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Effect of Anthropogenically Induced Environmental Alterations on Lung Parasites of Amphibians: A Preliminary Study

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Abstract

One hundred and ninety-two amphibians comprising fourteen species were collected from the environs of a gas flare facility at Agbada and also from a residential area at Rumuesara, both in Rivers State, Nigeria. The amphibians were sampled using visual encounter and acoustic survey method, between January, 2016, and October, 2016. They were dissected using standard protocols and lung parasites were isolated, fixed and identified. Five species of lung parasites were recovered from the host specimens. They were *Haematoloechus exoterorchis, H. micrurus*, *Rhabdias* sp., *Rhabdias africanus*, and *Raillietiella* sp. It was observed that *H. exoterorchis* and *H. micrurus* were more prevalent in infected *Hoplobatrachus occipitalis* specimens from the gas flare site though there was no statistically significant difference between their prevalence rates in both sites. However, prevalence of infection with *Rhabdias* sp. was significantly higher in *Ptychadena* spp. from Agbada gas flare site, while the prevalence of infection with *Rhabdias africanus* and *Raillietiella* species were significantly higher in *Amietophrynus maculatus* collected from the residential area. In fact, *Raillietiella* sp. was never recovered from any of the host specimens collected from the Agbada gas flare site. It was concluded that the environmental alterations brought about by the gas flare facility increased and enhanced the prevalence and intensity of *Haematoloechus* and *Rhabdias* sp. infections in *H. occipitalis* and *Ptychadena* spp., respectively, while preventing the establishment of *Raillietiella* and *R. africanus* infections in *A. maculatus* specimens resident in the site.

Keywords: environment, lung parasites, amphibians.

1.0 Introduction

Human alteration of the environment can lead to an increase in the transmission of certain parasites in amphibians (Johnson *et al*., 2007; Mckenzie, 2007, both cited in Aisien *et al*., 2011). Aisien *et al*., (2009) stated that environmental alteration can also affect the ability of some parasites to complete their life cycles and maintain infection in their normal hosts. The most prevalent human activities that disturb natural environments in Nigeria include deforestation, farming, as well as housing development, and oil exploration. These all result in habitat and environmental alterations, which impact negatively on the amphibian species and the parasites infecting them.

Some of the effects of habitat alteration on hosts and their parasites include increase in paratenic hosts, increase in parasite reproductive rate, and decrease in host immunity. Therefore, it is expected that hosts from disturbed environments should harbor more parasites than those from pristine or less disturbed environments. LaFonte and Johnson (2013) stated that both natural and anthropogenic factors can alter host stress hormones and the potential efficacy of their immune response. Such factors included environmental contaminants resulting from industrial activities, which have been shown to increase stress in amphibians (Rohr *et al.*, 2008; McMahon *et al*., 2011), and they concluded that changes in stress hormones in nature could result in increased host susceptibility.

In the present research, the impact of two major habitat alterations on the lung parasites of amphibians was evaluated. The first site was a gas flow station, while the other was a residential area with increasing housing development activities.

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2.0 Method

The study sites were the flow station at Agbada (E 4° 55' 57.006", N 7° 1' 13.692") in Ikwerre Local Government Area (LGA) and Rumuesara Community (E4° 54' 26.37", N7° 1' 58.128") in Obio-Akpor LGA, both of Rivers State (Nigeria). Sampling for amphibian specimens was carried out using the visual encounter and acoustic survey (VEAS) method, combined with auditory surveys for calling anurans at night, between 7.00 pm and 10.00 pm, with the aid of flashlights. Both stations were visited once each month for a period of ten months (January, 2016 to October, 2016). All specimens were properly labelled noting the sampling location and date of collection.

The amphibians were transported to the parasitology laboratory in the Department of Applied and Environmental Biology, Rivers State University of Science and Technology, Port Harcourt, in plastic containers with perforated caps, containing small amounts of water, for identification and laboratory analyses. They were sacrificed by exposure to Chloroform vapour in a tightly closed jar, and identified using appropriate keys (Minter *et al*., 2004; Rodel, 2000).

Lung parasites were collected by longitudinally dissecting the ventral side of the host specimens and excising the lungs which were then placed in a Petri dish containing 0.72% normal saline. Nematodes were fixed in hot 70% alcohol and preserved in fresh 70% alcohol. Trematodes were flattened under a cover slip on a microscope slide, fixed and preserved in 5% formol saline. Pentastomids were preserved in 70% alcohol saline. Parasites were identified to the possible taxonomic level with the aid of keys from Prudhoe and Bray (1982).

