PLASMA BIOCHEMISTRY, HAEMATOLOGICAL AND BIOMETRIC PARAMETERS OF *PROTOPTERUS ANNECTENS* OF ANAMBRA RIVER, NIGERIA

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TITLE PAGE

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CERTIFICATION

Ebeh, Ngozi Helen, a postgraduate M.Sc. student in the Department of Zoology and Environmental Biology, has satisfactory completed the requirements for the award of the degree of Masters of Science (M.Sc.) in Zoology and Environmental Biology (Fisheries). The content of the Thesis is original and has not been submitted in part or full for other certificate, Diploma, or Degree of this University or any other University elsewhere.

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External Examiner Date

DEDICATION

This work is dedicated to the most gracious God for His extravagant grace towards me.

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ABSTRACT

Biochemical, haematological and biometric studies of the African lungfish, *Protopterus annectens* were carried out in order to establish their mean and reference values which would serve as baseline data for assessment of the health status of the fish as well as reference point for future comparative surveys. The study was carried out between March and August, 2015. In the present study, blood was analyzed using standard techniques, and differences in haematological parameters including haemoglobin concentration, red blood cell count (RBC), white blood cell count (WBC), packed cell volume (PCV), mean corpuscular volume (MCV), mean corpuscular haemoglobin (MCH) and mean corpuscular haemoglobin concentration (MCHC) were determined. Biochemical parameters such as alkaline phosphate (ALP), aspartate transaminase (AST), alanine transaminase (ALT), total protein, albumin, globuline, glucose, cholesterol level, and urea were determined. Biometric parameters such as condition factor (K), hepatosomatic index, and gonadosomatic index were also determined. These haematological, biochemical and biometric parameters of fish were compared according to sex and seasons. Analysis of variance showed that there were significant differences ($P < 0.05$) in ALP, AST, ALT, Protein, PCV, RBC, WBC, Hb and MCV, between sex and season. The results indicated that blood parameter level such as PCV, RBC, WBC, Hb, MCV, and MCHC between sexes in dry season were significantly different from those measured in wet seasons. The result also indicated that there were significant ($P < 0.05$) differences in condition factor (K), hepatosomatic index (HSI) and gonadosomatic index (GSI) between the seasons. There were no significant differences in MCH and MCHC values between the sexes. The values of WBC and PCV were found to be higher in female fish especially in dry season, while the level of haemoglobin and MCV values were higher in male *Protopterus annectens*. This may be related to the aggressiveness of the male fish. All the biochemistry parameters were higher in dry season except cholesterol and urea, but no significant differences were found among the sexes. The length of the fish varied significantly with season. The highest length was recorded in the March and June, while the highest mean weight frequency was obtained in the month of August. The length-weight relationship showed a negative allometric growth and the condition factor indicated that the fish were in a good condition in the months of April and July. There was increase in Hepatosomatic index (HSI), as the fish length increased. The results of the present study provide useful information for monitoring changes in the health status of fish.

CHAPTER ONE

INTRODUCTION AND LITERATURE REVIEW

1.1 Introduction

It is recognized that the blood component value exhibit genetic and physiological variations. The genetic variation may be due to site-specific factors within species. Blood comprises 1.3-7% of the total body weight of fish and it represent one of the most active components that contribute to metabolic processes by ensuring gas exchange between the organism and the environment. For this reason, blood parameters are increasingly used as indicators of the physiological condition of sub-lethal stress response in fish to endogenous or exogenous change (Belanger *et al.,* 2001; Mohammadizadeh *et al*., 2012). In live fish, heamatology and plasma biochemistry provide a minimally invasive tool that can support health of fish, especially in relation to determining potential effects associated with such factors as pollution, disease, age, sex, seasonality and reproduction (Pradhan *et al.,* 2012). Changes in hematological parameters depend upon the aquatic biotope, fish species, age, sexual maturity and health status. The evaluation of physiological condition of fish depends on the availability of reference values. These should be as close as possible to normal values of various blood components considered as reliable descriptors of healthy fish under natural conditions (Pradhan *et al.,* 2012). It is clear that the environment in which fish live influences the metabolic content in blood. Thus, haematological, biochemical and biometric parameters are closely related to the response of the animal to the environment, an indication that the environment where fishes live could exert some influence on the heamatological characteristics (Gabriel *et al.*, 2012). Taking into account the long evolutionary history of fishes and the adaptation to different environment, it is obvious that no species can be used as a representative model for all fishes. One of the difficulties in assessing the state of

propagation of natural fish population has been the paucity of reliable reference values in healthy animals under natural habitat (Pradhan *et al.,* 2012).

The African lungfish, *Protopterus annectens* is a highly prized food fish in Nigeria (Otuogbai, 2001; Otuogbai and Ikhenoba, 2012). It is distributed in shallow parts of rivers and lakes of some West African countries ranging from Senegal to Cameroon where it contributes to a relatively high percentage of artisanal fisheries (Otuogbai, 2001; Otuogbai and Ikhenoba, 2001; Okafor, 2004). *Protopterus* are omnivores in nature, feeding on fish, shellfish, amphibians and plant matter (Otuogbai, 2001).

The determination of biochemical, haematological and biometric parameters of fishes are carried out for a variety of purposes to;

- establish a ñnormal rangeò of blood parameters (Pradhan *et al.,* 2012);
- investigate condition that might lead to alterations of some of these values such as sampling methods, temperature, sex, maturity, disease condition or nutrition of the fish (Okafor and Chukwu, 2010; Etim *et al.*, 2014). and
- ascertain the effects of certain chemical pollutants (e.g insecticide) and sublethal strength of some toxicants (such as heavy metals example, lead) on blood values (Gaafar *et al*., 2010; Abedi *et al*., 2013; El-Boshy *et al*., 2014).

Hematological and plasma biochemistry values of fishes have been studied in many parts of the world (Njidda and Isidalomen, 2010; Fazio *et al*., 2013; Okorie *et al.,* 2014). Recent studies disclosed that diseases and environmental conditions are the major constraints of aquaculture especially in the developing counties (Thien *et al*., 2007; Chi *et al.,* 2008; Phan *et al.,* 2010; FAO, 2012). Another study revealed that plasma biochemistry and hematology of fish enables us to differentiate the normal physiological condition of the animal under research from the eventual pathological modifications (Asadi *et al*., 2006;

Bahmani *et al.,* 2010; Kataria *et al.,* 2010). Haematological studies are of biological, ecological and veterinary interest and can help in the diagnosis of stress and fish diseases. Changes in blood parameters indicate the occurrence of hemo-concentration or hemo-dilution due to Osmoregulatory dysfunction (Tavares-Dias and Moraes, 2004).

There is appreciable documentation of plasma biochemistry, haematological and biometric reference values of *Protopterus annectens* in Nigeria. One of such report in haematological profile of the African lungfish, *Protopterus annectens* of Anambra River, Nigeria was that of Okafor and Chukwu (2010). They documented that the wide range of blood osmolarity observed in *Protopterus annectens* is an indication of high degree of tissue tolerance and this is of great value when encountering the estuarine or brackish water environment.

However, in a similar study, Okorie *et al.* (2014), which carried out their study in Umudike, Nigeria, observed that species, age, sex, stress and different management system have effects on haematological and blood biochemical values of fishes. Another study by Bankole *et al.* (1994), carried out in Idah area of River Niger, Kogi State, Nigeria revealed that *Protopterus annectens* is the only specie of primitive family lepidosirenidae found in West African freshwaters. Similar works have also been done in Nigeria by Owolabi (2011) in Jeba lake, Okafor and Chukwu (2005a) in Anambra River, Okafor (2006) in Anambra River, Okafor and Chukwu (2005b) in Anambra River,

The Anambra River is the largest tributary of the lower Niger below Lokoja, and often regarded as a component part of the lower Niger lowlands (Udo, 1975). The river is of great importance as it forms a commercial fishing center, supplying fishes to populace from southern Nigeria and beyond. A considerate biological and ecological studies have been undertaken and documented on some economically important tropical fish fauna from the

river basin (Ezenwaji, 2002; Nwani, 2004; 2006; Odo, 2004; Okafor and Chukwu, 2005a; Okafor, 2006; Odo *et al.,* 2012).

Therefore as part of the study on the improvement of fishery and fish production in Anambra River basin, more so, because of the importance of African lungfish in aquaculture industry and to assess health status and subsequent diagnosis of disease, there is a need to establish haematological, biochemical and biometric reference values of the fish.

1.1.2 Statement of the Problem

Blood has been regarded by man as the essence of life, the seat of the soul and progenitor of pschyic and physical strength. Fish blood is a pathophysiological indicator of the whole body function. Unfortunately, diseases and adverse environmental conditions are the major constraints of aquaculture especially in developed countries. The environment in which fish live influences the metabolic content in blood (Pradhan *et al*., 2012). The fish live in very intimate contact with their environment and are very susceptible to physical and chemical changes which may be reflected in their blood components. Continuous stress affects the behavior, growth and normal development of fish (Etim *et al.*, 2014) and increase in susceptibility to infections. This may cause mortality. Research has demonstrated that changes in blood parameter indicate the occurrence of hemo-concentration or hemo-dilution due to osmoregulatory dysfunction. (Tavares Dials and Moraes, 2004). Recent research by Tanti *et al.* (2011) revealed that age, sex, environmental conditions and diet can significantly influence biochemical parameter of the fish. Moreso, Okorie *et al.* (2014) revealed that diseases and adverse environmental conditions are the major constraints of aquaculture. Okafor and Chukwu, (2010) have performed an extensive work on haematological profile of the African lungfish, *Protopterus annectens.*

Indeed, many studies have demonstrated the usefulness of heamatology, plasma biochemistry and bioindicators in the assessment of fish health and as a biomarker of exposure to pollution (Coles, 1986; Togun *et al*., 2007; Isaac *et al*., 2013). Thus, haematology, plasma biochemistry and biometric parameter of fish continues to offer the valuable diagnostic tool and progress in establishing normal range values for blood parameters of different fish species (Khan and Zafar, 2005), but little is known about the normal physiology and response of the African lungfish (*Protopterus annectens*) to disease. However, basic research that provides evidence for plasma biochemistry, hematological and biometric parameters of *Protopterus annectens* is lacking.

1.1.3 Justification of the Study

A major part of the world's food is being supplied from fishery sources, and it is estimated that around 60% of people in many developing countries including Nigeria depend on fish for their protein requirement (Pradhan *et al.,* 2012), thus, it is essential to secure the propagation of fishes. Fish health management entails active regulation of the host, pathogen and environment to maximize the optimal condition for sustained growth and health. Fish must be free from diseases and mishandling so as to get better nutrition. It is in view of this, that the study attempts to investigate the plasma biochemistry, haematological and biometric values of the fish. The findings will be beneficial as it will serve a baseline for data assessment of health status of the African lung fish *Protopterus annectens,* determine potential effects associated with such factors as size, seasonality and sex; provides reliable information on metabolic disorders, deficiencies and chronic stress status, offer the valuable diagnostic tool and progress in establishing normal range values for plasma parameters of the African lungfish (*Protopterus annectens*) as well as provide governing bodies with information useful in management of aquatic ecosystems.

1.1.4 Objectives of the Study

The general objective of this study is to evaluate the normal plasma biochemistry, haematological, and biometric parameters of *Protopterus annectens* of Anambra River.

