

MORPHOLOGICAL AND PARASITOLOGICAL VARIATIONS OF AFRICAN LUNGFISH, *PROTOPTERUS ANNECTENS* IN DRY AND RAINY SEASONS

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ABSTRACT

*This study was carried out to determine the seasonal morphological and parasitological variations of African Lung fish (*Protopterus annectens*) from Upper River Benue, Nigeria. Twenty specimens each of the fish samples from the two seasons were collected monthly for a period of ten months and transported fresh (alive) to the Veterinary laboratory Teaching Hospital, University of Agriculture, Makurdi in plastic jars. The fish samples were identified and sorted into male and female. The total and standard lengths measurements were recorded while the weight was measured. Length weight relationship was determined. Examination for parasites on the fish samples was carried out. Parasitic indices (prevalence (%), and mean intensity, percentage parasite load and percentage frequency of occurrence of each parasite species per location in fish were calculated. The mean total length, total body weight and condition factor of *Protopterus annectens* in dry season were $36.25\text{cm} \pm 0.67$, $515.60\text{g} \pm 25.90$ and 0.91 ± 0.023 , respectively while in rainy season, the mean total length, total body weight and condition factor of $34.74\text{cm} \pm 0.63$, $460.60\text{g} \pm 24.20$ and 0.91 ± 0.019 , respectively. There was no significant difference ($p > 0.05$) between the mean total lengths, total body weight and condition factor of the fish sample in both seasons. Out of the 400 samples of *P. annectens* used for the study in dry and rainy seasons, 31.75% were infested with 242 different parasites. Of the total parasites, 50.83% parasites were recorded in dry season from 67.00% infested fish samples while 48.40% parasites were recorded from 32.00% infested fish. Of the parasites species, *Contracaecum* sp was the most prevalent (34.15%) in dry season. This parasite accounted for 18.70% in the intestine and 15.45% in the stomach while *Eustrongyloids* sp was the most prevalent (27.36%) in rainy season accounting for 22.64% in the intestine and 4.72% in the stomach. The least prevalent parasite load (3.25% in dry season and 2.83% in rainy season) was recorded for *Trichodina* sp. Among the body organs of the fish samples, intestine recorded the highest percentage parasite load (50.41%) in dry season while stomach had the highest percentage parasite load (49.06%) in rainy season. The least (3.25% in dry season and 2.83% in rainy season) were recorded for skin. Generally, intestine had the highest percentage parasite load (98.52%) with 50.41% in dry season and 48.11% in rainy season. Female *P. annectens* from both seasons had more percentage parasite loads (61.79% in dry season and 53.77% in rainy season) than the male *P. annectens* in both seasons (38.21% in dry season and 46.23% in rainy season). There were variations in percentage parasite load among the length and weight groups of the fish samples and conclusively, higher percentage parasite load (50.83%) was recorded in dry season than the rainy season (48.40%).*

Key words: *Protopterus annectens*, parasites, dry and rainy seasons and upper River Benue

INTRODUCTION

Protopterus annectens, a demersal and potamodromous fish species is found in marginal swamps and backwaters of rivers and lakes. It

normally lives on flood plains, and when these dry up it secretes a thin slime around itself which dries into a cocoon. These organisms are omnivorous, feeding on fish, insects, crustaceans, worms,

molluscs, amphibians and plant matter (Purkerson *et al.*, 2005). All lungfish demonstrate an uninterrupted cartilaginous notochord and an extensively developed palatal dentition. Basal ("primitive") lungfish groups may retain marginal teeth and an ossified braincase, but derived lungfish groups, including all modern species, show a significant reduction in the marginal bones and a cartilaginous braincase (Piper, 2007).

Fish parasites and diseases are some of the major problems confronting the fish culturists. They are considered as hindering fish production as they affect the survival, growth and development of fishes, inhibiting their digestive activity and directly or indirectly prevent vitamin and blood sugar metabolism which could lead to mortality of the fish as well as loss in economic value (Emere and Egbe, 2006). Infestation of fish by parasites is caused as a result of several factors among which are the seasons. This work therefore aimed at determining the morphological and parasitological variations of *Protopterus annectens* in dry and rainy seasons from Lower River Benue.

