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Survey of Helminth Parasite Species Infecting Anurans of Azuabie and Woji Communities, Port Harcourt, Rivers State, NigeriaAmuzie, C.C.^{*1} and Wokem, G.N.²

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Abstract

A survey of Azuabie and Woji communities in Port Harcourt, Rivers State, Nigeria, was conducted for a period of seven months with the view of identifying the anuran species and their associated gastro-intestinal helminth parasites in both locations. Standard sampling protocols were adopted and identification of both the host and parasite species was accomplished using appropriate keys. A total of three anuran species were recovered (*Sclerophrys maculata*, *Ptychadena pumilio* and *Hoplobatrachus occipitalis*). Seven helminth parasite species were recovered from the hosts, consisting of three nematodes, one trematode, two cestodes and one monogenean. Overall prevalence of infection was 82.86% and 97.14% at Azuabie and Woji, respectively. More parasite species (Azuabie = 6; Woji = 5) were recovered during the dry season than the rainy season (Azuabie = 4; Woji = 3). Likewise, more male (Azuabie = 27; Woji = 26) than female hosts (Azuabie = 8; Woji = 9) were infected. However, differences in prevalence of infection were not significantly affected by both season (Azuabie, $p=1.4204E-50$; Woji, $p= 6.2638E-23$) and gender (Azuabie, $p=2.9966E-288$; Woji, $p= 2.9956E-288$). Although diversity of both anurans and their helminth parasites was poor, there was high parasite endemicity in both communities. This research has added to the growing literature of species of both anurans and their helminth parasites found in parts of the Niger Delta Region of Nigeria.

Key words: Helminth parasites, gastro-intestinal, amphibians, Niger Delta, Port Harcourt.

Introduction

The order Anura includes frogs and toads (Raven and Johnson, 2002) and makes up over 90% of amphibians (McDiarmid and Mitchell, 2000). They are used as food items and for the treatment of

wounds and ailments like tuberculosis (Amuzie and Akani, 2017; Imkongwapang *et al.*, 2012; Anosike and Keke, 2002). They are hosts to several parasites (Aisien *et al.*, 2017; Gonzalez *et al.*, 2012; Densmore and Green, 2007; Pough *et al.*, 2001) as well as some pathogenic micro-organisms (Douglas and Amuzie, 2017).

Pough *et al.* (2001) reported that helminth parasites are the most common parasites of amphibians. These include nematodes, trematodes and cestodes (Densmore and Green, 2007; Patterson-Kane *et al.*, 2001). Hirudinians and pentastomids have also been reported infesting amphibians (Tinsley, 1995). Koprivnikar *et al.* (2012) stated that amphibian helminth parasites exhibited preference for certain hosts based on their ecology. For instance, nematodes were reported to be dominant in more terrestrial amphibian species while trematodes were dominant in more aquatic ones. Reports on helminth parasites of anurans from several locations in Nigeria revealed that nematodes were dominant over trematodes, with cestodes and monogeneans occurring less frequently (Aisien *et al.*, 2017; 2004; 2001; Amuzie *et al.*, 2016). This paper examines the helminth parasites infecting anuran species collected from locations in Port Harcourt, Nigeria, in order to add to the growing literature on the parasitic helminth infections of anurans from the Niger Delta Region of Nigeria.

Materials and Methods

Study Area: Two communities, Azuabie (N04°48.682', E007°03.112') and Woji (N04°49.385', E007°03.710'), were surveyed for the research. The location at Azuabie Community was by the banks of Azuabie River which was used by the residents for disposal of household and biological wastes. Vegetation around the river bank was composed of tall grasses including *Panicum maximum*.

Woji community is a residential location. There were domestic waste dump sites in the neighbourhood; economic (*Carica papaya*, *Citrus sinensis* and *Mangifera indica*) and medicinal plants (such as, *Occimum gratissimum* and *Vernonia amygdalina*), were common. Also, *Commelina nudiflora*, *Eragrostis tenella* and *Panicum maximum* grasses were abundant there.

Sampling Protocol: Both locations were surveyed for a period of seven months, June to December, 2006. Monthly searches were made at each location between the hours of 7.00pm to 10.00pm, with the aid of torches, as described by Andreone *et al.* (2003). For conservation purposes, a predetermined number of five specimens were collected from each location during each sampling expedition.

