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Helminth Parasites of Gobies from Two Creeklets of the New Calabar River, Rivers State, Nigeria

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Authors' contributions

Authors APU and SO designed the study, wrote the protocol, and wrote the first draft of the manuscript. Authors SO, WC and GMS managed the analyses of the study and performed the statistical analysis. Author MSOA managed the parasite identification and slides. All authors read and approved the final manuscript.

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ABSTRACT

Aim: An animal species either serves as a definitive, a paratenic or an intermediate host to helminth parasites which may be species or site specific. This study was undertaken to investigate the intestinal helminth parasites of gobies (*Bostrychus africanus* and *Periophthalmus papilio)* collected from two creeklets of the New Calabar River.
Study Design: Fifteen samples of each species were collected (from two stations fortnightly) measured and weighed. All fish was dissected and intestinal parasites were collected, identified and counted. Physico-chemical parameters (temperature, dissolved oxygen (DO) and salinity) of the study areas were also measured in dry and rainy seasons. A total of 240 fish samples were examined of *B. africanus* (Standard length (SL) of 3 - 12 cm, weight of 30 - 36.1g), and *P. papilio* (SL of 5.9 - 15 cm, weight of 4 - 28.2g).
Study Period: Study was between October 2016 and May 2017.
Results: A total of three hundred and eigthy nine (389) nematodes (Ascaridida) were isolated from 25.4% of the sampled fish. *Bostrychus africanus* had higher percentage prevalence and mean intensity (91.6 % and 23 in station 1, 21.8 % and 20.5 in station 2). *P. papilio* had a lower

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percentage prevalence of 0.7 % and mean intensity of 4 in station 1 and 5.7 %, 8 in station 2. The nematodes were recovered from the stomach, small and large intestine of infected fish. The total lengths of infected and non-infected *B. africanus* from station 1 were different at P < 0.05 revealing that size affects the prevalence of parasites.

Conclusion: There was no significant difference in the relative condition factor (Kn) of infected and non-infected *B. africanus* and *P. papilio* from both stations indicating that the parasite did not affect the condition of the fish. Sex appeared to play a role in parasite prevalence in *B. africanus*. *B. africanus* and *P. papilio* from Rumuolumeni and Bakana creeks in the Niger Delta were infected with Ascaridida nematodes in the GIT. The prevalence and intensity of infection was higher in *B. africanus* and at Rumuolumeni.

Keywords: Gobies; periophthalmus; bostrychus; New Calabar River; Helminths; Nematodes.

1. INTRODUCTION

Helminths are the most diversified group of parasites that infect freshwater and saltwater fishes. They have very high complex ecological inter-relationship with their hosts. Endoparasitic helminths possess an indirect life cycle, which usually involves two or more hosts. Fishes are known to be definitive, paratenic (transport) or intermediate host to these helminthic parasites [1]. Nematodes which are usually called roundworms are common in marine fishes [2]. Fish ingestion of larval helminth parasites occurs regularly because of the abundance and diversity of these parasites in the aquatic ecosystem [3]. The health of fish is affected by parasites which make them susceptible to secondary infection by disease causing agents (e.g. bacteria, fungi and viruses). Parasites also compete for food, thereby depriving fish of essential nutrients and inhibiting growth, leading to morbidity and mortality with consequent economic losses [4].

Gobies are fishes of the family Eleotridae, Gobiinae, Periophthalmidae and Gobiidae, which make up one of the largest fish families, comprising of more than 2,000 species in more than 200 genera. Most of them are relatively small, typically less than 10 cm in length. Some large gobies such as *Gobiodes* or *Periophthalmus* can reach over 30cm in length in exceptional cases [5]. The Eleotridae are usually known as sleeper gobies which are small to medium-sized fishes, closely related to gobies in the family Gobiidae. Eleotrids are found worldwide in fresh water, estuarine and marine environments in tropical and warm temperate regions. A few small-sized species occur on rocky or coral reef environments. They are small to medium-sized, ranging from 2 cm to 50 cm. Most species live on the bottom where they feed on benthic invertebrates, especially crustaceans; a few species swim in the water column and feed on zooplankton. Periophthalmidae is the family of mudskippers. They are also small to medium-sized fishes which have cylindrical bodies and reach 27cm in length. They possess large heads and bulging eyes, which are very mobile and are adapted for easy vision in water and on land [6].