Prevalence and intensity of infection of the parasite species collected were determined according to Bush *et al.,* 1997.

3.0 Results

One hundred and ninety-two individual amphibian specimens were captured over the ten month period; ninety-two from the environs of the gas flow station and one hundred from the residential area. Thirteen host species were collected from the flow station while nine species were from the residential area. The species included *Ptychadena bibroni, P. mascareniensis, P. oxyrhynchus, P. pumilio, P. schubotzi, Amietophrynus maculatus, A. camerounensis, A. regularis, Hoplobatrachus occipitalis, Hylarana galamensis, Hyperolius fusciventris burtoni, Hyperolius sp., Silurana tropicalis*, and *Afrixallus fulvovittatus*. Table 1 shows the host species collected and their distribution in both sites, as well as the overall prevalence of lung infections in the infected hosts.

Four of the thirteen host species captured at the gas flare zone were infected with four species of lung parasites- *Haematoloechus exoterorchis, H. micrurus, Rhabdias* sp., and *Rhabdias africanus. H. exoterorchis, H. micrurus* and *Rhabdias* sp. were recovered from *H. occipitalis* whereas only *Rhabdias* sp. was recovered from the lungs of *P. pumilio*, and *P. oxyrhynchus*, and *R. africanus* from *A. maculatus*.

Five of the nine host species examined at the Rumuesara residential area were infected with five lung parasites. *H. occipitalis* specimens collected from this site were infected with *H. exoterorchis, H. micrurus, Rhabdias* sp. and *Raillietiella* sp. All three *Amietophrynus* species collected from this site were infected with *R. africanus* and *Raillietiella* sp., either singly or as a mixed infection. *Rhabdias* sp. was also recovered from the lungs of *Hylarana galamensis*.

Both *H. exoterorchis* and *H. micrurus* were recovered from the lungs of *H. occipitalis* specimens collected from both sites, also, either singly or as a mixed infection. The prevalence and mean intensity of infection with *H. exoterorchis* were higher in host specimens from the gas flare site. However, the prevalence of *H. micrurus* infection was higher in *H. occipitalis* specimens from the gas flare site while its mean intensity was higher in host specimens from the residential area. *Rhabdias* sp. was also recovered from the species collected from both sites, but prevalence of infection was higher at Rumuesara while mean intensity of infection was higher at the Agbada gas flow station.

Table 1: Overall prevalence (%) of lung infections in the amphibian host specimens collected from both sites.

Host	Agbada gas flow station			Rumuesara residential area		
	Number examined	Number infected	Prevalence (%)	Number examined	Number infected	Prevalence (%)
Ptychadenidae						
Ptychadena bibroni	7	0	0.00	3	0	0.00
P. pumilio	3	1	33.33	5	0	0.00
P. mascareniensis	8	0	0.00	11	0	0.00
P. oxyrhynchus	9	2	22.22	-	-	-
P. schubotzi	5	0	0.00	-	-	-
Dicroglossidae						
Hoplobatrachus occipitalis	43	23	53.49	27	13	48.15
Pipidae						
Silurana tropicalis	3	0	0.00	-	-	-
Bufonidae						
Amietophrynus cameroonensis	2	0	0.00	15	8	53.33
A. maculatus	8	1	12.50	18	9	50.00
A. regularis	-	-	-	13	6	46.15
Hyperoliidae						
Hyperolius fusciventris burtoni	1	0	0.00	-	-	-
Hyperolius sp.	1	0	0.00	1	0	0.00
Afrixallus fulvovittatus	1	0	0.00	-	-	-
Ranidae						
Hylarana galamensis	1	0	0.00	7	3	42.86
Total number of hosts examined	92	27		100	38	

Rhabdias species infected both *P. pumilio* and *P. oxyryhnchus* from the gas flow station, whereas both host specimens were free of any lung infections at the Rumuesara residential area. So, higher prevalence of infection with *H. exoterorchis* and *H. micrurus* was obtained in *H. occipitalis* specimens from the gas flare site, and mean intensity of infection with *H. exoterorchis* was also higher in host specimens from Agbada gas flare site while that of *H. micrurus* infections was higher at Rumuesara residential area. However, there were no significant differences between both sites with respect to the prevalence (t_2=1.67, P=0.12) and mean intensity (t_2=0.51, P=0.33) of infection with both parasites. Both prevalence (t_2=5, P=0.04) and mean intensity of infection (t_2=3, P=0.05) with *Rhabdias* sp. were significantly higher in *Ptychadena* species from the Agbada gas flare site.

