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Distribution, Morphometrics, Feeding Evaluation and the Reproductive Capacity of *Polypterus Bichir* from the River Niger at Agenebode in Edo State Nigeria.

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Abstract
Background: *The global need to provide food, fish food and cheap sources of protein rich food sources is of the essence. Vast knowledge of the anatomy, morphometric, reproductive biology, and nutrition biology of a fish species is scientifically relevant to provide suggestions and solutions for its propagation and management. Polypterus bichir is a highly nutritious fish with essential elements needed for maximal intake by invalids, children and the aged, highly priced commercial fish distributed across West Africa commonly found in River Niger and caught in quantities along Agenebode River. Little is known about its biology, species variation and seasonal abundance of most ornamental fishes. The information gotten from this study will provide necessary background knowledge on its biology and provide opportunity for the culture of this fish species especially in its natural environment (cage culture) or pond culture.*
Main Findings*: A sex ratio of 1:1.4 (male to females) were obtained, stomach analyses revealed that P. bichir consumed shrimps, crabs with a preference for fish food. A negative allometric growth pattern was observed though fishes were in good condition in the river. It is a moderate fecund fish that spawns at the peak of floods.*
Conclusion: *Polypterus bichir exhibits a negative allometric growth pattern. It is found to be in good condition in the River Niger at Agenebode. P. bichir is a carnivore, a predator with a preference for live fish meals. P. bichir has a moderate fecundity with mean GSI of 951 eggs of 0.5mm in diameter. P. bichir has a great potential as a readily available culture-able fish species in Nigeria*
Key words: *negative allometric growth, carnivorous, relative gut length, feeding habit, fecundity Gonadosomatic index*

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I. Background

Understanding the reproductive biology, morphometrics and meristic biology, nutrition biology of fish is of relevance to scientific investigations to provide suggestions and solutions for the management, culture and production of any fish species. *Polypterus bichir* is a commercial fish distributed across West Africa. It is a living representative of the family Polypteridae, found in River Niger and caught in quantities along Agenebode River. It is highly priced when compared to other ornamental fish species at the Bode Market. Mbawike, (2011) stated that Polypterus is highly prized value when compared to other Nigerian ornamental fishes at the international markets. *P. bichir* is a highly nutritious fish with essential elements needed for maximal intake by invalids, children and the aged (WHO 2015; Tut et al., 2019), its flesh is very tasty, its flesh is thick and the muscles are tough thus gives the fish an advantage of satisfying mans' quest for animal protein especially one of adequate cholesterol. Little is known about the biology, species variation and seasonal abundance of most ornamental fishes (Areola, 2003). Knowledge of the biology especially on reproduction of these fish in the wild is required for successful breeding and production. In recent past, reports on the aspect of biology of fish species have been documented by Ayoade and Akponine (2016) on *P. senegalus* in Eleiyele Lake, on *Pagellus Erthrinus* Collected from the Coast off Benghazie. Previous studies on aspect of biology of Polypterus species in Nigeria include (Raji et al., 2004) on the food habits of *P. endlicheri* and *P. senegalus* in Lake Chad; (Offem et al., 2008) on the length-weight relationship, condition factor and sex ratio in the Cross River. (Adebisi, 1987) in upper Ogun River; (Fawole and Arawomo, 2000) in Opa Reservoir and (Issa et al., 2005) in Kigera reservoir reported on some aspects of biology of many fish species including Polypterus species. Other studies on the biology and attributes of other fish species include the study on the biology of *Pomadasys jubelini*, the *Proptopterus annectens*, *Gymnarchus niloticus* and on the anatomy of the bichir by (Agbugui et al., 2014; Agbugui and Oniye 2016, Agbugui et al., 2016; Agbugui and Oniye 2019a; Agbugui and Oniye 2019b); Agbugui et al., 2021a; Agbugui et al., 2021b). Available and reliable information on the different aspects of

biology of *Polypterus bichir* are often available only at taxonomy level. Scientific investigation into the biology of *P. bichir* in the River Niger at Agenebode will provide recent and viable information on the natural diet, feeding habit, growth structure, morphology, productivity and reproductive viability which will be useful for the possible breeding, rearing, culture and management. The information gotten from this study will provide necessary background knowledge on its biology and provide opportunity for the culture of this fish species especially in its natural environment (cage culture) or pond culture.

