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Normal Haematological Profile of *Parachanna obscura* as a Diagnostic Tool in Aquaculture

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Abstract: *Parachanna obscura* popularly called snakehead, a fresh water teleost belonging to the family channidae, is highly predatory no wonder it is adopted in Tilapia population. Its high quality flesh together with its good taste made it high priced fish in the local markets and hence improved its commercial and aquacultural potentials. Previous studies on the biology of the fish were concentrated on its food and feeding habits as well as age and growth. Information on its haematological characteristics is non-existent. The blood characteristics determined were leucocytes and erythrocytes count heamoglobin concentration and packed cell volume. Blood indices which include Mean Cell Heamoglobin Concentration (MCHC), Mean Corpuscular Haemoglobin (MCH), Mean Corpuscular Volume (MCV) and Erythrocyte Sedimentation Rate (ESR) were determined. There was no positive correlation between the physical parameters of length and weight, the leucocytes and erythrocyte counts. However significant and positive correlation existed among the haemoglobin concentration and the packed cell volume. The influence of stress due to capture, transportation, sampling age and sex on the heamatological characteristics of the fish were discussed establishment of the heamatological characteristics of *P. obscura* will help to determine the health status and provide basic information and data for comparative heamatological studies with other fish species as well as disease, toxicological and parasitological investigations.

Key words: *Parachnna obscura*, heamatology, snakehead, toxicology

INTRODUCTION

Snakeheads are bony fishes inhabiting freshwater of tropical Asia and Africa (Cruz and Landercia, 1980; Guseva, 1990) reported that snakeheads could be found in a variety of habitats including lowland streams and canals, lakes, rivers, ponds and swamps. Snakeheads are of high commercial value this is a result of good taste and high qnality flesh. Being a voracious predator, it is used in Tilapia population control, they are also good potential in fish culture.

The generic name snakeheads are *Parachanna* of the Teleost family channiade. Wee (1981 and 1983) reported that names like Ophicephalus and Ophiocephalus are still very commonly used. Two species of *Parachanna* are found in West Africa, they include *P. africana* and *P. obscura* (Syndenham, 1976). The body of *P. obscura* is elongated, cylindrical and covered with cycloid seales. Present on its round snout are two small nasal tentacles, the teeth are pointed and cardiform on the plate and both jaws. The caudal and pectoral fins are rounded and the dorsal and anal fins are soft-rayed.

P. obscura possesses accessory breathing organs similar to those of claridae family with which they are able to breathe air Reed *et al.* (1967). The use of heamatological parameters in diagnosing the health condition of fish is acquiring acceptance worldwide, as a tool in the management of fish farms (Blaxhall and Datisley, 1973).

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Changes in heamatological parameters due to unfavourable exogenous factors like poor water quality, overstocking etc. are indices of the ill health of cultivated fish. Regular monitoring of the heamatological parameters of farmed fish can be used to enhance fish production.

Report from Kelly (1979) pointed out that dehydration and other similar derangements of tissue fluid balance give rise to elevated erythrocyte counts because of a decrease in circulating plasma volume. Radujkovic *et al*. (1984) reported that haematocrit value and erythrocyte counts also reduce as a result of infections. Infection also can lead to elevated levels of glucose as reported by Jacob (1982).

These are several research conducted on different fish species by different researchers in Nigeria, as far as it is know, nobody has worked on heamatological parameters of *P. obscura* in Nigeria.

MATERIALS AND METHODS

Twenty five sub-adult and adult samples of *P. obscura* were collected from four freshwater bodies in South-western Nigeria, between March, 1998 and February 1999. They were caught as by catch in gill nets of 2 cm mesh size set for Tilapia the fish specimens comprised males and females and none of them showed symptoms of stress or diseases.

A measuring board was used to lake the standard length in cm, which Mettler's balance (PB 8001), Toledo top-load brand was used to measure the body weights in grams.

Also the condition factor or Fulton's factor was calculated using the formula:

$$K = \frac{100W}{L^b}$$

where, W denotes fish weight (g) and L represents standard length of fish (cm).

