Length - Weight Relationship and the Distribution of Intestinal Helminth Parasites in Freshwater Fishes from Amansea and Ebenebe Rivers in Anambra State, Nigeria

Abstract

Aims: The current study was carried out to evaluate the prevalence and distribution of intestinal helminths in freshwater fishes.

Study design: The study is a survey done to establish the type of intestinal parsite found in freshwater fishes in the Rivers**.**

Place and Duration of Study: The fish samples were collected from Amansea and Ebenebe Rivers from November 2021 to January 2022.

Methodology: Intestinal parasites of three (3) fish species commonly found in these rivers (*Parachanna obscura, Clarias gariepinus and Ctenopoma kingsleyae*) were examined. A comparative evaluation of the prevalence and distribution of the parasite was done in relation to their gender, location, weight and length.

Results: A total of 24 fish samples were collected in the Amansea River and 26 samples were collected in Ebenebe River. Seventeen (17) samples of *P. obscura*, 21 samples of *C. gariepinus* and 12 samples of *C. kingsleyae* caught with the cast nets of various mesh sizes and gill traps by artisanal fishermen in Rivers were bought early in the morning, transported to the Laboratory and identified based on morphological features. Out of a total of 50 fish sampled, 17 fishes (34% of the total sample) were infected by intestinal helminths. *P. obscura* had the highest parasite prevalence (47.06%) followed by *C. gariepinus* (42.86%). However, no parasite was recorded in *C. kingsleyae*. Prevalence of intestinal parasites in Ebenebe (18.00%) was higher than in Amansea (16.00) with a significant difference in prevalence ($p<0.05$), while parasite abundance was higher in Amansea (50.94%) than in Ebenebe (49.06%) with significant difference ($p<0.05$). Females had the highest prevalence (35.00%) against male fishes (33.33%) with no significant difference. A total number of 53 parasites belonging to 7 genera were recovered in this study. Parasites identified were nematodes: *Procamallanus* sp. (41.5%), *Camallanus* sp. (9.43%), *Rhabdochona* sp. (26.42%), *Contracaecum* sp. (7.55%), and *Spinitectus* sp. (5.66%); cestodes: *Polyonchobothrium* sp. (5.66%); and trematodes: *Clinostomum* sp (3.77%). The highest parasite abundance was recorded in *Procamallanus* sp. (41.5%), while *Clinostomum* sp had the least (3.77%).

Conclusion: This study reveals that the prevalence of intestinal parasites in freshwater fishes is relatively high therefore; fish consumers should subject the fish to proper processing before consumption, as some of these parasites are of zoonotic importance.

Keywords: Parasites, Freshwater fish, Ebenebe, Amansea, Nigeria.

1. Introduction

Freshwater is essential to human life. Freshwater bodies are known for their diversity of aquatic foods, their potential to contribute to sustainable healthy diets and their potential as a solution to address the "triple burden of malnutrition" (micronutrient deficiencies, under-nutrition, overweight and obesity) [1]. Fish ranked high in the contribution of essential protein in Nigeria [2]. As the human population inevitably increases, the demand for fish as a source of protein also grows. In recent times, there has been a tremendous increase in the development of fish farming and culture which is attributed to the increased need for affordable animal protein, especially in the tropics [3]. Fishes are a rich source of protein for man and other animals. They contain lipids, mineral oils, and vitamins which all have a remarkable impact on the lives of many individuals and communities. Fish oils which are known to aid the proper functioning of the heart, brain and immune system contain omega-3 essential fatty acids [4]. Fish are also good sources of vitamins B6 and B12. It is also a good source of fluorine and iodine which are needed for the development of strong teeth and the prevention of goitre [5].

Fish harvesting, handling, processing, and distribution provide a livelihood for millions of people as well as providing foreign exchange earnings to many countries [6]. Domestic fish production in Nigeria takes place in natural water bodies such as rivers, lakes, coastal water, and seas and artificial fish farming (pond rearing of fish in many urban areas). During the period 2007-2011, the average annual domestic fish production was put at 0.77 million metric tons [7].