2. MATERIALS

2.1 Sample collection, determination of sampled parameters and morphological measurements:

400 *Protopterus annectens* of different sizes comprising of 200 each in dry and rainy seasons were bought from fishermen along Lower River Benue, Wadata, Benue State.

Twenty specimens each of the fish samples from the two seasons were collected monthly for a period of ten months and transported fresh (alive) to the Veterinary laboratory Teaching Hospital, University of Agriculture, Makurdi in plastic jars with good aeration.

The fish samples were identified using the identification keys by Kottellat *et al.*, Kottleelat *et al.*, (1993), Lerssutthichawal *et al.*, (2005). The standard lengths of the fishes were measured from the head to the region where the tail develops while the total length was measured from the entire length of the fish to the end of the tail using a metre rule. The weight of each fish was obtained using an electronic weighing balance. The sexes of

the fishes were determined based on the examination of their genital papillae.

2.2 Length-Weight relationship and Condition factor of the sampled fish

The fish samples were identified and sorted into male and female (Idodo-Umeh, 2003). The total and standard lengths measurements were recorded to the nearest centimeters (cm) while the weight was measured to the nearest grams (g) using an electronic weighing balance. Length weight relationship was determined using the formula: $W = aL^b$. This formula was logarithmically transformed for the purpose of data analysis as thus:

$$\text{Log } W = \text{Log } a + b \text{ Log } L$$

Where 'a' = proportionality constant, 'W' = weight of fish in grams (g), 'L' = total length of fish in centimeter (cm) and 'b' = growth coefficient.

2.3 Condition factor (k)

Condition Factor (K) of the fish samples was calculated using the formula: $K = 100W/L^3$

Where: W = Fish weight and L = Fish Length

Examination of fish samples for parasites

2.4 Examination for parasites on the skin, fins and gills

Skin: Fish were placed laterally, the skin was examined for detection of parasitic manifestations, and skin smear was made using scalpel blade according to (Bichi and Ibrahim, 2009). Skin smears from the head to the tail mucus mixed with epidermal cells were made using a spatula. The scraped samples of mucus together with the tissues were placed on a Petri-dish containing 3mls of 0.9% saline solution and stirred using a mounted pin (Omeji *et al.*, 2010, Emere and Egbe, 2006). Some drops of the mixed solution were collected using dropper, placed on a clean slide and examined under phase-contrast microscope.

Gills: Detection of parasites from the gills was made using the methods described by Omeji *et al.*, (2010, Bichi and Ibrahim, 2009). Gills were cut by scissors, placed in a Petri-dish and gill filaments were dissected using anatomical needle and

examined under the microscope. Gill scrapings were further placed on few drops of water previously placed on to glass slides then covered with cover-slide and examined under the phase-contrast microscope

Fins: Examination of parasites on the fins was carried out using the techniques of Emere and Egbe, (2006) and Omeji *et al.*, (2010). Fins were firstly examined by the naked eye for detection of any macroscopically visible lesions using hand lens. Samples of mucus were later scraped gently from the fins using scalpel blade. The tissues were placed on a Petri-dish containing 3mls of 0.9% saline solution and stirred using a mounted pin. Some drops of the mixed solution were collected using dropper, placed on a clean slide and freshly examined under phase-contrast microscope.

2.5 Examination for parasites in the blood, liver, kidney, stomach and intestine of the sampled fishes.

Blood: Examination for parasites in the blood of the sampled fish was carried out using the method described by Eneyat *et al.*, (2011). Blood samples were collected from the arteria caudalis (caudal region (vein) and hearts) using heparinized syringes. Thin blood films were thereafter made by placing a drop of blood on clean slide, spread on, air-dried, fixed with absolute methanol and then stained with 10% Giemsa stain for (20-30) minutes.

$$i. \quad \text{Prevalence (\%)} = \frac{\text{No of fish Infected}}{\text{No of fish Examined}} \times 100$$

$$ii. \quad \text{Mean Intensity} = \frac{\text{Total No of Parasites}}{\text{No of fish infested}}$$

$$iii. \quad \% \text{ parasite load on each location} = \frac{\text{No. of each parasite}}{\text{Total no. of parasites observed}} \times 100$$

$$iv. \quad \% \text{ host part infested} = \frac{\text{No. of each host part infested}}{\text{Total no. of all parts of the host infested}} \times 100$$

Statistical Analysis

Chi – square was used for differences in parasite load between the sexes and sapling sites. Correlation matrix was used to establish the

Liver and Kidney: Examination for parasites in the livers and kidneys was carried out using the method of Adam *et al.*, (2009). Impression smears were prepared from the livers and kidneys of the sampled fishes after proper dissection of the fish samples and allowed to dry for 20-30 minutes. They were later examined under microscope for parasites.

