

Full Length Research Paper

The biology of *Parachanna obscura* (Osteichthyes: Channidae) in Anambra River, Nigeria

Odo, Gregory E.^{1*}, Onoja S. U.² and Onyishi Grace C.¹

¹Department of Zoology, University of Nigeria, Nsukka, Nigeria.

²Department of Home Science and Nutrition, University of Nigeria, Nsukka, Nigeria.

Accepted 17 May, 2012

The biology of a commercially important teleost, *Parachanna obscura* was studied for a period of twelve months. Monthly samples of the species were randomly collected from fishermen in the lower reaches of Anambra River, Southeastern Nigeria for the twelve months. The haematological profile established were mean haematocrit of 22.5%, mean blood haemoglobin concentration of 7.23 g/dl, mean red blood cell sedimentation rate 31.25 μm , mean red blood cell count 3.72 ($10^6/\text{L}$), white blood cell count $2.81 \times 10^4/\text{L}$, mean cell volume 60.75 μm^3 and mean corpuscular haemoglobin 19.5 pg. The blood groups are O⁺ (10%), O⁻ (90%), genotype AA (88%) and AS (12%). Digestive enzymes assays in the different gut regions of *P. obscura* revealed an array of glycosidase, proteases and lipases. The pattern of distribution and relative activity of the enzymes is consistent with its predatory diet. Total length (TL) ranged from 23.4 to 28.5 cm and weight from 86.0 to 140 g; males were not longer or heavier than females. Maturity occurred earlier in males than females; 50% of both sexes matured at 24.7 cm TL. Fecundity ranged from 126 to 1580 oocytes (mean 896 ± 477 oocytes). Total length was a better predictor of fecundity than ovary weight. Index of food significance (IFS) showed that insect (51.67%) was the dominant food group, followed by fish (15.66%) and frog eggs (8.02%). Food items of primary importance were ephemeroptera nymphs, chiromomidae, unidentified insects and fishes.

Key words: Anambra River, *Parachanna obscura*, digestive enzymes, haematological profile, blood groups.

INTRODUCTION

The snakeheads (Baily, 1994) are members of the freshwater perciform fish family Channidae, native to Africa and Asia. In parts of Africa, the snake head is considered a valuable food fish and is produced in aquaculture (Teugels et al., 1984). It is commonly available in local fish markets and it is one of the cheapest sources of proteins. One of the difficulties in assessing the state of health of natural fish population has been the paucity of reliable references of the normal condition. In pursuit of this goal, many fish physiologists have turned to the studies of hematology, probably because it has

proved a valuable diagnostic tool in evaluating human health. Although fish hematology continues to offer the potentials of a valuable tool, progress in establishing normal range values for blood parameters has been slow and literature in this area is isolated and often incomplete (Mawdesley-Thomas, 1971). Perhaps, further confounding these data are variables such as age, sex, dietary state, and stress, all of which may alter blood values (Barnhart, 1969; McCarthy et al., 1973).

The hematological profile of some tropical African fish species have been reported, namely, *Clarias isheriensis* (Kori-Siakpere, 1985), *Clarias gariepinus*, *Heterobranchus longifilis* and *Clarias nigrodigitatus* (Erondu et al., 1993; Sowunmi et al., 2003) *Oreochromis niloticus* (Fagbenro et al., 1999), *Hemichromis fasciatus* and *Tilapia zillii* (Egwuyenga et al., 1999), *Sarotherodon*

*Corresponding author. E-mail: odogreg@yahoo.com. Tel: +234-07037205640.

melanotheron (Gabriel et al., 2007) and *Heterobranchus bidorsalis* (Fagbenro et al., 1993). Meanwhile, there are no reports on the haematological profile of *Parachanna obscura* from Anambra River Basin. Hence there is need to study the hematological profile to provide some useful information on aspect of its biology. Moreover, the ability of an organism to digest a given material is dependent on the presence of appropriate enzymes. No information is available on the quantitative and qualitative assays of digestive enzymes in the gut of *P. obscura*, from Anambra River Basin in contrast to other African fresh-water fishes whose digestive enzymes assays have been established (Olatunde et al., 1988; Fagbenro, 1990; Fagbenro et al., 1993). Considerable biological studies have been undertaken and documented on some economically important tropical fish families in Anambra River Basin; for instance, Clariidae et al. (1992), Mgbenka and Eyo (1992), Ezenwaji and Inyang (1998), Distochontidae (1998) and Clupeidae et al. (2003). Nevertheless, studies of the *P. obscura* feeding biology are scanty. To be able to manage a resource of such commercial importance, knowledge of its biology is imperative.

The study of dietary habits of fish based on stomach content analysis is widely used in fish ecology as an important means of investigating trophic relationships in aquatic communities. Thus, this study, which forms part of a larger and on-going investigation on the fish and fisheries of the river basin, addresses aspects of the biology of the species viz. relative activities of enzymes in the different gut regions, the haematological and serological profiles of *P. obscura* in the Anambra River Basin.

MATERIALS AND METHODS

Study area

The Anambra River (Figure 1) has its source in Ankpa highlands of Kogi state of Nigeria, about 100 km North of Nsukka (Azugo, 1978). It lies between latitudes 6°10' and longitude 7°15' East of the River Niger. Essentially, the river has a southward course crossing the Kogi / Enugu state boundary, and then meanders through Ogorugu to Otuocha, from where it flows down to its confluence with the River Niger at Onitsha. The main river channel, which has a total length of about 207.40 km (Azugo, 1978), has its bank covered by such plants like *Echinochloa* spp., *Salvinia nymnellula*, *Ludwigia decurrens*, *Imperata cylindrica*, *Andropogon* spp., *Jussica* spp., *Pennisetum* spp. and *Cynodon* spp. There is a rainy season (April - September/ October) and a dry season (October/ November-March). From December to January/February, the basin is influenced by the harmattan but their effect is not well marked. Agricultural activities are very high and crops such as yam, cassava, rice, millet, vegetables, groundnuts, potatoes, banana and plantain are produced in large quantities. Fishing methods in the river basin include bailing out of water or pumping out water from ponds with water pumps, construction of fish fences, the use of "atalla", hooks and line, set lines, lift nets, dragnets, beach seines, cast nets, among others (Awachie and Ezenwaji, 1981; Eyo and Akpati, 1993). Species of fish found in the river include *Channa* (*Parachanna obscura*), *Labeo*, *Distichodus*, *Alestes*, *Mormyrids*, *Clarias*, and *Heterobranchus* among others.

Fish collection, morphometrics and stomach content analysis

Monthly samples of *P. obscura* were randomly collected from fishermen in the lower reaches of Anambra River, Southeastern Nigeria, between May 2007 and March 2008 inclusive. Total length (TL) to the nearest 0.1 cm and body weight (g) to the nearest 0.1 g of each *P. obscura* were measured and the sex determined. The length-weight relationships (LWR) were determined using the power curve: $W = aTL^b$ (Ricker, 1973). The parameters of the LWR were determined for the same species collected at different periods. These different estimates were considered separate 'populations'. Fulton's condition factor, K, was calculated as: $K = W/L^3 \times 100$. To determine gonad maturation, gonads were evaluated macroscopically and four maturation stages recognized: I-immature, II-mature, III-ripe and IV - spent. Size at maturity was determined as the length at which 50% of individuals were in gonad stage II.

Fecundity is defined as the number of spawnable oocytes in both ovaries of the species. The relationship between fecundity and gonad weight and total length was determined by the least squares method. The gonad somatic index (GSI) was determined as: $GSI = W_1/W_2 \times 100$, where W_1 = gonad weight (g) and W_2 = fish weight (g) (less gonad weight). The stomach of each *P. obscura* was dissected and silt open, and its degree of fullness estimated by an arbitrary 0 - 20 point scale: thus 0, 2.5, 5, 10, 15 and 20 points were allotted to empty, trace, quarter-full, half-full, three quarter-full and fully distended stomachs. Stomach contents were sorted into categories and analyzed using relative frequency (RF) and point's method (Hynes, 1950; Hyslop, 1980). In the RF, the frequency of a particular food item in all stomachs was expressed as a percentage of the frequencies of all food items. For the point's scheme, each stomach was allotted 20 points regardless of the fish size and these were shared amongst the various contents, taking account of their relative proportion by volume. The points gained by each food item in all stomachs examined were computed and expressed as a percentage of the total points of all food items. The point scheme gave an indication of bulk contribution of each food category to the diet composition. % RF and % PP (percentage of total points) were then used to determine the index of food significance IFS as follows:

$$IFS = \frac{\%RF \times \%PP}{\sum (\%RF \times \%PP)} \times 100$$

Food with IFS $\geq 3\%$ was regarded as primary, ≥ 0.1 to $<3\%$ as secondary, whereas food with $<0.1\%$ was regarded as incidental. The IFS of each were used to compute diet breadth based of

Shannon-Wiener function \bar{H} percentage of total points percentage of total points as follows:

$$\bar{H}_{(IFS)} = \sum_{i=1}^s \left(\frac{n_i}{N} \right) \log_e \left(\frac{n_i}{N} \right)$$

Where n_i = IFS of each food item, N =total IFS of all food items.

