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THE PREVALENCE OF HELMINTHOFAUNA AND FECUNDITY STUDIES IN WEST AFRICAN LUNGFISH, *Protepterus annectens* OWEN, 1839 (DIPTERIFORMES: LEPIDOSERINIDAE) IN RIVER ANAMBRA, NIGERIA

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Abstract

An investigation was conducted on the helminthofauna and some aspects of reproductive biology of the West African lungfish, *Protopterus annectens* in River Anambra, Nigeria, between May and July, 2008. The sample comprised forty two fishes purchased from fishermen and fish mongers at Otuocha fish market, a major fish landing location in the Anambra River Basin. The sex composition of the sample which was 1:41 (male: female) is thought to reflect a preponderance of female fish in the natural population of *P. annectens* in the river system. *Heterorchis protopteri,* a digenean, was the only helminth parasite recovered, infesting 6 (six) out of the 42 fish.33 worms were recovered, giving a prevalence of 14.29 %, mean intensity of infection of 5.5 and mean abundance of 0.8 for the parasite in the fish population. The infection parameters increased with size of fish (weight and length). No parasite was recovered from the lone male fish examined, and this could be attributed to sample size. *H. protopteri* is reported from *P. annectens* in Nigeria for the first time. All the female fish examined were gravid while the lone male fish had ripe testes which indicate that *P.annectens* spawns in the river system in the wet season. Fecundity of the species was estimated to be about 1423 eggs per female and *P. annectens* is categorized as a mesofecund species. It is suggested that some form of regulation of cropping of *P. annectens* in the river system should be put in place pending a clearer understanding of its ecology, in order to reduce the chances of harming the recovery capacity of the species.

Keywords: *Protopterus annectens,* River Anambra, *Heterorchis protopteri,* fecundity, spawning.

Introduction

River Anambra is a major tributary of the River Niger and fed by numerous tributaries which together with it form an extensive drainage basin. *P. annectens* is one of the commercially important species in the River basin. The flesh of *P. annectens* is cherished by communities of the river basin and the species is sold in the fish markets, particularly at Otuocha, all year round. The fish sold in the markets are either caught by fishermen or dug out of their underground aestivation chambers by farmers in the course of hoeing their farms. The harvest is unregulated, and fish of all sizes, irrespective of their physiological states are sold and eaten.

In addition to the commercial importance of *P. annectens* is River Anambra basin, knowledge of the status of relic species such as lungfishes in their natural habitats is important in order that they could be effectively monitored for purposes of conservation. A report by Goudswaard *et al.* (2002) which indicated that the population of the East and Central African lungfish, *Protepterus aethiopicus* in Lake Victoria is declining should be seen as an alert signal on the need to assess the stocks of lungfishes in water bodies in which they occur in Africa and elsewhere.

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Different aspects of the biology of fish species in the River Anambra basin have been investigated (Ilozumba and Ezenwaji, 1985; Ezenwaji, 1992; 1993; 2002; Ezenwaji and Ilozumba, 1992; Ezenwaji and Inyang, 1999; Ezenwaji *et al.*, 2005), but *P. annectens* was not a subject of any of the studies. Paucity of literature on the species in Nigeria also suggests that its neglect by ichthyologists is not limited to the River Anambra basin, since the species is also known to occur in some other river systems in the country (Holden and Reed, 1972). Although the neglect of *P. annectens* is not limited to the River Anambra basin, the need to monitor the species in the river basin has become something of urgent necessity, because the river basin is the base of a newly incorporated Orient Petroleum Resources PLC and the company has begun to lay its operational infrastructure in the trough of the river basin. The notoriety of the petroleum industry in Nigeria for ecological devastation makes a programmed monitoring of the aquafauna and aquaflora of the basin, but particularly species such as *P. annectens* which constitute a critical but fragile biological resource an urgent necessity.

The paper reports a preliminary investigation on helminthofauna and reproductive biology of *P. annectens* in the river basin.