Stomachs and intestines: Examination of fish parasites in the stomachs and intestines was carried out using the techniques of Bich and Dawaki (2010). The abdominal cavity of each fish was cut open and the gastrointestinal parts was removed and cut into parts. The gastrointestinal parts were separated from the other visceral organs and placed in petri dishes containing physiological saline. The intestines were further carefully slit open to aid the emergence of the parasites. The emergence of any worm was easily noticed by its wriggling movement in the saline solution. Some of the worms however remained permanently attached with their attachment organs to the gut walls. They were carefully removed and put into the physiological saline.

2.6 Determination of parasitic indices

Parasitic indices (prevalence (%), and mean intensity, percentage parasite load on each location and percentage frequency of occurrence of each parasite species per location in fish were calculated according to [15] as thus;

relationship between weight, length and the total number of parasites in the infested fishes using SPSS, version 16.0.

RESULTS

The mean total length, weight and condition factor (K) of the *P. annectens* in dry and rainy seasons are presented in Table 1 while Table 2 shows the results of the regression co-efficients of *Protopterus annectens* in dry and rainy seasons. In dry season, the total length, total body weight and condition factor of *P. annectens* ranged from 21.00-58.50, 109.52-1148.40 and 0.37-2.34 with the mean total length, total body weight and condition factor of 36.25 ± 0.67 , 515.60 ± 25.90 and 0.91 ± 0.023 , respectively while in rainy season, the total length, total body weight and condition factor ranged from 21.70-55.80, 104.30-1093.70 and 0.39-1.95 with the mean total length, total body weight and condition factor of 34.74 ± 0.63 , 460.60 ± 24.20 and 0.91 ± 0.019 , respectively. There was no significant difference between the mean total length, total body weight and condition factor of the fish sample in the two seasons. There was

no significant difference between the mean total length, total body weight and condition factor of the fish samples in the two seasons.

The regression co-efficients (r) of *P. annectens* for male, female and combined sexes in dry season are 0.934, 0.930 and 0.931 with “b” values of 3.058, 3.122 and 3.090, respectively. Contrastingly in rainy season, the regression co-efficients (r) of *P. annectens* are 0.934, 0.940 and 0.936 with 3.094, 3.027 and 3.053, respectively. There was no significant difference ($p > 0.05$) in “r” values of the sexes of *P. annectens* in the two seasons and also, there was no significant difference ($p > 0.05$) in “b” values of the sexes of *P. annectens* in the two seasons.

Table 1. Mean Total length and Weight of *Protopterus annectens* in dry and rainy seasons.

Parameters	Seasons		P-values
	Dry season	Rainy season	
Mean total length	36.25 ± 0.67	34.74 ± 0.63	0.099
Mean weight	515.60 ± 25.90	460.60 ± 24.20	0.122
Mean condition factor	0.91 ± 0.023	0.91 ± 0.019	1.000

Table 2. Regression co-efficients of *Protopterus annectens* in dry and rainy seasons

Sex	Dry season			Rainy season		
	A	b	R	A	b	R
Male	-2.173	3.058	0.934	-2.185	3.094	0.933
Female	-2.240	3.122	0.930	-2.115	3.027	0.940
Combined sexes	-2.205	3.090	0.931	-2.140	3.053	0.936

a = Regression constant, b = Regression co-efficient, r = Correlation co-efficient