There are reports of helminthic parasite infestation in freshwater fishes from a few localities in Nigeria, but there appears to be a dearth of information on parasites of fish from the brackish water ecosystem. A few reports on brackish water fisheries are those on Lagos lagoon [7] and Warri River [8], the study of the gastrointestinal helminth parasites of Sciaenid species [9] and the threadfin *Polydactylus quadrifilis* [10] from Buguma Creek, Niger Delta. The aim of this study was to investigate the helminth parasites of gobies from two creeklets of the New Calabar River.

2. MATERIALS AND METHODS

2.1 Description of the Study Area

The New Calabar River located in Rivers State is a part of the Niger Delta basin which covers all the land between latitude 4°14’N and 5°35’N and the longitude 5°26’E and 7°37’E with a total area of 20,000 km^2 [11]. The vegetation is mangrove *Rhizophora mangle, R. racemosa, Avicennia* and *Nypa fructicans* (Nipa palm) which grows from a muddy substrate characterised by its foul smell. There are many anthropogenic activities that have led to environmental stress on this river [12], which is also connected to the Bonny River [13]. The present study was conducted at the Rumuolumeni and the Bakana creeks (Fig. 1) of the New Calabar River and provides information on the helminth parasites of two gobiid species belonging to two families.

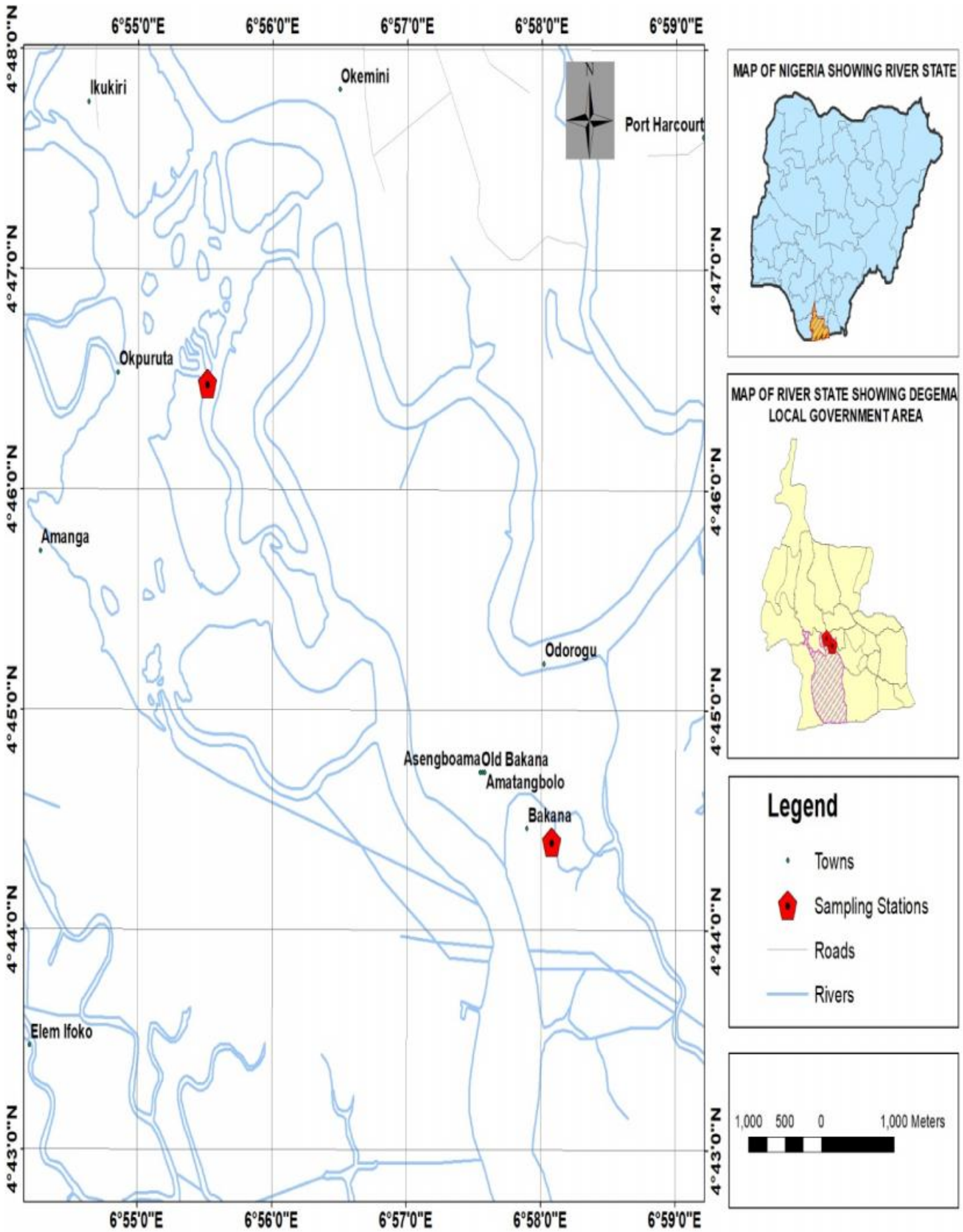

Fig. 1. Map of the study area showing Rumuolumeni and Bakana Communities in Rivers State

2.2 Collection of Samples

Sampling was for a period of eight months, from October 2016 to May 2017. Fish samples were collected from both creeks fortnightly between 6.00 am and 8.00 am using cone basket traps. Two hundred and forty (240) samples of each fish species *(B. africanus* and *P. papilio*) were collected from the intertidal zones of Rumuolumeni and Bakana creeks. Fish samples were conveyed to the laboratory in estuarine water collected from the creeks. Physico-chemical parameters (temperature, dissolved oxygen (DO), salinity, turbidity and pH were measured at both creeks using standard methods [14].

In the laboratory fish samples were weighed to the nearest g and length measured in cm to the nearest mm. The fish were examined for both ecto- and endo- parasites. For ectoparasites, the external surface of the fish was examined using a hand lens. For endoparasites, the fish was dissected and the entire gastrointestinal tract (GIT) was removed and placed in a Petri dish containing normal saline. The GIT was then divided into sections: oesophagus, stomach, small intestine and large intestine in separate petri dishes**.