Haematological profile

Twenty-three live specimens (TL 27 - 41 cm) were kept undisturbed in large glass aquarium (120 liter capacity) supplied with filtered and aerated tap water for 2 weeks of acclimation to laboratory conditions (pH 7 - 8: dissolved oxygen concentration, >6 mg/L: water, temperature, 28 - 30.5° prior to blood sampling. During this period, the fish were fed to satiation twice daily with 380 g/kg body weight of crude protein pellets. All fish were considered healthy on the basis of their appearance and absence of obvious signs of

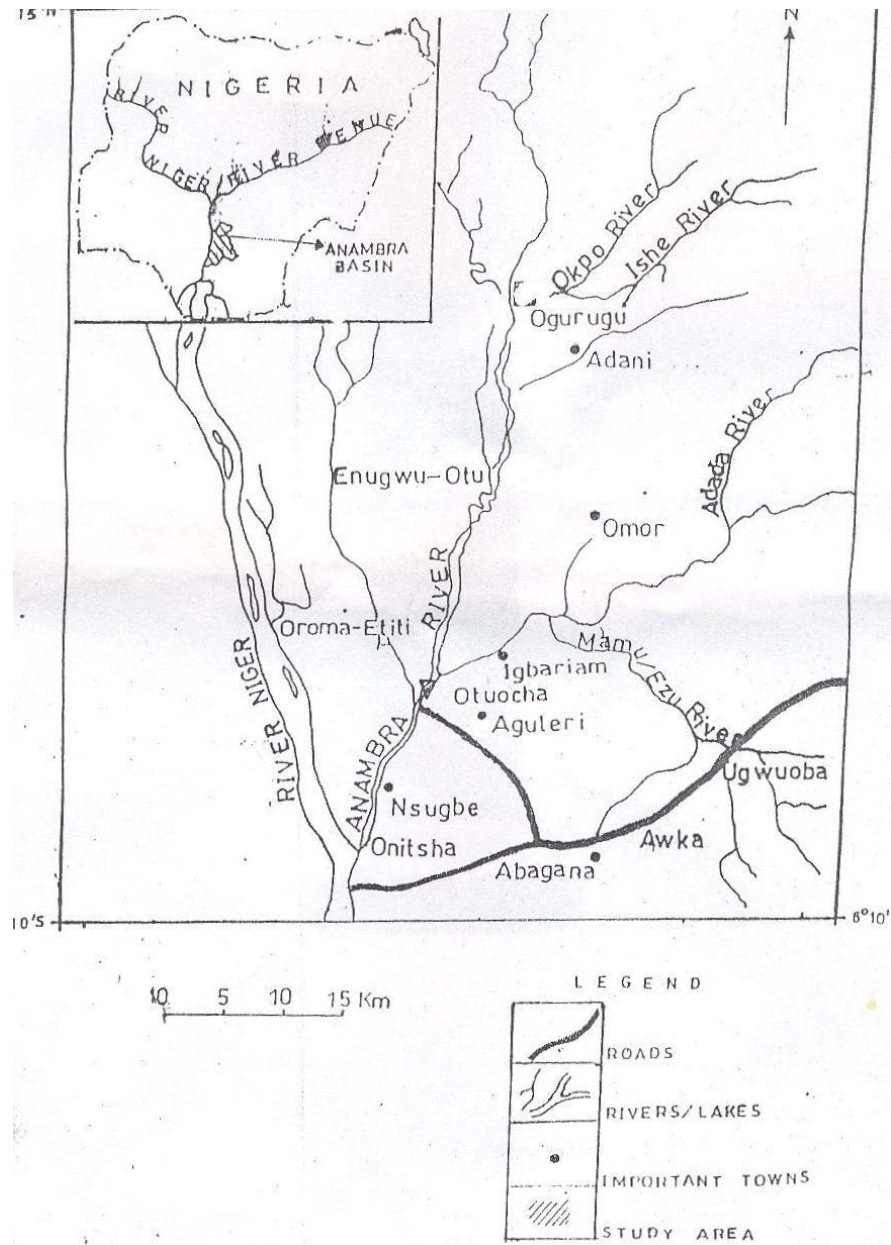


Figure 1. Map of Anambra River.

disease. No sex selection was made. Blood was collected from the caudal vein of each fish using separate heparinized disposable syringes and hypodermic needles. Haematocrit packed cell volume (PCV) was measured after centrifugation at 15000 rpm (MSE Microcentrifuge). Preparations were made for the determination of haemoglobin (Hb) content, leukocyte count (WBC), and erythrocyte count (RBC).

Serological profile

Blood grouping was performed by the test tube techniques based on agglutination tests, while the genotype was determined by haemoglobin electrophoresis (Delany and Garratty, 1969).

Digestive enzyme assays

Twenty-three adult *P. obscura* specimens (TL 29 - 37 cm) were kept unfed for 72 h inside out door concrete cisterns in order to bring them to a similar physiological state as well as to ensure the emptiness of the entire gut. They were anaesthetized with benzocaine and dissected to remove the entire gut, and later separated into the anatomically distinct regions. The different gut regions were pooled and homogenized; the homogenates were then centrifuged at 1200 rpm (Fisher Scientific Company, USA) at 4°C. The supernatants were used as crude extracts without further purification. Benedict's qualitative reagents were used for the qualitative assays of glycosidase following the method of Olatunde et al. (1988), while quantitative assays were conducted using the

dinitrosalicylate (DNS) method (Plumber, 1978). Qualitative and quantitative assays of proteases followed the method of Balogun and Fisher (1970).

Data analysis

Data for corresponding months were pooled together for analysis. Food richness was defined as the number of food items in the diet with IFS $\geq 0.1\%$. Abundance data were analyzed by two-way analysis of variance (ANOVA). Food composition and sex ratio were analyzed by students-test and " χ^2 " test, respectively (Bailey, 1994). Differences were considered significant at 5% level of probability. The methods described by Ogunbiyi and Okon (1976) were used to determine lip activity both qualitatively and quantitatively. Controls were run simultaneously for all assays. Regression analysis was carried out between the various haematological parameters and standard length. The coefficient of regression (r) was then analyzed for statistical significance by Student's t-test. Results were presented as mean with standard deviation (SD). The results were also analyzed using students t-test. The level of significance was $p < 0.05$ at 95% confidence limit.

RESULTS

Distribution and abundance

Of the 40 species of fin fish and three shell fish caught in Anambra River fishery totaling 2435 individuals and weighing 36.81 kg, *P. obscura* was the most abundant in both number (860, 35.32%) and weight (8.52 kg, 23.14%). The *P. obscura* was significantly more numerous in the rainy season months of June to October than in the dry months of November to March ($P < 0.05$).

Size range

The length of *P. obscura* ($n = 550$) ranged from 26 - 37 cm total length (mean 28 ± 88 cm TL). Males ranging from 23 - 28 cm TL (25 ± 0.84) were not longer than females that range from 27 - 39 cm TL (mean 28 ± 0.84 cm TL). Weight ranged from 86 - 140 g (mean 110 ± 0.76 g). The weight of the males ranging from 87 - 151 g (mean 89 ± 0.72 g) was not significantly heavier than the weight of females that range from 45 - 69 g, (mean 54 ± 0.76 g)

Population structure

The population structure is presented in Figure 2. From this figure, it can be seen that both sexes had one mode ($>53\%$ individuals) at 26 cm TL. The 25 - 27 cm TL size classes (represented by 3-5) constitute over 90% of the total sample (Figure 2). The overall monthly sex ratio was significantly different from 1:1 ($\chi^2 = 5.21$, $df=1$; $P < 0.05$, (Table 1; Figure 3). Within the months, females dominated in March ($\chi^2 = 9.68$, $df = 1$, $P < 0.05$) and May ($\chi^2 = 5.33$, $df = 1$, $P < 0.05$). There is seasonal variation in

the number of individuals depending on the sexes. For example, during the dry season the sexes (male = 178, 42.5%; female = 211, 57.5%) showed significant difference in the number collected ($\chi^2 = 5.4$, $df = 1$, $p = 0.05$). However, during the rainy season the difference was not significant as the number of males collected (141, 47.2%) did not differ markedly from the number of females collected (158, 52.8%) ($\chi^2 = 0.96$, $P > 0.05$).