Laboratory examination of specimens: Specimens were taken to the Environmental Parasitology Laboratory of the Department of Animal and Environmental Biology, Rivers State University, Port Harcourt, Nigeria, in wet, perforated plastic buckets. They were euthanized in chloroform vapour, weighed using a top-load electronic balance (Model: OHAUS Scout 11), and their snout-vent length measured using a metre rule.

Anurans were identified according to Rodol (2000) and Minter *et al.* (2004) technique. Voucher specimens were preserved in 10% formalin and deposited in the museum of the Department of Animal and Environmental Biology, Rivers State University, Port Harcourt, Nigeria.

Gastro-intestinal tract helminths of each specimen were collected by dissecting the ventral surface of the organism to reveal its intestinal tract and body cavity. The tract was cut into the regions: oesophagus/stomach, small intestine, and large intestine/rectum. Each of these sections were kept in separate Petri dishes containing 0.72% normal saline and severed to expose the contents.

Nematodes were stretched in hot 70% alcohol and fixed in fresh 70% alcohol, while trematodes and cestodes were flattened in 5% formol saline between a microscope slide and a cover slip for about 15 minutes and then fixed in the same solution. Parasites were identified according to Prudhoe and Bray (1982). Parasite quantitative measures, prevalence and mean intensity, were computed according to Bush *et al.* (1997).

Statistical Analysis: Data was entered into MS

Excel. Statistical differences between prevalence of infection between locations and based on gender and seasons in each location were computed using Chi-square test.

Results

Three anuran species were collected from each of both locations in the study area namely: *Sclerophrys maculata*, *Ptychadena pumilio* and *Hoplobatrachus occipitalis*. A summary of their morphometric characteristics is presented in Table 1. At Azuabie, the anuran species counts were as follows: *S. maculata*, sixteen; *P. pumilio*, thirteen and *H. occipitalis*, six. *Sclerophrys maculata* from this location was infected with two nematode parasite species (*Amplificum africanum* and *Cosmocerca ornata*) and one digenetic trematode (*Mesocoelium monodi*). *Hoplobatrachus occipitalis* was infected with only a nematode, *Chabaudus leberrei* while *P. pumilio* was infected with two nematode parasite species (*Amplificum africanum* and *Cosmocerca ornata*), one digenetic trematode (*Mesocoelium monodi*), one monogenetic trematode (*Polystoma aeschlimanni*), and two cestode parasite species (*Cephalochlamys compactus* and *Cylindrotaenia jaegerskioeldii*). Overall prevalence of infection at Azuabie was 82.86%.

At Woji, a total of thirty-two specimens of *S. maculata*, two of *P. pumilio* and one of *H. occipitalis* were collected. *Sclerophrys maculata* from this location was found to be infected with four species of helminth parasites comprised of one trematode (*M. monodi*), two nematodes (*A. africanum* and *C. ornata*), and one cestode (*C. jaegerskioeldii*). The *Ptychadena pumilio* specimens were infected with only nematode, *C. ornata*, whereas the only *H. occipitalis* collected was infected with only ten individual specimens of the cestode, *C. compactus*. Overall prevalence of infection at this location was 97.14%.

There was no statistically significant difference ($\chi^2_{0.05,11} = 299.36$, $p = 1.1946E-57$) in prevalence of the two communities. The overall prevalence and mean intensity of infection with the helminth parasites are presented in Table 2.

Prevalence and mean intensity of infection were also computed based on season and gender (Table 3 -6). More parasite species were recovered during the dry season than in the rainy season. For instance, both cestodes, *C. compactus* and *C. jaegerskioeldii*, were recovered during the dry season from both locations. Chi-square analysis showed no significant

differences between the prevalence rates of the parasites in both seasons at both locations (Azuabie, $\chi^2_{0.05,9} = 258.74$, $p = 1.4204E-50$; Woji, $\chi^2_{0.05,4} = 110.31$, $p = 6.2638E-23$).