** Helminths were collected using a pipette, killed in warm alcohol and preserved in 70 % alcohol. The parasites were cleared in lactophenol, examined under a compound microscope and identified using standard protocols [15,16]. The number of parasites from each fish was counted and preserved in 70 % alcohol. Parasite prevalence and mean intensity of infection were calculated as follows:

$$Mean\ Intensity\ of\ infection = \frac{Number\ of\ parasites\ collected\ from\ a\ fish\ species}{Number\ of\ infected\ host\ species}$$

$$Prevalence(\%) = \frac{Number\ of\ infected\ fish}{Total\ number\ of\ fish\ examined} \times 100$$

The relative condition (Kn) of fish was calculated from the equation

Kn = W / a*Lb = Observed weight / Expected weight

where

W = weight of fish (g)
L = Length of fish (cm)
a = a constant
b = Growth factor

2.3 Statistical Analysis

The statistical test for parasite prevalence, infection intensity and Kn was analyzed using the student's T- test of JMP statistical software.

3. RESULTS

The descriptive statistics of the length and weight of fish samples are presented in Table 1. The range of the standard length of *B. africanus* was 3 - 12 cm, and 5.9 - 15 cm for *P. papilio* samples. The *P. papilio* samples were generally bigger than the *B. africanus* samples collected.

The Kn (Relative condition factor) of *P. papilio* from Bakana was between 0.98 - 1.05 (mean of 1.0) and between 1.20 - 8.37 (mean of 3.5) for Rumuolumeni. *Bostrychus africanus* samples from both creeks had a Kn of 1.0. The length - weight relationship gave b values of < 3 for both species at both sampling stations (Table 2).

The Kn of infected against non-infected fish were not different at $P \leq 0.05$ for both *B. africanus* and *P. Papilio*. The length of infected *B. africanus* was different from non-infected at $P \leq 0.05$. The length of infected *P. papilio* against non-infected were not significantly different at $P \leq 0.05$.

Table 1. Descriptive statistics of measured morphometric parameters of *B. africanus* and *P. papilio* from Station 1 and Station 2

Descriptive statistics	*B. africanus*		*P. papilio*	
	Rumuolumeni	Bakana	Rumuolumeni	Bakana
Mean standard length (cm) ± S.D	7.92 ± 1.28	6.97±0.93	8.48± 1.54	7.88 ± 0.82
Range of standard length (cm)	5 – 12	3 - 8.5	5.9 - 15	6.7-10.1
Mean weight (g)± S.D	11.07± 5.91	7.66±2.38	9.53 ± 5.15	7.74 ±2.52
Range of Weight (g)	3 - 36.1	3.2- 13.7	4 - 28.2	3.6 - 14

Table 2. The length-weight relationship (b) and relative condition factor (Kn) of *B. africanus* and *P. papilio*