Materials and methods

A total of 42 fish were used for the study which was carried out from May to July, 2008. The fish were purchased from fishermen and fish mongers at Otuocha, a major landing port and fish market in the river basin. All the fish were alive when purchased and were transported in plastic buckets which contained water from the river to the laboratory of the Department of Zoology, Nnamdi Azikiwe University, Awka where the investigations were undertaken.

In the laboratory the fish were thinned out. Fish sample was separated in buckets to minimize cannibalism. Effort was made to examine fish on the day of purchase. However, those that could not be examined were kept in river water until the next day when they were autopsied. The fish were killed by pithing, after which each fish was weighed to the nearest gramme (g) and the length was measured to the nearest centimetre (cm), using an electronic weighing balance (ADAM AQT-1500), and a 1 metre measuring board respectively.

The external surface of each fish was thoroughly searched for helminth parasites using a hand lens. Thereafter, the visceral cavity of the fish was opened by means of a pair of dissecting scissors. The skin on the ventral part of fish was lifted up with a pair of forceps and a small slit was made on the wall with a small pair of scissors. Thereafter, the scissors was inserted into the slit and the wall was slitted in an anterior direction up to level of the pectoral fins. The visceral cavity and the organs contained there in were thoroughly searched for helminth parasites with the aid of a hand lens. Then the entire gut was removed and placed in a Petridish which contained normal saline pending examination for presence of helminths.

The gut was opened starting from the cloaca and working towards the oesophagus. The continuous slit approach was adopted to minimize the possibility of severing long helminths such as cestodes which may extend from one section of the gut into another. The contents of each section of the gut (oesophagus, stomach, intestine and rectum) were emptied into a separate Petridish containing normal saline and searched thoroughly for helminth parasites. The gut wall in each section was also carefully inspected for attached or adhering helminths with the aid of a hand lens.

Parasites seen were picked up with a small paint brush and placed in specimen vials which contained normal saline. The vial was shaken vigorously for a while to remove mucus and adhering debris from the worms and also to cause fatique in the parasites to minimize contraction on contact with the fixative (Lucky, 1977). The parasites were thereafter quickly transferred into vials which contained 4% formaldehyde solution as fixative. Infection parameters were defined in accordance with the terminologies of Bush *et al*. (1997) as follows:

$$P = \text{Prevalence} = \frac{\text{No. of fish infested by the parasite}}{\text{No. of fish examined}} \times 100\%$$

$$\text{Mean intensity of infection} = \frac{\text{No. of parasites recovered from all fish infested by the parasite}}{\text{No. of fish infested by the parasite}}$$

$$\text{Mean abundance} = \frac{\text{No. of parasites recovered from all fish infested by the parasite}}{\text{No. of fish examined}}$$

Sex of fish was determined through gonad examination because there are no external characteristic features to differentiate between male and female lungfish (Greenwood, 1987). Fecundity, defined as the number of eggs per brood (Winberg, 1971; Lagler *et al.*, 1977) was estimated by making a total count of eggs in gravid ovaries of 10 randomly selected female fish, in normal saline. The contents of the stomach of the fish were identified using low power of microscope where necessary.

Results

Prevalence of helminth parasites in *P. annectens* in River Anambra is summarized on Tables 1 and 2. No ectoparasitic helminths were recorded. Six out of the 42 fishes were infested by one species of digenetic trematode namely, *Heterorchis protopteri* (Thomas, 1958). (Family: Fellodistomatidae), and a total of 33 worms were recovered from infested fish, giving a prevalence (P) of 14.29 %, a mean intensity of infection (M.I.I.) of 5.5 and a mean abundance (M. A.) of 0.8.

Table 1. Prevalence of *Heterorchis protopteri* in *Protopterus annectens* in River Anambra by length of fish

Fish length (cm)	N. E.	N.I.	P (%)	N.P.R.	M.I.I.	M.A.
20 – 29	2	0	0.00	0	0.0	0.0
30 – 39	33	4	12.12	18	4.5	0.5
40 – 49	6	1	16.67	11	11.0	1.8
50 -59	1	1	100.00	4	4.0	4.0
Total	42	6	14.29	33	5.5	0.8

N.E. = Number of fish examined; N.I. = Number of fish infested by the parasite
P = Prevalence; N.P.R = Number of parasites recovered from fish infested by the parasite.
M.I.I. = Mean intensity of infection; M.A. = Mean abundance.