At both locations, infection was more common in male host specimens than in the females. All the

helminth parasites recovered from both locations were present in male specimens, as opposed to the females. There was however, no significant difference between parasite prevalence based on gender at both locations (Azuabie, $\chi^2_{0.05,9} = 0$, $p = 2.9966E-288$; Woji, $\chi^2_{0.05,6} = 0$, $p = 2.9956E-288$).

Table 1: Summary of morphometric characteristics of anuran species from both study locations

Amphibian Species Examined	Azuabie				Woji			
	SVL (cm)		TBW (g)		SVL (cm)		TBW (g)	
	Mean±sem	Range	Mean±sem	Range	Mean±sem	Range	Mean±sem	Range
<i>S. maculata</i>	6.28±0.29	4.2-8.1	25.14±4.85	8.0-90.7	6.83±0.30	3.9-9.8	27.73±3.36	4.5-77.0
<i>H. occipitalis</i>	8.00±0.62	5.4-9.4	47.73±9.18	16.0-81.7	9.15±0.05	9.1-9.2	93.75±6.55	87.2-100.3
<i>P. pumilio</i>	4.93±0.37	3.5-6.0	15.20±6.00	4.3-36.1	5.35±0.45	3.5-5.8	11.5±2.40	9.1-13.9

Legend: SVL means snout-vent length; TBW means total body weight; sem = standard error of the mean

Table 2: Helminth species-related prevalence and mean density of anurans recovered from the study area

Recovered Parasite Species	No. of Hosts Examined (n)	Azuabie		Woji		
		No. Infected n(%)	MD (±sem)	No. of Hosts Examined (n)	No. Infected n(%)	MD (±sem)
CESTODA						
<i>C. compactus</i>	<i>P. pumilio</i> (13)	2 (15.38)	4.5±0.5	<i>H. occipitalis</i> (1)	1 (100.00)	10.0±0.00
<i>C. jaegerskioeldii</i>	<i>P. pumilio</i> (13)	1 (7.69)	7.0±0.00	<i>S. maculata</i> (32)	3 (9.38)	2.0±1.33
Total	Cestoda (16)	3 (11.54)	5.33±0.88	Cestoda (17)	4 (12.12)	4.25±2.14
MONOGENEA						
<i>P. aeschlimanni</i>	<i>P. pumilio</i> (13)	1 (7.69)	1.0±0.00			
Total	Monogenea (1)	1 (7.69)	1.0±0.00			

DIGENEAE

<i>M. monodi</i>	<i>S. maculata</i> (16)	6 (37.50)	23.83±13.28	<i>S. maculata</i> (32)	14 (43.75)	18.92±5.01
	<i>P. pumilio</i> (13)	7 (53.85)	12.71±5.05			
Total	Digenea (232)	13 (44.83)	17.85±6.58	Digenea (256)	14 (43.75)	18.29±5.01

NEMATODA

<i>A. africanum</i>	<i>S. maculata</i> (16)	14 (87.50)	4.29±1.08	<i>S. maculata</i> (32)	25 (78.13)	14.0±3.06
	<i>P. pumilio</i> (13)	8 (61.54)	2.88±0.77			
<i>C. leberrei</i>	<i>H. occipitalis</i> (5)	2 (33.33)	2.0±0.00			
<i>C. ornata</i>	<i>S. maculata</i> (16)	15 (93.75)	4.47±1.00	<i>S. maculata</i> (32)	23 (71.88)	6.35±1.25
	<i>P. pumilio</i> (13)	8 (61.54)	3.75±1.28	<i>P. pumilio</i> (2)	1 (100.00)	1.0±0.00
Total	Nematoda (186)	47 (74.60)	3.88±0.50	Nematoda (405)	49 (74.24)	9.42±1.74
Grand Total	Helminths (435)	64 (48.85)	6.69±1.50	Helminths (678)	67 (51.15)	11.11±1.74

Legend: MD means mean density; sem means standard error of the mean

Table 3: Seasonal effects on the prevalence and mean density of helminth parasites in anurans, Azuabie Community

Recovered Parasite Species	No. of Hosts Examined (n)	Rainy		Dry		
		No. infected n (%)	MD	No. of Hosts Examined (n)	No. infected n (%)	MD
CESTODA						
<i>C. compactus</i>				<i>P. pumilio</i> (13)	2 (15.38)	4.5±0.50
<i>C. jaegerskioeldii</i>				<i>P. pumilio</i> (13)	1 (7.69)	7.0±0.00
MONOGENEA						
<i>P. aeschlimanni</i>				<i>P. pumilio</i> (13)	1 (7.69)	1.0±0.00