Like other businesses, domestic fish production in Nigeria is faced with challenges and one of them is the effects of parasitic infection on the growth and consumption of freshwater fishes. These freshwater organisms harbour parasites either external or internal which may affect their growth. Parasitic infection constitutes a major threat to the well-being and productivity of fishery industries in Nigeria. The effects of parasitism ranged from the infliction of injuries on organs, reduction in the population of fish, impairment of proper organ functions, and disturbance in the physiology of the fish. Its effects also include reduction of both biomass and weight through parasites feeding on the fish host [8].

Fish parasites are also medically important [9]. Parasites of *Clarias gariepinus* are of medical importance and this raises public health concerns in the different geopolitical zones in Nigeria. This brings to question the importance of food safety in the country. Zoonotic parasites infecting fishery products are one of the several concerns in food safety. Wild fish populations are affected by parasitic diseases that directly and indirectly affect their growth and survival, hence a decline in production [10].

2. Materials and Methods

Study Area

The study was carried out in the Ezu River of Amansea and Ebenebe Town, Awka-North Local Government Area (LGA), Anambra State, South-eastern part of Nigeria. The river runs through Amansea and Ebenebe towns. Amansea lies between latitude 6o21'40" N and longitude 6o51'38" E while Ebenebe lies between 6o20’02" N and 7o07’45" E. Ebenebe is 25km from Awka, the capital city of Anambra State and is bordered by Amansea in the south, Odoli River and Mgbakwu in the north. These areas have typical semi-tropical rainforest vegetation, characterized by freshwater swamps. They have a humid climate with a temperature of about 30.60^{c} (870^{F}) and a rainfall between 152 and 203cm annually [11].

Fish Sample Collection and Identification

A weekly collection of samples was done for a period of eight (8) weeks with the help of artisanal fishermen living in the study area that specializes in the use of fishing traps, cast and gill nets of various sizes. The fishes were collected in batches in a plastic container containing water from the river and transported to Zoology Laboratory, Nnamdi Azikiwe University Awka for analysis. The fish samples were identified with the aid of a qualified ichthyologist using the dichotomous identification method of fish determination adopted by [12]. This was achieved by taking account of the meristic features of the various fish species such as dorsal, anal, caudal, pectoral and ventral fin rays. Spines present on sampled fish were also counted and measured to aid the identification process. The morphometric data of each individual fish was taken which included the total length of the fish, head length, body depth and body girth. Fish morphometric measurements (measuring of weight and length) prior to observation of external fish condition and dissection were done. The standard lengths were measured and recorded to the nearest 0.5 centimetres (cm) using a transparent ruler on a measuring board. The standard length of each fish was taken from the tip of the snout to the end of the caudal peduncle. The weight of each fish was taken using a sensitive electronic weighing balance and recorded appropriately [13]. The total number of fish caught from the rivers was recorded; this also enabled the determination of the relative abundance of the various species in the rivers.

Sex Determination

The sex of each fish was determined by physical observation of the urogenital papillae which are long or distended in males while it is round and reddish in matured females. The sex was also confirmed by internal examination after dissection to expose the paired testes in the males and paired ovaries in the females [14].

Sample Analysis

The ventral surface of each sample was cut open lengthwise to expose the alimentary canal. The stomach and intestine of each of the fish were cut open, and the contents were washed into Petri-dish containing normal saline. The lining of the gut lumen was properly washed in normal saline with macroscopic helminths picked with a soft camel hair brush and thereafter, the remaining contents were separated by sedimentation through centrifugation using Techmel Centrifuge 800D and then decanted. One to two drops of the preparation were placed on a microscopic slide covered with slip and observed under an x4 and x10 binocular light microscope for various intestinal parasites. The parasites were identified with the help of a qualified parasitologist according to the methods [15]. Fish specimens found with the parasite were given separate serial numbers to differentiate them from those without parasites. Parasites obtained were counted and labelled with the serial number of the fish. The recovered parasites were fixed in heated 70% ethanol, counted, and recorded. The hot fixative caused them to straighten out making them easier for further studies and examination.

Ethical Approval

This study followed guidelines for the care and use of experimental animals established by the Animal Care and Use Committee of Nnamdi Azikiwe University, Awka, Nigeria, for the control and supervision of experiments on animals (RE: NAU/AREC/2021/00046).