Specifically, the objectives of this study are to:

1 establish the haematological profile of the African lungfish of Anambra River basin;
2 determine the leucocyte differentials of *Protopterus annecten*s of Anambra River;
3 establish a normal range of plasma biochemistry in *Protopterus annectens* of Anambra River;
4 determine the mean weight frequency variation of *Protopterus annectens* of Anambra River;
5 compare the mean weight frequency variation of *Protopterus annectens* of Anambra River;
6 determine the condition factor of *Protopterus annecten*s
7 determine the hepatosomatic and gonadosomatic index of *Protopterus annectens* of Anambra River
8 determine the seasonal variations in haematological, biochemical, biometric and leucocyte differentials of *Protopterus annectens;*
9 compare the plasma chemistry, haematology and biometric parameters of the African lungfish of Anambra River basin based on sex and season;

1.2 Literatures Review

In aquaculture, as in other sectors where work is done on live organisms, to get a high production is conditioned by awareness and keeping an unaltered health condition of the biological materials (Csep *et al.,* 2010; Farah *et al.,* 2010). That is why to know the values of plasma biochemical parameters enables us to differentiate the normal physiological condition of the animals under research from the eventual pathological modifications having occurred due to the disease in the organisms (Asadi *et al.,* 2006; Bahmani *et al.,* 2010).

Fish and fisheries products provide protein and essential micronutrients for balanced nutrition and health (FAO, 2012). Globally, fish accounted for 16.6% of animal protein intake and 6.5% of all protein consumed in 2009 (FAO, 2012). While world productivity of fish captured dropped slightly, there was a remarkable increase in aquaculture productivity in the last decade with Africa recording the highest animal increase in the number of people engaged in fish farming followed by South America and Asia respectively (FAO, 2012).

Table 1: Main channel length, basin area and catch from African rivers

River	Channel length (km)	Basin area (km²)	Catch (t)
Nile	6 669	3 000 000	40 840
Zaire	4 700	4 014 500	82 000
Ubangi	1 060	772 800	4 670
Kasai	1 735	342 116	7 750
Niger	4 183	1 125 000	30 000
Benue	1 400	219 964	12 570
Zambezi	2 574	1 300 000	21 000
Senegal	1 641	335 000	16 000
Gambia	1 120	77 000	3 000
Volta B.	650	45 324	1 560
Volta R.	260	6 871	370
Volta W.	255	6 602	70
Pendjari	330	11 226	140
Oueme	700	40 150	646
Mono	360	22 000	533
Tana	600	38 000	500
Bandama	950	97 000	3 408
Sassandra	650	75 000	1 518
Comoe	1 160	78 000	2 142
Rufigi/Ruaha	750	17 700	3 600

SOUTH AMERICA			
Orinoco	228	1 000	43.80
Amazon (Peru)	9 960	13 700	13.80
ASIA			
Mahaweli	121	413	34.13
Bangladesh	93 000	727 000	78.17
Lubuk Lampan	12	29	24.17
Lower Mekong	54 000	220 000	40.74
Ganges 1958ï 61	296	1 538	51.96
Ganges 1962ï 69	296	1 430	48.31

Fish live in very intimate contact with their environment and are therefore susceptible to physical and chemical changes, which may be reflected in their blood components .Blood is therefore recognized as a potential index of fish response to water quality (Olafedehan *et al.*, 2010) and it can be used to ascertain the effects of pollutants in the environment. Blood parameters in fish have been studied to elucidate physiological adaptation and to assess the health of fishes (Vazquez and Guerrero, 2007; Yildiz, 2009).

1.2.1 Plasma

Plasma is the fluid portion of the blood. It is made up of water (90%) and dissolved substances 10% (Idodo, 2010). The substances dissolved in the plasma include:

- Plasma proteins like; albumins, globulines - including antibodies, Fibrinogen, clotting factors.
- Inorganic salt (mineral salts)
- Nutrients from digested foods.
- Organic waste materials: example, urea, creatinine and uric acid
- Hormones
- Enzymes
- Gases (Anne and Allison, 2002).

Plasma acts as a pathological reflector of the status of exposed animals to toxicant, stress and other environmental conditions (Olafedehan *et al.,* 2010). As reported by Isaac *et al.* (2013), animals with good blood composition are likely to show good performance. The examination of blood gives the opportunity to investigate the presence of several metabolites and other constituents in body of animals and it plays a vital role in the physiological, nutritional and pathological status of an organism (Aderemi, 2004; Doyle and Williams 2006). Olafedehan *et al.* (2010) reported that examining blood for their constituents can provide important information for the diagnosis and prognosis of diseases in animals.

Fish blood is a pathophysiological indicator of the whole body function and therefore blood parameters are important in diagnosing the structural and functional status of fish exposed to a toxicant (Mohammadizadeh *et al.,* 2012). Fish blood is being studied increasingly in toxicological research and environmental monitoring as a possible indicator of physiological and pathological changes in fishery management and disease investigations (Asadi *et al*., 2006; Charoo *et al*., 2013).

According to Maxwell *et al.* (1990), blood parameters are important in assessing the quality and suitability of feed ingredients in farm animals. Animashahun *et al.* (2006) affirmed that the comparison of blood chemistry profile with nutrient intake might indicate the need for adjustment of certain nutrient upward or downward for different population groups.

1.3 Haematological Indices in *Protopterus annectens*

Haematology refers to the study of the numbers and morphology of the cellular elements of the blood- the red cells (erythrocytes), white cells (leucocytes), and the platelets (thrombocytes) and the use of these results in the diagnosis and monitoring of diseases (Merck Manual, 2012). Haematology also implies rapid and practical analysis to assist the

diagnosis of homeostatic imbalance. Haematological studies are useful in the diagnosis of many diseases as well as investigation of the extent of damage to blood (Togun *et al*., 2007; Mmereole, 2008). Haemotological studies also are of ecological and physiological interest in helping to understand the relationship of blood characteristics to the environment (Ovuru and Ekweozor, 2004) and so could be useful in the selection of animals that are genetically resistant to certain diseases and environmental conditions (Mmereole, 2008; Isaac *et al.,* 2013). The techniques of haematology are concerned with cellular formed elements of blood, their numbers or concentration.

Haematological parameters are those parameters that are related to the blood and blood forming Organs (Bamishaiye *et al.,* 2009). Qualitative and quantitative variations in haematological parameters including the red blood cell (RBC) and white blood cell (WBC) numbers, cell proportions of leucocytes, the amount of haemoglobin, and the size of RBC and WBC are the most significant findings as regards diagnosis (Bamishashiye *et al*., 2009). Haematocrit, erythrocytes count, and hemoglobin concentrations are the most readily determined haematological parameters for both the fish and hatchery conditions (Predham *et al.,* 2012). Haematological parameters can be affected by bacterial infection (Martins *et al.,* 2008), parasitic infection (Ugbor, 2015*)*, and poor water quality (Charoo *et al*., 2013). Variations in haematological parameters of fishes are caused by environmental stress (Afolabi *et al*., 2010), malnutrition, gender, fish size, seasonal difference and breeding efficiency (Predhem *et al.,* 2012). Haematological values would serve as baseline information for comparison in conditions of nutrient deficiency, physiology and health status of fishes. Haematological tests and the analysis of serum constituents provide crucial information for monitoring the health of fish (Mohammadizadeh *et al*., 2012). Specifically, these approaches provide reliable information on metabolic disorders, deficiencies and health

status before they become disease problems in cultivated fish. Regular monitoring of the haematological parameters of African lung fish can prevent losses due to fish diseases (Etim *et al*., 2014).

Haematological components, which consist of red blood cells, white blood cells or leucocytes, mean corpuscular volume, Mean corpuscular haemoglobin and Mean corpuscular haemoglobin concentration, are variables in monitoring health status of fishes.

1.3.1 The Red blood cells (Erythrocytes) transport oxygen to the body's tissues in exchange for carbon (iv) oxide, which is carried to, and eliminated by the lungs. It serves as a carrier of haemoglobin. It is this haemoglobin that reacts with oxygen carried in the blood to form oxyhaemoglobin during respiration (Chineke *et al.,* 2006; Isaac *et al*., 2013). Red blood cell count is a blood test that measures how many red blood cells an organism have. The result of red blood cell count can be used to diagnose blood-related conditions, such as iron deficiency anaemia (Chineke *et al.,* 2006; Isaac *et al*., 2013). A reduced red blood cell count implies a reduction in the level of oxygen that would be carried to the tissues as well as the level of carbon (iv) oxide returned to the lungs.

1.3.2 White blood cell (Leucocytes)

The major functions of the white blood cells (leucocytes) and its differentials are to fight infections, defend the body by phagocytocis against invasion by foreign organisms and to produce or at least transport and distribute antibodies in immune response. Thus, animals with low white blood cells are exposed to high risk of disease infection, while those with high counts are capable of generating antibodies in the process of phagocytocis and have high degree of resistance to diseases and enhance adaptability to local environmental and disease prevalent conditions (Isaac *et al.,* 2013).

1.3.3 Blood platelets are implicated in blood clotting. Low platelet concentration suggests that the process of clot-formation (blood clotting) will be prolonged resulting in excessive loss of blood in the case of injury.

1.3.4 Packed Cell Volume (PCV): which is also known as haematocrit (Ht or Hct) or erythrocyte volume fraction (EVF), is the volume percentage (%) of the red blood cells in blood. Packed cell volume is a measure of the ability to transport oxygen and nutrients. The measure of a fish blood sampleôs haematocrit levels may expose possible diseases in the fish. Increased PCV shows a better transportation and thus results in an increased primary and secondary polycythemia. Anaemia refers to an abnormally low haematocrit as opposed to polycythemia which refers to an abnormally high hematocrit.

1.3.5 Haemoglobin is the iron-containing oxygen-transport metalloprotein in the red blood cells of all vertebrates (Isaac *et al*., 2013) with the exception of the fish family, channichthyldae as well as tissues of invertebrates. Haemoglogbin has the physiological function of transporting oxygen to tissues of the animal for oxidation of ingested food so as to release energy for the other body functions as well as transport carbon (iv) oxide out of the body of animals (Isaac *et al.,* 2013). Previous reports from Peters *et al.* (2011) indicated that packed cell volume, haemoglobin and mean corpuscular haemoglobin are major indices for evaluating circulatory erythrocytes, and are significant in the diagnosis of anaemia and also serve as useful indices of the bone marrow capacity to produce red blood cells as in mammals. Furthermore, Chineke *et al.* (2006) posited that high packed cell volume (PCV) reading indicated either an increase in number of red blood cells (RBCs) or reduction in circulating plasma volume. Mean corpuscular haemoglobin and Mean corpuscular

haemoglobin concentration indicate blood level conditions. A low level is an indication of anaemia.

1.4 Biochemical Indices in *Protopterus annectens*

Biochemical parameter can be used to detect health of fish. Determination of reference values of these parameters in *Protopterus annectens* is of utmost importance since so many factors including species, age, sex, season and management system has been reported to have effect on the heamato-clinic chemistry values in fishes. Some of the parameters include:

1.4.1 Glucose (GLU) represents a permanent and immediate source of energy necessary for the operation of heart and of the muscles. Environmental stress can be also the cause of marked elevations in serum glucose concentration (Katari *et al*., 2010). Changes in serum glucose level are especially associated with renal injury (Katari *et al*., 2010). Further, nutritional condition can have an important influence on glucose level. To keep the glucose within certain normal limits is one of the mechanisms with the finest homeostatic adjustment, to which the hepatopancreas participates, as well as some extrahepatic tissues and a series of endocrine glands (Velisek and Svobodova, 2004; Dobsikova *et al.,* 2009; Nordlie, 2009; Patriche *et al.,* 2010; Shahsavani *et al.,* 2010).

1.4.2 Total protein (TP) represents the most important indicator of the nutritional condition of the organism and of fish health condition. Plasma proteins are responsible for transportation of lipids, vitamins and minerals in the circulatory system and the regulation of acellular activity and functioning of the immune system. Plasma proteins make up about 7% of plasma are normally retained within the blood, because they are too big to escape through the capillary pores into the tissues. They are largely responsible for creating the osmotic pressure of blood which keeps plasma fluid within the circulation. The concentration of total

protein decreases in many disease states due to decreased capacity of synthesis, reduced absorption or protein loss (Yang and Chen, 2003). From the chemical point of view, the plasmatic proteins represent a heterogeneous mixture of about 100 components with physical-chemical proprieties and different functions; some of them are molecular chaperones, directly involved in fish homeostasis (Petrescu-Mag *et al*., 2007a-c; Petrescu-Mag, 2010).

