Parasitofauna of ground-dwelling anurans from cocoa plantations in Ugboke, Edo State, Nigeria

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Abstract

The parasitofauna of ground-dwelling anurans from pesticide-treated cocoa plantations (CP) in Ojo Camp, Ugboke, Edo State of Nigeria were investigated and compared with those recovered from host specimens collected from the village settlement (VS). The anurans were caught by hand following visual or acoustic location. The anurans encountered in both the VS and the CP included Aubria subsigillata, Hylarana spp. (H. albolabris and H. galamensis), Sclerophrys spp. (S. maculata and S. regularis), Ptychadena spp. (P. aequiplicata, P. longirostris, P. mascareniensis, P. oxyrhynchus and P. pumilio) and Hoplobatrachus occipitalis. Hylarana galamensis, Ptychadena spp. and Sclerophrys spp. were encountered in the VS and the CP while Aubria subsigillata, H. albolabris and H. occipitalis occurred only in the CP. The helminth parasites recovered included four cestode species (adult of Cylindrotaenia jaegerskioeldi and three encysted proteocephalid larvae), five Polystoma spp. 11 species of digeneans and 19 nematode species. More parasite species were recovered from toads collected from the VS; parasite prevalence was generally low in both habitats but the intensity of infection was higher in the specimens collected from the VS. Although A. subsigillata and H. occipitalis both occurred in the CP, A. subsigillata was the more susceptible host of the two, harbouring 16 helminth parasites as against four from H. occipitalis. Polystomes were recovered from H. albolabris and H. galamensis in addition to Diplodiscus fischthalicus and Mesocoelium spp. Infections occurred mostly among the Ptychadeniidae collected from the CP, with prevalence ranging from 12.5% to 100% and infection intensity from 1.0 to 13.0. The generally low parasite burden in anurans from the CP can possibly be attributed to the pesticide contamination of this habitat which may have hindered the development of the free-living stages of parasites in this milieu.

Keywords: Anurans; cocoa plantation; pesticides; parasitofauna; prevalence; intensity.

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Introduction

Agriculture remains the largest proportion of human land use in the tropics because it constitutes the economic mainstay of many African countries. Farming activities range from small subsistence holdings by families to plantations owned by wealthy individuals and multinational organizations. Monoculture plantations (Oil Palm, Rubber, Cocoa and Coffee) alter the landscape in a major way, as it involves the removal of natural forests with their assorted flora to make way for a single crop that in most cases do not provide adequate cover for the animals that live in this new environment. Cocoa-farming is a major economic activity in the south-west and south-south geopolitical zones of Nigeria, most of which lie in the rainforest zone of the country, a biotope known to support high diversity of amphibians (Meijaard et al 2005). Although cocoa plantations alter the natural landscape, they are also known to harbour diverse species of amphibians which find safe haven in the diverse habitats provided by the native and cocoa trees, the deep leaf litter on the plantation floor and other microhabitats therein (Texeira *et al* 2015). However, the frequent use of pesticides in controlling cocoa pests and diseases impacts negatively on amphibian diversity and health, and also affects the survival and transmission of free-living stages of amphibian parasites to their hosts (Pietrock and Marcogliese, 2003).

The aim of this study was to determine the pattern of helminth parasitic infection in anurans from cocoa plantations in southern Nigeria and to determine the possible effect of pesticide use on the parasite transmission dynamics in the different microhabitats within the cocoa plantation. The anurans under consideration include the ground-dwelling, the arboreal (tree frogs) and those inhabiting the leaf litter on the plantation floor. An earlier publication (Edo-Taiwo and Aisien, 2020) examined the helminth parasitic infections of leaf litter frogs (*Arthroleptis* and *Phrynobatrachus* spp.) from cocoa plantations and the village settlement at Ojo Camp of



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Ugboke in Edo State, Nigeria. The present paper deals specifically with the parasites of ground-dwelling anurans collected from the same locality.

Materials and methods

Study area

The study was conducted in a number of contiguous cocoa plantations at Ojo Camp, Ugboke in Ovia North-East Local Government Area of Edo State, Nigeria (lying between 6°32 and 6°45N; 5°15 and 5°17E) covering a total area of 28.522 km², at an altitudinal range of 28.5 to 64.6 m above sea level (Figure 1). The area is a transitional vegetation, consisting partly of derived savannah and partly of rainforest. The plantations had the characteristic strata of canopy trees, made up of an upper canopy of native trees, followed by the cocoa trees and the undergrowth made up of shrubs, especially around the streams and rivulets within the plantations. The wet season in the area is from April to October while the dry season lasts from November to March. There is a dry harmattan spell between December and mid February. Monthly temperature ranged from 25°C to 29°C, with a mean temperature of 26°C during the sampling period. Besides cocoa farming, logging activities also takes place in the study area, with the haulage trucks passing through parts of the plantations. The depressions created by the wheels of these trucks served as water retention points which formed aggregation points for amphibians. As a pest control measure, the cocoa trees were sprayed with pesticides (Gammalin, Avesthrin (Cypernithrin 10% EC), Scorpion, Best, Instakill and Ridonul Gold 66WP). The herbicide, Weed Crusher was used by the farmers to eliminate weeds.



Figures 1A-C. Maps of Nigeria, Edo State, and the studyarea showing the sampled-locations.