On the other hand, prevalence of infection with *R. africanus* and *Raillietiella* sp. was significantly higher in *A. maculatus* collected from the residential area (t_2=4.33, P=0.05), but there were no statistically significant differences in the mean intensity of both infections in the same species from both sites. In fact, *Raillietiella* species were never found

Table 2: Prevalence (%) and Mean intensity of lung infection in the infected amphibian host species according to site.

Host	Parasite	Site			
		Agbada		Rumuesara	
		P (%)	MI	P (%)	MI
Ptychadenidae					
P. pumilio	*Rhabdias sp.*	33.33	1.00	-	-
P. oxyrhynchus	*Rhabdias sp.*	22.22	2.00	-	-
Dicroglossidae					
H. occipitalis	*Haematoloechus exoterorchis*	39.53	11.18	25.93	6.86
	H. micrurus	27.91	4.17	14.81	4.75
	Rhabdias sp.	2.33	6.00	11.11	4.00
	Raillietiella sp.	-	-	3.70	2.00
Bufonidae					
A. maculatus	*Rhabdias africanus*	12.50	8.00	33.33	5.17
	Raillietiella sp.	-	-	33.33	6.83
A. regularis	*R. africanus*	-	-	15.38	1.00
	Raillietiella sp.	-	-	38.46	9.00
A. cameroonensis	*R. africanus*	-	-	6.67	3.00
	Raillietiella sp.	-	-	46.67	8.71
Ranidae					
H. galamensis	*Rhabdias sp.*	-	-	42.86	1.33

infecting any host from the gas flare zone, but were readily recovered from *Amietophrynus* species collected from Rumuesara residential area and were even recovered from a *H. occipitalis* specimen collected from the same site. Table 2 shows the prevalence and mean intensity of the lung infections in infected host specimens from both sites.

Prevalence and mean intensity values were also computed for infected host specimens in relation to sex (Table 3). In the *H. occipitalis* specimens from Agbada gas flare zone, prevalence and mean intensity of *H. exoterorchis* were higher in male specimens. Prevalence of *H. micrurus* infection was higher in females while mean intensity was higher in males. The only *Rhabdias* sp. from this species was recovered from a female specimen. *Rhabdias* species were also recovered from male *P. oxyryhnchus* and female *P. pumilio*.

In host specimens from Rumuesara residential area, the prevalence of *H. exoterorchis* infection was higher in female specimens but mean intensity was higher in males. *H. micrurus* was recovered only from male *H. occipitalis*. The *Rhabdias* sp. and *Raillietiella* sp. recovered from this species in this site were from male specimens. Prevalence and mean intensity of infection with *Raillietiella* sp. were generally higher in male specimens of *A. maculatus* and *A. regularis*. In *A. camerounensis*, both prevalence and mean intensity were higher in female host specimens. Comparing all the *Amietophrynus* species from this site with respect to infection with *R. africanus*, the parasite was found infecting only male specimens of *A. camerounensis*; prevalence was higher in male *A. regularis* but mean intensity was same as in female specimens. However, the prevalence was same in both sexes of *A. maculatus*, but mean intensity was slightly higher in female specimens.

Specimens of *H. galamensis* collected from the residential area were also found to be infected with *Rhabdias* sp.; while prevalence of infection was higher in males, mean intensity was higher in females.

Table 3: Prevalence (%) and Mean intensity of lung infections in the infected amphibian host species according to sex.

Host	Parasite	Site			
		Agbada Male (Female)		Rumuesara Male (Female)	
		P (%)	MI	P (%)	MI
Ptychadenidae					
P. pumilio	*Rhabdias sp.*	- (100.00)	- (1.00)	-	-
P. oxyrhynchus	*Rhabdias sp.*	100.00 (-)	2.00 (-)	-	-
Dicroglossidae					
H. occipitalis	*H. exoterorchis*	39.39 (30.00)	11.31 (15.67)	17.39 (100.00)	10.75 (1.67)
	H. micrurus	27.27 (30.00)	4.67 (2.67)	17.39 (-)	4.75 (-)
	Rhabdias sp.	- (10.00)	- (6.00)	13.04 (-)	1.00 (-)
	Raillietiella sp.	-	-	4.35 (-)	2.00 (-)
Bufonidae					
A. maculatus	*R. africanus*	20.00 (-)	8.00 (-)	33.33 (33.33)	5.25 (6.00)
	Raillietiella sp.	-	-	33.33 (33.33)	9.75 (1.00)
A. cameroonensis	*Rhabdias* sp.	-	-	33.33 (-)	3.00 (-)
	Raillietiella sp.	-	-	35.71 (66.67)	8.2 (10.00)
A. regularis	*Rhabdias sp.*	-	-	20.00 (12.50)	1.00 (1.00)
	Raillietiella sp.	-	-	40.00 (37.5)	10.00 (8.33)
Ranidae					
H. galamensis	*Rhabdias sp.*	-	-	66.67 (25.00)	1.00 (2.00)