Statistical Analysis

The results were analyzed descriptively to calculate the prevalence and distribution of the parasite. The abundance of intestinal parasites and the prevalence of intestinal parasites in the samples were calculated using Microsoft Excel. The chi-square test ($\chi 2$) was used to determine the differences in parasitaemia amongst the fish species from the two locations. The level of significant difference was set at $P<0.05$.

3. Results

Table 1 revealed that *Procamallanus sp*. (68.2%), *Contracecum sp*. (100%), *Camallanus sp*. (80.00%), *Rhabdochona sp*. (71.4%), and *Spinitectus sp*. (100.0%) were more abundant in the fish *P. obscura* in comparison to *Clinostomum sp*. (100.0%) and *Polyonchobothrium sp*.

(100.00%) was found in *C. gariepinus*. There was a significant difference in the abundance of parasite species compared to the fish species sampled (P<0.05).

Intestinal parasites of fish species were more abundant in Amansea (50.94%) than in the Ebenebe River (49.06%). *Procamallanus sp*. was more abundant (41.51%) followed by *Rhabdochona sp*. (26.42%) while *Clinostomum sp*. had the least (3.77%). There was a significant difference in the number of parasite species recovered between locations (P<0.05) (Table 2).

Table 1: Parasites distribution by species of fish sampled.

Species of parasites	**Group of helminths**	***P. obscura***	***C. kingsleyae***	***C. gariepinus***	**Total**
Procamallanus sp.	Nematode	15(68.2%)	0(0.00%)	7(31.8%)	22(41.51%)
Contracecum sp.	Nematode	4(100%)	0(0.00%)	0(0.00%)	4(7.55%)
Camallanus sp.	Nematode	4(80.0%)	0(0.00%)	1(20.0%)	5(9.43%)
Rhabdochona sp.	Nematode	10(71.4%)	0(0.00%)	4(28.6%)	14(26.42%)

Spinitectus sp.	Nematode	3(100.0%)	0(0.00%)	0(0.00%)	3(5.66)
Clinostomum sp.	Trematode	0(0.00%)	0(0.00%)	2(100.0%)	2(3.77%)
Polyonchobothrium sp.	Cestode	0(0.00%)	0(0.00%)	3 (100.0%)	3(5.66)
Total		**36(**67.9%)	0(0.00%)	**17(**32.1%)	**53(100)**

$\chi 2 = 14.308$, df =6, P =0.03

Table 2: Abundance of parasites in relation to location.

	Abundance of parasites by location		
Parasites Identified	**Amansea**	**Ebenebe**	**Total (%)**
*Procamallanus*sp.	16(59.26)	6(23.08)	22(41.51)
*Camallanus*sp.	4(14.81)	1(3.85)	5(9.43)
*Rhabdochona*sp.	3(11.11)	11(42.31)	14(26.42)
Contracaecum sp.	4(14.81)	0(0.00)	4(7.55)
Polyonchobothrium sp.	0(0.00)	3(11.54)	3(5.66)
Clinostomum sp.	0(0.00)	2(7.69)	2(3.77)
Spinitectus sp.	0(0.00)	3(11.54)	3(5.66)
Total	**27 (50.94)**	**26(49.06)**	**53(100)**

$\chi 2 = 22.906$, df =6, P =0.001

The result in table 3 revealed that out of the 50 fish sampled and sacrificed, 17(34.00%) were infected with intestinal parasites. The females had a higher prevalence (35.00%) than the male fishes (33.33%). There was no significant difference in the prevalence of intestinal parasites in fish sampled in relation to sex (P>0.05).

P. obscura had the highest parasite prevalence, 8(47.06%) when compared to *C. gariepinus,* 9(42.86%) and *C. kingsleyae,* 0(0.00%). Also, the prevalence of intestinal parasites in

Ebenebe, 9(18.00%) was higher than in Amansea 8(16.00%). However, there was a significant difference in the prevalence of intestinal parasites in relation to fish species sampled ($P<0.05$) (Table4).

Table 3: Prevalence of intestinal parasites in relation to the sex of fish.

Sexes	No. Examined	No. Infected	Prevalence
Male	30	10	33.33
Female	20	7	35.00
Total	**50**	**17**	**34.00**

$\chi2 = 0.015$, df =1, P =0.903

Table 4: Prevalence of intestinal parasites in relation to fish species sampled from the two Rivers.