DIGENEA						
<i>M. monodi</i>	<i>S. maculata</i> (14)	4 (28.57)	5.0±3.03	<i>S. maculata</i> (2)	2 (100.00)	61.5±21.5
				<i>P. pumilio</i> (13)	7 (53.85)	12.71±5.05
NEMATODA						
<i>A. africanum</i>	<i>S. maculata</i> (14)	12 (85.71)	4.83±1.19	<i>S. maculata</i> (2)	2 (100.00)	1.0±0.00
				<i>P. pumilio</i> (13)	8 (61.54)	2.88±0.77
<i>C. leberrei</i>	<i>H. occipitalis</i> (6)	2 (33.33)	2.00±0.00			
<i>C. ornata</i>	<i>S. maculata</i> (14)	14 (100.00)	4.71±1.04	<i>S. maculata</i> (2)	1 (50.00)	1.0±0.00
				<i>P. pumilio</i> (13)	8 (61.54)	3.75±1.28
Total	Helminths (150)	32 (66.67)	4.55±0.70	Helminths (285)	32 (38.10)	8.91±2.94

Legend: sem means standard error of the mean; MD means mean density

Table 4: Seasonal effects on the prevalence and mean density of helminth parasites in anurans, Woji Community

Recovered Parasite Species	No. of Hosts Examined (n)	Rainy		Dry		
		No. Infected n(%)	MD	No. of Hosts Examined (n)	No. Infected n(%)	MD
CESTODA						
<i>C. compactus</i>				<i>H. occipitalis</i> (1)	1 (100.00)	10.0±0.00
<i>C. jaegerskioeldii</i>				<i>S. maculata</i> (12)	3 (25.00)	2.33±1.33
DIGENEA						
<i>M. monodi</i>	<i>S. maculata</i> (20)	7 (35.00)	21.43±7.26	<i>S. maculata</i> (12)	6 (50.00)	17.67±7.27
NEMATODA						
<i>A. africanum</i>	<i>S. maculata</i> (20)	15 (75.00)	12.07±3.57	<i>S. maculata</i> (12)	10 (83.33)	16.9±5.57
<i>C. ornata</i>	<i>S. maculata</i> (20)	17 (85.00)	4.47±0.37	<i>S. maculata</i> (12)	7 (58.33)	6.14±1.75
				<i>P. pumilio</i> (2)	1 (50.00)	1.0±0.00
Total	Helminth (340)	39 (65.00)	9.19±1.90	Helminth (345)	29 (56.86)	12.32±2.79

Legend: sem means standard error of the mean; MD means mean density

Table 5: Gender related prevalence and mean density of helminth parasites in anurans, Azuabie Community

Recovered Parasite Species	No. of Hosts Examined (n)	Male		Female		
		No. Infected n (%)	MD	No. of Hosts Examined (n)	No. Infected n(%)	MD
CESTODA						
<i>C. compactus</i>	<i>P. pumilio</i> (10)	2 (20.00)	4.5±0.50			
<i>C. jaegerskioeldii</i>	<i>P. pumilio</i> (10)	1 (10.00)	7.0±0.00			
MONOGENEA						
<i>P. aeschlimanni</i>	<i>P. pumilio</i> (10)	1 (10.00)	1.0±0.00			
DIGENEA						
<i>M. monodi</i>	<i>S. maculata</i> (13)	3 (23.08)	2.0±0.58	<i>S. maculata</i> (4)	3 (75.00)	45.7±20.12
	<i>P. pumilio</i> (10)	5 (50.00)	8.4±4.35	<i>P. pumilio</i> (3)	2 (66.67)	23.5±13.5
NEMATODA						
<i>A. africanum</i>	<i>S. maculata</i> (13)	11 (84.62)	5.2±1.25	<i>S. maculata</i> (4)	3 (75.00)	1.0±0.00
	<i>P. pumilio</i> (10)	6 (60.00)	2.5±0.85	<i>P. pumilio</i> (3)	2 (66.67)	4.0±2.00
<i>C. leberrei</i>	<i>H. occipitalis</i> (1)	1 (100.00)	2.0±0.00	<i>H. occipitalis</i> (1)	1 (100.00)	2.0±0.00
<i>C. ornata</i>	<i>S. maculata</i> (13)	13 (100.00)	4.9±1.10	<i>S. maculata</i> (4)	2 (50.00)	1.5±0.5
	<i>P. pumilio</i> (10)	6 (60.00)	3.3±1.36	<i>P. pumilio</i> (3)	2 (66.67)	5.0±4.0
Total	Helminths (226)	49 (49.00)	4.43±0.63	Helminths (210)	15 (65.22)	14.00±5.92