Generally, then, prevalence of lung infection was higher in male than in female specimens.

4.0 Discussion

More diversity of host species was observed at the Agbada gas flow station environs than from the Rumuesara residential area. Usually, more host diversity is expected from pristine or less disturbed environments (Akani *et al*. 2004; Aisien *et al*., 2009; Aisien *et al*., 2011). Though the Agbada gas flare site may not be qualified to be called a pristine zone, it was more forested and obviously less disturbed than the Rumuesara site, which was experiencing urbanization, and a fast rate of housing development.

In the present research, it was observed that *H. exoterorchis* and *H. micrurus* preferentially infected amphibian species from the gas flow station while *Rhabdias africanus* and *Raillietiella* spp. preferentially infected the lungs of those from the residential area. In fact, *Raillietiella* sp. was never recovered from any host specimen from the gas flow station, but the infection was so prevalent at Rumuesara that it was also recovered from the lungs of a *H. occipitalis* specimen from that site. Aisien *et al*. (2011) found that *R. bufonis* was recovered only from *A. maculatus* collected from the agricultural zone of the Pendjari Forest reserve,

Benin Republic. Similarly, *Haematoloechus johnsoni* and *H. phrynobatrachi* were recovered only from *H. occipitalis* and *Phrynobatrachus latifrons*, respectively, collected from the agricultural zone of the forest reserve, while a *Rhabdias* sp. was recovered from *P. latifrons* collected from the buffer and agricultural zones of the forest reserve. As such, neither *Rhabdias* nor *Haematoloechus* species were recovered from host specimens collected from the near pristine national park zone.

In his research in the Gelegele forest reserve, Aisien *et al*., (2009) reported *Rhabdias bufonis* from *Amietophrynus maculatus, H. micrurus* from *H. occipitalis*, and *Haematoloechus aubriae* from *Aubria subsigillata* at a prevalence of 0.6%, 0.6% and 1.2%, respectively. It is worthy of note that in both studies cited above, *Raillietiella* sp. was not recovered in hosts from both forest reserves. The prevalence of *Haematoloechus* infection in *H. occipitalis* and *R. africanus* in *A.maculatus* from the gas flow station, in the present research, were both higher than those of Aisien *et al*., (2009), though the Agbada gas flow station was also a forested area. For instance, the prevalence of *H. exoterorchis* and *H. micrurus* infection in *H. occipitalis* from the gas flow station were 39.53% and 27.91%, respectively; and that of *R. africanus* in *A. maculatus* from the same site was 12.5%. It is therefore, thought that the alterations resulting from the continual flare of gas at the flow station increased the prevalence and mean intensity of infection with the lung trematodes, *H. exoterorchis* and *H. micrurus*; while hindering the establishment of *Raillietiella* species in the lungs of resident amphibian host specimens.

With regards to sex, prevalence and mean intensity of lung parasites were generally higher in male host specimens. Begum and Banu (2012) reported that the level of parasitisation was often higher in male hosts than in female ones. It is thought that female sex hormones inhibited the establishment of parasites in female amphibians. Hamann *et al*., (2010) mentioned other researchers, such as Gillilland and Muzzall (1999), Bolek and Coggins (2001), and McAlpine (1997), who reported that host sex has effect on the number and abundance of parasitic species in vertebrates which could be as a result of the physiological, behavioural and morphological differences between host sexes. Folstad and Karter (1992) had reported that high testosterone levels could cause immunosuppression in males, and Poulin (1996) supported by noting that such hormonal levels could operate to create more infection levels in males than females. Hamann *et al*., (2010) also stated that females and males differ with respect to behaviour, e.g. differing reproductive behaviours between sexes can influence exposure to parasite infective stages. Hamann *et al*., (2010) found that larger males were more parasitized (in terms of species richness) than larger females, suggesting that reduced immune function and behavioural differences between males and females may also explain the increasing levels of parasites.

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