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The occurrence of heterophyid metacercariae in a stream linked aquatic reservoirs, Southeast Nigeria

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Abstract. The study of teleost parasites in an ecologically altered ecosystem through dam construction that evidently facilitate parasitic infections and aggravate public health concerns is essential. The study of stream linked dam showed that *Tilapia zillii* (Gervais, 1848), *Pelmatolapia mariae* (Boulenger, 1899) and *Clarias gariepinus* (Burchell, 1822) form the major fish composition of these reservoirs. They serve important food resource globally especially places bereft of extensive aquaculture practices. Although, knowledge about the gill parasites of *P. mariae* is inexistent the examined individual *P. mariae* showed that it had 54% *Heterophyes heterophyes* infection, *C. gariepinus* had no parasitic infection whereas *T. zillii* had a relatively very low *Batrachobdelloides* spp (1.7%). Besides, the highest DO levels in the dam occasioned by high agitation and floatation the physical factors conditions - pH and DO (> 6 $mg.L^{-1}$) had suitable levels for biodiversity.

Keywords: Refugia; Metacercariae; Community health; Spotted tilapia; Physical factors.

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Introduction

The present study was done to ascertain the hitherto gill parasites of *Pelmatolapia mariae* (Boulenger, 1899) and their possible health concerns at stream linked dam of the National Root Crop and Research Institute (NRCRI), Umudike, where an assortment of agricultural activities subsist. Besides benefits of damming rivers and streams to create reservoirs for useful purposes such as agricultural production, recreation, irrigation, fishing, etc, it is fraught with consequences that may support alterations of original ecological state of the aquatic ecosystem such as development of favourable environment that perhaps guarantees success of intermediate hosts of diseases that were initially inexistent in such dammed areas (Waddy, 1975; Gilles, 1980; Jewsbury and Imevbore, 1988; Ofozie, 2002).

The spread of heterophyiasis for instance, can only occur in aquatic ecosystems either altered or not wherein

molluscs and fish intermediate hosts co-inhabit. It is rife in areas where predilection for eating raw and or improperly cooked fish favors its spread (Annett et al., 1999; Bradford et al., 2011). In as much as human heterophyiasis presents asymptomatic situation, heavy infection may cause mucosal damage and some gastrointestinal disturbances that hardly associated to fish in medically less developed areas. The metacercariae of heterophyid fluke in fish cause direct infection in other vertebrates such as birds and mammals including humans (Abou-Basha et al., 2000; Lobna et al., 2010).

Nowadays, *P. mariae* has a global distribution sequel to its introduction, proliferation and establishment in the North America (USA), Asia (Australia), Europe (Russia) and the inhabitant West African ecosystems where they serve important resource to communities everyplace aquaculture is not broadly used. The successes of these species stem from omnivorous habit and flexibility in reproductive behaviours especially the care for their brood by usually monogamous parents (Arnold et al., 1968; Fagade, 1971).

Generally, the biology of *P. mariae* in the various habitats they now exist in has been well documented (Hildebrand et al., 1988; Nmor et al., 2004; Olurin and Somorin, 2006; Brooks and Jordan, 2010). But little attempts have been made to study the parasites of *P. mariae*, especially in its inhabitant ecosystems (Arthur and Albert, 1994; Nithikathkul and Wongsawad, 2008; Morenikeji and Adepeju, 2009).

Unfortunately, in the available studies information on its gill parasites and the influence of basic abiotic factors on the occurrence of such parasites still lack in knowledge. Therefore, the present study was to fill the gap in knowledge.

Due to lack in knowledge of gill parasites of *P. mariae*, a wild parasitic survey was necessary firstly to investigate them especially in an ecologically altered ecosystem sequel to dam construction to provide hydrological needs of crops, farm animals and surrounding communities at the dam of NRCRI using standard methods for both parasitic and abiotic surveys. Secondly, to determine other hosts spectrum of the dam. To the best of our perceptive there is little or no study on these considerations.