Out of the 400 samples of *P. annectens* used for the study in dry and rainy seasons, 273 (68.25%) were not infested while 127 (31.75%) were infested with 242 different parasites. Of the total parasites, 123 (50.83%) parasites were recorded in dry season from 134 (67.00%) infested fish samples while 106 (48.40%) parasites were recorded from 64 (32.00%) infested fish. Of the parasites species, *Contracaecum sp* was the most prevalent (34.15%) in dry season. This parasite accounted for 18.70% in the intestine and 15.45% in the stomach while *Eustrongyloids sp* was the most prevalent (27.36%) in rainy season accounting for 22.64% in the intestine and 4.72% in

the stomach. The least prevalent parasite load (3.25% in dry season and 2.83% in rainy season) was recorded for *Trichodina sp* (Fig. 1). Among the body organs of the fish samples in both seasons, intestine recorded the highest percentage parasite load (50.41%) in dry season while stomach had the highest percentage parasite load (49.06%) in rainy season. The least (3.25% in dry season and 2.83% in rainy season) were recorded for skin (Fig. 2). Generally, intestine had the highest percentage parasite load (98.52) with 50.41 in dry season and 48.11 in rainy season. There was no parasite on the fins and lung of the fish samples.

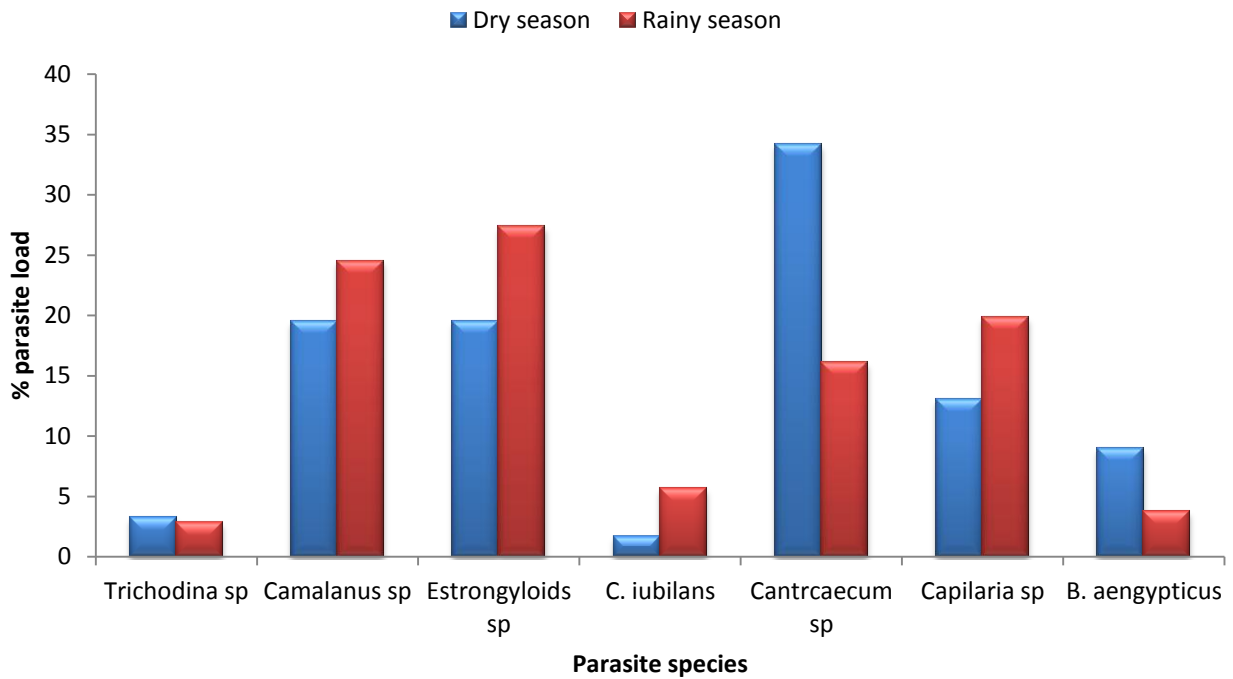


Fig. 1: Percentage parasite species spectrum in *P. annectens* from Lower River Benue