Morphometric parameters

The LWR analyses of the 12 populations are presented in Table 2. The analyses further indicated that the calculated corrections was significant ($P < 0.05$) with coefficient of determination ranging from 48 to 98.5%. These values showed that the intercept 'a' exhibited moderate variation among the populations (CV = 52.23%) and ranged from $a_{\min} = 8.6 \times 10^{-3}$ (overall) to $a_{\max} = 31.7 \times 10^{-3}$ (March). Conversely, the exponent 'b' showed low variations among the population (CV = 9.64%) and varied from $b_{\min} = 2.192$ (June) to $b_{\max} = 2.939$ (overall). The mean exponent ($b = 2.66$; SD. = 0.257) was significantly less than 3 (t-test, $df = 11$, $P < 0.05$), indicating negative allometric function for the populations. Table 2 also showed that LWR exhibited in both the rainy and dry seasons were isometric.

Fulton's conditions factor, K

The mean monthly K was 0.78 ± 0.11 and varied from 0.61 ± 0.16 in June to 0.92 ± 0.10 in March, (Figure 4). The fish species were in very good condition in September, October, February and March, whereas the poorest conditions occurred in January, June and July.

Reproductive biology

The mean monthly gonad somatic index (GSI) of 94 female *P. obscura* varied from 1.1 - 3.3% (mean $2.05 \pm 0.72\%$). There were only slight variations in the mean monthly GSI indicating an all year round reproductive pulse, although three peaks in June, September and January were evident (Figures 4 and 5). The monthly dynamics in the percentage male and female at each maturation stage (Table 3) showed that immature, mature, ripe and spent gonads were present throughout the year indicating an all year round gonad recrudescence, breeding period and recruitment. There were significantly more females than males in immature stage ($\chi^2 = 4.4$, $df = 1$, $P < 0.05$) and spent stage ($\chi^2 = 19.6$, $df = P < 0.05$). Males and female did not depart from a 1:1 sex ratio in mature and ripe *P. obscura* (Table 4). The size at maturity in females and males differed. The smallest female matured at 24.3 cm TL, whereas in the males it was at 23.4 cm TL. Over 50% of both sexes matured a

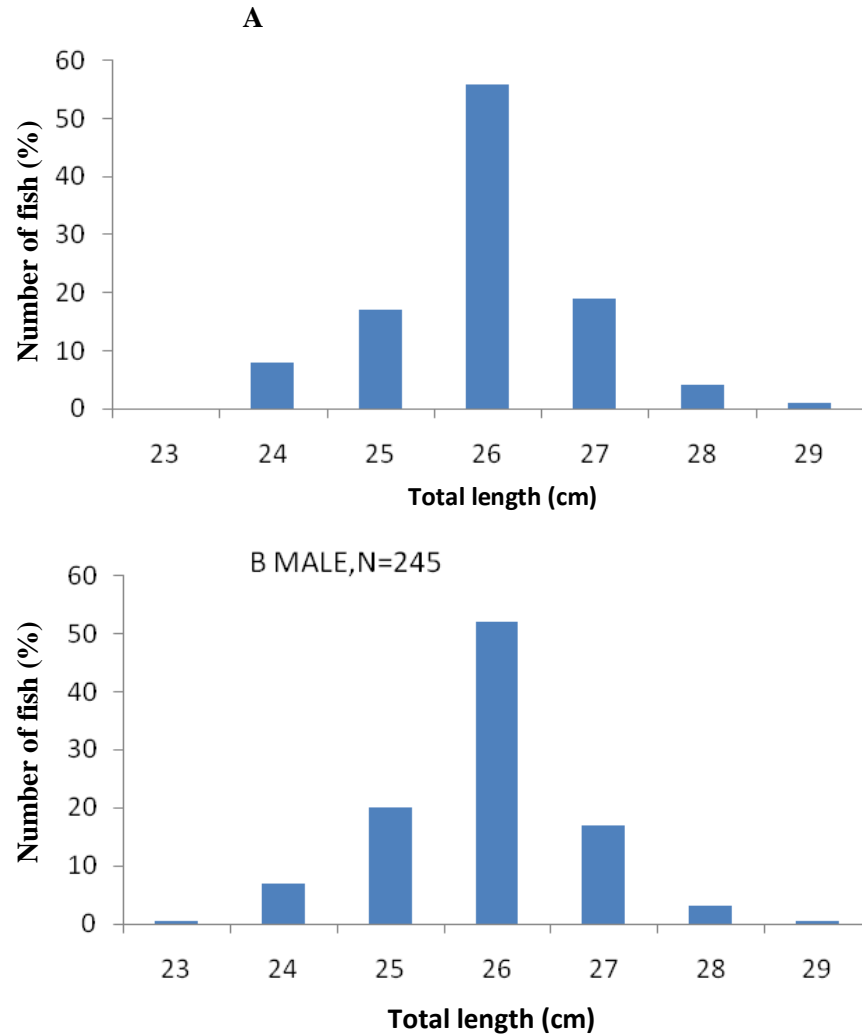


Figure 2. Length-frequency distribution of (A) female and (B) male *P. obscura* in Anambra River.

Table 1. The overall monthly sex ratio of *P. obscura* in Anambra River.

Month	Number collected		Sex ratio (M : F)
	M	F	
M	30	54	1:2.6
J	39	18	1:1.2
J	46	36	1:0.7
A	40	40	1:1.2
S	44	37	1:0.8
O	39	44	1:1.1
N	38	39	1:1.1
D	34	46	1:1.5
J	37	43	1:1.3
F	38	37	1:1.1
M	1631	46	1:2.0
Total	420	440	1:1.3

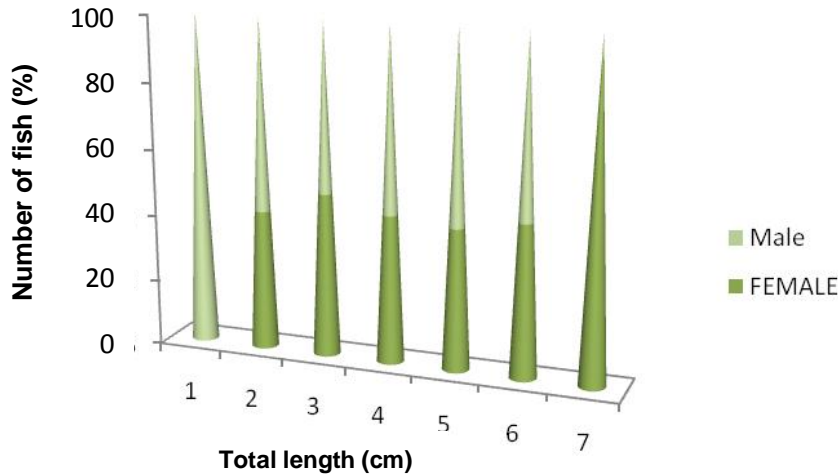


Figure 3. Variation in sex ratio of *P. obscura* in Anambra River.

Table 2. Length-weight relationships and related statistics in *P. obscura* (May 2007 - March 2008).

Month	Total length (cm)				Length-weight relationships			
	Mean	S.D	Min	Max	A	B	r	N
M	26.00	0.67	25.0	28.4	0.0099	2.821	0.946	50
J	26.37	0.73	23.4	26.7	0.0227	2.192	0.735	50
J	26.56	0.56	25.9	28.5	0.0087	2.890	0.992	50
A	26.80	0.46	26.1	28.2	0.0112	2.906	0.977	50
S	25.45	0.59	24.6	27.3	0.00153	2.704	0.972	50
O	26.48	0.65	25.6	28.3	0.0307	2.348	0.949	50
N	26.04	0.43	25.3	27.3	0.0113	2.801	0.933	50
D	24.53	0.51	23.8	26.0	0.0135	2.612	0.968	50
J	25.70	0.28	25.1	26.3	0.0133	2.564	0.692	50
F	25.78	0.81	24.7	28.0	0.0116	2.859	0.967	50
M	26.20	0.50	25.4	27.9	0.0317	2.317	0.884	50
Overall	25.99	0.63	23.4	28.4	0.0086	2.939	0.941	550
Rainy season	26.3	0.57	23.4	28.4	0.0081	2.942	0.945	300
Dry season	25.6	0.62	23.8	28.3	0.0060	3.169	0.941	250

24.7 cm TL. Oocyte count of 15 females ranged from 126 to 1580 (mean 896 ± 477 oocytes). The regression equations for the relationships between fecundity and total length, and ovary weight ($F = aX^b$, where X stands for either total length or ovary weight) were: $F = 21.93TL^{1.82}$, $r = 0.37$; $F = 847.65OW^{0.04}$, $r = 0.02$. The correlations were positive but low; total length had a better predictive value of 0.37 than ovary weight (0.02).