Material and methods

The stream linked dam is located at NRCRI (05° 29.461’ N and 07° 32.315’ E). The stream supply by run offs to various inter-community reservoirs to River Anya at Amaba to Akwa Ibom and Cross River States of Nigeria where they join Atlantic Ocean. The constructed dam at the stream caused establishment of reservoirs at the adjoining side that undergo fullness and contraction during rainy and dry season months, respectively. These aquatic reservoirs receive water mainly from precipitation and the dam during rainy season months when the banks are full (05° 29.277’ N and 07° 32.256’ E). The mid deposit is mud with soft organic debris from thick natural vegetation around, anthropogenic influence by the surrounding population collect water for domestic purposes and agricultural activities mainly along the inflowing stream location all seasons (05° 29.072’ N and 07° 32.400’ E) and the dam proper (05° 29.461’ N and 07° 32.315’ E) (Figure 1).

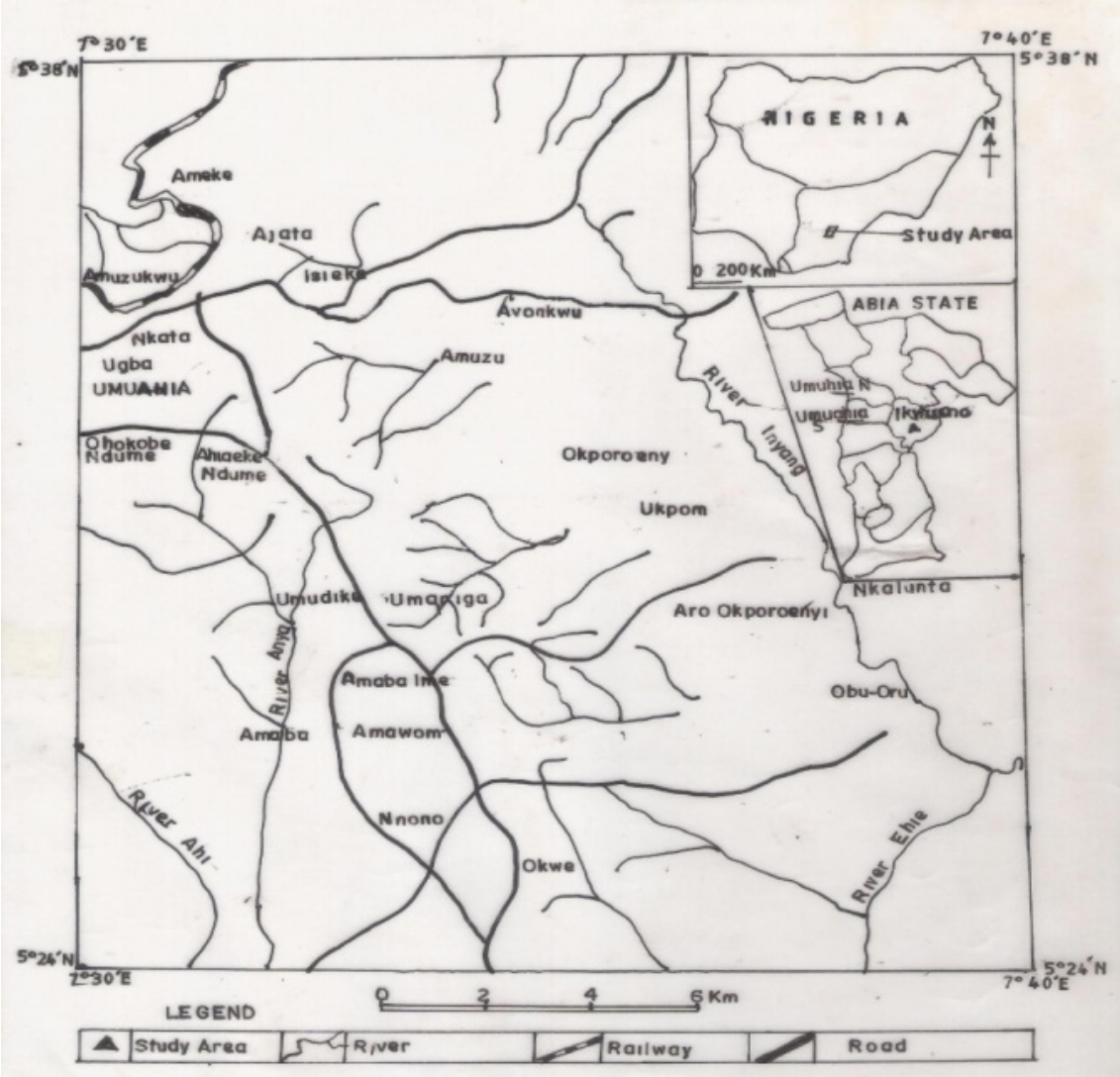

Figure 1. Water ways of the study area and the surrounding communities (Source: Federal Survey, 1967).

The abiotic factors were determined using the details in Gupta and Gupta (2013). In every case, only freshly caught fish was examined for parasites and the procedure followed details in Arthur and Albert (1994). The buccal cavity, surface of the gills, body and scales were thoroughly examined. In addition, smears of scrapings from the gill and external body parts were made and examined under a microscope. The smears were put on microscope slides, air-dried, fixed in 95% methyl alcohol for 5 min, stained in Giemsa for 20 min and examined under oil immersion. The fish were examined for the presence of external bumps called yellow grubs, then dissected and studied internally for metacercarial cyst in the muscles and visceral organs. The locations of cyst and number of cyst were recorded. Cysts were opened using two sharp needles. Metacercariae were transferred into saline (6.5%). The abdomen of each fish was opened for examination of the internal organs (heart, liver, and gall bladder, spleen, digestive