1.4.3 Albumins are the most common plasma proteins found in the blood. Albumins maintain colloid osmotic pressure, create osmotic pressure and transport insoluble molecules. They also provide the body with the protein needed to both maintain growth and repair tissues (Davita, 2005). The main function is to maintain a normal plasma osmotic pressure. A plasma albumin test measures the amount of protein in the clear liquid portion of the blood.

1.4.4 Globulines are also formed in the liver and the remainder in lymphoid tissue. Their main functions are to transport some hormones and mineral salt; inhibition of some sproteolytic enzymes and as antibodies (immunoglobulines), which are complex proteins produced by lymphocytes that play an important part in immunity (Anne and Allison, 2002). Globulines are usually classified into four groups;

Gamma globuline; primarily associated with immune system functioning;

Beta globuline; primaril***y*** associated with hormone transport;

Alpha -1 and

Alpha -2 globulines: which are primarily associated with clotting factor.

1.4.5 Blood Urea Nitrogen (BUN)

Blood urea nitrogen is an indication of renal (kidney) health. The liver produces urea in the urea cycle as a waste product of the digestion of protein.

Increased BUN levels suggest impaired kidney function. This may be due to acute or chronic kidney disease, damage or failure. It may also be due to a condition that results in decreased blood flow to the kidney.

Low BUN levels are not common and are not usually a cause for concern. They may be seen in severe liver disease and malnutrition.

1.5 Biometric Parameters in *Protopterus annecteens*

Biometric parameters are techniques as well as tools used to obtain biological information in a system and could be used to manage contaminated sites (Pastor *et al.,* 2004). Most commonly used bioindicators are corporal indices such as condition factor (K), hepatosomatic index (HSI), and gonadosomatic index (GSI) (Bastardo *et al*., 2006).

1.5.1 Condition factor (K)

Analysis of condition factor (K) could offer information on the general health condition of the fish. Pollution may damage organisms directly by increasing their mortality or interfering with the processes of food acquisition and uptake, and reducing their growth and reproduction rates. Growth represents the integration of feeding, assimilation and energy expenditure over a period of time. Poor growth means less energy is available for reproduction, which will in turn reduce the species fitness and lead to a decline in population. Growth and reproduction therefore can serve as a time-integrated indicator of the general well being of the organism (Shalaka and Pragna, 2013). Condition factor (K) calculated by weight/body length has been used to compare growth conditions of fish. A high condition factor reflects good environmental quality, while a low condition factor reflects poor environmental quality.

1.5.2 Hepatosomatic Index (HSI)

Hepatosomatic index is defined as the ratio of liver weight to the body weight (Shalaka and Pragna, 2013). It provides an indication on the status of energy reserve in an organism. It is associated with the liver energetic reserves and metabolic activity. When the food is available in large amount and condition is favourable, it causes increase in the hepatosomatic index value. Increase in the daily weight of the body is related to the increase in the hepatosomatic index value and it is also observed that hepatosomatic index depends upon seasonal cycle (Shalaka and Pragna, 2013). That is to say that the hepatosomatic index values are indirect indices of energy status on a seasonal basis. As the liver is a vital organ in the body and it performs various physiological functions such as it converts excess sugar into glycogen, it detoxifies the toxic substances, it also acts as a haemopoietic organ, destroy the old red blood cell etc. So, its healthy condition is essential for growth of fish.

As weight of the body increases, weight of the liver will also increase. The hepatosomatic index gives us information about the condition of liver and body. It also provides an indication on status of energy reserve in fish. In poor environment, fish usually have a smaller liver with less energy reserved in the liver. Hepatosomatic Index has been reported to decrease in fish exposed to water pollution (Shalaka and Pragna, 2013). Hepatosomatic Index value also provides information about the healthy condition of fish and also about the quality of water. Higher hepatosomatic value means that fishes are growing rapidly and have a good aquatic environment but lower value indicates fish not growing well and is facing unhealthy environmental problems (Shahaka and Pragna, 2013).

1.5.3 Gonadosomatic index (GSI)

Gonadosomatic index (GSI) is a tool for measuring the sexual maturity of a fish in correlation to ovary development and testes development. GSI may provide more specific information related to the fuction of the selected organ (Martin-Diaz *et al*., 2005). Gonadosomatic index is the calculation of the gonad mass as proportion of the total body mass (Martin-Diaz *et al.,* 2005).

1.6 Description of *Protopterus annectens*

Protopterus, the African lungfish is elongated cylindrical, more or less eel-like. It has prominent snout, small eyes, and its diameter 9 ï 15 cm at the head region, it has a soft cycloid scales, small and completely enclosed in the skin. There are thread- like pectoral and pelvic filametious fins without fin-rays always 4 in number. *Protopterus* is widespread, being found in Sierra Leone, Guinea, Togo, Ivory coast, Cameroon, Niger, Nigeria, Burkina Faso, Gambia, Ghana, Central African Republic, Chad, Benin, Senegal, Kenya, Mali and Sudan.

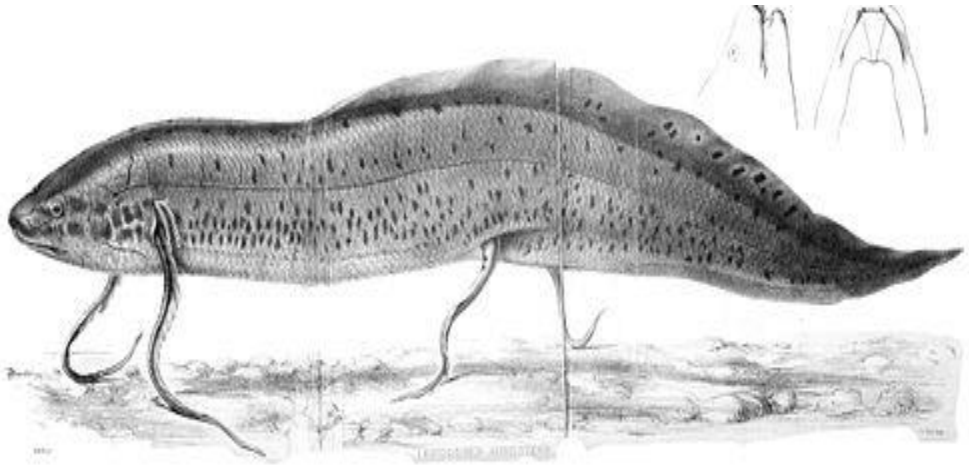

Fig. 1a: ***Protopterus annectens***

Fig. 1b: ***Protopterus annectens*** (head region)

Source: (Snoeks *et al*., 2007)

The African Lungfish is classified as belows;

Kingdom:	Animalia
Phylum:	Chordata
Sub-phylum:	Vertebrata
Super class:	Gnathostomata
Class:	Sarcopterygii
Sub-class	Dipnoi
Order:	Lepidosireniformes
Family:	Protopteridae
Genus:	*Protopterus*
Species:	*Protoperus annectens*

Protopterus has a maximum length of 100 cm and maximum published weight of 4.0kg. Its natural habitat is wet for only part of the year, and occurs in swamps, marshes and backwater along the Amazon River system. During the wet season of June to August, the African lungfish, *Protopterus annectens* live in the swamps, oxygen-poor and weed covered edges of some Nigerian Rivers and Lake such as Oguta Lake, Anambra River (Ndukuba, 2007). But in the dry season of November to April, when the water dries up, *Protopterus annectens* is adapted to prevent desiccation by digging a tunnel in the mud (river banks) and dwells there, though in an inactive state but secretes a water proof cocoon which wraps its body entirely probably until the next season (Okafor, 2008; 2009). This is in the form of aestivation. It can remain in this state of aestivation with neither food nor water until the next wet season when the entire aestivation area is flooded by the rain (Okafor, 2012). The genus *Protopterus annectens* is of great economic importance as it is a highly prized food fish in Nigeria with a great source of protein and food source for human. *Protopterus annectens* was selected for the study because it is of commercial importance, an aestivating specimen, capable of withstanding stress for a long time, as well as ability to survive more than three

years of starvation (Otuogbai, 2001) thus, the species serve as an attractive model evaluating the plasma heamatology, biochemistry and biometric parameters.

Research has shown that to establish normal haematological characteristic of a particular species of fish would serve as reference for future comparative studies (Davish *et al.,* 2010; Etim *et al.,* 2014), for instance Blaxhall and Daisely, (1973) have reported the essence of using haematocrit to detect factors affecting fish health. Several reported values for fish haemacrit fall between 20% and 35% and rarely do values above 50% reported (Etim *et al.*, 2014). Another study on the same species revealed that the mean haematocrit values for *Protopterus annectens* of all sizes (fingerlings, juveniles, intermediates, and large) fall within this range; 27.7%, 28.1%, 28.8% and 29.2% for fingerlings, juveniles, intermediate and large specimen respectively (Okafor and Chukwu, 2010).

Previous studies have shown that both the haemoglobin contents and erythrocytes counts tend to increase with length and age of the fish (Isaac *et al*., 2013). However prior to aestivation, *Protopterus annectens* stored quite a large quantity of fats, which were gradually being broken down to provide energy for the maintenance of life processes during aestivation as the fish was starving (Okafor and Odiete, 2002).

CHAPTER TWO
MATERIALS AND METHODS

2.1 Study Area

The study area of this research work is Anambra River located at Otuocha, in Anambra State. The Anambra River (Figure 2) has its source in Ankpa highland of Kogi State of Nigeria about 100 km North of Nsukka (Odo *et al.,* 2012). The River lies between latitude $6^0\ 10^1$ and longitude $7^0\ 5^1$ East of the River Niger. The River crosses the Kogi/Enugu State boundary, and passes through Ogurugu to Otuocha from there it flows down to River Niger at Onitsha where it has its confluence. The main River channel has a total length of about 207.40 km (Okafor and Chukwu, 2010). The bank of the River is covered by such plants like *Andropogon* spp, *Imperata cylindrica, Echinochloa* spp., *Jussica* spp*, Salvinia nymnellula*, *Ludwigia decurrens, Pennisetum* spp*,* and *Cynodon* spp. (Okafor and Chukwu, 2010). The mean annual rainfall is between 150 cm and 200cm. The basin is influenced by harmattan from December to January/February but their effect is not well marked. The water temperature reading in the River ranges from 24^0C to 31 ^{0}C while the Secchi Disc reading is from 5cm to 85 cm (Odo, 2004).

The fishing methods in that particular River basin include construction of fish fences, the use of ñatallaò, hooks and line, set lines, lift nets, dragnets, beach seines, cast nets, pumping out water from ponds with water pump, among others (Eyo and Akpati, 1995). Apart from *Protopterus annectens,* some other species of fishes are found in the River; they include Clarias, Labeo, Distichodus, Alestes, Heterobranchus, Mormyrid, Channa *(Parachanna obscura)* among others (Odo *et al.,* 2012).

Crops such as cassava, millet, rice, yam, groundnut, potatoes, vegetables, banana and plantain are produced in large quantities around the area, thereby making agricultural activities very high.