Diet

Out of 550 stomachs examined, 56(10.2%) had full stomachs (FS), 396 (72%) had partially-filled stomachs (PS), whereas 97(17.6%) had empty stomachs (ES). Full

stomachs were highest in May (6.46%) and lowest in September, January and March (0.0%). Among the partially-filled stomachs, 41(10.35%) were 1/4 full, 120 (30.30%) 1/2 full, 193 (48.74%) 3/4 full and 42 (10.61%) obtained traces of food. Twenty-four different food items were ingested (Table 5). Insects (51.67%) were the dominant food group followed by frog eggs (15.66%) and fish (8.02%). Foods of primary importance were *Ephemeroptera* nymph, Chironomidae, unidentified insects and fishes. The most important food item was Chironomidae (IFS = 36.95%); which occurred more regularly in the stomach and constituted a large proportion of the food taken. Of all the food items ingested, only *Ephemeroptera* nymphs and chironomids were consumed in all the months. Food of primary

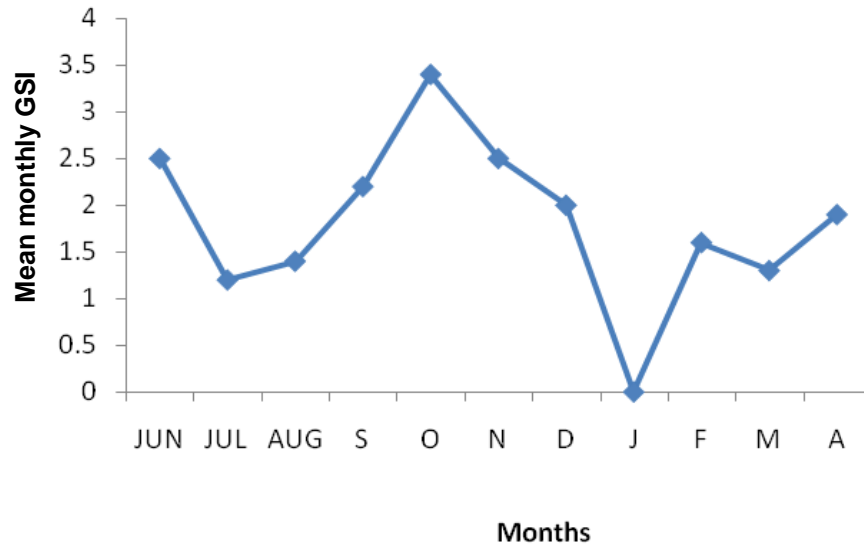


Figure 4. The mean monthly GSI of 94 mature female of *P. obscura*.



Figure 5. Mean monthly conditional factor of *P. obscura* from Anambra River.

importance varied May 1st to October 6th (Table 6). The total mean and range were also estimated

Hematological parameters

The general mean values with the standard deviation of the blood parameters of *P. obscura* are presented in Tables 7. The haematological profile established were mean haematocrit of 22.5%, mean blood haemoglobin concentration of 7.23g/dl, mean red blood cell

sedimentation rate 31.25 μm , mean red blood cell count $3.72 (10^6/\text{L})$, white blood cell count $2.81 \times 10^4/\text{L}$, mean cell volume $60.75 \mu\text{m}^3$ and mean corpuscular haemoglobin 19.5 pg. Table 10 shows the correlation analysis of the blood parameters. The Linear regression and prediction equation of blood parameters as functions standard length is presented in Table 11, while the regression and prediction equation of blood parameters as functions of standard length and body weight are presented in Table 12. From the correlation analysis, RBC has a weak positive relationship with WBC and MCHC

Table 3. The monthly variation in the percentage female (N=440) and male (N = 420) *P. obscura* at each maturation stage.

Month	No. of females examined	Maturation stage								No. of males examined
		Female				Male				
		I	II	III	IV	I	II	III	IV	
M	36	61.1	36.1	2.8	-	28.6	71.4	-	-	14
J	27	37.0	51.9	11.1	-	21.7	56.5	21.7	-	23
J	20	25.0	25.0	45.0	5.0	16.7	46.7	33.3	3.3	30
A	27	14.8	25.9	51.9	7.4	34.8	43.5	13.0	8.7	23
S	22	22.7	31.8	27.3	18.2	46.4	28.6	21.4	3.6	28
O	26	46.2	34.6	7.7	11.5	30.4	39.1	30.4	-	23
N	24	41.7	16.7	33.3	8.3	54.5	27.3	18.2	-	22
D	28	50.0	25.0	21.4	3.6	36.8	36.8	26.3	-	19
J	28	42.9	42.9	7.1	7.1	27.3	40.9	31.8	-	22
F	26	26.9	19.2	15.4	38.5	17.4	26.1	47.8	8.7	23
M	32	3.1	9.4	59.4	28.1	12.5	6.3	-81.3	-	16
Total	296									

Table 4. The dynamics of female and male *P. obscura* in maturation stages.

Maturation stage	Female	Male	Sex ratio (M.F)
Immature	102	73	1: 0.7
Mature	86	93	1: 1.1
Ripe	74	71	1: 1
Spent	34	6	1: 0.2
Total	296	243	1: 0.8

($P < 0.05$). There is high positive relationship between RBC and PCV at $P < 0.05$ level of significance but there was a negative correlation with ESR. Moreover, there is a negative correlation between RBC and ESR. RBC has a negative correlation with MCV but has no significant correlation with MCH. WBC has a significance correlation with PCV ($P < 0.05$) WBC has a significance correlation with HB ($P < 0.05$) but no significance correlation with ESR, MCV, MCH and MCHC $P > 0.05$.

PCV has a high positive correlation with Hb at the ($P < 0.01$), but has a negative correlation with ESR. On the other hand, PCV has no significant correlation with MCV. It has significant correlation with MCH and MCHC ($P < 0.01$). Hb had a negative correlation with ESR but no significant correlation with MCV. PCV had a significant high positive correlation with MCH and MCHC ($P < 0.01$); ESR had a significant correlation with MCV at $P < 0.01$ level of significance but no significant correlation with MCH and MCHC. MCV had a high positive relationship with MCH and MCHC at the 0.01 level of significance. MCH also had a positive correlation with MCHC which was also very high at ($P < 0.01$; $P < 0.01$), while weight has little or no significance with it.

Serological parameters

The blood group and genotype are presented in Tables 8 and 9, respectively. Among the 64 samples of *P. obscura*, 6 fishes were O+ with 10% prevalence, while 58 were O⁻ with 90% prevalence. There is no group A, group B or group AB; the prevalent blood group is O+ genotype. Among the fish samples, 56 had AA genotype with 88% prevalence, while eight fish had AS genotype with 12% prevalence. Moreover, none expressed SS genotype.

Digestive enzyme assays

Table 12 shows the various enzymes detected in the different regions of the *P. obscura* gut, their distribution and activity varying along the gut length. A variety of glycosidase was detected (Table 14). Cellulase activity was recorded only in the pyloric caeca (Table 12). The protein hydrolyzing enzymes found in the stomach are pepsin-like while those in the pyloric caeca are alkaline proteases, possibly trypsin and /or chymotrypsin. Lipase activity occurred along the entire gut length with peaks in

Table 5. Trophic spectrum of the diet of all sizes of *P. obscura* in Anambra River.

Food species/group	%RF	%PP	IFS
Crustacean			
Ostracoda	2.31	2.48	0.53
<i>Macrobrachium felcium</i>	2.31	2.73	0.58
Insecta			
Ephemeroptera nymph	6.45	6.66	3.97
<i>Coenagrion</i> sp.	0.10	0.11	+a
Water bugs	1.15	0.89	0.09
Dystiscidae	0.48	0.55	0.02
Formicoid Hymenoptera	1.06	0.99	0.10
Mosquito larvae	0.29	0.31	36.95
Chironomid larvae and pupae	17.90	22.33	36.95
Chaoborus larvae	0.29	0.29	0.01
Terrestrial Orthoptera	2.89	3.22	0.86
Neuropteran larva	1.25	1.03	0.12
Unidentified insect	10.78	9.57	9.54
Fish			
Alestes.	0.29	0.32	0.01
<i>Barbus callipterus</i>	0.10	0.11	+
Epiplastys	0.38	0.60	0.02
<i>Chrysichthys</i> sp.	0.19	0.29	0.01
Fish remains	7.31	7.80	5.27
Fish fry	11.93	13.82	15.24
Fish egg	1.54	1.68	0.24
Nematodes	1.35	1.47	0.18
<i>Macrobrachium felcium</i>	10.39	8.35	8.02
Tadpole	2.02	2.29	0.43
Frog eggs	16.75	11.48	17.77

^a+ <0.01

generally survival strategies and adaptations aimed at perpetuating the species in response to high fishing and/or natural mortality. The survivors prey on the rich variety of food available in the river; they grow fast and become recruited into the fishery. It seems probable that it is in this way that large numbers of *P. obscura* are maintained in the Anambra River. Ezenwaji (1999, 2002) has recorded similar situations in *Clarias albounclatus* and *C. ebriensis* in the same river system.