II. Materials and Methods

Study Area:

The River Niger at Agenebode is located at 706'N 642'E. Agenebode is a waterside town located by the banks of the River Niger. It is located at the lower River Niger, typically with a humid climate and weather of approximately 32 ^{o} C. The area is marked by 2 seasons, the wet season and the dry season. The wet season is from April to October while the dry season is from November to March. The River serves the people of Edo and its environs with a route for transportation to other neighboring communities; a fishing spot and fishery; market and a source of water for domestic purposes, farm, irrigation, industrial purposes and trading location for local traders and the riparian localities at the popular Agenebode Market.

Collection of specimens and sampling

A total of sixty eight (68) fish species were collected fortnightly for 12 months from November 2019 to October 2020 from three stations (based on the landing sites of fishing localities) from catch landings of fisher folks by the use of canoes, gill nets, cast nets, drag nets, fish traps and calabashes from the River Niger at Agenebode. Samples of fishes were transported to the Laboratory of the Department of Biological Sciences, Edo State University Uzairue, Edo State. Fishes were rinsed, wiped dry and identified to species level using guides, keys and pictures provided by (Pandey and Shukla 2005; Froese 2006; Froese and Pauly 2020) then preserved in 5% formalin.

Morphological Parameters

The standard length (cm), total length (cm), head length (cm), gape mouth (cm), girth length, and weight (g) of *P. bichir* were obtained by using a graduated ruler and tape, a measuring board and a digital electronic scale (Storius 177). These were recorded and analysed. Fin counts and measurements were also taken and recorded.

Determination of Sex

Sex was determined by external observation of the caudal tail and anal fin in addition to visual and microscopic observation of the gonads. Where there was the presence of two ovaries the fish was referred as females or the absence of ovaries and connoted as males.

Length – weight relationship

The total length (TL) and body weight (W) were measured from the fresh samples to the nearest 0.1 cm and 0.01 g respectively. The length – weight relationships were estimated from the formula, W = a L b, where W is total body weight (g), L is the total length (cm), a and b are the coefficients of the functional regression between W and L. This relationship was transformed into a linear form by the equation:

$$Log\, W = Log\, a + b\, log\, L$$

Condition Factor

The condition factor 'K' was calculated by the following formula given by (Pauly, 1983):

$$\frac{(K = 100\, W)}{L^3}$$

where: W = total weight of fish in grams; K = condition factor; L= total length of fish in centimeters. It was calculated for both sexes separately and then for the combined sexes. Variations of K with season and size groups were also determined.

Gonadosomatic Index (GSI)

The Gonadosomatic Index of samples of *P. bichir* were determined according to Ugwamba et al., (1991) as follows

$$\frac{GSI = weight\ of\ gonad\ X\ 100}{weight\ of\ fish}$$

The monthly catches were sorted into sexes. The mature female ovaries were analysed and used to determine the GSI.

Fecundity: Both ovaries of the mature fish were removed and placed gently in boiling water and allowed to boil for 20 minutes. The eggs became hard enough for easy counting. The boiled eggs were then stored in 5% formalin. 1.00g of the whole ovaries was cut off. The cut section of the eggs were carefully counted and multiplied by the total weight of the ovary to give the total number of eggs.
The maturity stages of the ovaries were classified according to Nikolsky (1963):
Stage 1 – Immature, Stage II-Quiescent, Stage III - Maturing, Stage IV-Mature,
Stage V - Running, Stage VI -Spent.
Egg diameter: Egg diameter (mm) was measured with an ocular micrometer. A stage micrometer was earlier used to calibrate the microscope. Diameters of twenty eggs randomly selected from each ovary were measured and their mean was taken as the average egg diameter.
Determination of Food and Feeding Habits
Each stomach was split open and the contents emptied into a petri dish. The contents were observed under a hand lens and the food materials and identified.
Frequency of occurrence method (FO): In the frequency of occurrence method the individual food matter in the stomach were sorted and identified. The number of stomachs in which each food item occurred was expressed as a percentage of the total number of stomachs with food examined.
Numerical method (NO): This method involves counting the number of each food item present in the stomach of a fish and summing these numbers to obtain the total number of all food items found in the stomach. The number of each food item is then expressed as a percentage of the total number of all food items. It was expressed as

$$Percentage\ number\ of\ a\ food\ item\ = \frac{Total\ number\ of\ a\ particular\ food\ item}{Total\ number\ of\ a\ particular\ food\ item}\ x\ 100$$