Species-station	Length-weight relationship (b)	Mean condition factor ± S.D
B. africanus Rumuolumeni	2.94	1.0 ± 0.08
B. africanus – Bakana	1.51	1.0 ± 0.16
P. papilio – Bakana	2.95	1.0 ± 0.01
P. papilio Rumuolumeni	2.52	3.5 ± 0.08

3.1 Identification of Parasites

The parasites from the GIT of both fishes were identified as ascarid nematodes (Plates 1-3) whole males and females could be distinguished by their sizes and the presence or absence of some bodily structures. The female worms were longer and larger and grew up to 2- 4 cm in length, while males were smaller and ranged from 1.5-3 cm in length. Examination of posterior opening showed that male ascarid had pineal spicules or spine – like extensions near its opening. It also had papillae or bump – like protrusions, in front and behind its posterior opening (Plate 3) while the females lacked these structures (Plate 2). The female worm had a reproductive opening on the posterior third of its body, while the males lacked such opening. Examining the posterior region of the body cavity under a light microscope, a tube – shaped reproductive organ was seen. The females had two tubes that joined together to form a “Y”, while the males had one straight tube. The plates show that there are 3 different types of the nematode but due to difficulties in identifying to species they were pooled as ascarids.

3.2 Percentage (%) Occurrence of *Ascaris* in *B. africanus* and *P. papilio* at Rumuolumeni and Bakana Rivers in Rivers State

In Bakana, the percentage of occurrence of the ascarids in *B. africanus* and *P. papilio* was 91.6% and 0.7% respectively, while in Rumuolumeni, the percentage occurrence in *B. africanus* and *P. papilio* were 21.8% and 5.7% respectively (Table 3). *B. africanus* had the highest percentage occurrence from both stations. The mean intensity of the worms in *B. africanus* and *P. papilio* were 23.0 and 4.0 respectively in Bakana, and 20.5 and 8.0 respectively in Rumuolumeni. The mean intensity was higher in *B. africanus* at both stations. In both gobies, these ascarid nematodes were found in their large intestine, small intestine and stomach. The cummulative parasite burden for both fish species was 25.4 %.

Plates 1. A– E. Five variations in the anterior ends of ascaridida nematodes from gobies. Scale Bar: 0.25mm

Table 3. Incidence of parasite in *B.africanus* and *P. papilio* from Rumuolumeni and Bakana, Rivers State

Station	Species	% (Percentage) occurrence of parasite	Parasite mean intensity	Location of parasite in fish host
Rumuolumeni	*B. africanus*	91.6%	23.0	Small intestine, large intestine, Stomach.
	P. papilio	0.7%	4.0	Small intestine, Stomach
Bakana	*B. africanus*	21.8%	20.5	Small intestine, Stomach.
	P. papilio	5.7%	8.0	Small intestine, Large Intestine

Plates 2. A – C. Sections of female ascaridida nematodes from gobies. A. Uterine region of gravid female with eggs. B, C. Posterior end of female parasites Scale: 0.25mm

Plates 3. A – C: Posterior regions of male ascaridida nematodes from gobies. A. Ventral view showing spicules; B, C. Side views showing spicules. Scale Bar: 0.25mm

The students T- test analysis of parasite prevalence of *B. africanus* from Bakana and Rumuolumeni revealed significant difference @ P = 0.05 (Fig. 2), while the parasite prevalence of *P. papilio* from Bakana and Rumuolumeni were not significantly different @ P = 0.05 (Fig. 3).

4. DISCUSSION

Approximately 25.4 % of 240 fish investigated from this study were infested with ascaridida nematodes indicating a low level of parasite burden in the fish population from both sampling stations. This does not conform to the report by [17] on the parasite load of *Ascaris* in *Battygobius soporator* from Lagos lagoon which had a high level (47 %) of parasite burden in the fishes investigated. The parasite burden of the individual species however revealed a higher burden on *B. africanus*. The presence of only one family of nematode from this study, in both species of gobies from both sampling stations indicated low parasite species diversity which is not in agreement with the report by [18] on the Niger Delta tidal creek in Nigeria where they recorded no infestation in the gobies species collected from Buguma creek. The parasite *Ascaris* and *Anisakis* have been recorded from