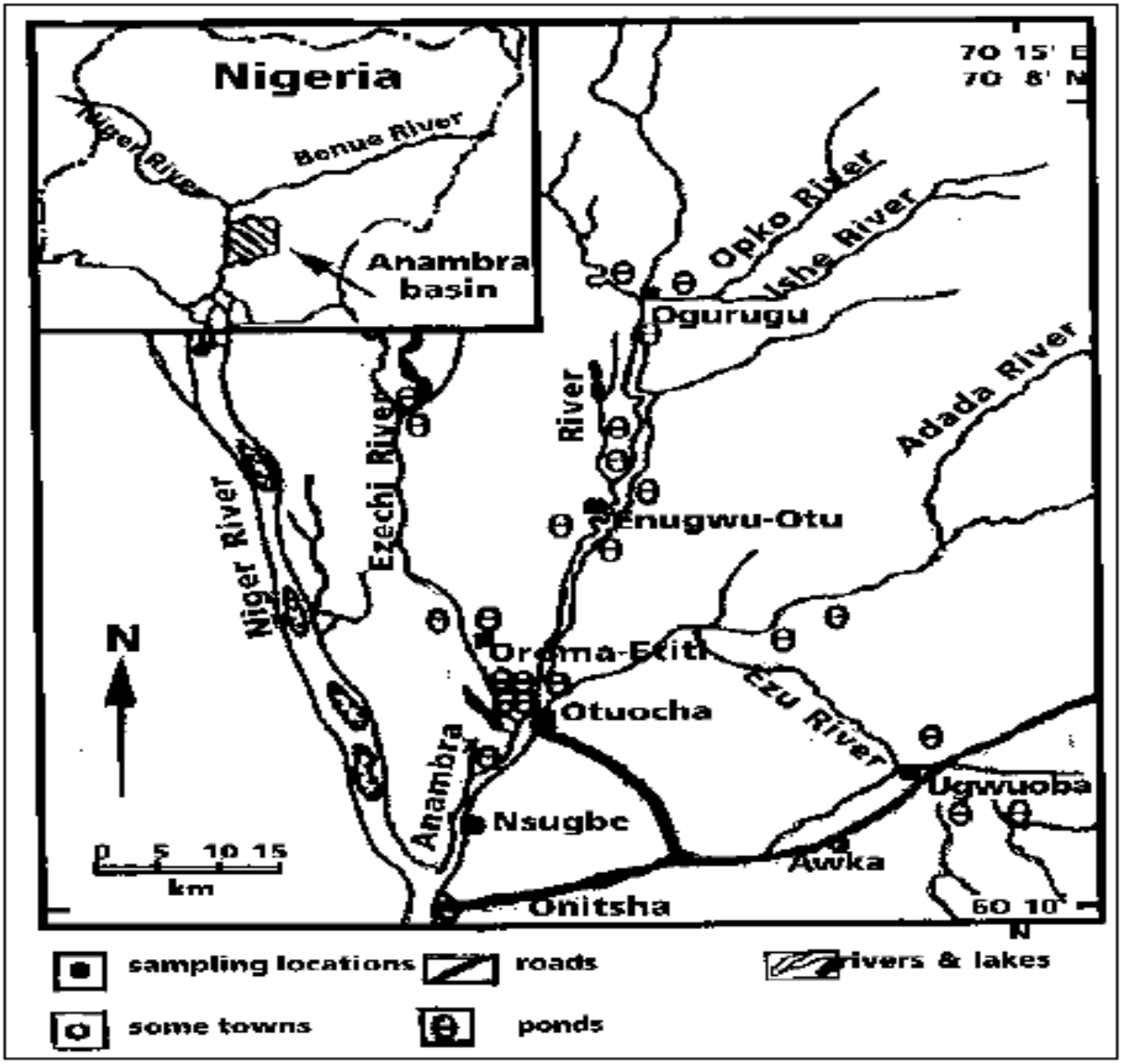

Fig. 2: Map of Anambra River
Source: (Ezenwaji, 1999)

2.2 Fish Sampling

Fish samples of different sizes (fingerlings, intermediate and large) were procured monthly from Otuocha station along the Anambra River for six consecutive months starting from March to August, 2015. The fish specimen, (*Protopterus annectens*) procured were transported in a plastic container with water from that River to the Fisheries Laboratory, Department of Zoology and Environmental Biology, University of Nigeria, Nsukka for analysis. In the laboratory, preliminary data records were taken as follows: date caught, sex of the specimens, the standard length using a measuring ruler, weight of the specimens using electronic weighing balance and the values recorded in grams. Blood was collected from the caudal vein of each fish using separate heparinized disposable syringes and hypodermic needles. Later the liver and gonads were dissected out and weighed to the nearest 0.1g using an electronic balance.

2.3 Haematological Analysis

The haematological parameters were determined as follows;

2.3.1 Packed cell volume (PCV)

The packed cell volume (PCV) was determined by the microhaematocrit method as described by Coles (1986). A heparinized capillary tube was filled with well-mixed anticoagulated blood approximately three fourth (3/4) of its length. The end of the capillary tube was sealed with plasticin and then centrifuged for five minutes in a heamatocrit centrifuge set at 10,000 revolutions per minute (rpm). The height of the packed cell and the total height in millimeter were measured using the haematocrit reader. The Packed Cell Volume (PCV) was calculated and expressed in percentage using the formular:

$$\text{PCV (\%)} = \frac{Height\ of\ red\ cell\ (mm)}{Total\ height\ (mm)} X\ 100$$

2.3.2 Haemoglobin estimation

Haemoglobin concentration was determined using the method of Sood (2006). Using a dropper, a graduated tube was filled with N/10 HCL, up to the mark 2 on the red graduation. The blood sample was carefully sucked up to 20 cm mark, the end of the pipette was wiped and blown into the acid in the mixing tube. The acid was sucked up and blown back into the tube 3-4 times, thereby rinsing the pipette and then allowed to stand for 10 minute for formation of acid haematin. Likewise, the acid haematin was diluted using distilled water in drop-wise manner. The addition of each drop of distilled water to the solution was stirred and its colour matched with that of the standard sealed tubes. The addition of distilled water continued until the colour of the acid haematin solution fades away as compared with the standard by holding the comparator towards light. The haemoglobin (Hb) was read out in gram percent from the level of the fluid in the mixing tube.

The haemoglobin concentration is calculated thus,

$$\text{Hb concentration (g/100ml)} = \frac{\text{OD of test solution} \times \text{Hb concentration of Standard solution}}{\text{OD of Standard solution} \times \text{blank} \times 100}$$

(Sood, 2006)

2.3.3 Red Blood cell count (RBC)

Erythrocyte count was determined using the method of Sood (2006) as follows. The blood was carefully sucked up to 0.5 mark in the red cell diluting pipette and was diluted in a special glass bublet. The pipette was plunged into the diluting fluid and sucked up to the mark, 101. The micropipette (end of the pipette) was rinsed several times by gripping it between the finger and the thumb and shook thoroughly for 3 minutes. The diluted blood was

introduced into a Neubauer counting chamber, and was counted using a light microscope at a magnification of X 100

The Red blood cells for each sample were calculated using the formula below

$$\text{RBC } (mm^3) = \frac{Cell\ counted\ X\ dilution\ \ factor}{Volume\ conted\ in\ mm^3}$$

(Sood, 2006)

2.3.3.1 Erythrocyte indices

Erythrocyte indices like, Mean Cell Volume (MCV), Mean Cell haemoglobin Volume (MCH) and Mean Cell haemoglobin concentration (MCHC), were calculated with the following formulae:

$$MCV\left(l\,cel^{-1}\right)\ PCV\ \text{X}\ \frac{10}{RBC}^{=}\ count\,in\,millions\,mm^{-3}$$

$$MCH\,(pg\,cell^{-1}) = (g\,dl^{-1})\ \text{X}\ \frac{10}{RBC}\ \ count\,in\,million^{-3}$$

$$\text{MCHC}\left(\text{g dl}^{-1}\right) = Hb\ \left(g\,dl^{-1}\right) / PCV\ \%\ \text{X}\ 100$$

$$\text{That is MCHC} = \frac{\text{Hb}}{\text{PCV}}\ \text{X}\ \frac{100}{1} \quad \left(Dacie\,and\,Lewis\,1984\right)$$

2.3.4 White blood cell count (WBC)

Leucocyte count was also determined using the method described by Sood (2006). Fish blood was sucked slowly up to the mark 0.5 in the red diluting pipette, and was diluted in a special glass bublet. The pipette was plunged into the diluting fluid and sucked up to the mark, II. The micropipette was rinsed by gripping it between the finger and the thumb and shook thoroughly for 3 minutes. The resulting mixture was introduced into a Neubauer counting Chamber, and was counted using a light microscope at a magnification of x 100.

The white Blood cells (WBC) for each sample were calculated using the formula:

$$\text{WBC } (mm^3) = \frac{Cell\ counted\ X\ dilution\ \ factor}{Volume\ counted\ in\ mm^3}$$

(Sood, 2006)

2.3.5 Leukocyte differential count

Differential white cell count or differential leukocyte count was determined using a method known as Leishman Technique. Blood was collected from the caudal vein using heparinised syringe and stored in small EDTA bottles at 4^{o}C. The blood sample was shaken gently and a drop of blood was placed on a clean grease-free-slide. The drop of blood was carefully smeared on the slide using a cover slip to make a thin smear. The smear was air-dried and thereafter stained slides are later examined with an immersion objective using a light microscope. Two hundred cells were counted by the longitudinal counting method and each cell type was identified and scored using the differential cell counter. Results for each white blood cell type were expressed as a percentage of the total count and converted to the absolute value per microlitre of blood.

2.4 Biochemical Analysis

Part of the whole blood collected was centrifuged at 10, 000 x g, at 4^{o}C for 20 minutes to separate the plasma for estimation of plasma glucose, protein, alanine aminotransferase (ALT), alkaline phosphate (ALP), and aspartate aminotranferase (AST)

2.4.1 Determination of alkaline phosphate (ALP)

The plasma ALP was determined using commercial reagents manufactured by Quimica Clinica Aplicada (QCP Spain) with Phenolphthalein Monophosphate Method (Babson *et al.,* 1984).

Reagents

i. Standard solution of alkaline phosphate in water (equivalent to 30 IU/L)

ii. Alkaline phosphate chromogenic substrate

iii. Colour developer.

Sample = Plasma

Procedure

1. The colour developer was prepared by adding one vial of colour developer salt to 250 m1 of de-ionized water.
2. The 1.0 ml of de-ionized water was added to the clean test tube.
3. To the test tube containing water was added 1 drop of chromogenic substrate.
4. It was well mixed and incubated at 37^0 for 5 minutes.
5. About 0.1m1 of plasma sample was added to the sample test tube.
6. It was mixed thoroughly and incubated at 37^0 for 20 minutes.
7. 5 m1 of colour developer was also added, after which the absorbance was read against a water blank at wavelength 550 nm.
8. For the standard, 1.0 ml of water was added to test tube and 1 drop of the chromogenic substrate. It was well mixed and incubated at 37^0C for 5 minutes, 0.1ml of standard was then added. It was mixed again and incubated at 37^0C for 20 minutes.
9. About 5 ml of colour developer was added and absorbance read at 550 nm.

   The following formular below was used to obtain the alkaline phosphate concentration in the plasma sample:

$$ALP = \frac{Absorbance\ of\ Sample}{Absorbance\ of\ S\tan dard} \quad X \quad \frac{30}{1} \qquad (Babson\ et\ al., 1966)$$

2.4.2 Determination of aspartate aminotranferase (AST)

The plasma AST was determined using commercial reagent manufactured by Quimica Clinica Applicada (QCA) test kit, according to the methods of Reitman and Frankel (1957).