Amphibians were collected from the village settlement and cocoa plantations from August 2012 to October 2013, during the wet and dry seasons using the Visual Acoustic Encounter Surveys method (Crump and Scott 1994). The amphibians were collected by hand at night between 7.00

pm and 1.00 am). The specimens collected were transported to the laboratory in plastic bottles with 2 to 5 ml of water and covered with perforated screw caps. The anurans were identified using appropriate protocols (Roedel 2000, 2007), euthanized with Benzocaine solution and the snout-vent length (SVL) measured. The specimens were dissected and the various sections of the gastrointestinal tract (oesophagus/stomach, small intestine, large intestine/rectum) were isolated and transferred to Petri dishes containing 0.72% NaCl solution. Other organs examined included the lungs, liver/gall bladder, urinary bladder and the body cavity. The parasites recovered from these organs were isolated and preserved using appropriate procedures. The flatworms (cestodes, monogeneans and digeneans) were flattened under cover slip pressure, fixed and preserved with 5% formol-saline. Nematodes were fixed with hot 70% ethanol and preserved in fresh preservative. Acanthocephala cysthacanths were preserved in 70% ethanol.

The flatworms were washed free of the preservative (5% formol-saline) and stained with a dilute solution of acetocarmine. The parasites were washed to remove excess stain and then dehydrated in alcohol series, cleared in xylene and mounted in Canada balsam. Nematodes were cleared in lactophenol and examined as temporary mounts under a binocular microscope. Parasites were identified with appropriate keys (Yamaguti 1961, 1971; Prudhoe and Bray 1982; Khalil *et al* 1994). Photomicrographs were taken using the Imaging Source Microscope Digital Camera (DFK MKU 130-10x22) attached to a binocular research microscope.

Results

The ground-dwelling anurans collected either from the cocoa plantation (CP) and the village settlement (VS) at Ojo Camp included *Sclerophrys* spp. (*Sclerophrys* maculata and *S. regularis*), *Ptychadena* spp. (*P. aequiplicata*, *P. longirostris*, *P. mascareniensis*, *P. oxyrhynchus* and *P. pumilio*), *Hylarana* spp. (*H. albolabris* and *H. galamensis*), *Aubria subsigillata* and *Hoplobatrachus occipitalis*. The sites of infection in these hosts are presented in Table 1.

A total of 212 S. maculata (53 from the CP and 159 from the VS) and 116 S. regularis (4 from the CP and 112 from the VS) were examined. The prevalence and mean intensity of parasites in these toads are shown in Table 2. The helminth parasites recorded in these bufonids included Cestoda: adults of Cylindrotaenia jaegerskioeldi, Ophiotaenia sp. larva and Proteocephalus sp. 2 larva; Monogenea: Polystoma africanum; Digenea: Mesocoelium spp. (tentatively designated as spp.1-6); Nematoda: Amplicaecum africanum, Amplicaecum sp., Aplectana sp., Cosmocerca commutata, C. ornata, Foleyellides sp., Physaloptera sp., Oswaldocruzia hoepplii, Rhabdias africanus and an Ascaridida larva. Among the cestodes, C. jaegerskioeldi was recorded only in toads collected from the VS with low prevalence and infection intensity (Table 2). Ophiotaenia sp. larva (Figure 2A)

Table 1: Parasites of ground-dwelling anurans from Ojo Camp, Ugboke, Edo State, Nigeria.

Parasites	Host	Site of infection
Acanthocephala		
Acantocephala cystacanth	A. subsigillata	Body cavity
Costada	in subsignitud	
	G I I	0 11
Cylindroteania jäegerskiöeldi	S. maculata	Small intestine
	S. regularis	Small intestine
	P. longirostris	Small intestine
Onhiotaenia sp. larva	S regularis	Attached to liver stomach and small intestine
Dustassenhalus en 1 (lamus)	D. mumilia	Attached to inver, stomating and liver
<i>Froieocephalus</i> sp. 1 (laiva)		Attached to small mestile and liver
Proteocephalus sp. 2 (larva)	S. maculata	Attached to small Intestine
	A. subsigillata	Attached to small and Large intestines
	P. aequiplicata	Attached to small intestine and liver
Monogenea	1 1	
Robertoma accellinguni	D numilio	Uringry bladder
Torystoma aeschumanni .		
P. africanum .	S. regularis	Urinary bladder
P. ebriensis	P. aequiplicata	Urinary bladder
P. galamensis.	H. galamensis	Urinary bladder
P nerreti	H albolabris	Urinary bladder
Diamaa	11. 4100140115	erinary bladder
Digenea	** ** * * *	• • • • • • • •
Diplodiscus fischthalicus	H. albolabris	Large intestine/rectum
	P. pumilio	Large intestine/rectum
Metahaematoloechus aubriae	A. subsigillata	Lungs
M micrurus	H occinitalis	Lings
II. Incrurus .	D. munilio	Ossenhagua/atamash
Haupegus sp.	P. pumilio	Oesophagus/stomach
Mesocoelium sp. 1	S. maculata	Small intestine
	S. regularis	Small intestine
	A. subsigillata	Small and large intestine
	H galamonsis	Small intestine
	D	Small intestine
	P. oxyrnynchus	Small intestine
	P. pumilio	Small intestine
Mesocoelium sp. 2	S. maculata	Small intestine
	S. regularis	Small intestine
	A subsigillata	Small and large intestine
	II. alkolahuia	Small intesting
	H. albolabris	Small intestine
	H. occipitalis	Small intestine
	P. aequiplicata	Small intestine
	P. mascareniensis	Small intestine
	P orvrhynchus	Small intestine
	D pumilio	Small intestine
Mesocoelium sp. 3	S. maculata	Small intestine
	S. regularis	Small intestine
	A. subsigillata	Small and large intestine
	P aequiplicata	Small intestine
	P masaaraniansis	Small intestine
	1. muscuremensis	
	P. oxyrnynchus	Small intestine
Mesocoelium sp. 4	S. maculata	Small intestine
	S. regularis	Small intestine
	A. subsigillata	Small and large intestine
	H galamensis	Small intestine
	D acquimiticata	Small intestine
	P. aequipiicaia	
	P. longirostris	Small intestine
	P. oxyrhynchus	Small intestine
Mesocoelium sp. 5	S. maculata	Small intestine
*	S. regularis	Small intestine
	A subsigillata	Small and large intestine
	A. subsignation	
	P. aequiplicata	Small intestine
Mesocoelium sp. 6	S. maculata	Small intestine
	S. regularis	Small intestine
	A. subsigillata	Small and large intestine
	H occipitalis	Small intestine
		Sman musulu Small intesting
	n. gaiamensis	
	P. aequiplicata	Small intestine
	P. longirostris	Small intestine
	P. mascareniensis	Small intestine
	P oxyrhynchus	Small intestine
	D mumilia	Small intesting
	r. pumillo	Small mitesune