Legend: sem means standard error of the mean; MD means mean density

Table 6: Gender related prevalence and mean density of helminth infections in anurans, Woji Community

Recovered Parasite Species	No. of Hosts Examined (n)	Male		Female		
		No. Infected n(%)	MD	No. of Hosts Examined (n)	No. Infected n(%)	MD
CESTODA						
<i>C. compactus</i>	<i>H. occipitalis</i> (1)	1 (100.00)	10.0±0.00			
<i>C. jaegerskioeldii</i>	<i>S. maculata</i> (23)	2 (8.70)	3.0±2.00	<i>S. maculata</i> (9)	1 (11.11)	1.0±0.00
DIGENEA						
<i>M. monodi</i>	<i>S. maculata</i> (23)	9 (39.13)	14.78±5.84	<i>S. maculata</i> (9)	5 (55.56)	24.6±9.50
NEMATODA						
<i>A. africanum</i>	<i>S. maculata</i> (23)	19 (82.61)	12.79±3.65	<i>S. maculata</i> (9)	6 (66.67)	16.17±6.31
<i>C. ornata</i>	<i>S. maculata</i> (23)	18 (78.26)	7.0±1.57	<i>S. maculata</i> (9)	4 (44.44)	4.0±0.91
	<i>P. pumilio</i> (2)	1 (50.00)	1.0±0.00			
Total	Helminths (539)	50 (52.63)	9.98±1.73	Helminths (237)	16 (44.44)	14.81±4.16

Legend: sem means standard error of the mean; MD means mean density

Discussion

The size range of the species was somewhat larger than the reports of Rodel (2000). For instance, the snout-vent length (SVL) of *S. maculata* in this research ranged from 3.9-9.8cm while Rodel (2000) reported adult specimens of the species to range from 3.8-6.0cm. The SVL of *H. occipitalis* and *P. pumilio* ranged between 5.4-9.4cm and 3.5-6.0cm respectively, while Rodel (2000) reported size ranges of 5.2-10.4cm and 2.5-3.6cm, respectively, for adult specimens. However, Rodel (2000) cited other authors including Poynton and Haacke (1993) who

reported specimens of *S. maculata* ranging in SVL from 4.5-8.5cm, Schiotz (1964) who found a female *H. occipitalis* measuring 16.0cm, and Poynton (1964) who reported a female specimen of *P. pumilio* of 4.1cm. The larger size of the anurans examined may be due to the longer wet season characteristic of the Niger Delta region associated with availability of food organisms, and the low diversity of parasites infecting them.

Most of the catch was composed of *S. maculata*. The bullfrog, *H. occipitalis* and the Ptychadenids are

considered a delicacy among the inhabitants of both locations. This is speculated to have contributed to the scarcity of both species of anurans in the study locations. However, the habitat structure of both locations also played a part in the predominant anuran species collected- they have both been altered by man. *Ptychadena pumilio* prefers altered vegetation composed of grasses; *H. occipitalis* prefers temporary pools common around bush paths while *S. maculata* are found in drier habitats around human habitations (Amuzie and Ekerette, 2017; Minter *et al.*, 2004; Rodel, 2000).

In other locations in Rivers State, Nigeria, Amuzie *et al.* (2016) found five anuran species (*S. maculata*, *S. camerunensis*, *H. occipitalis*, *P. oxyrhynchus* and *P. pumilio*) at Rumuji-Emohua, while Aisien *et al.* (2017) found nine amphibian species on the campus of Rivers State University, which were *Afrixalus fulvovittatus*, *Sclerophrys regularis*, *S. camerunensis*, *Hyperolius concolor* phase B, *Hyperolius concolor* phase C, *H. occipitalis*, *Phrynobatrachus* sp., *Ptychadena mascareniensis* and *Ptychadena pumilio*.