While a fairly good knowledge of the breeding biology of *P. obscura* is beginning to emerge in Nigerian lentic and lotic habitats, we still need as Marshal (1993) noted in *Limnothrissa miodon* to ascertain the environmental factors determining reproductive success, the effect of fishing on the sexually mature individuals and the relationship between stock and recruitment. Furthermore, knowledge of other demographic characteristics (such as growth and mortality) of *P. obscura* is also important in order to gain an overall understanding of factors

determining its abundance in the Anambra River. The size range *P. obscura* is a good reflection of its population structure. The preponderance of the fish between 25 and 27 cm TL is probably because the shallow water habitat of fish less than 25 cm TL may not have been adequately sampled, and fish up to 9 cm TL would have been exploited as a result of the high fishing and natural mortality.

In fishes, generally, the annual production of oocytes accounts for a large proportion of the body weight. *P. obscura* of the Anambra River appears to be a notable exception. With an average of $2.05 \pm 0.72\%$, the oocyte weight/body weight ratio of this clupeid appears to be one of the lowest in fishes.

This is slightly different from the average ovary weight/body weight ratio; 4.6% in *P. afzeius* and 7.4% in *Siermtlirissa leonensis* in Kainji lake (Otobo, 1978), and $6.1 \pm 2.5\%$ (range 2:2 -1: 2.2%) in *C. ebriensis* in the Anambra River system (Ezenwaji,

Table 6. The monthly IFS of *P. obscura* in Anambra River.

Food species/group	M	J	J	A	S	O	N	D	J	F	M
Crustacean											
Ostracoda	-	-	-	-	-	9.29	3.17	0.50	1.78	0.64	0.05
<i>Macrobrachium felcium</i>	-	-	-	0.01	-	11.29	0.87	0.12	-	0.28	10.10
Insecta											
Ephemeroptera nymph	0.43	41.38	0.49	3.75	0.78	0.29	0.29	0.56	6.12	5.28	6.13
<i>Coenagrion</i> sp	-	-	-	-	-	1.10	-	-	-	-	-
Water bugs	-	-	0.11	0.01	0.05	-	-	9.78	0.44	0.05	-
Dystiscidae	-	-	-	-	-	0.86	-	-	-	-	0.34
Formicoid Hymenoptera	-	-	-	-	-	0.18	0.04	-	-	0.08	-
Mosquito larvae	-	-	-	-	-	0.86	-	0.08	-	-	-
Chironomid larvae & pupae	99.57	55.19	8.26	35.22	0.31	1.62	0.76	1.66	0.69	1.77	20.15
Chaoborus larvae	-	-	-	-	-	0.18	0.09	-	-	-	-
Terrestrial Orthoptera	-	-	-	-	-	-	-	-	0.55	0.53	1.79
Neuropteran larvae	-	-	-	-	-	2.02	0.55	-	0.52	0.08	0.46
Unidentified insect	-	-	-	1.11	35.37	45.70	57.24	28.42	36.01	3.04	9.50
Fish											
<i>Brycinus</i> sp	-	-	0.22	0.03	-	-	0.09	-	-	-	-
Alestes	-	-	2.32	0.05	-	-	-	-	-	-	-
<i>Chrysichthys</i> sp	-	-	0.54	0.09	-	-	-	-	-	-	-
<i>Barbus callipterus</i>	-	-	-	0.03	-	-	-	-	-	-	-
Fish remains	-	1.52	36.56	8.30	3.45	1.84	0.02	1.48	13.46	3.39	-
Nematodes	-	1.27	34.31	40.34	22.78	5.60	5.09	6.79	7.58	30.41	11.62
Frog eggs	-	-	-	-	-	-	-	0.15	0.07	-	0.05
Prawns	-	-	-	-	-	1.47	-	0.32	1.18	0.05	0.25
Cray fish	-	0.07	1.88	0.59	-	7.04	3.68	28.67	11.66	41.70	4.07
Mud	-	-	-	-	-	4.86	15.65	0.08	0.25	0.25	0.20
Sand	-	0.57	15.30	10.46	37.26	5.80	12.37	20.37	19.69	12.37	35.29
Food richness	2	6	10	13	7	17	15	15	14	16	14
Diet breadth	0.03	0.85	1.47	1.40	1.25	1.94	1.40	1.71	1.83	1.55	1.85

Table 7. Summary of estimated haematological characteristics of *P. obscura*.

Haematological parameters	Mean \pm SD	Range
Standard length (SL) (cm)	25.13 \pm 2.18	22.00 – 29.00
Total length (TL) (cm)	29.88 \pm 2.78	26.00 – 37.00
Body weight (wt) (g)	110.00 \pm 16.11	86.00 – 140.00
RBC (10^6)	3.72 \pm .74	1.50 – 5.10
WBC (10^4)	2.81 \pm .22	25800.00 – 31800.00
PCV (%)	22.50 \pm 4.17	16.00 -32.00
Hb (g/dl)	7.23 \pm 1.72	3.70 - 12.20
ESR (%)	31.25 \pm 11.22	12.50 – 49.00
MCV (μm^3)	60.75 \pm 3.18	57.00 - 68.00
MCH (pg)	19.50 \pm 2.83	15.00 – 26.00
MHCH (%)	32.00 \pm 2.89	26.00 – 40.00

Table 8. The haematological characteristics of blood group of *P. obscura*.

Blood group	No of fish positive	Prevalence
A	None	None
B	None	None
B	None	None
O ⁺	6	10%
O ⁻	58	90%

Table 9. The haematological characteristics of the genotype of *P. obscura*.

Genotype	No. of positive Fishes	Prevalence (%)
AA	56	88
AS	8	12
SS	0	-

2002). A similar situation obtained for *L. miodon* in the northwestern waters or Lake Tanganyika (Muhmbua, 1993). Thus, this phenomenon appears typical of fishes that mature early and breed all the year round.

The length (24.27 cm TL) at which 50% of the sample matured ensures that the largest number of the most fish (those in the 24 - 28 cm TL bracket) is in reproductive state. This agrees with the size at maturity in *P. obscura*. As size at maturity of fish depends on the growth rate, it does seem that there are no significant differences in the size of this teleost in Anambra River and in Kainji lake environments. A large number of autochthonous and allochthonous insects constitute important proportion of the food or many fishes inhabiting the Anambra river system (Ezenwaji and Inyang, 1998; Ezenwaji, 1999). Thus, *P. obscura* of this study, although omnivorous, subsists mainly on insects (Table 6). As ephemeropterans, chironomids, algae, fish, terrestrial Orthoptera and formicoid Hymenoptera are largely ingested, it implies that *P. obscura* feeds in or on the substratum in mid-water and in the air-water interface. Apart from eating other fish (e.g. *8 m-bus callipterus*), the exhibition of the phenomenon of cannibalism by *P. obscura*, even in the face of a rich variety of other high quality food items, is attributed to light attraction employed by fishers which Gliwicz (1984) points out may lead to this kind of abnormal behavior in fish. Thus, *L. miodon* inhabiting lake Kariba, Zimbabwe, is omnivorous, feeding predominantly on plankton, followed by insects (particularly chironomids, ephemeropterans, trichopterans and hemipterans) and fish, and exhibits cannibalism (Chifamba, 1993). *Microtiltrissa acutirostris* is omnivorous in lake Mweru-Wantipa, Zambia (Mubamba, 1993); it feeds mainly on zoo- and phyto-plankton and rotifer. It appears, therefore, that those teleosts are able to exploit all food niches (bottom, mid-water and water surface) in their habitats. Thus, they exhibit wide plasticity in their feeding,

primarily consuming a combination of two or more of insects, crustaceans, plankton, fish algae, plant detritus sand rotifers, depending on availability and abundance of these food items.