tract, gonads, kidney, and urinary bladder). The organs were separated and thoroughly examined. Each organ, except the gut, was then be put into a separate Petri dish containing physiological saline. The gut was separated into stomach, pyloric caeca, small and large intestines; each of these sections will be put into a Petri dish containing physiological saline,

cut longitudinally and examined for parasites with a hand lens. These gut sections, were further separately rinsed into beakers to which solution of sodium bicarbonate (one spoonful per litre) had been added to remove mucus and allow parasites to settle. The filtrate will be decanted and the residue examined with a hand lens. The body cavity was rinsed with physiological saline and examined. The brain, eye socket and sliced musculature were examined.

Procedures used in further treatment, fixation and preservation of parasites followed details in Ash and Orihel (1987). The Heterophyid metacercariae were shaken in normal saline to remove mucus and other host's debris. They were shaken in cold 4% formaldehyde until they died; then fixed in FAA (5% formal - 90% alcohol - 15% glacial acetic acid) for 2 h prior to staining the parasites. The parasites were stained in carmine - Alum washed in acetic acid and ethanol solution, dehydrated in an alcohol series, cleared in xylene and mounted on permanent slides using Canada balsam. The parasites were identified using details in Yamaguti (1971) and Paperna (1991).

Results

Out of 35 *P. mariae* examined nineteen had *Heterophyes heterophyes* infection 54%, excysted forms affecting the gills and the heart, the only *Clarias gariepinus* (Burchell, 1822) collected had no parasites whereas out of fifty nine *Tilapia zillii* examined only one had infection of two leeches - *Batrachobdelloides* spp (1.7%).

The spectrum of infection indicates that the *Tilapía zillii* with size 38 cm had the leech infection and the *P. mariae* individuals with standard length 6-8 cm size had the highest infection by size as those within 9-10 cm had highest percentage intensity of 0.26 (Table 1).