		Amansea	Ebenebe	
Fish species	**No. Examined**	**No. Infected**	**No. Infected**	**Total**
P. obscura	17	6(35.29)	2(11.76)	8(47.06)
C. gariepinus	21	2(9.52)	7(33.33)	9(42.86)
C. kingsleyae	12	0(0.00)	0(0.00)	0(0.00)
Total	**50**	**8(16.00)**	**9(18.00)**	**17(34.00)**

$\chi2 = 8.208$, df = 2, P = 0.02

The study revealed that fish weighing 400-499.9g had the highest prevalence of intestinal parasites, 3(100.00%) than those weighing 200-299.9g, 6(35.29%) and 100-199.9g 7(28.00%) while those weighing 300-399.9g, had the least, 0(0.00%). There was a significant difference in the prevalence of intestinal parasites in relation to the weights of fishes sampled ($P<0.05$) (Table 5).

Table 6 revealed that fish with lengths ranging from 30-39.9 cm had the highest prevalence of parasites, 4(66.67%) than those from 20-29.9 cm, 9(28.13%) while those with lengths between 10-19.9cmhad the least, 5(16.67%) infection. There was no significant difference in the prevalence of intestinal parasites in relation to the length of fish sampled.

Table 5: Prevalence of intestinal parasites in relation to weights.

Weight range (g)	No. Examined	Number of fishes infected (%) *P. obscura*	*C. gariepinus*	*C. kingsleyae*	Total
100-199.9	25	4(16.00)	4(16.00)	0(0.00)	8(32.00)
200-299.9	17	1(5.88)	5(29.41)	0(0.00)	6(35.29)
300-399.9	5	0(0.00)	0(0.00)	0(0.00)	0(0.00)
400-499.9	3	3(100.00)	0(0.00)	0(0.00)	3(100.00)
Total	**50**	**7(16.00)**	**9(18.00)**	**0(0.00)**	**17(34.00)**

$\chi 2 = 8.457$, df = 3, P = 0.04

Table 6: Prevalence of intestinal parasites in relation to lengths.

Length of fish (cm)	Number examined	Fish species: *P. obscura* No. Infected (%)	*C. gariepinus* No. Infected (%)	*C. kingsleyae* No. Infected (%)	Total (%)
10-19.9	12	2(16.67)	3(25.00)	0(0.00)	5(16.67)
20-29.9	32	3(9.38)	5(15.63)	0(0.00)	8(28.13)
30-39.9	6	3(50.00)	1(16.67)	0(0.00)	4(66.67)
Total	**50**	**8(16.00)**	**9(18.00)**	**0(0.00)**	**17(34.00)**

$\chi 2 = 4.323$, df = 2, P = 0.12

4. Discussion

A total of fifty-three (53) helminths belonging to seven (7) helminth genera were recovered in this study which includes trematode: *Clinostomum* sp., cestode: *Polyonchobothrium* sp. and nematodes: *Procamallanus* sp., *Camallanus* sp., *Contracaecum* sp., *Rhabdochona* sp. and *Spinitectus* sp. The recovery of similar helminth parasites has been reported previously from the same or related species elsewhere [16 and 17. In Ebonyi River in Ebonyi State, Nigeria the four freshwater fishes studied harboured helminth parasites [18].

The high prevalence of helminth parasites in freshwater fishes may be attributed to anthropogenic activities by humans who harbour the eggs of the parasites. Secondly, freshwater fish tend to have more parasites (e. g. in the United States) compared to their oceanic counterpart [19] due to factors such as ecological and biological factors that shape the pattern and prevalence of infection amongst fish species and location. These factors are summarized as human activity and natural factors [20].

The prevalence of helminth parasites was higher in the Ebenebe community compared to Amansea with a significant difference ($p<0.05$). Freshwater (FW) fish inhabit aquatic environments that vary greatly in their ionic composition and pH, but uniformly these environments have much lower concentrations of total salts than the fish's body fluids [21]. Furthermore, the type of habitat, water pollution, over-exploitation, the spread of invasive species, the spread of alien parasites and pathogens, salinization, acidification and climate change may play vital role in the prevalence differences [22].