Clinical haematological studies are of great importance in the epidemiology, management and husbandry practices, veterinary diagnosis; treatment and prognosis in many disease conditions (Benjamin, 1961; Cole, 1967). Undoubtedly, these haematological characteristics of fish are affected by age, sex, nutrition, seasons as well as other physiological conditions of blood such as composition of serum protein and pathological state. The ranges of blood parameters determined for *P. obscura* are generally similar to those determined for African fresh water fishes reported. The mean haematocrit value of *P. obscura* is comparable to those of African Clariid fishes *C. isheriensis*, (Kori-Siakpere, 1985), *C. gariepinus* (Adeyemo, 2007) *H. longifilis* (Valenciennes) (Erondu et al., 1993) and *Sarotherodon melanotheron* (Gabriel et al., 2007). Badawi and Said (1971) listed some blood parameters of Nile Tilapia, *Tilapia zillii* and *Tilapia aurerurs* and concluded that blood picture is not a dull and static phenomenon, but it depends on what is happening to the fish at any particular locality and time and so it is very dynamic. Lagler et al. (1977) stated that whereas red blood cells range from 20,000 to 3,000, 000 per mm³ of blood; white blood cells vary between 20,000 and 50,000 per mm in different group of fishes. The values obtained for *P. obscura* fall between these parameters. The number of circulating red blood cells is a response to both internal and external stress and in the relation to oxygen demand (Maclean, 1978). The permeability of the red blood cells is a function of the lipids and proteins of the membranes (Ness and Stengle, 1974). It is natural for the body of animals to fight infections of parasites and diseases through the immune system made up of immunoglobulin or antibodies in the blood,

Table 10. Correlations of the blood parameters.

Blood parameter	Source of variation	RBC	WBC	PCV	Hb	ESR	MCV	MCH	MCHC
RBC	Pearson Correlation	1							
	Sig. (2-tailed)								
	N	64							
WBC	Pearson Correlation	0.317(*)	1						
	Sig. (2-tailed)	0.011							
	N	64	64						
PCV	Pearson Correlation	0.900(**)	0.328(**)	1					
	Sig. (2-tailed)	0.000	0.008						
	N	64	64	64					
Hb	Pearson Correlation	0.826(**)	0.253(*)	0.894(**)	1				
	Sig. (2-tailed)	0.000	0.043	0.000					
	N	64	64	64	64				
ESR	Pearson Correlation	-0.648(**)	0.171	-0.634(**)	-0.475(**)	1			
	Sig. (2-tailed)	0.000	0.177	0.000	0.000				
	N	64	64	64	64	64			
MCV	Pearson Correlation	-0.088	0.005	0.086	0.214	0.442(**)	1		
	Sig. (2-tailed)	0.491	0.971	0.501	0.089	0.000			
	N	64	64	64	64	64	64		
MCH	Pearson Correlation	0.206	0.170	0.413(**)	0.502(**)	0.215	0.842(**)	1	
	Sig. (2-tailed)	0.102	0.179	0.001	0.000	0.088	0.000		
	N	64	64	64	64	64	64	64	
MCHC	Pearson Correlation	0.297(*)	0.069	0.447(**)	0.526(**)	0.093	0.655(**)	0.840(**)	1
	Sig. (2-tailed)	0.017	0.586	0.000	0.000	0.464	0.000	0.000	
	N	64	64	64	64	64	64	64	64

** Correlation is significant at the 0.01 level (2-tailed); * Correlation is significant at the 0.05 level (2-tailed).

the white blood cells or leucocytes play this all-important defensive role.

Weinreb (1958) used leukocytes count as a means of assessing the systematic response of the rainbow trout, *Salmo gairdneri* (R), to various injections. Leukocyte counts seem to have wide range of variation 20000 – 63000/mm³ for brown trout, *Salmo trutta* (L). In the present work, the range of Leukocyte counts was 2.58 – 3.18 × 10⁴/mm³. There is variation between these results and those obtained by some other workers like Kori-Siakpere et al. (2005). However, the variation has no statistical significance. The PCV of *P. obscura* has a high significant positive correlation with RBC, Hb, MCH and MCHC. This is expected since PCV of blood depends on the number of RBC, shape and size of cells and the haemoglobin concentration. Consideration of the intra-

species relation between blood parameter as indicated by the correlation coefficient (r) in Table 11 and the intraspecies variations as indicated by the wide range of some of the parameters in this study have been attributed to any of the many factors such as season, spawning, migration, sex and genetic variation that affect the blood picture of fish. Stress factors due to capture, handling and sampling procedures of which capture is most important are factors, which can cause intra-species haematological variation (Bouck and Ball, 1966). Stress of handling has been shown to produce a low haemo-concentration. It has also been shown that the haemoglobin concentration and haematocrit of fish blood decreases after the stress of capture and transportation (Hattingh and Van Pletzer, 1974). Positive and useful correlation between haematocrit, haemoglobin and red

Table 11. Linear regression blood parameter with standard length (SL) and body weight (WT).

Blood parameters	Regression analysis
RBC	= 2.703 + 0.044 sl (cm) - 0.001 wt (g) $r^2 = 0.015$ ($p < 0.05$) ($P > 0.05$)
WBC	= 1.446 + 0.040 sl (cm) - .003 wt (g) $r^2 = 0.318$ ($p < 0.05$) ($p > 0.05$)
PCV	= 13.602 + 0.376 sl (cm) - 0.005 wt (g) $r^2 = 0.035$ ($p > 0.05$) ($p > 0.05$)
Hb	= 2.246 + 0.200 sl (cm) + 0.000 wt (g) $r^2 = 0.063$ ($p > 0.05$) ($p > 0.05$)
ESR	= -37.720 + 2.090 sl (cm) + 0.149 wt (g) $r^2 = 0.303$ ($p < 0.05$) ($p > 0.05$)
MCV	= 41.151 + 0.898 sl (cm) - 0.027 wt (g) $r^2 = 0.308$ ($p < 0.05$) ($p < 0.05$)
MCH	= 1.130 + 0.729 sl (cm) + 0.000078 wt (g) $r^2 = 0.316$ ($p < 0.05$) ($p > 0.05$)
MCHC	= 15.608 + 0.637 sl (cm) + 0.003 wt (g) $r^2 = 0.245$ ($p < 0.05$) ($p > 0.05$)

Table 12. Summary of qualitative and quantitative assays of digestive enzymes in the gut of *P. obscura* (n = 23).

Enzymes	Oesophagus	Stomach	Pyloric caeca	Duodenum	Ileum	Rectum	SE
Glycosidases ¹							
Amylase	7.1	9.4	112.2	140.1	87.3	ND	2.34
Sucrase	11.1	11.6	16	32.6	29	ND	0.42
Maltase	19	18.7	49.1	40.6	48	ND	0.59
Lactase	ND	26.2	34.1	37	31.2	ND	0.64
Cellulase	11	9.1	21.8	ND	ND	ND	-
Proteases ²	ND	125.8	240.8	161	102.7	N	2.29
Lipase	ND	40.5	184.4	131	101.4	ND	2.67

ND = Not detected; ¹mg glucose/min/mg protein at 37°C; ²change in optical density at 595 nm/h/mg of L-tyrosine/h at 37°C; SE=standard error.

cell count used in the computation of the haematological indices, have been demonstrated for several species (Eisler, 1965; Summerfelt et al., 1967; Houston and Dewilde, 1968).

One of the fundamental needs of a fish, like other animals, is to have an adequate supply of oxygen in the tissues so that oxidation can occur and provide the necessary energy for life. Oxygen transport in blood depends on the compound, haemoglobin, which is the respiratory pigment of the blood. According to Lagler et

al. (1977), the haemoglobin content of the fish blood varies with the number of erythrocytes present, expressed as percentage of dry weight of erythrocytes. They found the values of 14 to 19% in the smooth dogfish, *Mustelus canis* and 37 to 79% in a number of marine and fresh water teleosts. The mean values of *P. obscura* studied seem to deviate from the above relationship since PCV has a negative correlation with ESR. Guyton (1991) postulates that when Hb formation is deficient in the bone marrow, the percentage of haemoglobin in the cells may

fall considerably below the normal value and the volume of red blood cell decreases as well because of diminished haemoglobin to fill the cell, which also affects the MCV; this could explain this deviation. The mean value for Hb conc. of *P. obscura* was 7.23 g/dl. This value falls within the range reported for other fishes like *Labeo rohita*; 6.0 g/dl for males and 6.3g/dl for females (Seddiqui and Mishra, 1979), *Hypophthalmichthys molitrix*, 6.11 g/dl (Pieterse et al., 1981) and *C. gariepinus*, (Adeyemo, 2007; Kori-sakpere et al., 2005). The Hb value of *C. obscura* is higher than that obtained for *P. obscura* (Fagbenro et al., 1998). In fish blood, oxygen is carried in physical solution and in combination with haemoglobin. So physiologically, haemoglobin is crucial to the survival of the fish as its role is directly related to the oxygen building capacity of blood. Despite this importance, *Chaenocephalus aceratus* has been reported as having no haemoglobin (Rund, 1954), indicating that oxygen in such species is transported by blood in physical solutions only.