Importance of food index (IRI): The importance of various food items were determined with the index of food importance following a method Ugwumba and Ugwumba (2007)

$$IRI\ =\ (Cn\ +\ Cw)\ X\ F \ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ldots\ (1)$$

Where IRI = index of relative importance
Cn = percentage of numerical composition
Cw = percentage of gravimetric composition
F = percentage of frequency of occurrence
The dietary compositions for the species examined were expressed as percentages, that is

$$\%\ IRI\ =\ (Cn\ +\ Cw)X\ F\ X\ 100 \ldots\ldots\ldots\ldots\ldots\ldots 2$$

Food item with %IRI ≥ 3 are regarded as primary, ≥ 0.1 to <3 are secondary whereas ≤ 0.1 are considered as incidental food items.
The Relative Gut Length (RGL): This was expressed as the ratio of total length of gut to total body length (Biswas, 1993).

$$RGL = \frac{total\ length\ of\ gut}{total\ length\ of\ fish}$$

Where fish can be classified as herbivorous (RLG>1), carnivorous (RLG<1) or omnivorous (RLG =, or>, or <1).
Prey-predator relationship: The relationship between the total body length and total weight of *P. bichir* and prey body weight was determined and described by the equation;

$$YL\ =\ a\ +\ bXL\ (Ogari, 1988)$$

Where YL = Prey body weight (g), XL = *P. bichir* body length (cm) or body weight (g).
Statistical analyses
The Microsoft Excel 2010 was used to analyse the data obtained for this study.

III. Results

The Length weight relationship (LWR), of *P. bichir* obtained in the study revealed the correlation coefficient (r) was above 0.84. Total weight and standard length of the 68 specimens of *P. bichir* examined were within the ranges of 468 - 2015g (1359 ± 346.68) and 8.0 – 61.0 cm (36.71cm ± 13.15) respectively. The value obtained for b was 2.12 and was highest during the dry season. The Length Girth Relationship of *P. bichir* showed a correlation coefficient (r) of 0.91 and a 'b' value of 1.20 this was also at its highest (0.95 and 1.41) respectively in the dry season. The condition factor 'K' was 0.73. (t-test, P>0.05).

29 males and 39 females were examined with a ratio of 1:1.3. All of these specimens examined for variety of food items, 33 had empty stomachs while 35 had food items in their stomachs. The percentage composition of food items obtained from *P. bichir* from November 2019 to October 2020 is shown on Table 1.

The dominant food items found were fish flesh (35.79%) and Shrimps (18.95%) by %FO. Other food items of low importance were shells, plant parts and grasses 5.26%, 2.11% and 1.05% respectively (Table 2). The food item with the most relevance was fish with 13.81%

Table 1: Percentage composition of various food species consumed by *Polypterus bichir*

Food items	**%NO**	**%FO**	**%W**	**IRI**	**%IRI**
Fish flesh	23.38	35.79	45.67	2761.81	13.81
Shrimps	20.78	18.95	27.12	1014.25	05.07
Crabs	12.99	10.53	08.56	253.53	01.27
Scales	32.47	26.32	08.56	724.07	03.62
Shells	06.49	05.26	05.71	71.78	00.36
Tree parts	02.60	02.11	02.67	12.39	00.06
Grass	01.30	01.05	01.71	03.54	00.02
TOTAL	**100.00**	**100.00**	100.00	20000.00	100.00

Key: NO = Numerical method FO = Frequency of occurrence method
W = Gravimetric method IRI = Index of relative importance

(IRI): Food item with %IRI > 3 are regarded as primary, > 0.1 to <3 are secondary where as < 0.1 are considered as incidental food items

The Relative Gut Length (RGL) was expressed as 0.56.