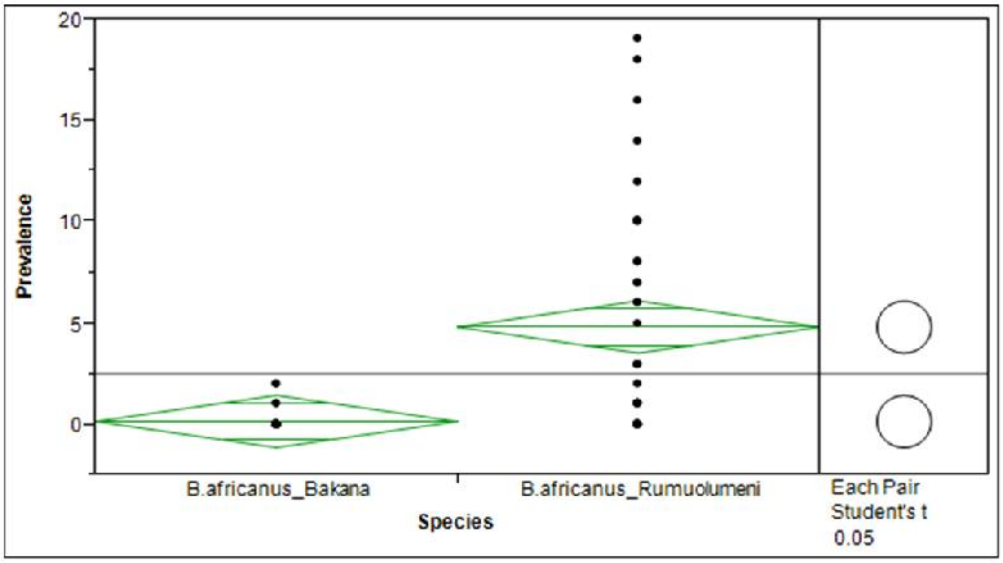

Fig. 2. Student's T- Test analysis of parasite prevalence of *B. africanus* from Bakana and Rumuolumeni

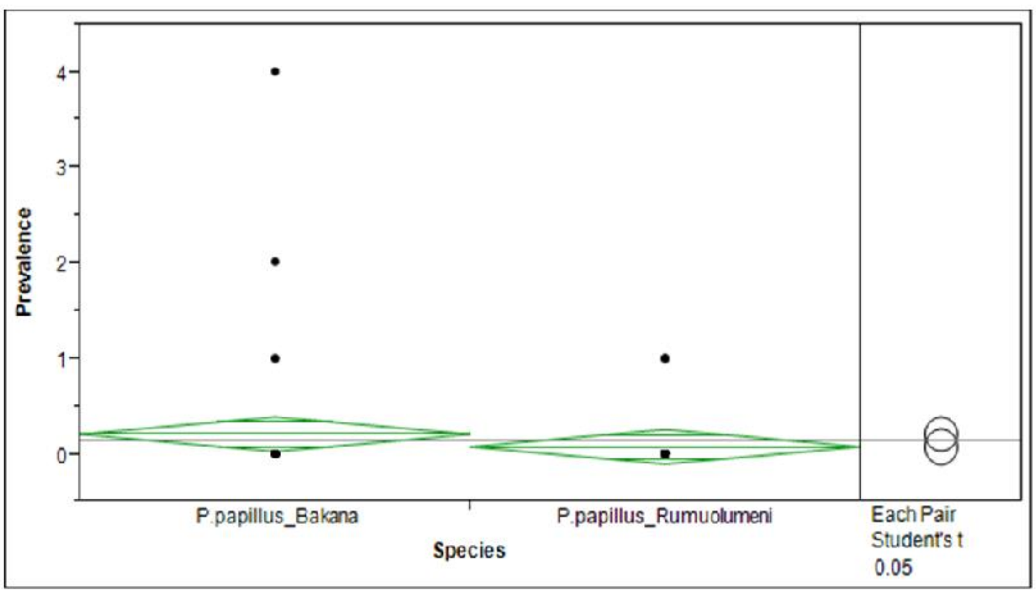

Fig. 3. Student's T- Test analysis of parasite prevalence of *P. papilio* from Bakana and Rumuolumeni