Table 1 ((cont'd):	Parasites of g	ground-dwelling	g anurans from C	ojo Camp	, Ugboke,	Edo State,	Nigeria.
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Nematoda		
Amplicaecum africanum.	S. maculata	Oesophagus/stomach and small intestine
	S. regularis	Oesophagus and small intestine
	A. subsigillata	Oesophagus/stomach
	H. galamensis	Small intestine
Amplicaecum sp.	S. maculata	Oesophagus and small intestine
	S. regularis	Oesophagus and small intestine
	A. subsigillata	Oesophagus/stomach
	H. galamensis	Small intestine
	H. occipitalis	Small intestine
	P mascareniensis	Oesophagus and small intestine
	D complementaria	Occophagus and sman mestine
	P. oxyrnynchus	Oesophagus
	P. pumilio	Oesophagus and small intestine
Aplectana sp.	A. subsigillata	Large intestine/rectum
	H. albolabris	Large intestine/rectum
	S. maculata	Small and large intestine
	S regularis	Small and large intestine
	H aglamonsis	Large intestine/rectum
	n. guiamensis	
	P. aequiplicata	Large intestine/rectum
	P. oxyrnynchus	Large intestine/rectum
	P. pumilio P. maga anonionaia	Earge intestine/rectum
A	P. mascareniensis	De de acciter
Ascaridida larva 1	H. galamensis	Body cavity
	P. mascareniensis	Body cavity
Association longer 2	S. regularis	Body cavity
	n. albolabris	Lorge intesting/regtum
Cosmocerca commutata	S. maculala	Large intestine/rectum
C. omaia.	n. albolabris	Small and large intesting
	S. maculatia	Large intesting/reaturn
	S. regularis	Large intestine/rectum
	A. subsignata	Large intestine/rectum
	H aglamonsis	Large intestine/rectum
Folmullidae en 1	11. guidmensis	Pody covity
Poleyelides sp. 1	S. macularis	Body cavity
Folguellides sp 2	H aalamansis	Oesophagus/stomach
Folevellides sp. 2	A subsigillata	Body cavity
Aswaldocruzia komplij	S regularis	Small intestine
Oswalaberazia hoeppili	S. maculata	Small intestine
Paracosmocerca sp	H albolabris	Large intestine/rectum
Physalontera sp.	S maculata	Oesophagus/stomach
Thysulopiera sp.	S. nacularis	Oesophagus/stomach
	A subsigillata	Oesophagus/stomach
	H. galamensis	Oesophagus/stomach
	P. aeauiplicata	Oesophagus/stomach
	P. mascareniensis	Oesophagus/stomach
	P. oxyrhynchus	Oesophagus/stomach
	P. pumilio	Oesophagus/stomach
Rhabdias africanus	S. maculata	Lungs
	S. regularis	Lungs
<i>Rhabdias</i> sp. 1	H. albolabris	Lungs
Rhabdias sp. 3	H. galamensis	Lungs
Unid. oxyurid nematode	P. mascareniensis	Small intestine

occurred only in *S. regularis* from the VS but *Proteocephalus* sp. 2 (Figure 2C) occurred in the *S. maculata* from both habitats. *Polystoma africanum* was recorded in a single toad among those caught from the VS. The six *Mesocoelium* spp. recorded were recovered from the toads examined, with *Mesocoelium* spp. 1 and 6 (Figures 3A and 3F) occurring in toads from the VS and the CP. In the CP the prevalence of the two species was

higher in *S. regularis* (25% each), but the infection intensity was slightly higher in *S. maculata*. Other species (*Mesocoelium* spp. 2, 3, 4 and 5) were recorded mostly in host specimens collected from the VS. In the VS, prevalence and infection intensity were both higher in *S. regularis*.

Of the nine nematodes species recorded, only five (Aplectana sp., C. ornata, Foleyellides sp.,

Table 2: Prevalence and	mean intensity	y of	parasites o	of Bufo	nids	from C)jo	Camp,	Ugl	boke.