The prey –predator relationship of *P. bichir* revealed that fish weight was more related to prey size. Fig 1 and Fig 2

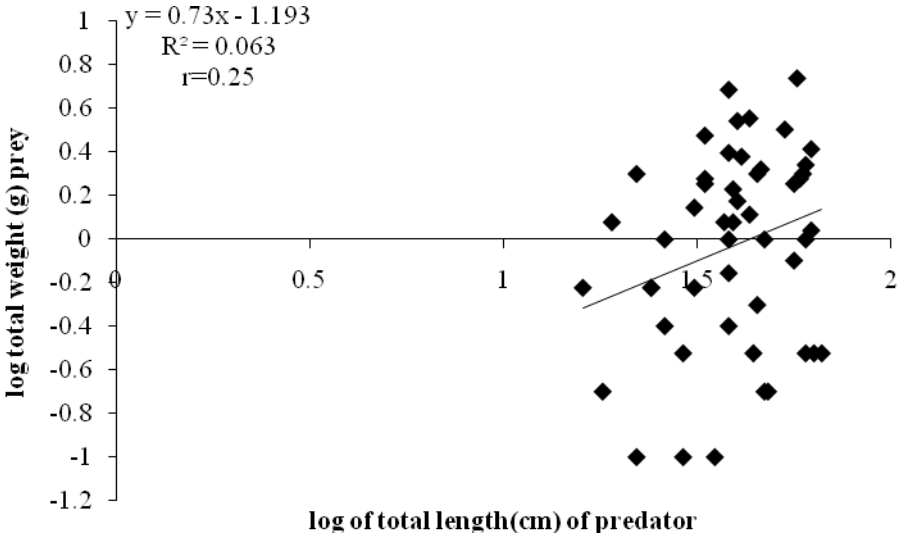

Fig 1. Relationship between prey body weight and total length of *P. bichir*

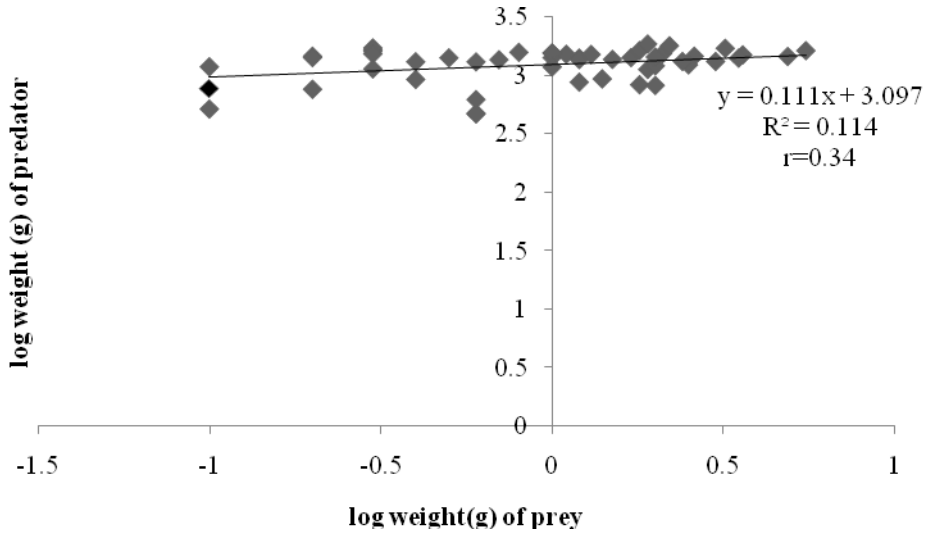

Fig. 2. Relationship between prey body weight and total weight of *P. bichir*

Polypterus bichir possesses two ovaries, one on each side of internal gut. Four stages of gonad development were found in *P. bichir* during the sample period; the quiescent, maturing, mature and running stages in both males and female fishes. (Table 2).

Table 2: Stages of gonad developments observed in *P. bichir*

Gonad stage	Macroscopic character	
	Testis	Ovaries
I. Immature	Not encountered	Not encountered
II. Quiescent	Testis were small and creamy in colour	The quiescent stage had ovaries that were translucent and creamy in colour, oocytes were not visible to the naked eye, blood vessels were seen on the surface of ovaries.
III. Maturing	The males had small milt that was milky in colour.	The maturing stage had ovaries that were amber in colour with reddish patches indicating the presence of blood vessels. Oocytes were translucent and were not visible to the naked eye, blood vessels were seen on the surface of ovaries
IV. Mature	The mature stage had larger milt that could be released with some pressure while the	Oocytes were amber and sometimes reddish in colour. Oocytes could be seen with naked eye and counted easily.
V. Running	Milt could be released with little pressure	Eggs could be released with little pressure
VI. Spent	Not encountered	Not encountered

Table 3. Variation in Gonadosomatic Index of Polypterus bichir from the River Niger at Agenebode in Edo State Nigeria