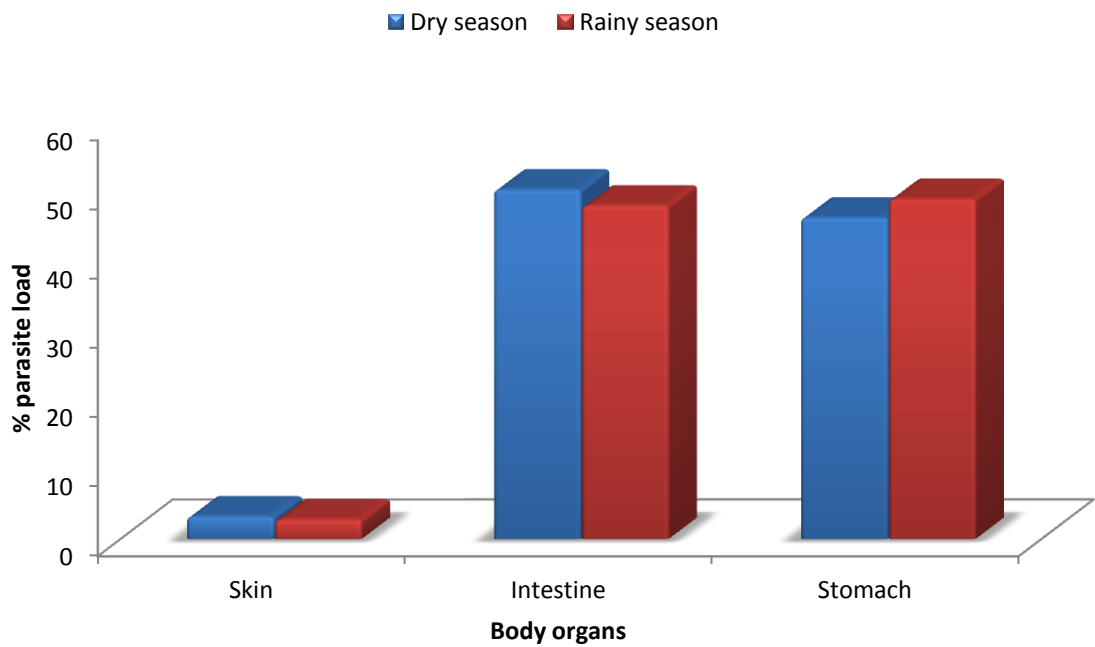


Fig. 2: Percentage parasite load per body organs of *P. annectens* from Lower River Benue

Results of the relationship between sex and percentage parasite infestation in *P. annectens* in dry and rainy seasons from Lower River Benue are shown in Figure 3. Female *P. annectens* from both seasons had more percentage parasite loads

(61.79% in dry season and 53.77% in rainy season) than the male *P. annectens* in both seasons (38.21% in dry season and 46.23% in rainy season).