Blaxhall and Daisely (1973) have reported the possibility of using haematocrit as a tool in aquaculture and fishery management for checking anaemia condition. Reported values for fish haematocrit are usually between 20 - 35% and scarcely attain values greater than 50% (Clarks et al., 1979). The mean haematocrit value in this work was within this range ($22.5\% \pm 4.7$). Das (1965) stated that both the red cell number and haemoglobin concentration tend to increase with length and age. Increase haematocrit have been observed for male fishes approaching spawning, but this increase seems to be of a limited and of transient nature (Poston, 1966; Summerfelt 1967). A snake head (*Parachanna*) can live out of water for more than 24 h and be restored to normal life even after the skin has become dry (Lagler et al., 1977). Thus, the high Hb value in *P. obscura* is indicative of its air breathing character and activity. The predominant blood group in *P. obscura* is 90% prevalence. The prevalence of this blood group is advantageous to the fish since the risk of death especially of the fingerlings is minimized in case of fertilization with the positive group; the genotypes are AA (88%) and AS (12%). This is also advantageous among the species since there will be little probability of generating sickle cell off springs. It can also be deduced that this genotype group is favored by natural selection being able to withstand environmental stress.

The linear regression analysis has shown that standard length affects the blood parameters, while body weight has little or nothing to offer. In conclusion, therefore, it should be realized that blood picture is not a dull and static thing; It depends on what is happening to the fish at any particular locality and time and so it is very dynamic. For this and other reasons there is a great need to monitor with broad limits the blood values in normal healthy fish in order to improve the management and treatment practice since these variables affect the

total yield and ultimately the productivity of these fishes.

Except for the occurrence of amylase in the oesophagus, Akintunde (1985) observed a similar general pattern of enzyme distribution in *Sarotherodon galilaeus* (Linnaeus, 1758), which like *P. obscura* has a microphagous dietary. Worthy of note is the occurrence of enzyme secretion in the oesophagus of *H. niloticus* (Table 12) which is rare, having been reported in only a few species (Kawai and Ikeda, 1971). The variety of glycosidase indicates the ability of *P. obscura* to digest a wide range of carbohydrate food components. Cellulase activity was recorded only in the pyloric caeca and its origin is attributed to gut micro flora ingested along with the detritus which featured prominently in the diet. The relatively high activity levels of proteases, particularly in the duodenum (Table 12), are not surprising in view of the large proportion of protein components (mainly zooplankton) in the diet. Pepsin would hardly be expected to occur in the two distal gut regions since it is active only in strongly acid media found in the stomach. Lipase distribution and activity along the entire gut was also reported in *C. isheriensis* by Fagbebro (1980). From the foregoing, it is evident that *P. obscura* is well equipped to digest the carbohydrate, protein and lipid components of its diet.

The conclusion may be reached therefore that plasticity and resilience of biological attributes of *P. obscura* enables it to survive and thrive on both remote sahelian and coastal environments. Early sexual maturity, rapid growth and high recruitment compensate the large mortality of the species resulting from predation and fishing. Thus, the high abundance of *P. obscura* in the Anambra River is sustained.

REFERENCES

- Adeyemo O K (2007). Haematological profile of *Clarias gariepinus* (Burchell, 1822) exposed to lead. *Turk. J. Fish. Aquat. Sci.* 7:163-169.
- Akintunde EA (1985). Digestive enzymes in the gut of *Sarotherodon galilaeus* (syn. *Tilapia galilaea*, family Cichlidae) of Lake Kainji. *Nigeria. J. Sci.* pp. 1822-1825.
- Awachie JBE, Ezenwaji HMG (1981). The importance of *Clarias* spp. in the Fisheries development of the Anambra river basin, Nigeria. *CIFA Tech. pap.* 8:212-233.
- Azugo WI (1978). Ecological studies of the helminth parasites of the fish of Anambra river system. *M. Phil Thesis, University of Nigeria, Nsukka.* p. 178.
- Badawi HK, Said MM (1971). A Comparative Study of the Tulip species (pisces). *Marine Biol.* 8:202-204.
- Bailey NTJ (1994). *Statistical Methods in Biology*, 3rd Edition. Cambridge University Press Cambridge, p. 255.
- Balogun RA, Fisher O (1970). Studies on the digestive enzymes of the common African toad, *Bufo regularis* Boulger. *Comp. Biochem. Physiol.* 33:813-820.
- Barnhart RA (1969). Effects of certain variables on haematological characteristics of rainbow trout, *Salmo gairdneri* (Richardson). *Trans. Am. Fish Soc.* 98:411-418.
- Benjamin MM (1961). *Outline of veterinary clinical pathology*. 2nd Ed. IOWA State University Press, America. IOWA, p. 250.
- Blaxhall PC, Baibley KW (1973). *Routine haematological methods for*