The plasma glutamate = oxaloacetate transaminase, is based on the principle that oxaloacetic acid is formed from the reaction,

$$\alpha - \ Oxoglutarate + L - aspartate \longrightarrow L - glutamate + oxaloacetate$$

Sample = Plasma

i. AST substrate solution (Reagent A), containing phosphate buffer P^H 7.4 and a Ketoglutaric acid and L-Aspartic acid

ii. Colour developer (Reagent B), containing 2-4 dinitrophenyl hydrazine (DNPH).

iii. NaOH 4N. This will be diluted $1/10$ with deionized water prior to use (Reagent C).

iv. Standard (reagent d), aqueous solution of sodium pyruvate

Procedure

1 About 0.5ml of reagent A (AST substrate solution) was added in a test tube, incubated for 5 minutes at 37^{0c}.

2 After incubation, 0.1 ml of the plasma sample was added and the two were incubated for 60 minutes.

3 The standards were prepared as follows:

Tube 1: 0.1 ml deionized water + 0.50 ml Reagent A

Tube 2: 0.1ml deronized water + 0.45 ml Reagent A $\pm$ 0.05 ml of standard.

Tube 3: 0.1 ml deionized water + 0.40 ml Reagent A + 0.10 ml of standard.

Tube 4: 0.1 ml deronized water + 0.35 ml Reagent A + 0.15 ml of standard

Tube 5: 0.1 ml deionized water + 0.30 ml Reagent A + 0.20 ml of standard

Tube 6: 0.1 ml deionized water + 0.25 ml Reagent A + 0.25 ml of standard.

0.5ml of colour developer (reagent B) was added to both sample tubes and the standards, and then allowed to stand for 20 minutes at room temperature.

4. 5 ml of NaOH working solution (diluted Reagent C) was also added and allowed to stand for 15 minutes at room temperature.
5. The transmittance of both samples and standards was read at 505 nm against deionixed water blank.
6. In other to get the activity value of the samples, the transmittance obtained for the samples was interpolated in the calibration curve made from the standards; the results were expressed in SI units (International units per litre (IU/L).

However, to convert them to SI units, the following formular was used.

$$IU/L = U/ML \quad X \quad 0.483$$

2.4.3 Determination of alanine aminotransferase (ALT)

The plasma ALT was determined using a Randox commercial Enzyme Kit according to the methods of Reitman and Frankel (1957).

Reagents

i. ALT substrate solution (Reagent A), containing phosphate buffer P^H 7.4 and a-ketoglaric acid and L-alanine.

ii. Colour developer (Reagent B), containing 2, 4 dinitropheny/hydrazine (DNPH)

iii. NaOH 4 N -, This was diluted $1/10$ with deionized water prior to use (Reagent C).

iv. Standard (Reagent D), aqueous solution of sodium pyruvate.

Procedure

1. About 0.5 ml of Reagent A (ALT substrate solution) was added to a clean test tube, and incubated for 5 minutes at 37^{0c}.
2. After incubation, 0.1ml of serum sample was added. Incubate again the two for 30 minutes at 37^{0c}.
3. The standards were prepared as follows

Tube 1: 0.1ml deionized water + 0.5ml Reagent A

Tube 2: 0.1ml deionized water + 0.45 Reagent A + 0.05 ml of standard.

Tube 3: 0.1ml deionized water + 0.40 ml Reagent A + 0.10ml of standard

Tube 4: 0.1ml deionized water + 0.35 ml Reagent A + 0.15 ml of standard

Tube 5: 0.1 ml deionized water + 0.30 ml Reagent A + 0.20 ml of standard

4. 0.5ml of colour developer (Reagent B) was added to sample tubes and the standards, and then allowed to stand for 20 minutes at room temperature.
5. After which, 5 ml of NaOH working solution was added and stood for 15 minutes at room temperature.
6. The transmittance of both sample and standard was read at 505 nm against deionized water blank.
7. To get the ALT activity/value of the samples, the transmittance obtained for the samples was interpolated in the calibration curve made from the standards; the results were expressed in SI units (International Units per litre, (IU/L)

2.4.4 Determination of total plasma protein

Total plasma protein was determined by the Direct Biuret method of Lubran (1978)

Materials

i. Biuret reagent containing NaOH, potassium iodide, copper (11) sulphate and sodium-potassim tantarate

ii. Standard, containing aqueous solution of protein equivalent to 5g/dl (50g/l)

Procedure

1. A series of clean test tubes were arranged and labeled according to the sample identifications. Two standards and two blanks (SD_1, SD_2, BL_1 and BL_2) were also labeled.
2. 0.02 ml (20 microlitres) of each sample was added to the appropriate labeled test tube, and also 0.02 ml (20 microlitres) of the standards was added to the test tube labeled standard (SD) SD, and SD_2, nothing was added to the test tubes labeled blank 1 and 2.
3. About 1.0 ml of Biuret reagent was added to all the test tubes (both those containing samples, standards and blank. Each of the test tubes was well mixed and stood for 10 minutes and room temperature (20-25^{0c}).
4. The absorbance of samples and standard against the contents of the blank was read up at 540nm.

The total protein is calculated as follows:

$$\text{Total protein (g/dl)} = \frac{Absorbance\ of\ \ sample\ \ x\ 5}{Absorbance\ of\ \ s\tan dard\ \ x\ 1}$$

6. The result can be converted to SI units as follows

$$g\ \ protein/dl\ \ x\ 10 = g\ \ protein/L$$

2.4.5: Determination of plasma albumin

Plasma albumin was measured using *bromocresol green method* for the in-vitro determination of albumin in plasma using Quimica Clinica Applicada (QCA) Albumin test kit (QCA, Spain) Doumas and Peter (1997).

Procedure

1. Clean test tubes was arranged and labeled corresponding to the number of groupings of the samples to be determined. Also, label test tubes for two standards (SD_1 and SD_2) and two blanks (BL_1 and BL_2).
2. 2.5 ml of bromocresol reagent was added into each of the test tubes
3. 0.01ml (10ul) of the sample was added into the corresponding test tubes and 0.01 ml of standard into the test tubes labeled SD_1 and SD_2. No sample or standard was added to the test tube labeled blank.
4. The contents of the tube were mixed well and left to stand at room temperature for 5 minutes
5. The absorbance of the test samples and standards was read against the blank at 630 nm wavelength.
6. The reading was taken immediately at 5 minutes of keeping, because delay will give a wrong reading as there may be further colour changes consequent upon binding to other proteins.

Calculation

The albumin content of the plasma sample is calculated thus:

$$\frac{Absorbance\ \ of\ \ Sample}{Absorbance of\ s\tan dard}\ x\frac{5}{1}\ \ = g\ \ albu\min/\ dl$$

2.4.6: Determination of plasma cholesterol

Plasma cholesterol was determined using enzymatic colorimetric test (CHOD-PAP method) for the *in vitro* determination of cholesterol in serum or plasma, using Quimica Clinica Applicada (QCA) cholesterol test kit (QCA, S.A. Spain) (Allain *et al.,* 1974).

Procedure

1. 1 ml of the working reagent was added to a set of clean labeled test tubes ñStandard 1ò and standard ñ2ò while another was labeled ñBlankò part from the samples to be tested.
2. 0.01 ml of the sample was added to the appropriately labeled test tube, they were mixed well and let stand for 10 minutes at room temperature. Also 0.01 ml of the standard was added to the each of the test tubes labeled Standard ñ1ñ and ñStandard ñ2ò, they were mixed well and left to stand for 10 minutes of room temperature.
3. The absorbance of the samples and standard was read against the reagent blank at 505 nm.
4. The cholesterol content of each of the sample was calculated using the following formular.

$$\frac{Absorbance\ of\ Sample}{Absorbance\ of\ S\tan dard} \quad x \quad \frac{200}{1}$$

The result is expressed in Mg cholesterol/ dl

To convert to SI Units: (Mg/100dl) x 0.0259 = mm 0 $\frac{l}{l}$

2.5 Biometric Analysis

2.5.1 Condition factor (K)

The condition factor (K) of the fish was calculated using the formula,

$$K = \frac{\text{Weight of the fish (g)}}{\text{Length of the fish (L}^3\text{cm)}} \; x\; 100$$

2.5.2 Hepatosomatic index

Hepatosomatic index was calculated with the formular;

$$H.S.I = \frac{weight\ of\ the\ liver(\text{g})}{weight\ of\ body(\text{g})} \; x\; 100$$ (Jelodar and Fazli, 2012)

2.5.3 Gonadosomatic Index

Gonadosomatic index was calculated with the formular;

$$K = \frac{\text{Weight of the gonad (g)}}{\text{Weight of the fish (g)}} \; x\; 100$$ (Jelodar and Fazli, 2012)

2.6 Statistical Analysis

The data to be obtained will be analyzed statistically using the statistical package for social sciences (SPSS) version 20.0. One way analysis of variance (ANOVA) was used to compare haematological and biochemistry parameters of fish samples. Regression analysis was used to evaluate the length ï weight relationship of fish, with the formular; $W=aL^b$; where

W= weight of the fish in (g)
a= intercept
L =length of the fish in (cm)
b =slope

All analysis was done at 5% level of significant.

CHAPTER THREE

RESULTS

3.1 Haematological Parameters

The variation in haematological parameters in male, female and combined sex of *Protopterus annectens* is shown in Table 2. The male PCV value recorded in the month of May was significantly ($P < 0.05$) higher than the other values in the other months. The least PCV value was observed in the month of July.

The female PCV values of March, April, May and August were significantly ($P < 0.05$) higher than the values observed in June and July. However, there were no significant ($P > 0.05$) differences observed in PCV values according to sex

No significant ($P > 0.05$) differences were observed in RBC values according to months and sex. The male WBC count recorded in the month of May was significantly ($P < 0.05$) higher than the other values in the other months. The male and the female WBC counts recorded in the month of August were significantly ($P < 0.05$ higher than that of June. The least WBC count was observed in the month of June. However, the least WBC count was recorded among the female fish in the month of June. There were significant ($P < 0.05$) differences observed in WBC count according to sex.

There were no significant ($P < 0.05$) differences in other months apart from March in the female Hb concentration. However, no significant ($P > 0.05$) differences were observed in Hb concentration according to sex. The male MCV values recorded in the month of July were significantly ($P < 0.05$) lower than the other values in the other months.

The female MCH value recorded in the month of Mareh was significantly ($P < 0.05$) lower than the other values in the other months. Meanwhile, there was a significant ($P < 0.05$) difference of female MCH value in the month of August, lower than the other months.

The male MCHC value recorded in the month of April, June and July were significantly ($P < 0.05$) higher than the other values in the other months. The male MCHC value recorded in the month of May and the female in the month of August were significantly ($P < 0.05$) higher than MCHC male value recorded in the month of Mareh. However, there were no significant ($P > 0.05$) differences observed in MCHC values according to sex.

Table 2: Variations in haematological parameters in male, female and combined sex of *Protopterus annectens* of Anambra River, Nigeria.