Parameters	N	Range of GSI	Mean ±S.D
Dry season	41	0.7-5.3	3.2±0.22
Rainy season	27	5.6-15.8	10.88±0.36
Sex			
Male	29	0.52-4.28	2.5±1.39
Female	39	5.1-15.86	8.66±2.13

Gonadosomatic index: The mean GSI of the female (8.66±2.13) was higher than male GSI (2.5±1.39). The GSI increased with size of fish. Significant difference were recorded in the GSI between the seasons, size and sexes ($P<0.05$). The percentage of individuals with mature gonads (stages 3 and 4) and mean monthly GSI were correlated for both males.

Fecundity: The fecundity of the bichir ranged between 326 (for specimen with TL = 26.3 cm; total weight = 687g) and 3193 eggs (for specimen with TL = 57.4 cm; total weight = 2396.62 g). The relationship between absolute fecundity and TL and absolute fecundity and total weight of *P. bichir* was described by the following

regression equation respectively: Y = 14.26 X + 1485.5 (r=0.051) Y = -3.148 X + 1762.3 (r = 0.209) Where, Y = absolute fecundity and X = total length (cm) / total weight of fish (g). The relative fecundity for the species varied between 687g – 2392g with mean of 951 ± 1.8991 eggs/g. Egg diameter: The mean egg diameters of *P. bichir* were 0.52 ± 0.03 mm. Gravid females were found during the peak of the rainy season in June –July and August.

IV. Discussion:

The result shows that the length of *Polypterus bichir* increased with body weight and exhibits a negative allometric growth (b<3.0). Negative allometric growth implies that the length increases more than its weight. In line with findings of this study, similar reports on negative allometric growth (b<3) were reported for in *Sarotherodon melanotheron*, *P. senegalus*, for *H. fasciatus* (Atama et al., 2013) and *Chyysichythys nigrodigitatum* (Kareem et al., 2015) respectively. Negative allometric growths (b < 2.5) were also reported for adult *Gymnarchus niloticus*. (Carlander, 1969; Pauly and Gayanilo, 1997). Other reports of LWR were given for other pelagic fish species by (Koutrakis and Tsikliras 2003; Sinovcic et al 2004).

Differences in LWR of fish species could be as a result of changes through stages in its life cycle defined by changes in the body form with size, feeding habits and factors related to reproduction and state of the habitat.

The average condition factor obtained in this study was <1 for both males and females, indicating that *P. bichr* are in a good condition in the River Niger. Many factors could delineate the productivity of a river, pollution, turbidity, indiscriminate dredging could adversely alter the bottom condition of the river, as it is reported that *P. bichir* prefers clear waters because of its poor sight. These could impact negatively on habitat structure and feeding of sensitive and vulnerable fish species like the *P. bichir* in the River. It is of note then that during culture, *P. bichir* should be kept in clean waters. Raji et al (2004); Ayoade and Ikulala, (2007); Ayoade and Akponine (2016); Agbugui and Oniye 2019 reported that the increase in condition factor during the dry season could be as a result of breeding activities of the fish that occurs during rainy season which could lead to depletion of reserves. Of the 68 stomachs examined, a high proportion of empty stomachs (52%) of *P. bichir were* observed. This appears to be a common phenomenon in predatory species. Such reports has been availed by Ayoade 2018; Olawole et al., 2019; Timo and Ralph 2019). It is also reported that *P. bichir* is a slow swimmer and mostly feeds at night (Harrington 1899; Robert 2019; Freose and Pauley 2021). This action reduces its feeding periods hence the frequency of availability of food in its gut, again, fishing often takes place at night thus increasing the chances of this species of fishes to have empty stomachs when caught. Carnivorous species have short periods of feeding followed by rapid periods of digestion due to the well developed stomach activities maximum digestive capacities.