Parasite	Host	Location	No. examined	No. infected	Prev. (%)	No. of parasite	M.I±S.E
Cestoda							
C. jaegerskioeldi	S. maculata	CP	53	-	-	-	-
		VS	159	05	3.2	18	3.6±1.6
	S. regularis	CP	04	-	-	-	-
	0	VS	112	01	0.9	01	1.0
Proteocephalus larva sp. 1	S. regularis	CP	04	-	-	-	-
	0	VS	112	04	3.6	07	1.8 ± 0.48
Proteocephalus larva sp. 3	S. maculata	CP	53	03	5.7	03	1.0
		VS	159	06	3.8	07	1.2±0.17
Monogenea							
P. africanum	S. regularis	CP	04	-	-	-	-
	0	VS	112	01	0.9	01	1.0
Digenea							
Mesocoelium sp. 1	S. maculata	СР	53	02	3.8	25	12.5 ± 2.5
Ĩ		VS	159	02	1.26	06	3.0±1.0
	S. regularis	CP	04	01	25.0	10	10.0
		VS	112	12	10.7	1029	85.8±50.67
Mesocoelium sp. 2	S. maculata	CP	53	-	-	_	_
		VS	159	02	1.3	13	6.5±1.5
	S. regularis	CP	04	-	-	-	-
	21110	VS	112	14	12.5	413	29.5+15.71
Mesocoelium sp. 3	S. maculata	CP	53	-		-	-
niesococitani spi o	Simoona	VS	159	09	5.7	39	4.3+1.13
	S regularis	CP	04	-	-	-	-
	5. regularis	VS	112	14	12.5	576	41 1+16 73
Mesocoelium sp 4	S maculata	CP	53	-	-	-	-
mesoebenum sp. 4	5. macaiaia	VS	159	01	0.63	02	2.0
	S regularis	CP	04	01	25.0	16	16.0
	5. regularis	VS	112	12	10.7	111	0 3+2 51
Mesocoelium sp 5	S maculata	CP	53	02	3.8	19	95+55
mesocoenum sp. 5	5. macaiaia	VS	159	14	8.8	88	6 3+1 54
	S regularis	CP	04	14	0.0	00	0.5±1.54
	5. regularis	VS	112	17	15.2	312	- 18/1+2/77
Masacalium sp. 6	S maculata	CP	53	04	7.6	50	10.4 ± 2.77 12 5+3 10
mesocoetium sp. 0	5. macaiaia	VS	150	21	13.2	102	12.5 ± 3.10 1.0 ± 1.47
	C manulania		139	21	25.0	102	4.9±1.47
	S. regularis		112	40	23.0 12.9	2010	10.0
Nomotodo		v S	112	49	43.8	3010	/8.8±10.30
Auntica course africanous	C manulata	CD	52				
Amplicaecum ajricanum	S. macutata		150	-	- 2.1	- 27	-
	C manulania		139	03	5.1	57	7.4±3.30
	S. regularis	CP VC	04	-	-	-	-
Amplianoum	C manulata		52	24	21.4	500	12.3±2.55
Amplicaecum sp.	S. maculata	CP	55	-	-	-	-
	C	V S	159	12	7.5	155	12.9±7.20
	S. regularis	CP	04	-	-	-	-
A T .	G 1.	VS CD	112	11	9.8	27	2.5±0.41
Aplectana sp.	S. maculata	CP	53	-	-	-	-
	с I ·	VS	159	06	3.8	136	22.7±13.85
	S. regularis	CP	04	01	25.0	06	0.0
	а I	VS	112	06	5.4	191	31.8 ± 5.32
Cosmocerca commutata	S. maculata	СР	53	-	-	-	-
		VS	159	01	0.6	20	20.0
C. ornata	S. maculata	CP	53	02	3.8	17	8.5±7.5
		VS	159	04	2.5	74	18.5 ± 7.12
	S. regularis	CP	04	-	-	-	-
		VS	112	02	1.8	03	1.5 ± 0.5

Table 2 (cont'd): Prevalence and mean intensity of parasites of Bufonids from Ojo Camp, Ugboke.

Cosmocerca sp.	S. maculata	CP	53	-	-	-	-
-		VS	159	01	0.6	05	5.0
Foleyellides sp. 1	S. maculata	CP	53	01	1.9	02	2.0
		VS	159	03	1.9	18	6.0 ± 2.52
	S. regularis	CP	04	-	-	-	-
		VS	112	06	5.4	85	14.2 ± 4.89
Physaloptera sp.	S. maculata	CP	53	01	1.9	01	1.0
		VS	159	11	6.9	44	4.0 ± 1.29
	S. regularis	CP	04	01	25.0	03	3.0
		VS	112	11	9.8	30	2.7±0.75
Oswaldocruzia hoepplii	S. maculata	CP	53	-	-	-	-
		VS	159	01	0.6	03	3.0
	S. regularis	CP	04	-	-	-	-
		VS	112	11	9.8	172	15.6 ± 10.31
Rhabdias africanus	S. maculata	CP	53	17	32.1	122	7.2 ± 0.97
		VS	159	44	27.7	176	4.0 ± 4.36
	S. regularis	CP	04	-	-	-	-
		VS	112	19	17.0	79	4.2 ± 0.52
Ascaridida larva 1	S. regularis	CP	04	-	-	-	-
		VS	112	01	0.9	01	1.0



Figures 2A-C. Scoleces of proteocephalid cestode larvae infecting ground-dwelling amphibians at Ojo Camp, Ugboke. **A**, *Ophiotaenia* sp. infecting *S. regularis*; **B**, *Proteocephalus* sp. 1 infecting *P. pumilio*; **C**, *Proteocephalus* sp. 2 infecting *S. maculata*, *A. subsigillata* and *P. aequplicata*. Scale bar: A, C = 0.2 mm; B = 0.3 mm.