Pepsin tissue digested parts showed no presence of metacercariae. The abiotic factor conditions in the adjoining reservoir had DO (6-10 $mg.L^{-1}$) and pH slightly acidic (5.0), while in the dam had approximately neutral pH (6.8) and higher DO ($\geq$ 13 $mg.L^{-1}$), while the stream channel of flow had pH (5.7) and DO ($\geq$ 7.6 $mg.L^{-1}$). In addition, in both aquatic ecosystems, temperature posed inestimable influence (21-28 °C) as the conditions are relatively at par (Table 2).

Table 1. Prevalence and intensity of *Heterophyes heterophyes* infection in *P. mariae*.

Size of fish (cm)	No. of fish hosts	No. of infected hosts	No. of parasites	Prevalence (%)	Intensity
$\leq$ 5	9	4	6	11.4	0.17
6-8	15	7	5	20.0	0.14
9-10	7	5	9	14.3	0.26
$\geq$ 11	4	3	4	8.6	0.11
Total	35	19	24	-	-

Table 2. Abiotic contents of the three aquatic ecosystems.

Months	Adjoining reservoir			Dam			Stream		
	T (°C)	pH	DO ($mg.L^{-1}$)	T (°C)	pH	DO ($mg.L^{-1}$)	T (°C)	pH	DO ($mg.L^{-1}$)
March	27	5.1	6.0	27	6.8	14	27	5.8	7.7
April	28	5.1	7.8	28	6.9	13	28	5.8	7.6
May	22	5.0	9.0	22	6.9	15	21	5.7	8.9
June	21	5.1	10.0	21	6.9	14	21	5.8	9.0
July	21	5.0	10.0	21	6.9	16	21	5.8	9.0

The stream path of flow represents point source pollution due to influx of wastes into the stream by human and animal pollutions around. These include household and agricultural wastes consisting non biological materials or biodegradable materials which float along with biodiversity through the channel that is slightly acidic pH (5.8 ± 0.8). The stomach contents; small both aquatic and terrestrial insects, various plant materials etc, represent entirely omnivorous feeding mode.

Out of 35 individual species of *P. mariae* collected, 11 were collected from the adjoining reservoir and stream whereas 13 were collected from the dam respectively. The overall monthly prevalence showed that most occurrences were during late dry season months of March and April 2015 except in the dam that recorded no parasites in April. However, wet periods had lower infection rate and prevalence except in July in the dam that had 3 collected *P. mariae* out of which 2 were infected with 8 *Heterophyes heterophyes*. The adjoining reservoir had the highest overall monthly prevalence 36.3% whilst the least prevalence was 7.6% in May in the dam.

Table 3. Infection vs. month.

Months	Adjoining reservoir			Dam			Stream		
	No. of hosts collected and infected	No. of parasites	Prevalence by month (%)	No. of hosts collected and infected	No. of parasites	Prevalence by month (%)	No. of hosts collected and infected	No. of parasites	Prevalence by month (%)
March	4 (4)	5	36.3	6 (4)	13	30.7	2 (1)	6	9.0
April	3 (3)	7	27.2	1	-	-	4 (2)	12	18.1
May	1 (1)	4	9.0	2 (1)	7	7.6	2 (1)	5	9.0
June	2	-	-	1	-	-	1	-	-
July	1	-	-	3 (2)	8	15.3	2	-	-

Discussion

Although, *Batrachobdelloides* spp it is not considered a serious parasitic problem, it plays damaging role at facilitating secondary infections of both micro and macro - parasites. In most cases, skin ulcerations caused by ecto-parasites are secondarily affected by microorganisms (Van As et al., 1984). Ectoparasites species have been found to be either host specific or ubiquitous and opportunistic (Woo, 1995).

The present efforts represent the first occurrence of gills parasite in *T. mariae.* It portends serious public health concerns for the large farm animals, many wild Aves and of course large crowing human population of NRCRI including the surrounding communities. The parasite when ingested with undercooked infested fish meal can ulcerate the mucosa of the intestine of the definitive hosts to distort the process of their food digestion which may lead to loss in nutrients (Abou-Basha et al., 2000; Lobna et al., 2010). It is established fact that excysted fluke's intermediate hosts cause more histopathological damages than encysted individuals that is also an explanation of the inability of the host's immunity to cause encystment of the flukes in their tissues (Echi and Ezenwaji, 2009).