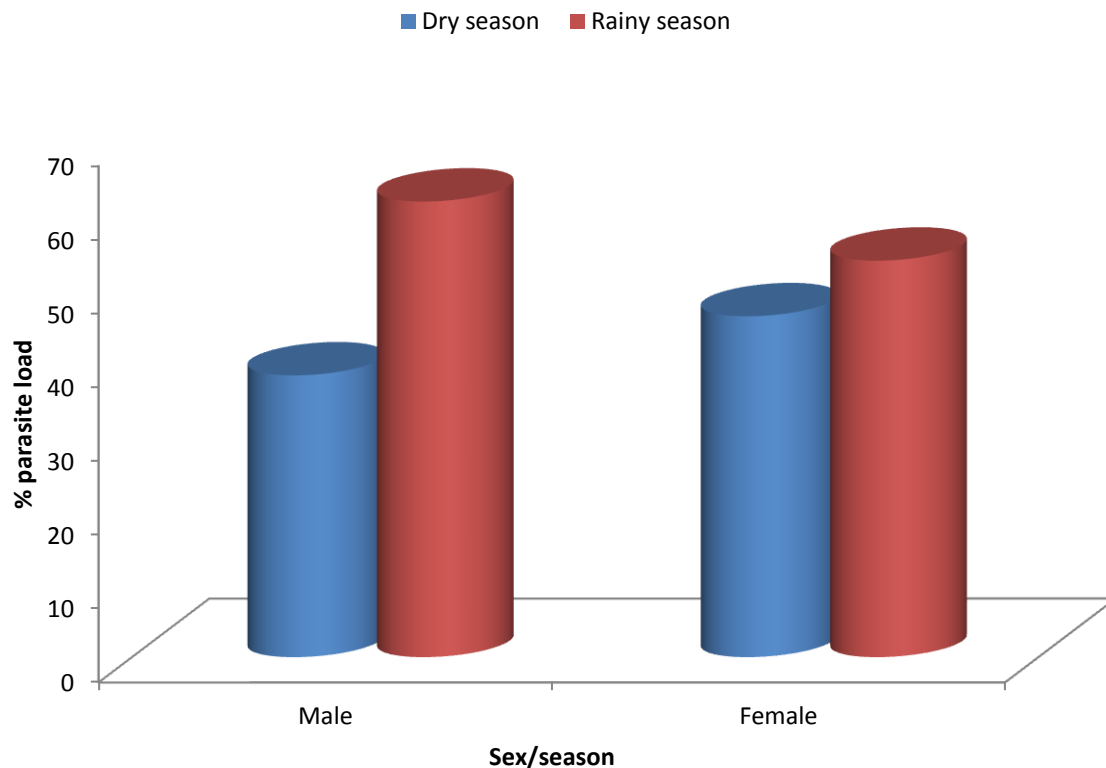


Fig. 3: Relationship between sex and parasite infestation in *P. annectens* in dry and rainy seasons from Lower River Benue

The size (Total length in cm) distribution and percentage parasite infestation in *P. annectens* in dry and rainy seasons from Lower River Benue are presented in Table 3 while Table 4 shows the relationship between body weight (g) and percentage parasite infestation in *P. annectens* in dry and rainy seasons from Lower River Benue.

In dry season, range in length of 40.1-49.0cm had the highest percentage parasite load while the lowest percentage parasite load was recorded in the length group of 58.1-67.0cm. no parasite was recorded in the length group of 22.0-31cm. Contrastingly in rainy season, the highest percentage parasite load (37.40%) was recorded in

the length group of 45.1-54.0 while the lowest percentage parasite load was recorded in the length group of 21.0-29.0cm.

From Table 4, the highest percentage parasite load (37.40%) in dry season was recorded in the weight range of 549.1-769g while the lowest percentage parasite load was recorded in the weight range of 329.1-549g. Contrastingly in rainy season, the highest percentage parasite load (51.89%) was recorded in the weight range of 319.1-534.0g while the lowest percentage parasite load (2.83%) was recorded in the weight range of 964.1-1179.0g.

Table 3: Size (Total length in cm) distribution and percentage parasite infestation in *P. annectens* in dry and rainy seasons from Lower River Benue

Dry season		Rainy season	
Range in length (cm)	% parasite load	Range in length (cm)	% parasite load
22.0-31	0.00	21.0-29	9.76
31.1-40	26.83	29.1-37	4.07
40.1-49	34.96	37.1-45	37.40
49.1-58	30.08	45.1-54	34.15
58.1-67	8.13	54.1-63	14.63

Table 4: Relationship between body weight (g) and percentage parasite infestation in *P. annectens* in dry and rainy seasons from Lower River Benue

Dry season		Rainy season	
Range in weight (g)	% Parasite load	Range in weight (g)	% parasite load
109.0-329.0	9.76	104.0-319.0	6.60
329.1-549.0	4.07	319.1-534.0	51.89
549.1-769.0	37.40	534.1-749.0	17.92
769.1-989.0	34.15	749.1-964.0	20.75
989.1-1209.0	14.63	964.1-1179.0	2.83

The correlation matrix for the total number of parasites found on *P. annectens* by size in dry and rainy seasons is shown in Table 5. There was a high correlation (0.917) in dry season (0.922) in rainy season between the total length and weight of fish samples. Low positive correlations (0.113 and 0.116) in dry season occurred between total

length (TL) and total number of parasite (TNP) and total number of parasite (TNP) and the weight of the fish respectively but in rainy season, low negative correlations (-0.101 and -0.070) existed between total length (TL) and total number of parasite (TNP), total number of parasite (TNP) and weight (WT) of the fish samples.

Table 5: correlation matrix for total number of parasites found on *p. Annectens* by size in dry and rainy seasons.

	<i>P.annectens</i> in dry season			<i>P.annectens</i> rainy season		
	TL	TNP	WT	TL	TNP	WT
TL	1.00			1.00		
TNP	0.113	1.00		-0.101	1.00	
WT	0.917	0.116	1.00	0.922	-0.070	1.00

DISCUSSION

The recovery of the different parasites belonging to different parasite groups observed in this present study from the different body organs of the fish species is not surprising as they have been recorded previously from the species or related species elsewhere (Paperna, 1996).