The relationship between infection parameters and length of fish is shown in Table 1. No parasite was recovered from the smallest length class (20–29cm). Four out of the 33 fishes in the 30–39cm length class were infested and 18 worms were recovered from the infested fish, giving a P of 12.12 %, M.I.I. of 4.5 and M.A. of 0.5 for that length class. One out of 6 fishes in the 40–49cm length class was infested and 11 worms were recovered, giving a P of 16.67 %, M.I.I of 11 and M.A. of 1.8. The 50–59cm length class had one fish and the lone fish in that class was infested by 4 worms, giving a P of 100.00%, M.I.I. of 4.0 and M.A. of 4.0. Fewness of fishes in some of the length classes made statistical tests inpracticable but it could be surmised that all the infection parameter measured but particularly prevalence and mean abundance tended to increase with length of fish.

Table 2. Prevalence of *Heterorchis protopteri* in *Protopterus annectens* in River Anambra by weight of fish

Weight of fish (g)	N. E.	N.I.	P (%)	N.P.R.	M.I.I.	M.A.
200 – 299	2	0	0.00	0	0.0	0.0
300 – 399	33	4	12.12	18	4.5	0.5
400 – 499	6	1	16.67	11	11.0	1.8
500 -699	1	1	100.00	4	4.0	4.0
Total	42	6	14.29	33	5.5	0.8

The relationship between weight of fish and infection parameters is shown in Table 2. Two of the 15 fishes in the 200- 299g were infested and 9 worms were recovered from the infested fish giving a P of 13.33%, M.I.I. of 4.5 and M.A. of 0.6 for the weight class. One out of the 14 fishes in the 300–39g weight class was infested and 4 worms were recovered, giving a P of 7.14%, M.I.I. of 4.0 and M.A. of 0.3 for that weight class. Also one of the 7 fishes in the 400–499g weight class was infested and 5 worms were recovered, giving a P of 14.29 %, M.I.I. of 5.0 and M.A of 0.7 for that weight class. None of the 2 fishes in the 500–599g weight was infested, while one of the 2 fishes in the 600–699g weight class was infested and 11 worms were recovered, giving a P of 50.00 %, M.I.I of 11 and M.A of 5.5 for that weight class. Two fish belonged to 700g and above weight class and one of the fish was infested with 4 worms, giving a P of 50.00 %, M.I.I. of 4 and M.A. of 2.0 for that weight class. Although the infection parameters could not be subjected to statistical tests because of fewness of fish in some of the weight classes, it could be said that both prevalence and mean abundance tended to increase with weight of fish.

All the parasites were recovered from the intestine. No parasite was recovered from the single male fish that was found in the sample.

All the 41 female fish in the sample were gravid with the paired ovaries bulging with pinkish brown eggs. The 10 fish used in fecundity estimate had a total 14,228 eggs giving a corrected mean fecundity of 1,423 eggs per fish. The single pair of testis of the single male

specimen was prominent and pinkish/ reddish in colour.

Remains of insects, bits of flesh of fish (*P. annectens)*, plant materials, small stones and sand were among the items found in the stomach of some of the specimens.