Although highest DO occurred in the dam largely due to increased agitation and floatation the physical factors conditions - pH and DO (≥ 6 mg.L^{-1}) had suitable levels for biodiversity. Many parasites have free living stages (cysts, eggs or larvae) which have complimentary behavioural reactions to the external environment with the free living communities such as variation in temperature, DO and pH. Thus, their distribution and abundance can be modified

by these conditions (Echi and Ezenwaji, 2009).

High water flow rate in the dam through constructed channels, although increases dissolved oxygen content, it affects the chance of associating parasites infecting the host as both suffer complimentary problems of the prevailing floatation from the dam through channels to other communities. What's more, parasitic intermediate hosts are washed away quickly before location, attachment, and infection can occur and this affects fauna diversity generally.

The ever flow of water in streams can significantly affect their nutrition; this is because enormous planktonic communities are removed along with the floatation. In the proximate time, the stream would become anorexic as the pH readings represented slightly acidic level that is typical of streams at the proximity of urban and agricultural areas (Willey et al., 2008).

In such aquatic ecosystems the pH range is an indication of predominating high carbonic acid content. Also, this is characteristic of water body that is heavily infested with heavy organic materials from its surroundings resulting in low alkalinity value/s. The carbonic acid formed after dissolution of carbon dioxide gets dissociated into bicarbonate (HCO_3^-) and carbonate ions (CO_3^{-2}) (Gupta and Gupta, 2013). During hot periods such the month of April - the month with highest infection rate, cercariae shedding and transmission to fish increase and under cooked fish may serve as a source of heterophyiasis in domesticated mammals and humans in this area.

This seasonal influence and other abiotic factors have been a reputable reality that many parasites have free living stages (eggs or larvae) which react to the surrounding environmental effects alike their adults and their fish hosts. Tropical Africa wet and dry seasons influence general biology of aquatic biodiversity. The parasites therein perhaps evolved the mechanism of infestations over years due to the opportunity offered by the climatic conditions in Tropical Africa (Echi and Ezenwaji, 2009). In streams seasonality is relatively reduced due to constant in - and - out flow of water and other materials into refugia they form. This encourages free movements of smaller fauna including larvae of parasites and their intermediate hosts into various outlets round the year. Knowledge on the seasonal occurrence of parasites in streams together with the consideration of complimentary effects of abiotic factors has little attention. Similarly, Africa has rich aquatic biodiversity that dart its landscape wherein little or no basic information exists on the parasites of some aquatic fauna and they are therefore conventionally data deficient (Echi and Ezenwaji, 2009).

The inflowing water source of the stream and high flow rate enriches oxygen content of the dam thereby promoting abundance of other organisms therein including snail intermediate hosts of various fluke infections of vertebrates such as birds and mammals including humans (Echi et al., 2015). Nevertheless, human inhabitants in the Research Institute, adjoining University of Agriculture and the surrounding communities utilize the water for fishing, recreation, domestic and agricultural uses. This is especially during frequent break-down of bore-holes that dart about the two academic communities. The soil is sandy and can severe intractable erosion menaces there. Runoffs of various locations in these surrounding communities collect in these natural aquatic ecosystems, which exacerbate distribution of eggs, larvae forms of zoonotic parasitic organisms through oral feacal route among definitive vertebrate hosts (Echi et al., 2015).

Conclusion

P. mariae harbours *Heterophyes heterophyes* in their gills and these parasites are zoonotic to birds and mammals including humans. Proper habits must always be instilled for farm animals, ecological consideration of wild Aves infestation as well as human consumption. Although DO in the dam increases (≥ 13 mg.L^{-1}) in this human altered environment, the dam construction reduces

biodiversity of both parasites and their host fish.

Conflict of interest statement

Authors declare that they have no conflict of interests.

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