pterophylli	Camallanus cotti
Temperature (OC)	1.0								
pH	**0.6**	1.0							
Dissolved Oxygen (mg/L)	0.3	0.0	1.0						
Transparency (cm)	0.0	-0.1	0.3	1.0					
Drain Depth (cm)	0.0	-0.3	0.5	0.0	1.0				
E. ignotus	-0.1	0.4	-0.3	-0.2	-0.4	1.0			
Trichinella spp.	0.3	0.1	0.2	0.0	0.3	0.0	1.0		
C. pterophylli	0.4	0.3	-0.1	-0.2	0.2	-0.1	-0.2	1.0	
C. cotti	0.2	-0.2	0.4	-0.4	0.3	-0.1	0.0	-0.2	1.0

Correlation of mean intensity of nematode parasites in *P. reticulata* obtained from Ahmadu Bello Road in relation to means of monthly physicochemical parameters of wastewater in drains.

	Temperature (OC)	PH	Dissolved Oxygen (mg/L)	Transparency (cm)	Drain Depth (cm)	*Eustrongylides ignotus*	*Trichinella spp.*	*Capillaria pterophylli*	*Camallanus cotti*
Temperature (OC)	1.0								
pH	-0.5	1.0							
Dissolved Oxygen (mg/L)	-0.2	0.1	1.0						
Transparency (cm)	-0.2	0.3	0.4	1.0					
Drain Depth (cm)	**0.6**	0.1	-0.5	-0.4	1.0				
E. ignotus	0.0	0.2	0.0	-0.2	0.5	1.0			
Trichinella spp.	0.1	-0.2	-0.3	-0.2	0.4	0.2	1.0		
C. pterophylli	-0.2	0.5	-0.1	0.4	0.0	0.1	0.2	1.0	
C. cotti	0.0	-0.2	0.5	0.0	-0.2	-0.1	0.4	-0.2	1.0

Correlation of mean intensity of nematode parasites in *P. reticulata* obtained from Adenaike Alagbe Street in relation to means of monthly physicochemical parameters of wastewater in drains.

	Temperature (OC)	PH	Dissolved Oxygen (mg/L)	Transparency (cm)	Drain Depth (cm)	*Eustrongylides ignotus*	*Trichinella spp.*	*Capillaria pterophylli*	*Camallanus cotti*
Temperature (OC)	1.0								
pH	-0.1	1.0							
Dissolved Oxygen (mg/L)	-0.3	0.1	1.0						
Transparency (cm)	-0.6	0.0	0.3	1.0					
Drain Depth (cm)	0.0	-0.1	0.5	0.3	1.0				
E. ignotus	0.1	0.2	-0.1	-0.4	-0.4	1.0			
Trichinella spp.	0.3	-0.2	0.6	0.0	0.2	0.2	1.0		
C. pterophylli	0.4	0.0	0.1	-0.3	-0.2	0.5	**0.8**	1.0	
C. cotti	0.5	-0.6	0.0	-0.4	0.3	0.2	0.5	0.5	1.0