Figures 3 A-F. *Mesocoelium* spp. infecting ground-dwelling amphibians at Ojo Camp, Ugboke. **A.** *Mesocoelium* sp. 1; **B.** *Mesocoelium* sp. 2; **C.** *Mesocoelium* sp. 3; **D.** *Mesocoelium* sp. 4; **E.** *Mesocoelium* sp. 5 and **F.** *Mesocoelium* sp. 6. Scale bar: A = 0.25 mm; B, F = 0.5mm; C, D, E = 0.3 mm.

Physaloptera sp. and *R. africanus*) occurred in the toads from the CP. In contrast, the toads caught in the VS harboured all the nine species recorded with higher prevalence in *S. regularis* but the mean intensity for the parasites varied in the two *Sclerophrys* spp.

Table 3 shows the prevalence and mean intensity of the helminth parasites recorded in *Aubria subsigillata* and *Hoplobatrachus occipitalis* collected from the CP (n=11 each). While *A. subsigillata* harboured 16 helminth species, only four were recorded in *H. occipitalis*. Parasite prevalence in both frogs ranged from 9.1% to 27.3%; infection intensity seldom exceeded 20 parasites/infected host (*Mesocoelium* sp. 6) in *A. subsigillata* and 6 parasites/infected host (*Mesocoelium* spp. 2 and 6) in *H. occipitalis*.

The prevalence and mean intensity of infection of parasites in Hylarana spp. from Ojo Camp are presented in Table 4. A total of 15 specimens of H. galamensis was collected, one from the CP and 14 from the VS. The 13 H. albolabris examined were all collected from the CP. Two monogeneans, Polystoma galamensis (from H. galamensis) and P. perreti (from H. albolabris) were recovered from these frogs. It was the H. galamensis from the VS that harboured P. galamensis. Five digeneans including D. fischthalicus and four Mesocoelium spp. (1, 2, 4 and 6) were recorded in the Hylarana spp. Diplodiscus fischthalicus was only recorded in. H. albolabris (prevalence: 15.4%; mean intensity: 3.0±1.03). Mesocoelium spp. were recorded in frogs from both habitats but with the different species occurring in either H. galamensis or H. albolabris. Mesocoelium spp. 1 and 6 infections were over-dispersed in the specimens infected, with infection intensity as high as 50 and 70 parasites/infected host, respectively. Nematodes recorded in these frogs included Amplicaecum africanum, Amplicaecum sp., Aplectana sp., Cosmocerca ornata, Paracosmocerca sp., Foleyellides sp., Physaloptera sp., Rhabdias spp. 1 and 3 (from H. albolabris and H. galamensis, respectively), two ascaridida larvae and an unidentified nematode sp. from H. albolabris (Table 4).

Most (08) of the nematodes were recorded in host specimens collected from the VS while the others (05) occurred in those from the CP. The prevalence ranged from 7.1% to 30.8% while the infection intensity ranged from 1.0 ± 00 to 13.7 ± 5.78 parasites/infected host.

The parasites recorded in the *Ptychadena* spp. are presented in Table 5. The two cestode genera (*Cylindrotaenia* and *Proteocephalus*) recovered were both from frogs caught in the CP. *Cylindrotaenia jaegerskioeldi* was recorded in *P. longirostris* while

Table 3: Prevalence and mean intensity of	f helminths in Aubra subsigillata and Hoplobatrachus occipitalis from
cocoa plantations in Ojo Camp, Ugboke.	

Parasite	Aubra s	ubsigillata	Hoplobatrachus occipitalis		
	Prevalence (%)	Mean intensity ± S.E	Prevalence (%)	Mean intensity ± S.E	
Cestode					
Proteocephalus larva sp. 3	18.2	14.5±13.5	-	-	
Digenea					
M. aubriae	9.1	5.0	-	-	
M. micrurus	-	-	9.1	1.0	
Strigeiod trematode larva	9.1	1.0	-	-	
Mesocoelium sp. 1	9.1	10.0	-	-	
Mesocoelium sp. 2	18.2	14.0 ± 1.0	9.1	6.0	
Mesocoelium sp. 3	9.1	2.0	-	-	
Mesocoelium sp. 4	18.2	10.0±2.0	-	-	
Mesocoelium sp. 5	9.1	10.0	-	-	
Mesocoelium sp. 6	18.2	23.5±3.5	9.09	6.0	
Nematoda					
A. africanum	9.1	1.0	-	-	
Amplicaecum sp.	9.1	1.0	9.1	4.0	
Aplectana sp.	9.1	1.0	-	-	
C. ornata	9.1	7.0	-	-	
Cosmocerca sp.	9.1	2.0	-	-	
Foleyellides sp. 3	9.1	2.0	-	-	
Physaloptera sp.	27.3	3.7±0.88	-	-	

Table 4: Prevalence and mean intensity of helminths in Hylarana spp. from Ojo Camp, Ugboke.