Parameter	Sex	March	April	May	June	July	August
PCV (%)	M	30.01 ± 1.08^{a1}	27.50 ± 0.85^{a1}	35.20 ± 1.05^{b1}	28.33 ± 0.95^{a1}	24.25 ± 1.02^{c1}	29.5 ± 0.96^{a1}
	F	29.05 ± 0.85^{a1}	30.75 ± 1.04^{a1}	33.67 ± 0.75^{a1}	26.67 ± 0.77^{b1}	27.20 ± 0.67^{b1}	29.30 ± 1.08^{a1}
	M & F	29.03 ± 0.67^{a1}	30.10 ± 0.79^{a1}	34.40 ± 1.06^{b1}	27.50 ± 0.85^{a1}	25.50 ± 0.91^{c1}	29.30 ± 1.07^{a1}
RBC ($x10^6$)	M	10.42 ± 0.25^{a1}	10.06 ± 0.18^{a1}	11.24 ± 0.73^{a1}	9.07 ± 0.60^{a1}	9.77 ± 0.50^{a1}	9.91 ± 0.80^{a1}
	F	10.63 ± 0.50^{a1}	10.02 ± 0.65^{a1}	11.28 ± 0.81^{a1}	9.52 ± 0.71^{a1}	9.66 ± 0.60^{a1}	10.03 ± 0.50^{a1}
	M & F	10.53 ± 0.45^{a1}	10.03 ± 0.62^{a1}	11.25 ± 0.93^{a1}	9.30 ± 0.65^{a1}	9.73 ± 0.19^{a1}	9.95 ± 0.70^{a1}
WBC ($x10^3$)	M	10133 ± 8.90^{a1}	10750 ± 6.65^{a1}	12867 ± 8.90^{b1}	9066 ± 6.70^{c1}	10025 ± 6.60^{a1}	9150 ± 7.01^{d1}
	F	10700 ± 4.50^{a2}	10800 ± 5.60^{a1}	11300 ± 6.70^{b2}	9057 ± 5.10^{c1}	10050 ± 4.80^{a1}	9950 ± 4.50^{d2}
	M & F	10416 ± 7.50^{a2}	10783 ± 6.50^{a1}	12083 ± 5.50^{b3}	9067 ± 8.20^{c1}	10033 ± 5.80^{d1}	9417 ± 6.50^{e3}
Hbc (g/dl)	M	5.57 ± 0.43^{a1}	10.30 ± 0.33^{a1}	11.47 ± 0.25^{a1}	10.37 ± 0.60^{a1}	9.40 ± 0.50^{a1}	6.23 ± 0.45^{a1}
	F	5.43 ± 0.23^{a1}	10.62 ± 0.15^{b1}	10.90 ± 0.50^{b1}	9.83 ± 0.49^{b1}	10.30 ± 0.62^{b1}	6.10 ± 0.28^{b1}
	M & F	5.50 ± 0.19^{a1}	10.60 ± 0.38^{b1}	11.20 ± 0.27^{b1}	10.10 ± 0.62^{b1}	9.70 ± 0.39^{b1}	6.20 ± 0.17^{b1}
MCV (fl)	M	28.79 ± 0.96^{a1}	27.34 ± 0.68^{a1}	31.14 ± 0.75^{a1}	31.23 ± 0.82^{a1}	24.56 ± 0.27^{b1}	29.77 ± 0.65^{a1}
	F	27.28 ± 0.80^{a1}	30.69 ± 1.04^{a1}	29.85 ± 0.89^{a1}	28.01 ± 0.91^{a1}	27.95 ± 0.71^{a1}	28.91 ± 0.67^{a1}
	M & F	28.04 ± 0.45^{a1}	29.02 ± 0.70^{a1}	30.50 ± 0.89^{a1}	29.62 ± 0.95^{a1}	26.26 ± 0.79^{b1}	29.34 ± 0.67^{a}
MCH (pg)	M	5.35 ± 0.12^{a1}	10.24 ± 0.23^{b1}	10.20 ± 0.48^{b1}	11.43 ± 0.51^{b1}	9.62 ± 0.48^{b1}	6.29 ± 0.19^{a1}
	F	5.11 ± 0.28^{a1}	10.60 ± 0.36^{a1}	9.66 ± 0.43^{a1}	10.33 ± 0.54^{a1}	10.66 ± 0.45^{a1}	6.08 ± 0.31^{b1}
	M & F	11.04 ± 0.28^{a2}	10.42 ± 0.26^{a1}	9.93 ± 0.45^{a1}	10.88 ± 0.38^{a1}	10.14 ± 0.18^{a1}	6.19 ± 0.19^{b1}
MCHC (g/dl)	M	18.57 ± 0.57^{a1}	37.45 ± 0.67^{b1}	32.77 ± 0.59^{c1}	36.60 ± 0.34^{b1}	39.17 ± 0.69^{b1}	21.12 ± 0.56^{d1}
	F	18.72 ± 0.37^{a1}	34.53 ± 0.45^{b1}	32.37 ± 0.66^{b1}	36.86 ± 0.59^{b1}	38.15 ± 0.38^{b1}	21.03 ± 0.80^{c1}
	M & F	18.65 ± 0.56^{a1}	35.99 ± 0.71^{b1}	32.57 ± 0.95^{b1}	36.73 ± 1.02^{b1}	38.66 ± 1.03^{b1}	21.08 ± 0.76^{c1}

*Values with different alphabetic superscripts differ significantly ($p < 0.05$) between durations within the same sex. Values with different numeric superscripts differ significantly ($p < 0.05$) between the sexes within the same duration.

3.2 Seasonal Variations in Heamatological Parameters of *Protopterus annectens* of Anambra River, Nigeria

The Seasonal variations in heamatological parameters of *Protopterus annectens* of Anambra River, is presented in Table 3. No significant ($P > 0.05$) differences were observed in PCV values according to seasons and sex. Also, there were no significant ($P > 0.05$) differences observed in RBC count according to seasons and sex.

The white blood cell count recorded in dry season were significantly ($P < 0.05$) higher than the other values recorded in wet season. Meanwhile, the WBC count recorded in males was significantly ($P < 0.05$) higher than that of females. There were no significant ($P > 0.05$) differences in Hbc values according season and sex.

The female MCV values recorded in wet season were observed to be significantly ($P < 0.05$) higher than that obtained in dry season. No significant ($P > 0.05$) differences were observed in MCH values according to season. However, the female MCH value showed a significant ($P < 0.05$) difference lower that of the male. No significant ($P > 0.05$) differences were observed in MCHC values according to season and sex

Table 3: Seasonal variations in haematological parameters of *Protopterus annectens* of Anambra River, Nigeria

Parameter	Sex	Dry Season	Wet Season
PCV (%)	M	30.83 ± 1.02^{a1}	27.28 ± 0.99^{a1}
	F	31.14 ± 1.01^{a1}	27.56 ± 0.89^{a1}
	M & F	31.30 ± 0.79^{a1}	27.30 ± 0.67^{a1}
RBC ($x10^6$)	M	10.57 ± 0.77^{a1}	9.58 ± 0.63^{a1}
	F	10.64 ± 0.45^{a1}	9.74 ± 0.62^{a1}
	M & F	10.61 ± 0.78^{a1}	9.50 ± 0.82^{a1}
WBC ($x10^3$)	M	11250.20 ± 8.50^{a1}	9414.50 ± 5.57^{b1}
	F	10933.50 ± 7.08^{a2}	9689.30 ± 6.55^{b2}
	M & F	11094.60 ± 6.65^{a1}	9506.20 ± 6.09^{b1}
Hbc (g/dl)	M	9.11 ± 0.56^{a1}	8.67 ± 0.78^{a1}
	F	8.98 ± 0.18^{a1}	8.74 ± 0.57^{a1}
	M & F	9.10 ± 0.63^{a1}	8.70 ± 0.39^{a1}
MCV (fl)s	M	29.09 ± 0.18^{a1}	28.52 ± 0.86^{a1}
	F	19.27 ± 0.72^{a2}	28.29 ± 0.71^{b1}
	M & F	29.19 ± 0.57^{a1}	28.41 ± 0.72^{a1}
MCH (pg)	M	12.90 ± 0.51^{a1}	9.11 ± 0.54^{a1}
	F	8.46 ± 0.39^{a2}	9.02 ± 0.37^{a1}
	M & F	16.13 ± 0.70^{a3}	9.07 ± 0.36^{b1}
MCHC (g/dl)	M	29.60 ± 0.70^{a1}	32.30 ± 0.60^{a1}
	F	28.54 ± 0.64^{a1}	32.01 ± 0.6^{a1}
	M & F	29.07 ± 0.37^{a1}	32.16 ± 0.70^{a1}

*Values with different alphabetic superscripts differ significantly ($p < 0.05$) between seasons within the same sex. Values with different numeric superscripts differ significantly ($p < 0.05$) between the within the same season

3.3 Leucocyte Differentials

Variation in leucocyte differentials in male, female and combined sex of *Protopterus annectens* of Anambra River, Nigeria, is shown in Table 4. The male Neutrophil value recorded in the month of May was significantly ($P < 0.05$) higher than the other values in the other months. The least Neutrophil value was observed in the month of August. The male Neutrophil values of June, July and August were significantly ($P < 0.05$) lower than the values observed in March, April and May. However, there were no significant ($P > 0.05$) differences observed in Neutrophil values according to sex.

The male lymphocyte value recorded in the month of May was significantly ($P < 0.05$) lower than the other values in the other months. The highest lymphocyte value was observed in the month of August (Table 4). No significant ($P > 0.05$) differences were observed in lymphocyte values according to sex.

There were no significant ($P > 0.05$) differences observed in monocyte value according to months however, there were significant ($P < 0.05$) differences observed in female monocyte values. No significant ($P > 0.05$) differences were observed in Basophil values according to months and sex. Likewise, there were no significant ($P > 0.05$) differences observed in Eosinophil values according to Months and sex (Table 4).

Table 4: Variations in leucocyte differentials in male, female and combined sex of *Protopterus annectens* of Anambra River, Nigeria

Parameter	Sex	March	April	May	June	July	August
Neutrophile (%)	M	27.33 ± 0.98^{a1}	29.51 ± 1.03^{a1}	33.40 ± 1.05^{b1}	23.33 ± 0.73^{c1}	22.75 ± 0.85^{c1}	20.25 ± 0.71^{c1}
	F	33.20 ± 1.00^{a1}	31.75 ± 1.05^{a1}	29.67 ± 0.88^{a1}	22.20 ± 0.76^{b1}	18.50 ± 0.69^{b1}	22.30 ± 0.63^{b1}
	M & F	30.2 ± 0.78^{a1}	31.50 ± 0.56^{a1}	31.30 ± 0.73^{a1}	23.30 ± 0.49^{b1}	21.30 ± 0.39^{b1}	21.20 ± 0.37^{b1}
Lymyhocyte (%)	M	71.50 ± 2.31^{a1}	68.20 ± 1.09^{a1}	65.67 ± 1.23^{b1}	75.20 ± 1.13^{a1}	76.20 ± 2.32^{a1}	78.25 ± 1.02^{c1}
	F	66.40 ± 1.23^{a1}	67.40 ± 1.15^{a1}	69.02 ± 1.18^{a1}	78.30 ± 1.17^{b1}	80.50 ± 2.38^{b1}	77.20 ± 1.67^{b1}
	M & F	68.50 ± 1.03^{a1}	67.30 ± 1.11^{a1}	67.35 ± 1.23^{a1}	76.50 ± 2.10^{b1}	77.50 ± 1.16^{b1}	77.83 ± 1.19^{b1}
Monocyte	M	1.01 ± 0.03^{a1}	1.02 ± 0.05^{a1}	1.05 ± 0.03^{a1}	1.33 ± 0.05^{a1}	0.75 ± 0.07^{a1}	1.05 ± 0.07^{a1}
	F	1.01 ± 0.08^{a1}	0.75 ± 0.02^{a1}	0.67 ± 0.08^{a1}	0.10 ± 0.02^{a2}	1.05 ± 0.01^{a1}	0.50 ± 0.02^{a2}
	M & F	1.01 ± 0.09^{a1}	0.80 ± 0.07^{a1}	0.80 ± 0.06^{a1}	0.72 ± 0.05^{a1}	0.80 ± 0.08^{a1}	0.80 ± 0.05^{a1}
Basophil	M	0.10 ± 0.02^{a1}	0.50 ± 0.03^{a1}	0.33 ± 0.05^{a1}	0.33 ± 0.03^{a1}	0.10 ± 0.01^{a1}	0.10 ± 0.01^{a1}
	F	0.10 ± 0.02^{a1}	0.10 ± 0.01^{a1}	0.33 ± 0.01^{a1}	0.10 ± 0.03^{a1}	0.10 ± 0.04^{a1}	0.50 ± 0.03^{a1}
	M & F	0.10 ± 0.01^{a1}	0.30 ± 0.05^{a1}	0.30 ± 0.05^{a1}	0.20 ± 0.03^{a1}	0.10 ± 0.01^{a1}	0.20 ± 0.01^{a1}
Eosinophil	M	0.67 ± 0.03^{a1}	0.50 ± 0.04^{a1}	0.10 ± 0.02^{a1}	0.10 ± 0.04^{a1}	0.50 ± 0.05^{a1}	0.50 ± 0.03^{a1}
	F	0.10 ± 0.02^{a1}	0.50 ± 0.01^{a1}	0.33 ± 0.05^{a1}	0.10 ± 0.01^{a1}	0.10 ± 0.02^{a1}	0.10 ± 0.02^{a1}
	M & F	0.30 ± 0.03^{a1}	0.50 ± 0.05^{a1}	0.20 ± 0.04^{a1}	0.10 ± 0.04^{a1}	0.30 ± 0.01^{a1}	0.30 ± 0.05^{a1}

*Values with different alphabetic superscripts differ significantly ($p < 0.05$) between durations within the samesex.
Values with different numeric superscripts differ significantly ($p < 0.05$) between the sexes within the same duration

3.4 Seasonal Variations in Leucocyte Differentials of *Protopterus annectens* of Anambra River, Nigeria

The seasonal variation in leucocyte differentials of *Protopterus annectens* of Anambra River is shown in Table 5. The neutrophil value recorded in dry season was significantly ($P < 0.05$) higher than the other values recorded in wet season. However, no significant ($P > 0.05$) differences were observed in neutrophil values according to sex.