Blood from every specimen was used separately for the analyses heparinized disposable syringes (10 mL) were sterilized and treated with 0.5 g of ethylene-diamine acetic acid (EDTA) to act as anti-coagulant and was introduced to specimen tubes. Blood was taking with the aid of syringe through cardio puncture from each fish immediately it was taken out of water.

The preserve the shape of the cells as well as large number of ht erythrocytes blood samples were diluted with Hayems fluid, in order to prevent agglutination, few drops of physiological saline solution were added.

A Neubauer improved heamocytometer mounted on a compound microscope was used to count for estimating the erythrocyte population. The number of cells counted was determined using the method describe by Svobodva *et al*. (1991).

Each smallest square has a volume of 1/4000 mm^3 and counting was done in 80 squares with the sum total value of 1/50 mm^3, taken dilution factor to be 200. The total number of erythrocyte therefore was obtained as 200×50×R cells. Where, R is number of erythrocyte counted. For leucocytes count 0.8 cm objective of the microscope was used and larger squares having volume of 0.1 mm^3 and dilution factor of 20 with four squares used the total count per mm^3 was also obtained as:

$$\frac{20 \times 1 \times L}{0.4} \text{cell} = 50 \times L \text{ cells}$$

Where, L is number of leucocytes counted.

In determining the heamoglobin concentration, the indirect acid heamatin (Sahli) method was used. The reagents and procedure used are described by Kelly (1979).

Heamoglobin concentration was converted to acid heamatin by the action of 0.1 M HCl using 0.02 mL pipette. Twenty milliliter, 0.1 M HCl and 0.02 mL of blood sample were used to fill the graduated tube. The mixture was allowed to stand for 5 min before introducing few drops of distilled water till colour matched the standard. The heamoglobin concentration was later estimated as:

$$\text{Haemoglobin concentration} = \frac{\text{Value obtained}}{100} \times 17.2\,\text{g}/100\ \text{mL}$$

The determination of packed cell volume was done using method describe by Wintrobe (1934). The 1 mL volume haemotocrit tube sealed at one end were filled with fish blood and set in Hawkley's micro-haematocrit centrifuge for 5 min at 5000 rpm. The packed cell volume was estimated using a micro haematocrit reader and expressed in percentages Wintrobe (1934).

Wintrobe haemaotcrit tube filled with the fish blood samples and then placed in perfectly vertical position using a wooden sedimentation rack for 1 h at ambient temperature (25°C) was used in Erythrocyte Sedimentation Rate (ESR) determination. This was done using a haematocrit reader. The erythrocyte column was estimated as a percentage of the total column of the fish blood and the erythrocyte sedimentation rate within a time interval of 1 h.

Erythrocyte values such as Mean Corpuscular Volume (MCV), Mean Corpuscular Haemoglobin (MCH) and Mean Corpuscular Haemoglobin Concentration (MCHC) were calculated as:

$$\text{MCV} = \frac{\text{PCV} \times 1000}{\text{Erythrocyte count}}\ (\text{Er})\ \text{expressed in fentolitres}$$

$$\text{MCH} = \frac{\text{Haemoglobin value}}{\text{Erythrocyte count}}\ \frac{\text{Hb}}{\text{Er}}\ \text{expressed in Picogrammes}$$

$$\text{MCHC} = \frac{\text{Hb}}{\text{PCV} \times 1000}\ \text{expressed in g L}^{-1}\ \text{and from the haematocrit}$$

value (PCV).

Regressing and correlation analysis using standard length as dependent variable were estimated. Haematological indices (absolute values) were estimated for the data obtained for Mean Cell Haemoglobin Concentration (MCHC). Mean Cell Volume (MCV) and mean cell haemoglobin (Hb) calculated as described by Delaney and Garatty (1969).

Correlation co-efficient, (r) was calculated from simple regression analysis and this was used to establish the relationships among the indices using computer with SPSS program.

RESULTS AND DISCUSSION

The mean weight of the 25 *Parachanna obscura* samples used was 120.67 g, while the mean standard length was 20.56 cm. The results of haematological characteristics of *P. obcura* are shown in Table 1.