Parasite	Host	Cocoa P	lantation	Village Settlement		
		Prevalence	Mean	Prevalence	Mean	
		(%)	intensity ±	(%)	intensity \pm	
			S.E		S.E	
Monogenea						
P. galamensis	H. galamensis	-	-	21.4	5.0 ± 3.50	
P. perreti	H. albolabris	7.7	1.0	-	-	
Digenea						
Diplodiscus	H. albolabris	15.4	3.0±1.03	-	-	
fischthalicus						
Mesocoelium sp. 1	H. galamensis	-	-	7.1	50.0	
Mesocoelium sp. 2	H. albolabris	7.7	13.0	-	-	
Mesocoelium sp. 4	H. galamensis	100.0	2.0	-	-	
Mesocoelium sp. 6	H. galamensis	-	-	7.14	67.0	
Nematoda						
A. africanum	H. galamensis	-	-	7.1	1.0	
Amplicaecum sp.	H. galamensis	-	-	7.1	1.0	
Aplectana sp.	H. albolabris	7.7	4.0	-	-	
	H. galamensis	-	-	21.4	13.7±5.78	
C. ornata	H. albolabris	30.8	1.8 ± 0.48	-	-	
	H. galamensis	-	-	7.1	2.0	
Foleyellides sp. 2	H. galamensis	-	-	14.3	2.5±1.5	
Paracosmocerca sp.	H. albolabris	7.7	2.0	-	-	
Physaloptera sp.	H. galamensis	-	-	7.1	1.0	
Rhabdias sp. 1	H. albolabris	15.4	3.5 ± 2.5	-	-	
Rhabdias sp. 3	H. galamensis	-	-	7.1	1.0	
Ascaridida larva 1	H. galamensis	-	-	14.3	4.0±0.0	
Ascaridida larva 2	H. albolabris	7.7	8.0	-	-	
Unidentified nematode	H. albolabris	23.1	6.0±2.08	-	-	

Parasite	Host	Location	No.	No.	Prev.	No. of	
			examined	infected	(%)	parasite	M.I±S.E
Costada							
C igagarskioaldi	P longirostris	СР	01	01	100.0	13	13.0
C. juegerskibelui	1. iongirosiris	VS	01	01	100.0	15	15.0
Proteocephalus larva sp. 2	P numilio	CP	05	03	60.0	11	3 7+1 67
1 Toteocephanas fui va sp. 2	1 . punnito	VS	07	-	-	-	-
Proteocephalus larva sp. 3	P aequiplicata	CP	08	01	12.5	03	3.0
i roleocephalas iai va sp. 5	1. acquipticata	VS	-	-	-	-	-
Monogenea		15					
P aeschlimanni	P numilio	СР	05	01	20.0	01	1.0
1 · desentindanti	1. punnito	VS	07	01	14.3	04	4.0
P. ehriensis	P. aeauiplicata	CP	08	01	12.5	03	3.0
	1 · acquipticata	VS	-	-	-	-	-
Digenea		15					
D. fischthalicus	P. pumilio	CP	05	01	20.0	01	1.0
		VS	07	-		-	-
Halinegus sp.	P. pumilio	CP	05	01	20.0	02	2.0
		VS	07	-	-	-	-
Mesocoelium sp. 1	P. oxvrhvnchus	CP	01	_	-	-	-
1		VS	05	02	40.0	31	15.5 ± 2.5
	P. pumilio	CP	05	-	-	-	-
	. <u>r</u>	VS	07	03	42.9	04	1.3 ± 0.33
Mesocoelium sp. 2	P. aeauiplicata	CP	08	03	37.5	15	5.0 ± 2.08
1	1 1	VS	_	_	-	_	_
	P. mascareniensis	CP	03	01	33.3	01	3.0
		VS	02	01	50.0	03	3.0
	P. oxyrhynchus	CP	01	01	100.0	07	7.0
	5 5	VS	05	01	20.0	23	23.0
	P. pumilio	CP	05	01	20.0	07	7.0
	. <u>r</u>	VS	07	05	71.4	42	8.4±6.42
Mesocoelium sp. 3	P. aequiplicata	CP	08	01	12.5	02	2.0
1	1 1	VS	_	_	-	_	_
	P. mascareniensis	CP	03	-	-	-	-
		VS	02	01	50	01	1.0
	P. oxyrhynchus	CP	01	-	-	-	-
		VS	05	02	40.0	22	11.0
Mesocoelium sp. 4	P. aequiplicata	CP	08	03	37.5	15	5.0±2.31
	1 1	VS	-	-	-	-	-
	P. longirostris	CP	01	01	100.0	01	1.0
	0	VS	-	-	-	-	-
	P. oxyrhynchus	CP	01	01	100.0	07	7.0
		VS	05	02	40.0	23	11.5±1.5
Mesocoelium sp. 5	P. aequiplicata	CP	08	03	37.5	16	5.3±1.76
		VS	-	-	-	-	-
Mesocoelium sp. 6	P. aequiplicata	CP	08	03	37.5	29	9.7±3.93
		VS	-	-	-	-	-
	P. longirostris	CP	01	01	100.0	01	1.0
		VS	-	-	-	-	-
	P. mascareniensis	CP	03	-	-	-	-
		VS	02	01	50.0	03	3.0
	P. oxyrhynchus	CP	01	01	100.0	07	7.0
		VS	05	03	60.0	39	13.0±7.94
	P. pumilio	CP	05	01	20.0	10	10.0
		VS	07	04	57.1	41	10.3±4.33
Nematoda							
Amplicaecum sp.	P. mascareniensis	CP	03	02	66.7	05	2.5 ± 0.50
		VS	02	01	50.0	01	1.0
	P. oxyrhynchus	CP	01	01	100.0	03	3.0
		VS	05	01	20.0	06	6.0
	P. pumilio	CP	05	-	-	-	-
		VS	07	03	42.9	04	1.3±0.33

Table 5: Prevalence and mean intensity of helminth parasites in the *Ptychadena* spp. from Ojo Camp, Ugboke.