- use with fish blood. *J. Fish Biol.* 5:771-774.
- Bouck RG, Ball RC (1966). Influence of Capture methods on blood characteristics and mortality in rainbow trout (*Salmo gairdneri*). *Trans. Am. Fish Soc.* 95:167-176.
- Chifamba PC (1993). The life history style of *Limnothrissa miodon* in Lake Kariba. CIFA Occas. Pap. No. 19:75-86.
- Clarks S, Whitmore DH, McMahan RF (1979). Consideration of blood parameters of large mouthbass, (*Micropterus salmoides*). *J. Fish. Biol.* 14:147-154.
- Cole EH (1967). *Veterinary Clinical pathology*. W. B. Saunders Company, Philadelphia, p. 235.
- Das BC (1965). Age-related trends in the blood chemistry and haematology of the Indian carp (*Catla catla*). *Gerontologia* 10:47-64.
- Delany JW, Garratty G (1969). *Handbook of hematological and blood transfusion techniques*. London: Butterworths, p. 165.
- Egwunyenga AO, Nmorsi OPG Igbinosun (1999). Haematological changes in Cichlidae of Ethiopia river (Niger Delta, Nigeria) due to intestinal helminthiasis. *Biol. Sci. Res. Commun.* 11:361-365.
- Eisler R (1965). Erythrocyte count and haemoglobin content of nine species of Marine teleosts. *Chesapeake Sci.* 6:116-120.
- Erondu E S, Nnubia C, Nwudukwe O (1993). Haematological studies on four catfish species raised in fresh water ponds in Nigeria. *J. Appl. Ichthyol.* 9:250-256.
- Eyo JE, Akpati CI (1993). Fishing gears and fishing methods. pp. 143-167. In: Ezenwaji, HMG Inyang NM, Orji EC (Eds). *Proceeding of the UNDP assisted Agricultural and rural development programme (Ministry of Architecture Awka, Anambra State). Training Course on Artisanal Fisheries development, held at University of Nigeria Nsukka, October 29, November 12, 1995.*
- Eyo JE, Mgbenka BO (1992). Aspect of the Biology of *Clarias gariepinus* in Anambra river basin. 1. Oocyte diameter, fecundity and Sex ratio. *J. Agric. Sci. Technol.* 2(1):47-51.
- Ezenwaji HMG (1999). The abundance and trophic biology of *Clarias albopunctatus* Nichols and La Monte, 1953 (Osteichthyes: Clariidae) in the Anambra flood river system. *Nig. Ecol. Freshw.* 8:22-25.
- Ezenwaji HMG (2002). The biology of *Clarias ebriensis* Pellegrin, 1920 (Osteichthyes: Clariidae) in an African rain forest river basin. *Fish. Res.* 54:235-252
- Ezenwaji HMG, Inyang NM (1998). Observations of the biology of *Clarias agboyiensis* Sydenham, 1980 (Osteichthyes: Clariidae) in the Anambra river flood system. *Nig. Fish. Res.* 36:47-60.
- Ezenwaji HMG, Inyang NM (1998). Observations on the biology of *Clarias agboyiensis* Sydenham, 1980 (Osteichthyes: Clariidae) in the Anambra flood river system. *Nig. Fish. Res.* 36:47-60.
- Fagbenro AO (1990). Food composition and digestive enzymes in the gut of pond-cultured *Clarias isheriensis* (Sydenham 1980). (Siluriformes: Clariidae). *J. Appl. Ichthyol.* 6:91-98.
- Fagbenro OA, Adedire CO, Owoseeni E A Ayotunde EO (1993). Studies on the Biology and aquacultural potential of feral catfish, *Heterobranchus bidosalis*. (Geoffrey Sst Hilaire 1809) (*Clariidae*). *Trop. Zool.* 6:67-79.
- Fagbenro O, Adedire CO, Ayotunde EO, Famino EO (1999). Haematological profile, food composition and digestive enzyme assay in the gut of African bony-tongue fish, *Heterotis (clupisudis) nitrocticus* (Cuvier, 1829) (osteoglossidae). *Trop. Zool.* 13:1-9.
- Gabriel UU, Anyanwu PE, Akinrotimi AO (2007). Haematological profile of Black chinned tilapia (*Sarotherodon melanotheron*) from Buguma creek, Niger Delta. *J. Fish Biol.* 2(2):186-189.
- Gliwicz ZM (1984). Limnological study of Cahora Bassa reservoir with special regard to sardine fishery expansion. *FAO/GCP/MOZ/OO6/SWE Field Document*, 8: 71.
- Gourene G, Teugels GG (1991). Revision du genre *Pellonula* des eaux deuces africaine (Pisces: Clupeidae). *Ichthyol. Explor. Freshw.* 131:213-225.
- Guyton MD (1991). *Textbook of medical physiology*. W. B. Saunders Company, Philadelphia, p. 1014.
- Hattingh J, Van Pletzer JJ (1974). The influence of capture and transportation on some blood parameters of fresh water fish. *Comp. Biochem. Physiol.* 49:607-609.
- Hickman CP, Roberts LS, Larson A (2001). *Integrated Principles of Zoology*. McGraw Hill, New York, pp. 899.
- Hora SL (1994). *Hints of the Culture of Murrel Industrial Farming*. Bombay Nat. Hist. Soc. 6:8.
- Houston AH, Dewilde MA (1969). Haematology and blood volume of thermally acclimated brook trout, *Salvelinus fontinalis* (Mitchill). *Comp. Biochem. Physiol.* 28:877-885.
- Hynes HBN (1950). The food of freshwater sticklebacks, *Gasterosteus aculeatus* and *Pygosteus pungitius*. with a review of methods used in studies of the food of fishes. *J. Anim. Ecol.* 19:36-58.
- Hyslop EJ (1980). Stomach content analysis-a review of methods and their applications. *J. Fish Biol.* 17:411-429.
- Idodo GU (2003). *Fresh water fishes of Nigeria (Taxonomy, Ecological Notes, Diet and Utilization)*. Idodo Umeh publishers Limited, Benin City, Nigeria, p. 232.
- Kewai SI, Ikeda S (1971). Studies on digestive enzymes of fishes. I. Carbohydrates in digestive organs of several fishes. *Bull. Jpn. Soc. Sci. Fish.* 37:333-337.
- Kori Siakpere O, Ake JEG, Idoge E (2005). Haematological characteristics of the African snake head, *Parachanna obscura*. *Afr. J. Biotechnol.* 4(6):527-530.
- Kori-Siakpere O (1985). Haematological characteristics of *Clarias isheriensis*. *Sydenham J. Fish Biol.* 27:259-263.
- Lagler KF, Bardach EJ, Miller RR, May Passino DR (1977). *Ichthyology*. John Wiley and sons, New York. p. 506.
- Le Danois E (1955). *Fishes of the world*. The Countryman Press, Paris. p. 350.
- Lelek A (1973). Sequences of changes in fish population in the New Tropical man-made lake Kainji, Nigeria, West Africa. *Archiv fur Hydrobiologie*, 71:381-420
- Linnaeus C (1758). *System Nature. Regnum Animale*. 10th ed. L. Salvii. Holmniae.
- Lishev MH (1950). The food of Amur Predatory fish. *Trudy Amurskoi ikhtologichskoi ekspeditsu.* 1:1945-1949.
- Maclean N (1978). Haemoglobin. *The institute of Biology. Series in Biology*. Edward Arnold Ltd, p. 69.
- Malcolm J (1995). *Environmental Biology of fishes*. Chapman & Hall, London, pp. 455.
- Marshall BE (1987). On the relationship between tigerfish and sardines in lake kariba. *J. Limnol. Soc. South Africa* 13:78-79.
- Marshall BE (1993). *Limnothrissa* in man-made lakes: do we understand the implications of their small size? CIFA Occas. Pap. No. 19:1-17.
- Mawdesley-Thomas LE (1971). Toxic chemicals-the risk to fish. *New Scientist.* 49:74-75.
- McCarthy DH, Stevenson, JP, Roberts, MS (1973). Some blood parameters of the rainbow trout (*Salmo gairdneri* Richardson). 1. The Kamloops variety. *J. Fish Biol.* 5:1-8.
- Mgbenka BO Eyo JE (1992). Aspects of the biology of *Clarias gariepinus* in Anambra river basin. 2. Maturation and condition factor of Aquaculture. *Sci. Technol.* 2(1):52-55.
- Miale JB (1982). *Laboratory Medicine Haematology*. The C.V Mosby company, London. pp. 1083.
- Ness PH, Stengle JM (1974). *Historical Introduction: The Red Blood cell.* (2nd ed) vol. 1 Surgenor DM (ed), Academic Press Inc, pp. 23.
- Norman JR (1947). *A History of Fishes*. Ernest Benn Limited, London. 463 pp.
- Nwani CD (1998). Aspects of the Biology of *Distichondus* sp in Anambra River, Nigeria. M.Sc Thesis Dept. of Zoology, University of Nigeria Nsukka, p. 133.
- Ogunbiyi AO, Okon EE (1976). Studies on the digestive enzymes of the African fruit bat, *Eilodon helve* (Kerr.) *Comp. Biochem. Physiol.* 55:359-361.
- Ogutu-Ohwalo R (1993). *Lates* spp. and other predators as a cause of mortality in small pelagic fish species in the African great lakes. CIFA Occas. Pap. No. 19:18-23.
- Olatunde AA, Ukoha AI, Oguntayo BF (1988). Digestive enzymes in the alimentary tract of *Clarias lazera* cuvier and valenciennes family clariidae (Osteichthyes: Siluriformes). *Arch. Far Hydrobiol.* 112(1):107-113.
- Otobo FO (1978). The reproductive biology of *Pellonula afzeliusi* Johnels and *Sierrathrissa leonensis* Thys Audernaerde in lake kainji, Nigeria. *Hydrobiologia* 61:99-122.
- Oyowale JO (1987). Haematological and plasma Biochemical values of two breeds of domestic fowl in tropical environment. *Anim. Technol.*

- 38(1):49-52.
- Petr T, Kapetsky JM (1983). Pelagic fish and fisheries of tropical and subtropical natural lake and reservoir. ICLARM Newslett. 6:9-11.
- Pieterse JJ, Smith GL, Van vilet K, Schoonbe HJ, Hattingh J (1981). Observation on the blood of the Chinese silver carp, *Hypophthalmichthys molitrix* (valenciennes). J. Fish Biol. 18:455-459.
- Plummer DT (1978). An introduction to practical Biochemistry. 2nd edition. London: McGraw Hill Book company, p. 283.
- Poston HA (1966). Effects of sex and reproduction stage on haemoglobin levels in brown trout. Fish Res. Bull. 29:28-29.
- Reynolds I (1969). The biology of the clupeids in the new Volta lake. In: *Man-made lakes: the Accra symposium*, Ed. by Obeng LE. Ghana University Press, Accra, pp. 195-203.
- Reynolds JD (1970). Biology of the small pelagic fishes in the new Volta in Ghana. 1. The lake and the fish: feeding habits. Hydrobiologia 35:569-603.
- Ricker WE (1973). Linear regressions in fishery research. Fish. Res. 30:409-434.
- Rund JJ (1954). Vertebrates without erythrocytes and blood pigment. Nature 173:848-850.
- Seddiqi AK, Mishra S (1979). Blood dyscrasia in a teleost, *Colisa fasciatus* after acute exposure to sub lethal concentrations of lead. J. Fish Biol. 14: 199-204.
- Sowunmic AA (2003). Haematology of the African catfish, *Clarias gariepinus* from Eleiyele Reservoir Ibadan South-West, Nigeria. Zoologist 2:85-91.
- Summerfelt RC, Lewis WM, Ulrich MG (1967). Measurement of some haematological characteristics of the gold fish. Prog. Fish Cult. 29:13-20.
- Teugels GG, Daget J (1984). *Parachanna* nom. Nov. for the African snakeheads and rehabilitation of *Parachanna insignis* (Sauvage, 1884) (Pisces, Channidae). Cybium. 8(4):1-7.
- Weinreb EL (1958). Studies on the histology and histopathology of the rainbow trout, *Salmo gairdneri* (Richardson). 1. Haematology. Under normal and experimental conditions of inflammations. Zoologica, New York, pp. 46-153.