Discussion

The helminthofauna of *P. annectens* in the river basin could be said to be poor, based on the facts that only one species of helminth was observed from the fish. Earlier studies (Ilozumba and Ezenwaji, 1985; Ezenwaji and Ilozumba, 1992; Ezenwaji *et al.*, 2005) had shown that fishes of different taxa in the Anambra river basin were infested by a large variety of helminths. Absence of nematode parasites from the fish is of interest because Boomker (1982) had noted that *Eustrongyloides* larvae are important parasites of lungfishes due to the predatory habits of the latter. The poor helminthofuna of *P. annectens* in the river may be due to the ecophysiology of the fish, particularly its habit of aestivation. The fish may remain in a state of aestivation for up to 12 months (Holden and Reed, 1972), during which it does not feed, and starvation is known to have deleterious effects on parasite population of a starving host. For example, Ilozumba and Ezenwaji (1985) reported that *Tenuisentis niloticus* an acanthocephalan parasite of *Heterotis niloticus* is expelled from starving fish. It could therefore be that helminths acquired by the fish prior to aestivation may not survive the physiological conditions that develop in the aestivating fish. If such be the case, the fish would emerge from aestivation in a relatively parasites free state and may acquire new populations of parasites post aestivation. This would then mean that infestation of *P. annectens* by helminths follows an annual cycle. More unequivocal statements on the scenerio can only be made possible by the results of all season studies of the fish in the river system.

Thomas (1958) reported infestion of *P. annectens* by *H. protopteri* in Ghana. To the best of the knowledge of the authors, the parasite has not been reported in the fish in Nigeria. The report of *H. protopteri* in the present study therefore constitutes a new geographical record for the parasite. In the absence of statistical tests, the observed relationships between host parameters and infection parameters can only be taken as pointer to trends for now. Nonetheless, the observed trends where the values of the different infection parameters increased with both the length and weight of fish could be said to be natural and normal for a fish species such as *P. annectens* in which diet would seem to be essentially the same for fish of different sizes/ages.

The state of the gonads in both male and female fish indicates that the *P. annectens* population was getting into a spawning state and since the investigation was conducted in the wet season, the situation corroborates the observation by Greenwood (1987) that lungfishes spawn during the wet season. A mean fecundity of 1,423 ripe eggs per fish as obtained in this study would categorize *P. annectens* as mesofecund on a fecundity scale which places species such as elasmobranchs which lay few eggs as oligofecund and those that lay many more eggs such as sunfish, *Mola mola* (28,000.000 eggs) and cod *Gadus morhua (*9,000.000 eggs) (Lagler *et al.*, 1977) in a brood, as hyperfecund. Against the background of relatively low fecundity and absence of information on population characteristics of *P. annectens* in the river basin and other river systems in Nigeria, it is important that caution be exercised in cropping the species, to avoid

possible over fishing. Although *P. annectens* is reported to exhibit some level of parental care (Holden and Reed, 1972), the rate of survival of the young fish is not known. It is possible that larger fish and other vertebrates which prey on juvenile lungfishes (Graham, 1997) could take heavy toll on the fries and fingerlings, so that the proportion of a brood that attain maturity may not be sufficient to sustain the population economically if cropping is left uncontrolled.

The sex composition of the sample which was 1:41 (male: female) may be a reflection of the situation in nature since the fish were purchased as they were available in the market and there is no external manifestation of sexual dimorphism that would make sorting of catches into sexes. Greenwood (1987) noted that females greatly out number males in lungfish populations and that a single male fertilizes eggs laid by many females. Since *P. annectens* is also a nest breeder (Holden and Reed, 1972), it could also be designated a polygamous species, distinct from communal spawning described by Ezenwaji (1992) for some species of clariids in the same river basin. A preponderance of females in natural populations of lungfishes underscores the need to regulate cropping in such a way that reproductive viability of such populations is not sabotaged through scarcity of males to fertilize eggs laid by female fish.

The status of the plant materials, small stones and sand which were present in the stomach of some of the fish as dietary items is not clear. They could be regular items in the diet of the fish because although lungfishes are known to be predatory (Holden and Reed, 1972; Boomker, 1994), fish generally make do with what they can assess in the water and some ingest bottom deposits (Lagler *et al.*, 1977).

In all, a clearer picture of the ecology and biology of *P. annectens* in River Anambra basin will emerge from more detailed studies as this is only a preliminary study. Studies of longer duration have to be undertaken and ecological parameters of the abiotic environment have to be monitored along side the fish so that the effect of such factors on the fish could be discerned. What seems obvious presently is that there is a need for some form of regulation of *P. annectens* fishery to reduce the chances of irreversible harm being done to the existing population by the indiscriminate cropping which is presently going on in the river basin.

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