Atlantic cod, *Gadus morhua* which was sampled in the Barents, Baltic and North Seas [19,20]. [21] and [22] recorded *Camallanus* and *Procamallanus* species from another Nigerian creek. This difference might be because parasites are focal and their prevalence are determined by prevailing environmental parameters. In fishes, host and pathogen interaction is modulated by environmental factors and this is a key determinant of susceptibility to disease [23]. Ascaridida nematodes are important helminths which have the potential of being zoonotic as was reported by [24] during a study, when they isolated the eggs of some helminth parasites (nematodes; *Capillaria sp., Ascaris sp., Enterobius sp.*, and hookworm) which had zoonotic potential, from sediments and samples of Carp in India. The presence of ascaridida nematodes in the sampled fish species is also in conformity with the study by [25] who identified Ascaridida nematode larvae (*Anisakis, Hysterothylacium, Raphidascaris* and *Terranova*) from the intestinal lumen and abdominal organs of marine fishes from New Caledonia. Their research reported those nematodes as being important and potential zoonotic parasites in that region. The ratio of female to male Ascarid nematodes isolated was 2:1 and the presence of matured females (gravid) and male nematode parasite in the fish samples indicates that the species of Gobies investigated were the definitive host of the parasites.

From this study, *B. africanus* species from Rumuolumeni were larger than those in Bakana and recorded more parasite intensity and prevalence (91.6 %). This result is in agreement with the study of [17] who recorded higher parasite prevalence (61.5 %) in *B. soporator* in bigger fish of 15 – 17 cm than the smaller 9 – 11 cm fish (19.4 %). This would mean that older fish may have higher parasite prevalence probably due to longer exposure and parasite recruitment. The observation regarding *P. papilio* samples collected from both sampling stations does not agree with the findings of [17] as it appears that size was not a determining factor on parasite prevalence. There was also no relationship between parasite load (intensity) and fish length and weight, which is in agreement with the findings of [26] on the helminth parasites of *Tilapia zilli* from River Oshun. The intensity should depend on prevailing environmental conditions that make the host susceptible to the parasite and also on the prevalence of larval stages in the intermediate host(s). The gobies (*B. africanus* and *P. papilio*) from this study inhabit the intertidal regions of the creeks and feed on crustaceans and planktons. Children are often seen on the tidal flats picking periwrinkles (*Tympanotonus*) and mudskippers (*P. papilio*). The possibility of infection when they ingest the eggs of these parasites cannot be ruled out. [17] has suggested that the presence of *Ascaris* species in *B. soporator* was because of feacal waste discharged into the Lagos lagoon. The significantly higher prevalence of ascarids in *B. africanus* during the wet season does not agree with previous work by [18], who recorded the highest dominance of parasites in *Polydactylus quadrifilis* during the dry season. This shows some variability in parasite prevalence for different seasons.

Many factors such as the change in physico-chemical properties of the water body, fish species, food, sex and stage of maturity [27] can be responsible for the change of b values for the length – weight relationships of gobies. The length-weight relationship analysis of both species of gobies from both sampling stations indicated negative allometric growth ($b < 3$). This implies more increase in length as growth proceeds, giving a slimmer fish as the increase in length does not give a corresponding increase in weight. These findings are similar to those made by [28] and [29] that observed negative allometric growth for different species of cichlids from the Anambra River in Nigeria.

The condition factor of a fish depends on a number of factors, availability of food, ideal temperature and salinity for growth. The *P. papilio* from Rumuolumeni appeared to be in a better condition with a Kn of 3.5 than *B. africanus* with a Kn of 1. [28] also recorded mean condition factors from cichlids which showed variations due to seasonal and environmental changes. Fishes thrive better in water bodies that have less anthropogenic activities and are less polluted. The condition factor of fish describes the physiological state of the fish with respect to its welfare and nutritional status [30]. *P. papilio* from Rumuolumeni with a mean relative condition factor of 3.5 with low parasite prevalence were in better condition. This agrees with the records from the study by [31] on the cat fish, *Synodontis filamentosus* and *Calamoichthys calabariscus* sampled from Lekki lagoon, which was in good condition due to the absence of parasites. The condition factor of infected and non – infected fish was not significantly different, implying that the infection with these parasites did not affect the length – weight relationship of the fish. However, it is possible that there could be pathological effects on the gastrointestinal tract (GIT) of the fish which may not result in weight loss. When nematodes are numerous, they can block the lumen of the GIT and affect food digestion and absorption.

5. CONCLUSION

In conclusion, *B. africanus* and *P .papilio* from Rumuolumeni and Bakana creeks in the Niger Delta were infected with Ascaridida nematodes in the GIT. The prevalence and intensity of infection was higher in *B. africanus* and at Rumuolumeni. Size appeared to be a factor in determining parasite prevalence for *B. africanus*. The mean Kn was greater than 1 for both species but *P. papilio* with lower parasite prevalence was in better condition.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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