The lymphocyte value recorded in wet season were significantly ($P < 0.05$) higher than the other values recorded in dry season. However, there were no significant ($P > 0.05$) differences observed in lymphocyte value according to sex. No significant ($P > 0.05$) differences were observed in monocyte, basophil and eosinophil values according to seasons and sex.

Table 5: **Seasonal variation in leucocyte differentials of *Protopterus annectens* of Anambra River, Nigeria**

Parameter (%)	Sex	Dry Season	Wet Season
Neutrophil (%)	M	29.94 ± 0.80^{a1}	22.11 ± 0.57^{b1}
	F	31.47 ± 1.01^{a1}	20.83 ± 0.40^{b1}
	M & F	31.50 ± 0.91^{a1}	22.05 ± 0.63^{b1}
Lymyhocyte (%)	M	68.22 ± 1.20^{a1}	76.42 ± 1.03^{b1}
	F	67.33 ± 1.23^{a1}	78.50 ± 1.22^{b1}
	M & F	67.73 ± 1.08^{a1}	77.60 ± 1.06^{b1}
Monocyte	M	1.02 ± 0.38^{a1}	1.03 ± 0.06^{a1}
	F	0.81 ± 0.18^{a1}	0.50 ± 0.19^{a1}
	M & F	0.60 ± 0.36^{a1}	0.80 ± 0.17^{a1}
Basophil	M	0.28 ± 0.06^{a1}	0.11 ± 0.03^{a1}
	F	0.11 ± 0.06^{a1}	0.17 ± 0.08^{a1}
	M & F	0.20 ± 0.07^{a1}	0.10 ± 0.06^{a1}
Eosinophil	M	0.39 ± 0.08^{a1}	0.33 ± 0.07^{a1}
	F	0.28 ± 0.05^{a1}	0.10 ± 0.02^{a1}
	M & F	0.30 ± 0.06^{a1}	0.25 ± 0.04^{a1}

*Values with different alphabetic superscripts differ significantly ($p < 0.05$) between seasons within the same sex. Values with different numeric superscripts differ significantly ($p < 0.05$) between the within the same season

3.5 Biochemical Parameters

Variation in biochemical parameters in male, Female and combined sex of *Protopterus annectens* of Anambra River, Nigeria is shown in Table 6. The male ALP values recorded in the months of May and July were significantly ($P < 0.05$) lower than that of March, April and August. The least ALP value was observed in the month of June. The female ALP value of July was significantly ($P < 0.05$) higher than ALP value of combined sex, recorded in the month of July. However, there were no significant ($P > 0.05$) differences observed in ALP value according to sex.

The AST values recorded in the month of July and August were significantly ($P < 0.05$) lower than the values recorded in the month of March, April and May. The least AST value was observed in the month of June. However, there were significant ($P < 0.05$) differences observed in the AST values of female fish in the month of June.

The male ALT value recorded in the month of April and May were significantly ($P < 0.05$) higher than that of June, July and August. The highest ALT value was observed in the month of March, and the least value recorded in the month of June. The ALT values recorded in July and August was significantly ($P < 0.05$) higher than ALT value obtained in June. However, there were significant ($P < .05$) differences observed in ALP values of female fish. No significant ($P > 0.05$) differences were obtained in protein values according to months and sex.

The male and female Albumin values recorded in the month of August were significantly ($P < 0.05$) lower than the other values in the other months. No significant ($P > 0.05$) differences were observed in Albumin content according to sex.

The male globuline values recorded in the month of June and July were significantly ($P < 0.05$) lower than the other values in the other months. The female

globuline values recorded in the month of April, May, and August were significantly ($P < 0.05$) higher than that of June and July.

The male glucose values recorded in the month of May, June, July and August were significantly ($P < 0.05$) higher than that of April. The highest glucose value was observed in the month of March. The female glucose values of June, July, and August were significantly ($P < 0.05$) higher than that of April and May. The female glucose value recorded in the month of March was significantly ($P < 0.05$) higher than other female glucose values in the other months.

The male cholesterol values recorded in the month of March, May, and August were significantly ($P < 0.05$) higher than that obtained in the month of April. The highest cholesterol value was observed in the month of July. The female cholesterol value of July and August were significantly ($P < 0.05$) higher than the values observed in the other months. No significant ($P < 0.05$) differences were observed in Urea values according to months and sex

Table 6: Variations in biochemical parameters in male, female and combined sex of *Protopterus annectens* of Anambra River, Nigeria

Parameter	Sex	March	April	May	June	July	August
ALP (g/dl)	M	66.67 ± 1.35^{a1}	63.05 ± 1.67^{a1}	53.33 ± 1.55^{b1}	40.67 ± 1.06^{c1}	47.20 ± 1.22^{b1}	60.50 ± 1.50^{a1}
	F	63.67 ± 1.24^{a1}	61.50 ± 1.35^{a1}	58.50 ± 1.03^{a1}	43.67 ± 0.97^{b1}	49.40 ± 1.09^{c1}	57.52 ± 1.12^{a1}
	M & F	66.17 ± 1.11^{a1}	62.20 ± 1.25^{a1}	55.67 ± 1.22^{b1}	42.17 ± 1.23^{c1}	47.67 ± 1.15^{d1}	59.51 ± 1.05^{b1}
AST (g/dl)	M	81.50 ± 2.32^{a1}	83.50 ± 1.80^{a1}	84.40 ± 1.15^{a1}	29.50 ± 0.78^{b1}	37.75 ± 0.85^{c1}	35.75 ± 0.45^{c1}
	F	84.40 ± 1.85^{a1}	87.20 ± 1.05^{a1}	81.67 ± 1.50^{a1}	20.50 ± 0.50^{b2}	38.50 ± 0.70^{c1}	40.52 ± 0.55^{c1}
	M & F	82.50 ± 2.00^{a1}	85.83 ± 1.25^{a1}	82.83 ± 1.10^{a1}	24.40 ± 0.35^{b2}	37.83 ± 0.35^{c1}	37.17 ± 0.60^{c1}
ALT (g/dl)	M	80.33 ± 1.03^{a1}	72.40 ± 1.60^{b1}	71.33 ± 2.03^{b1}	22.40 ± 0.60^{c1}	51.25 ± 0.95^{d1}	51.25 ± 1.22^{d1}
	F	78.33 ± 1.55^{a1}	70.50 ± 1.09^{b1}	80.07 ± 2.01^{a2}	30.61 ± 0.70^{c2}	52.10 ± 1.01^{d1}	53.20 ± 1.11^{d1}
	M & F	79.33 ± 1.25^{a1}	71.20 ± 2.11^{b1}	76.50 ± 1.28^{a1}	26.33 ± 0.45^{1}	51.50 ± 1.05^{d1}	51.83 ± 1.40^{d1}
Protein (g/dl)	M	6.75 ± 0.60^{a1}	6.20 ± 0.50^{a1}	6.60 ± 0.60^{a1}	5.18 ± 0.50^{a1}	5.95 ± 0.50^{a1}	6.58 ± 0.65^{a1}
	F	6.57 ± 0.50^{a1}	5.63 ± 0.35^{a1}	6.73 ± 0.50^{a1}	6.04 ± 0.65^{a1}	5.41 ± 0.55^{a1}	6.31 ± 0.45^{a1}
	M & F	6.63 ± 0.19^{a1}	5.82 ± 0.55^{a1}	6.67 ± 0.55^{a1}	5.61 ± 0.35^{a1}	5.77 ± 0.45^{a1}	6.49 ± 0.45^{a1}
Albumin (g/dl)	M	3.63 ± 0.15^{a1}	3.85 ± 0.40^{a1}	5.80 ± 0.25^{a1}	4.03 ± 0.54^{a1}	5.69 ± 0.35^{a1}	0.89 ± 0.50^{b1}
	F	3.63 ± 0.15^{a1}	4.05 ± 0.18^{a1}	5.47 ± 0.15^{a1}	4.05 ± 0.18^{a1}	5.29 ± 0.55^{a1}	0.85 ± 0.20^{b1}
	M & F	3.63 ± 0.20^{a1}	3.98 ± 0.35^{a1}	5.63 ± 0.18^{a1}	4.02 ± 0.58^{a1}	5.56 ± 0.28^{a1}	4.85 ± 0.45^{a1}
Globuline (g/dl)	M	2.62 ± 0.30^{a1}	5.35 ± 0.50^{a1}	4.17 ± 0.38^{a1}	0.62 ± 0.05^{b1}	0.82 ± 0.08^{b1}	4.79 ± 0.17^{a1}
	F	2.60 ± 0.25^{a1}	5.65 ± 0.60^{b1}	4.13 ± 0.80^{b1}	0.71 ± 0.05^{c1}	0.62 ± 0.06^{c1}	4.96 ± 0.60^{b1}
	M & F	2.65 ± 0.15^{a1}	5.55 ± 0.70^{b1}	4.15 ± 0.49^{b1}	0.65 ± 0.06^{c1}	4.53 ± 0.09^{b2}	0.88 ± 0.07^{c2}
Glucose (mg/dl)	M	85.33 ± 2.11^{a1}	54.50 ± 1.10^{b1}	65.67 ± 0.95^{c1}	62.50 ± 1.03^{c1}	64.45 ± 1.10^{c1}	65.25 ± 1.00^{c1}
	F	84.20 ± 2.07^{a1}	44.25 ± 1.05^{b2}	54.50 ± 1.06^{c2}	69.60 ± 1.11^{d2}	63.50 ± 0.80^{d1}	65.20 ± 1.22^{d1}
	M & F	84.67 ± 1.70^{a1}	55.17 ± 1.90^{b1}	59.83 ± 0.98^{b2}	65.50 ± 0.95^{c1}	63.83 ± 0.75^{c1}	65.17 ± 1.50^{c1}
Cholesterol (mg/dl)	M	84.33 ± 2.40^{a1}	76.50 ± 1.20^{b1}	80.33 ± 1.22^{a1}	64.33 ± 1.01^{c1}	91.73 ± 2.30^{d1}	84.75 ± 2.04^{a1}
	F	85.33 ± 2.90^{a1}	63.25 ± 1.80^{b2}	82.67 ± 2.10^{a1}	65.30 ± 1.00^{b1}	92.05 ± 1.25^{c1}	90.50 ± 2.35^{c1}
	M & F	84.83 ± 2.30^{a1}	67.33 ± 1.40^{b2}	83.50 ± 1.20^{a1}	64.67 ± 1.05^{b1}	91.83 ± 1.50^{c1}	86.51 ± 1.90^{a1}
Urea (mg/dl)	M	3.25 ± 0.20^{a1}	3.30 ± 0.60^{a1}	3.23 ± 0.19^{a1}	5.07 ± 0.35^{a1}	4.53 ± 0.35^{a1}	3.75 ± 0.50^{a1}
	F	3.13 ± 0.25^{a1}	3.43 ± 0.30^{a1}	3.67 ± 0.28^{a1}	4.27 ± 0.23^{a1}	4.53 ± 0.25^{a1}	3.54 ± 0.40^{a1}
	M & F	3.17 ± 0.50^{a1}	3.38 ± 0.25^{a1}	3.45 ± 0.15^{a1}	4.67 ± 0.40^{a1}	4.53 ± 0.15^{a1}	3.67 ± 0.55^{a1}

*Values with different alphabetic superscripts differ significantly ($p < 0.05$) between durations within the samesex. Values with different numeric superscripts differ significantly ($p < 0.05$) between the sexes within the same duratio

3.6 Seasonal Variation in Biochemical Parameter of *Protopterus annectens* of Anambra River, Nigeria.

Seasonal variation in biochemical parameter of *Protopterus annectens* of Anambra River, Nigeria is shown in Table 7..There were no significant ($P > 0.05$) difference in ALP, AST, and ALT values obtained in *Protoptenis annectens* according to sex, but the values obtained in dry seasons were significantly ($P < 0.05$) higher than those obtained in wet season. However, there were no significant ($P > 0.05$) differences observed in protein, globuline, glucose, urea and cholesterol values according to season and sex.