The dominant food items obtained from the stomach of *P. bichir* was fish. This was strongly followed by prawns (*Macrobrachium macrobrachion*), these were often found in the larger sizes of the bichir. Fish scales and shells of crustaceans were randomly sited. With regards to relative importance of diets, fish flesh and shrimps had higher values. This indicates that the river is rich in variety of food and feed specimens, availability of food prey. It also shows a restriction to prey specificity which is fish. Similar reports of fish prey specificity were reported by Raji et al., (2003); Adedokun and Falaye (2015), this result is however in contrast to that obtained by Ayoade et al., 2018 which showed that the diets of *P. senegalus* in Eleyele Lake were dominated by insects (71.65%) while *Hepsetus odoe* was purely piscivorous. It is of great interest to note that most of the fish items found in the stomachs of *P. bichir* were whole irrespective of sex of fish and season, revealing that it prefers it meal while alive and swallows them whole thus imposing maximum limit on the size of its prey. This is a relevant pointer during culture or propagation of the *P. bichir*, revealing that live feed will be preferable for food. Rose et al., (2001); Agbugui et al., 2014; Agbugui and Oniye 2019; Chhaya and Rahul, (2020), also showed that many carnivorous species specialize in their feeding habits as they approach maximum size. These changes in diet go with changes in biotope as adult fish can cover wider areas to feed whereas small fishes are restricted for various reasons; predation, energy saving and swimming abilities.

A linear and positive relationship between the predator body weight and prey body weight of *P. bichir* was observed. , larger fish had a preference for larger prey. Past studies have shown that predators, especially carnivorous species are prone to selecting larger food items as they get bigger as reported by (Victor and Akpacho 1992; Okon 2002; Agbugui 2014; Hussein et al., 2019).

The RGL of 0.56 is within the limits for carnivorous species, as described by (Biswas 1993). This limit again confirms its feeding group hence explains the reason for the diversity of food materials in its diet. In the study of gut morphology of the gobbies, it was reported that gut length or relative gut length did not necessarily reflect the trophic levels. While the omnivores *A. phalaena* and *V. sexguttata* exhibited higher GL and RGL than the carnivore *C. cinctus*, as would be expected, the highly specialised herbivore A. wardi had a short gut, which was related to species evolutionary history. Generally, gut length reflects diet as predators commonly have short and simple guts, while in omnivores and herbivores they are long and complex, reflecting digestibility and

nutrient content of a species' preferred food item (Karachle and Stergiou, 2010; Pogoreutz and Ahnelt, 2013), however, German and Horn (2006) stated that because gut length of fishes vary, the body mass must be taken into account in the comparison of the gut lengths of small and large individuals of the same species, in the ones having similar gut structure with different body mass, or the ones having different gut structure with different body structure.

A linear and positive relationship between the predator body weight and prey body weight of *P. bichir* was observed, larger prey were obtained as the fish size (weight) increased. Size of fish is often associated with age and growth. In essence older fish are prone to prey specificity. Similar results were obtained by Balogun (2000) and Adeyemi et al., (2009) in the diets of *Lates niloticus* and *P. jubelini*, in Lake Kianji, Jachi Dam, Katsina; Lake Bassa. The diet compositions obtained in this study were shown to depend mainly on seasonal availability and abundance of food rather than preference of diet.

Gonadosomatic Index (GSI): The higher GSI values during the rainy season gives an indication that gonadal development is at the peak (Ayoade and Akonine 2016). It is also reported that the peak of breeding season of Polypterus occurs between June and September (Offem et al., 2008; Ayoade and Akonine 2016). The absolute fecundity obtained in this study 687g – 2392g with mean of 951 ± 1.8991 is higher than those obtained from previous studies 622 (TL = 16.4 cm) – 2593 (TL = 27.7 cm}, 175 – 487 for size range 20.5 – 51.7 cm standard length recorded by (Offem et al., 2008; Ayoade and Akonine 2016) the differences in fecundity could be attributed to differences in species, availability of food nature of habitat, and environmental factors and sizes of fishes (Rose et al. (2001; Agbugui and Deekae 2013).

V. Conclusion:

Polypterus bichir exhibits a negative allometric growth pattern.
It is found to be in good condition in the River Niger at Agenebode
P. bichir is a carnivore, a predator with a preference for live fish meals
P. bichir has a moderate fecundity with mean GSI of 951 eggs of 0.5mm in diameter
P. bichir has a great potential as a readily available culture-able fish species in Nigeria

Declarations
Ethical Approval and consent to Participate: Not applicable in this section
Consent for publication statement: Not applicable in the section
Availability of data and material: Not applicable in this section
Competing interests: No competing interest.
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Authors' contributions: Dr AMO is the principal researcher, sample collection, analyses, script writer. Dr HMZ was the research guide and sample director. All authors have read and approved the manuscript, and ensure that this is the case.
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