The value of the erythrocytes ranged from 0.6×10^6 per mm^3 to 3.3×10^6 per mm^3 with mean of 2.004×10^6 per mm^3. There was correlation between the standard length and the amount of erythrocytes in each of the fish specimen used. There was considerable variation in the erythrocyte count within the species. The equation is:

$$\begin{aligned} L &= 22.03383 - 0.49195\ (\text{RBC}) \\ &\quad\ (2.60897)\quad (0.8457) \end{aligned}$$

Table 1: Haematological characteristics of *P. obscura*

Parameters	Mean	Range	Standard deviation
Standard length (cm)	21.01	13.8-24.2	2.92
Weight (g)	122.03	56.7-194.3	52.78
Fullons condition factor (k)	1.25	1.06-1.51	0.11
Erythrocyte count$\times 10^6$ mm^{-3}	2.004	0.6-3.3	0.71
Haemoglobin conc (g/100 mL^{-1})	11.48	9-14	1.55
PCV (%)	26.40	22-23	3.89
Erythrocyte sedimentation rate (mm h^{-1})	13.12	11-16	1.26
MCV (mm^3)	131.70	73.3-533.3	108.20
MCH (pg)	57.28	32-216	43.25
MCHC (%)	43.48	33-58	6.97
Lencocyte count$\times 10^3$ mm^{-3}	4.01	1.8-6.1	1.32

Where, L = Standard length, $R^2 = 0.0145$ while F = 0.33839

The result shows that the range of leucocytes is between 1.8-6.1$\times 10^3$ mm^{-3} with a mean of 4.01$\times 10^3$ mm^{-3}. Data analysis shows that:

$$L = 22.28068 - 0.34411\ (WBC)$$
$$(2.35541)\quad (0.45492)\ \text{and}\ F = 0.57216$$

Erythrocyte and leucocytes counts were discovered to vary with the individuals of *P. obscura*. The variation showed significant differences in the haematocrit value.

A mean haemoglobin concentration value of 11.48 g/100 mL was obtained. The value ranges from 9-14 g/100 mL with standard deviation ±1.55 (Table 1).

The blood parameter values of *Parachanna obsucra* presented in Table 1 based on the 25 fish samples are similar to those reported for other freshwater teleost species. There are variations in haematological values, which can be attributed to many factors such as age, size of the fish, nutritioual state, season, spawning, sex and genetic variation Larsson *et al*. (1984). Das (1965) discovered that the number of red blood cells and haemoglobin concentration tend to increase with length and age.

Nutritioual deficiencies, parasitic diseases, infectious diseases or organic and glandular disorders can result in situation known as dyshaemopoitic anaemia brought about by depression in erythrogenesis according to Kelly (1979). Nutritional deficiencies which depress bone narrow function include iron, copper, cobalt, protein and vitamins which include folic acid thiamin, riboflavin and cyanocobalamin.

In fishes where there is reduction below the normal range of value of the erythrocyte numbers, there is correspondent reduction in haemoglobin values per cell (Larsson *et al*., 1984).

Preston (1960) and Mahagen *et al*. (1979) observed seasonal fluctuations in haematological parameters while Smith (1977) noted a correlation of MCV to weight which ranged from highly negative to highly positive based on the season.

Poston (1966) equally noted increased haematocrit values for male fishes prior to the time of spawning. The blood of a healthy vertebrate shows correlation between the number of erythrocytes, haematocrit value and haemoglobin content (Badawi and Said, 1971). This same correlation was also observed in the value and haemoglobin content (Badawi and Said, 1971). This same correlation was also observed in the values of haematological parameters of *P. obscura* (Table 2).

The PCV is positively correlated to the haemoglobin content (Hb) and Erythrocyte Sedimentation Rate (ESR) while it is negatively correlated with erythrocyte and leucocytes numbers ($p<0.01$). The leucocytes counts in *P. obscura* show positive correlation with haemoglobin content, ESR and Erythrocyte counts while it is negatively correlated with PCV.

None of the coefficient of the variables was significant at 0.05 level. The standard length was found to be negatively correlated with haemoglobin content, ESR, erythrocyte and leucocyte counts.