The composition of the parasitic helminth species, comprised of seven species, was rather poor as compared to that from other locations in the State. Amuzie *et al.* (2016) reported eleven helminth species (a pentastomid, *Raillietiella* sp.; a cestode, *C. jaegerskioeldi*; trematodes, *M. monodi* and *Diplodiscus fischthalicus*; and nematodes, *Rhabdias africanus*, *Rhabdias* sp., *A. africanum*, *Oswaldocruzia hoepflii*, *C. ornata*, and ascaridida larvae). Aisien *et al.* (2017) reported ten helminth species including two trematodes (*D. fischthalicus* and *M. monodi*), four nematodes (*A. africanum*, *C. ornata*, *C. leberrei* and *Rhabdias* sp.), one monogenean (*Polystoma pricei*), one cestode (*Cylindrotaenia* sp.) and acanthocephalan cystacanths. However, Amuzie and Ekerette (2017) also reported only the trematode, *M. monodi*, in the *Sclerophrys* species they examined, but in addition to the three nematodes reported in the present research, they also found *Oswaldocruzia hoepflii* and *Rhabdias africanus*. They did not find any cestodes or monogeneans but reported the presence of the tongue-worm, *Raillietiella* species. In comparison, Aisien *et al.*, (2009), reported eleven species of amphibians (*S. maculata*, *H. occipitalis*, *Aubria subsigillata*, *P. longirostris*, *P. oxyrhynchus*, *P. bibroni*, *P. pumilio*, *Chiromantis rufescens*, *Leptopelis hyloides*, *Hyperolius fusciventris* and a *Phrynobatrachus* sp.) and nineteen species of helminth parasites from a pristine forest reserve in

Southwestern Nigeria. The helminth parasites reported in that research included three monogeneans (*Metapolystoma cachani*, *Polystoma baeri*, and an unidentified specimen from *P. oxyrhynchus*), two cestodes (*C. jaegerskioeldi* and an encysted cestode), six trematodes (*Mesocoelium monas*, *D. fischthalicus*, *Metahaematoloechus micrurus*, *Pleurogenoides tener*, *Haematoloechus aubriae*, *Halipegus* sp.) and eight nematodes (*Rhabdias bufonis*, *C. ornata*, *C. leberrei*, *Camallanus dimitrovi*, *Physalopteroides* sp., *Applectana* sp., ascaridida larva and an unidentified nematode species). Thus, habitat structure, developmental activities and the nutritional potentials of anurans have effect on the composition of both amphibians and their helminth endo-parasites.

In consonance with other studies in Nigeria, nematodes were dominant in the helminthofauna of the amphibians examined (Aisien *et al.*, 2001, 2004, 2017; Amuzie *et al.*, 2016). The fact that more parasites were recovered during the dry season could be because infection was actually acquired in the rains when exposure rate was higher and environmental condition was more favourable for the anurans. In contrast, the dry period seemed to have favoured the multiplication of the already acquired parasites due to increased temperature, and reduced flood, typical of the Niger Delta Region, which is capable of flushing away helminth eggs and larvae. Most literature report higher infections during the rainy season because that is when the animals become active in search of food and mates (Begum and Banu, 2012; Chandra and Gupta, 2007). We observed that more male than female hosts were infected with the helminth parasites. Our finding is in agreement with the reports of Haman *et al.* (2010) who reported that the male hormone, testosterone, caused a reduction in the immune status of amphibians, thereby resulting into higher infection rates in male host specimens.

Conclusion

The research has revealed the presence of three anuran species and the seven helminth parasite species found infecting them. Although the findings presented low diversity of both anurans and their helminth parasites, the parasite endemicity and prevalence were high. It is likely that their presence was a serious risk factor to the survival of the anurans. Again, anthropogenic activities seemed to have adverse effects on the anurans and the parasite species that are able to parasitize them. The research has contributed to the growing knowledge of anuran species and their helminthofauna in parts of Rivers State, Nigeria.



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