Henneguya sp was obtained as whitish cyst of different sizes which were attached to aborescent organ, few were observed on the gill filament of infected fish. Oniye *et al.*, (2004) reported high prevalence of the parasite in different fish species from various fish farms and rivers, respectively, in Zaria, Kaduna State. Haladu (2003) reported similar incidence in Tiga dam, Kano.

Infestation of the different body parts of *C. garipepinus* by protozoan parasites had been reported by Omeji *et al.*, (2010) and Omeji *et al.*,

(2011) from River Benue, Bichi and Yelwa (2010) Emere and Egbe (2006) from River Kaduna. Awa *et al.*, (1996) as well as Adeyemo and Agbede (2008) reported infestation of *Tilapia* species by trematodes. Amed (2007) observed higher prevalence of trematodes and Cestodes in *tilapia* species than in all the other species examined. Infestation of *O. niloticus* by protozoa and trematode parasites had also been reported by Bichi and Yelwa (2010).

The present study revealed that *Trichodina* species appeared on the skin, *C. iubilans* was found only in the stomach, while *Camalanus* sp, *Eustrongyloids* species, *D. latum*, *Contracaecum* species, *C. philipinensis*, *Bothriocephalus aegypticus* appeared in the intestine and stomach of the fish samples.

The highest number of parasites recorded by intestine compared to the other body organs could

be attributed to the fact that most digestion activity takes place in the intestine which could lead to the release of parasite ova/cysts in food items. This agrees with Onyedineke *et al.*, (2010) who reported higher number of helminth parasites in the intestine of some freshwater fish from River Niger at Illushi Edo State. Similarly, Dankishaya *et al.*, (2013) reported higher number of parasites in the intestine than the stomach and attributed that to several factors among which is the presence of digested food there or due to the greater surface area presented by the intestine. Reduced number of the parasites in the stomach of the fish samples compared to the intestine might be due to the muscular movement of the stomach, hydrochloric acid nature of the stomach and its omnivore habit (Akinsanya *et al.*, 2008).

Incidence and intensity of parasitism varied with the seasons being more prevalent in the dry season (48 %) than the rainy season (47.04%). In the dry season, which roughly corresponds to the dry phase of the hydrological cycle, there was virtually no precipitation and the flow and volume of water were very much reduced, resulting in much higher contact between the parasites and host fish leading to relatively higher prevalence that was observed during the dry season. similar observation had been made by Yakubu *et al.*, (2002) who reported 59% in his comparative study of gut helminths of *Tilapia zilli* and *Clarias gariepinus* from River Uke, Plateau State, Nigeria.

The overall percentage parasite infestation between the sexes indicated that the female fish had more percentage parasite infestation (51.66%) in dry season and (51.58%) in rainy season than

the male fishes (48.34%) in dry season and (48.42%) in rainy season. Differences in the incidence of infestation between the female and male fish could be due to the physiological state of the females, as most female fishes could have had reduced resistance to infestation by parasites than the male fishes. In addition, their increased rate of food intake to meet the development of their eggs might have exposed them to more contact with the parasites, which subsequently increased their chance of being infested. This agrees with Dankishaya *et al.*, (2013) who reported higher prevalence of helminth parasites in the gastrointestinal tract of wild female African sharptooth catfish (*Clarias gariepinus*) in Gwagwalada, Abuja, Nigeria than the male fishes, Omeji *et al.*, (2011) who reported higher parasite infestation in female *C. gariepinus* than male from cultured and wild environments in Benue State.

It was generally observed during the study period that small fish samples had lower percentage parasite infestation than the big fishes in both seasons. The higher incidence of infestation obtained in big fishes compared to small fishes is an indicator that size of the fish is important in determining the parasite load. Geets and Oliver (1996) and Tachia *et al.*, (2012) also reported increase in the abundance of parasites with host size. Aisoke *et al.*, (1992) reported that number of parasites and its diversity increase with size of fish, Mohammed *et al.*, (2009) also reported that prevalence was found to increase as the fish grows, and that could be attributed to the longer time of expose to the environment by body size.

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