This study also showed a high parasitic infection in *P. obscura*, followed by *C. gariepinus* while no parasite was recovered from *C. kingsleyae*. Freshwater fishes from River Niger *Proteocephalu*s species were recovered in *C. kingsleyae* [23] and this is in contrast to the result of this study. Forty-one per cent (41.67%) of infection in 120 examined *C. gariepinus* samples has been reported elsewhere [17] which when compared to this study is of lower prevalence, although a smaller number of samples were used in this study. Also, in another study conducted in Iran, 18.96% of the examined catfish were infected with digenean trematodes (39%) and cestode (34%) [24]. In Pakistan, a total of 43 specimens of four species of freshwater fish gut contents and visceral organs revealed the highest prevalence of nematodes (60.46%), followed by trematodes (53.48%). The differences in the prevalence of parasites in *P. obscura* and *C. kingsleyae* may be attributed to a number of factors which may include preferred location in the habitat, immuno-competence of the fish, the behavioural and feeding pattern of the fish as well as the high nutritional content of their intestine and suitability of the fish host in the provision of appropriate ecological requirements of the parasite [25].

Knowledge of the level of parasitisation of fish in a particular population is important because the damage associated with it is relative to the rate of infestation with the parasite. Heavily infected fish may have mechanical damage such as fusion of gill lamellae, tissue replacement, physiological damage such as cell proliferation, immune-modulation, detrimental behavioural responses, altered growth and reproductive damages. Changes in the environment, both anthropogenic and environmental, can alter the parasite/host equilibrium and cause disease or mortality in fish [26].

This study also shows that males have a slightly lesser prevalence rate than female fishes. There was no significant difference in prevalence in relation to gender. Higher prevalence in females (42.86%) compared to their male counterparts (40.00%) was reported in some other studies [17; 23]. It has been reported that infection of the four fish species in the Ebonyi River was not significantly sex-dependent [26]. Variations in parasitic infection among the sexes of fish may be by chance and not for any other reason [27]. The gender difference recorded in this study for the helminth-infected fishes could be due to alterations in the physiological status of sampled fishes, particularly males which could be a result of differential feeding either in quality or quantity of food eaten. The male may have a comparatively strong immune system than their female counterpart. It could also mean that more females were more readily available for parasitic infestation than males.

There is a relationship between parasitic prevalence infections and fish length and weight, which are equivalent to fish age. Fishes weighing 400-499.9g and having a standard length measurement of 30-39.9cm had a high prevalence of parasitic infection than their counterparts with lower weights and lengths. The relationship could be linked to differences exhibited in their feeding habit as a result of age, as older fishes tend to pick up more parasites than younger ones as they supposedly feed more [28]. Another reason could also be that younger fishes with supposedly active and better immunity were more resistant to parasitic infections than older fishes with compromised immunity.

This study also reported the highest abundance of nematodes (*Procamallanus sp*. *Rhabdochona* sp., *Camallanus* sp., *Contracaecum* sp. and *Spinitectus* sp), followed by a corresponding low abundance of cestodes (*Polyonchoboyhrium* sp.) and trematodes (*Clinostomum* sp). Similar helminth parasites were recovered in similar studies [16] however; *Polyonchobothrium* sp. and *Procamallanus* sp. recovered in this study were not among the parasite fauna, however, cestode (*Polyonchobothrium clarias* 28.18%) and nematode (*Procamallanus laevionchus* 11.82%) [17], has also been recovered in a similar study.

Conclusion

The study revealed that the helminth parasites distribution was higher in females with no significant difference in prevalence. The study also reported a higher prevalence of intestinal parasites in Ebenebe than in Amansea with a recorded significant difference. Seven parasite species, namely, *Procamallanus sp*. (nematode), *Camallanus* sp. (nematode), *Rhabdochona* sp. (nematode), *Contracaecum* sp. (nematode), *Polyonchobothrium* sp. (cestode), *Clinostomum* sp. (trematode), and *Spinitectus* sp, were the intestinal parasite found in 3 fish species from Amansea and Ebenebe Rivers.

Recommendation

It is necessary to always carry out surveys to determine and identify the parasite fauna and their distribution in different fish species in freshwater habitats such as Amansea and Ebenebe Rivers in Anambra State. Further studies should be encouraged to help establish the relationship between the physicochemical parameters and parasites assemblage

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