Table 2: Correlation analysis of haematological parameters of *P. obscura*

Correlation	HB	ESR	RBC	WBC	PCV
Length	-0.2244	-0.3663	-0.1204	-0.1558	0.219
HB	1.0000	0.9182**	-0.2070	0.0778	0.4856*
ESR	0.9182**	1.0000	-0.1059	0.0858	0.4778*
RBC	-0.2070	-0.1059	1.0000	0.1898	-0.4588
WBC	0.7780	0.8580	0.1898	1.0000	-0.1189
PCV	0.4856*	0.4778*	-0.4588	-0.1189	1.0000

No. of Fish sample = 25, 1-tailed significance level = *: -0.01; **: -0.001

These tally with the finding of Erondu *et al*. (1993) who work on four catfishes and also correlate with the findings of Fagbenro *et al*. (1993) on *Heterobranchus biosalis*. Erythrocyte counts that ranged between 0.6-3.3$\times$10^6 mm^{-3} in this study were within the normal values. The wide range could not be unconnected with the fact that the fish samples were collected from different enviroumental conditions especially since live fishes are known to be very sensitive to physical and chemical changes as observed by Blaxhall (1972). The high values of erythrocyte count in some of the fish samples are consistent with fish activity (Siakepere, 1984). A high early morning oxygen demand as a result of relatively high oxygen consumption by aquatic flora and fauna could create a stress situation and consequently lower fish blood values as obtained for some of the fish used in this study.

Boon *et al*. (1990) discovered that parasitic infections relatively reduced haematocrit and leucocytes count values. Stress due to capture, handling and sampling procedures was observed in 1966 by Bauck and Ball, to cause variation in intraspecies haematological values. Hattingh and Van Pletzen (1974) reported that decrease in the fish blood haemoglobin concentration and PCV. Stress due to capture had been reported by Hatingh (1976) to cause hyperglycaemia in fish.

More than 85% of *P. obscura* fish used in this study had between 1.1-3.3$\times$10^6 mm^{-3} erythrocyte counts. This shows the high capability of the blood to carry oxygen and perhaps this could be attributed to the piscivorous habit of the fish. The fact that the fish is able to stay out of water for some few hours, may be due to its air-breathing habit. Lenfant and Johansen (1976) established a tendency for haemoglobin concentration to be higher in air breathing fishes it has also been established by Kelly (1979) that a significant increase in erythrocyte numbers occurs in polycythaemia vera and in disturbed tissue fluid balance. During this period, there is a primary absolute elevation in the circulating erythrocyte population, due to decrease in circulating plasma volume. The incidence of hypoxia causes increased production of erythropoietin, which leads to increase in the circulating erythrocyte numbers.

The leucocytes count shows a negative correlation with the standard length and PCV. This was significant at 0.05 levels. There was a negative correlation with haemoglobin, erythrocyte and ESR values. Studies had shown that the leucocytes, after performing specific functions, are destroyed.

CONCLUSIONS

The basic haematological parameters which include erythrocyte and leucocytes counts, haematocrit and the erythrocyte sedimentation rate have been established based on the 25 fish specimens of *P. obscura* collected form four water bodies located in different places in south western part of Nigeria. The use of haematological values in disease diagnosis and nutritional status of fish has been documented. Knowledge of the various parameters is beneficial to the fish farmers, scholars and further development of the fishing industry in the country.

The baseline data presented by this study on the haematological indices of *P. obscura* will contribute to the comparative studies on fish haematology and aspects of fish physiology, pathology and toxicology.

A direct relationship exists between the fish standard length and body weight while there is pronounced interspecies variation in the haematological values obtained for *P. obscura* as earlier discovered in *Heterotis niloticus* (Ayotunde, 1997). *Heterobranchus bidosrsalis* (Fagbenro *et al*., 1993) and four catfishes (Siakepere, 1984). The correlation between the erythrocyte count, haematocrit and haemoglobin content is as close in *P. obscura* as in mammals and other vertebrates.

RECOMMENDATION

More research work should be done in the areas of ecological studies to ascertain the specific nich, roles and the position of *P. obscura* in the food chain in the freshwater environment. Also more work should also be done to include the serological study and reproductive biology of this species of fish in order to promote its propagation.

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