Aplectana sp.	P. mascareniensis	СР	03	-	-	-	-
		VS	02	01	50.0	40	40.0
	P. aequiplicata	CP	08	02	25.0	22	11.0 ± 4.00
		VS	-	-	-	-	-
	P. pumilio	CP	05	01	20.0	16	16.0
		VS	07	03	42.9	49	16.3 ± 8.41
	P. oxyrhynchus	CP	01	-	-	-	-
		VS	05	01	20.0	07	7.0
C. ornata	P. pumilio	CP	05	01	20.0	02	2.0
		VS	07	03	42.9	12	$4.0{\pm}1.00$
	P. aequiplicata	CP	08	01	12.5	01	1.0
		VS	-	-	-	-	-
Physaloptera sp.	P. aequiplicata	CP	08	04	50.0	08	2.0±0.41
		VS	-	-	-	-	-
	P. mascareniensis	CP	03	01	33.3	03	3.0
		VS	02	01	50.0	03	3.0
	P. oxyrhynchus	CP	01	01	100.0	03	3.0
		VS	05	01	20.0	01	1.0
	P. pumilio	CP	05	-	-	-	-
		VS	07	01	14.3	02	2.0
Ascaridida larva 1	P. mascareniensis	CP	03	-	-	-	-
		VS	02	01	50.0	03	3.0
Oxyurid nematode	P. mascareniensis	CP	03	01	33.3	01	1.0
		VS	02	-	-	-	-

Table 5 (cont'd): Prevalence and mean intensity of helminth parasites in the Ptychadena spp. from Ojo Camp, Ugboke.

Proteocephalus sp. larva 1 (Figure 2B) and 2 (Figure 2C) were recorded in P. pumilio and P. aequiplicata, respectively. Two Polystoma spp. were recorded among the Ptychadena spp.; Polystoma aeschlimanni from P. pumilio (CP and VS) and P. ebriensis from P. aequiplicata only from the CP. Irrespective of host habitat both monogeneans had low infection intensity. Eight digenetic trematodes (D. fischthalicus, Halipegus sp. and Mesocoelium spp. 1-6) were recorded in these grass frogs. Diplodiscus fischthalicus and Halipegus sp. infected P. pumilio specimens caught in the CP. Mesocoelium sp. 1 occurred in P. oxyrhynchus and P. pumilio taken in the VS. Except for Mesocoelium sp. 5 which was recorded only in P. aequiplicata from the CP, Mesocoelium spp. 2, 3, 4 and 6 infected more than two host species each, occurring in host specimens from either the CP or the VS and in some instances from both habitats (Table 5). The nematodes in these frogs were mostly generalists, infecting different host species either in the VS or the CP or both.

Discussion

Although *Sclerophrys* spp. (*S. maculata* and *S. regularis*) were encountered in the VS and the CP, a higher proportion of the species was recorded in the VS. This is probably an indication that the pesticide contaminated environment of the CP was not too conducive for these toads especially for the development of their tadpoles.

Pesticide contamination in the CP may also be responsible for the absence of some parasites in the toads caught from this habitat arising from the elimination of their intermediate hosts. For example *C. jaegerskioeldi* was only recorded in the VS albeit at low prevalence and intensity. Similarly, *Ophiotaenia* sp. larva was not recorded in *S. regularis* from the CP while *Proteocephalus* sp. larva 2 infected *S. maculata* in both environments with higher prevalence in the CP but with no observed differences in the infection intensity. The monogenean *P. africanum* was recorded in the VS with very low prevalence and mean intensity (0.9% and 1.0, respectively) compared to previous records of this parasite in northern Edo State, where Aisien and Du Preez (2009) recorded an overall prevalence of 18.7% and a mean intensity of 4.6, respectively.

Interestingly, only Mesocoelium spp. were the digeneans recorded in the two Sclerophrys spp. irrespective of the environment of collection (Table 1). This result is similar to that obtained by Aisien et al (2011) in the S. maculata (formerly Amietophrynus maculatus) collected in the Agricultural Zone of the Pendjari Biospere Reserve in Benin Republic. The Mesocoelium spp. recovered varied morphologically, especially with respect to the testes/ovary sizes, that it is perhaps safer for now to regard them as different species until otherwise determined. Irrespective of the Mesocoelium sp. infecting these toads, the prevalence and the infection intensity were generally higher in the toads collected in the VS (Table 2), an indication this habitat was more conducive for the arthropod intermediate hosts of these trematodes. In a recent publication, Imasuen and Aisien (2019) remarked that bush burning, a preparatory phase in farming eliminated most of the arthropod vectors of Mesocoelium monodi, hence the low prevalence of Mesocoelium in the anurans collected from a farm bush.

Infection with nematodes mostly followed the pattern observed for the *Mesocoelium* spp. as the prevalence and infection intensity were similarly higher in the VS. This is another indication that contaminated environments impact negatively on the free-living larval stages of parasites developing in such a milieu (Pietrock and Marcogliese 2003).

Unlike the Sclerophrys spp. which were represented in the VS and the CP, A. subsigillata and H. occipitalis were encountered only in the CP, which provided them with ponds and puddles, away from human habitations. Of the two frogs, A. subsigillata harboured more parasite species (16 parasites) than *H. occipitalis* in which only 4 parasite species were recorded. It is not clear why A. subsigillata was more susceptible to infection than *H. occipitalis*. It may be traceable to the immunosuppressive effects of pesticides, which according to Rohr et al (2008), induce higher trematode infection in amphibians exposed in their tadpole stage. Aisien et al (2011) however found that this phenomenon (immunosuppressive effect) was not restricted to trematode infections alone, but was also applicable to other parasite groups. Except for Mesocoelium spp. 2, 4 and 6, and Physaloptera sp., infection intensities for parasites recovered from both frogs were generally low. This again may be connected to the inhibitory effect of pesticides in this environment as observed by Aisien et al (2011) in the Agricultural Zone of the Pendjari Biosphere Reserve, Benin Republic.

The two Hylarana spp. encountered in this study had their preferred habitats; H. albolabris occupied the more humid cocoa plantations and H. galamensis, the village settlement, which was relatively drier because of its sparse vegetation. Hylarana galamensis has been more frequently encountered in the savannah biotope of Nigeria (Aisien et al 2003, 2004; Ozemoka 2012) but a population of this frog has also been reported in the humid environment of the Niger Delta of Nigeria (Aisien et al 2017). The parasites recovered from the two frog species showed a high degree of separation with only two nematode species (Aplectana sp. and C. ornata) common to them. While a few of them may be host specific (*P. galamensis*, P. perreti and Foleyellides sp. 2), some others (D. fischthalicus, A. africanum and Physaloptera sp. larva) are known generalists. It is therefore not clear why they selectively infected some hosts in Ojo Camp. Although P. galamensis frequently infects frogs from the drier environments as in the savannah and in the VS, this polystome has also been recorded in frogs from the humid environment of the Niger Delta, albeit with low prevalence and infection intensity (Aisien et al 2017). Polystoma perreti on the other hand seems to have a more restricted distribution. Up till now, this *Polystoma* sp. has only been recovered from H. albolabris taken at the Abraka wetlands in Delta State of Nigeria (Aisien, M.S.O. unpublished data). The higher prevalence and infection intensity recorded for Polystoma galamensis has shown that the environment in VS was more conducive for oncomiracidial development and host infection. Other parasites infecting frogs in the VS including Mesocoelium spp. 1 and 6, and Aplectana sp., had infection intensity higher than their counterparts in the CP, thus confirming the limiting influence of pesticide contamination in the plantations on parasites.

Infection among the Ptychadenidae were mostly recorded in specimens collected from the CP. It is likely that the anurans from this environment were again more susceptible to infection due to their exposure to pesticides in the CP. For example, the cestodes, represented by three species in two genera occurred only among the frogs collected from the CP. Whereas C. jaegerskioeldi had high infection intensity in P. longirostris, the two Proteocephalus spp. larvae recovered from P. pumilio and P. aequiplicata, respectively, had lower infection intensity in these hosts (Table 5). Despite the differences in the environmental conditions in the VS and the CP, the prevalence and infection intensities for P. aeschlimanni in both environments were generally low, although with a slightly higher infection intensity in the VS. Polystoma ebriensis which occurred only in P. aequiplicata taken in the CP also had low infection intensity. It is likely that conditions existing in both environments generally did not favour the development of the eggs/larvae of these monogeneans.

The digeneans were represented by only three genera, namely, Diplodiscus, Halipegus and Mesocelium. While D. fischthalicus and Halipegus sp. were exclusively recovered from host specimens from the CP, the Mesocoelium spp. had mixed distribution, sometimes occurring in host specimens from both environments and in other instances only from the CP. The mixed distribution of the Mesocoelium spp. could partly be attributed to the mobility of the arthropod intermediate host which consequently did not restrict them to a particular environment. Alternatively, these arthropods may belong to a species that is available in both habitats and are commonly consumed by the anuran species in both habitats. The apparently high prevalence values obtained for the nematode parasites can be accounted for by the low host number examined from both the CP and the VS. Except for *Aplectana* sp. with appreciable infection intensity in a few hosts, the mean intensity of infection for other nematodes was very low. This is an indication of the unfavourable milieu confronting the free living stages of these worms, which inhibit survival and their ability to reach and establish infections in their hosts (Pietrock and Marcogliese 2003).

In conclusion, this study has shown that the pesticidepolluted environment of the CP was not conducive for some ground-dwelling anurans and for the parasites infecting them. The prevalence and mean intensity of parasites recovered from Sclerophrys spp. were generally higher in the VS. In A. subsigillata and H. occipitalis which were encountered only in the CP, A. subsigillata harboured more parasites than H. occipitalis. The greater susceptibility of A. subsigillata is presumed to arise from the immunosuppressive effects of pesticides. The low prevalence and infection intensity recorded for some parasites are presumed to have arisen from the inhibitory effects of the pesticide contamination in the CP. The two Hylarana spp. encountered had distinct habitat preferences with *H. galamensis* preferring the drier environment of the VS while *H. albolabris* was restricted to the more humid CP. The parasites infecting these frogs also had a high degree of separation with only two parasites common to both frogs. Among the Ptychadenidae, infections with helminth parasites were mostly recorded in specimens collected from the CP. The higher susceptibility observed in the frogs from this environment is presumed to be as a result of their exposure to pesticides during development. The low intensity of infection recorded in them is also presumed to be a consequence of the inhibitory effects of the pesticide-polluted environment on the free-living stages of parasites infecting them.

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