Seasonal variation of combined sex in biochemical parameter of *Protopterus annectens* of Anambra River, Nigeria is also shown in Figure 3. The ALP value of combined sex recorded in dry season was significantly ($P < 0.05$) higher than the other value recorded in wet season. Likewise, the AST value of combined sex recorded in dry season was significantly ($P < 0.05$) higher than the other values recorded in wet season Moreso, the ALT value of combined sex recorded in dry season was significantly ($P < 0.05$) higher than the other values recorded in wet season. However, there were no significant ($P > 0.05$) differences observed in protein, albumin, globuline, glucose cholesterol and urea values according season (Fig. 3).

Table 7: Seasonal variation in biochemical parameters of *Protopterus annectens* of Anambra River, Nigeria

Parameter	Sex	Dry Season	Wet Season
ALP (g/dl)	M	61.50 ± 1.02^{a1}	49.39 ± 0.67^{b1}
	F	61.50 ± 0.95^{a1}	50.06 ± 0.85^{b1}
AST (g/dl)	M	82.83 ± 0.93^{a1}	35.75 ± 0.33^{b1}
	F	84.22 ± 0.67^{a1}	32.83 ± 0.65^{b1}
ALT (g/dl)	M	74.55 ± 2.33^{a1}	41.53 ± 0.95^{b1}
	F	76.50 ± 1.65^{a1}	42.22 ± 0.85^{b1}
Protein (g/dl)	M	6.50 ± 0.56^{a1}	5.95 ± 0.60^{a1}
	F	6.31 ± 0.45^{a1}	5.92 ± 0.54^{a1}
Albumin (g/dl)	M	4.43 ± 0.33^{a1}	3.54 ± 0.22^{a1}
	F	4.38 ± 0.23^{a1}	3.38 ± 0.23^{a1}
Globuline (g/dl)	M	4.04 ± 0.08^{a1}	2.08 ± 0.07^{a1}
	F	4.13 ± 0.07^{a1}	2.11 ± 0.04^{a1}
Glucose (mg/dl)	M	68.50 ± 1.33^{a1}	63.75 ± 0.96^{a1}
	F	60.75 ± 0.95^{a1}	65.83 ± 1.03^{a1}
Cholesterol (mg/dl)	M	81.22 ± 1.55^{a1}	80.28 ± 1.45^{a1}
	F	77.50 ± 1.04^{a1}	82.33 ± 1.86^{a1}
Urea (mg/dl)	M	3.24 ± 0.23^{a1}	4.45 ± 0.12^{a1}
	F	3.41 ± 0.16^{a1}	4.11 ± 0.35^{a1}

*Values with different alphabetic superscripts differ significantly ($p < 0.05$) between seasons within the same sex. Values with different numeric superscripts differ significantly ($p < 0.05$) between the within the same season

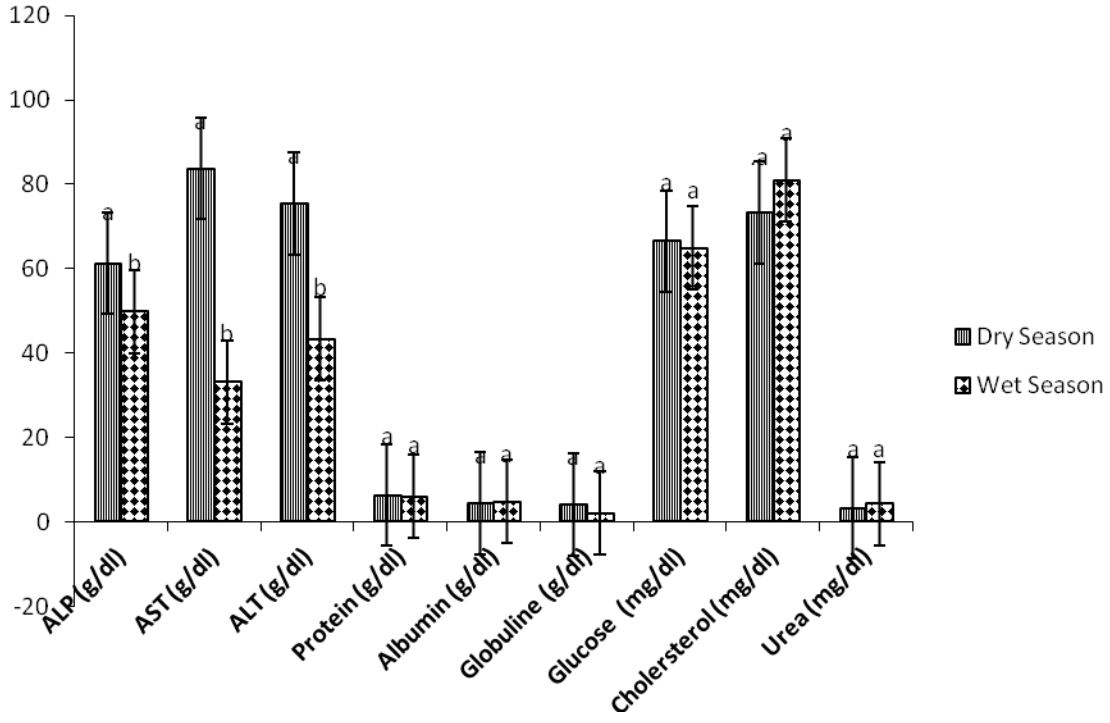

Fig. 3 Seasonal variation (combined sex) in biochemical parameter of *Protopterus annectens* of Anambra River, Nigeria

3.7 Mean Length Frequency Variations of *Protopterus annectens* of Anambra River, Nigeria

The mean length frequency variations of *Protopterus annectens* of Anambra River, is shown in Figure 4. The mean length-frequency variation of *Protopterus annectens* of Anambra River showed that the month of March and June recorded significant ($P < 0.05$) differences higher than the other months. However, the months of May and July recorded a significant ($P < 0.05$) value greater than that recorded in month of April.

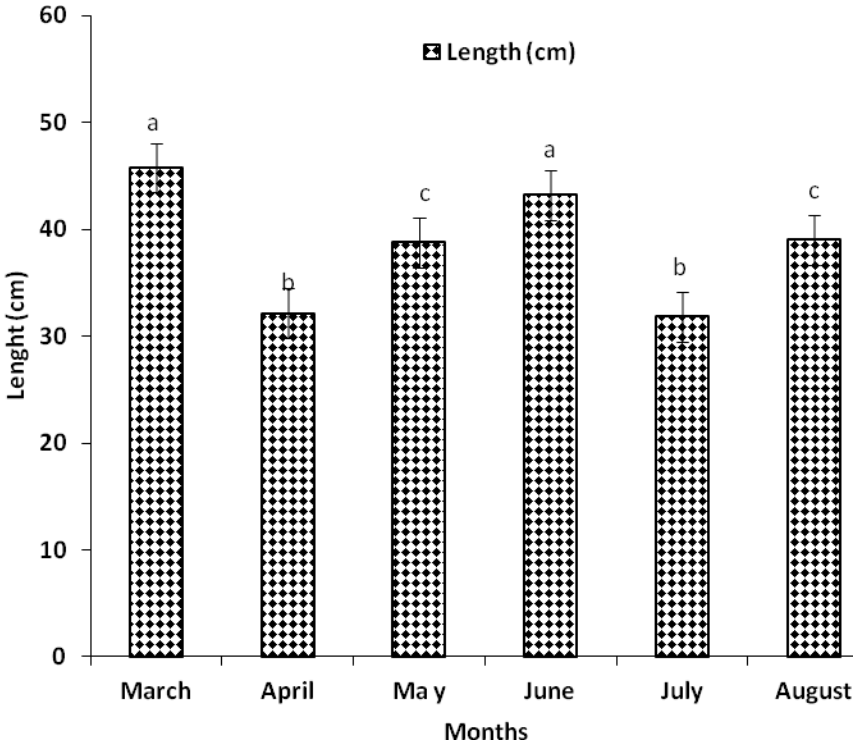

Fig .4: Mean length frequency variations of *Protopterus annectens* of Anambra River, Nigera

3.8 Mean Weight Frequency Variations of *Protopterus annectens* of Anambra River, Nigeria

The mean weight frequency variation of *Protopterus annectens* is shown in Figure 5. From the figure, the highest mean weight value was recorded in August. The mean values recorded in the month of May showed a significant ($p < 0.05$) difference lower than that of August.

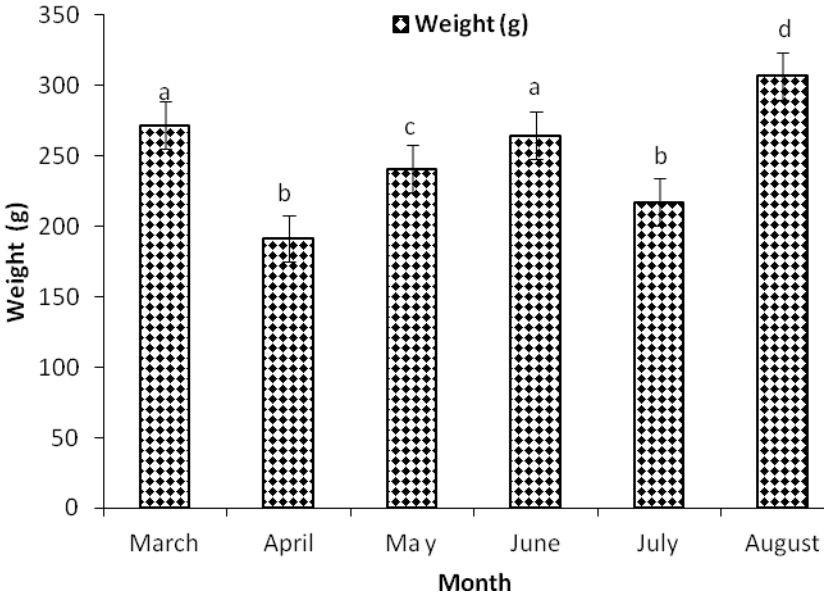

Fig. 5: Seasonal mean weight frequency variations of *Protopterus annectens* of Anambra River, Nigeria

3.9 Mean Monthly Variation in Condition Factors 'K' of *Protopterus annectens* of Anambra River

The mean monthly variation in condition factor ðKô of *Protopterus annectens* is shown in Fig 6. From the Figure, the mean values recorded in month of April and July were significantly ($P < 0.05$) higher than the values obtained in the other months.

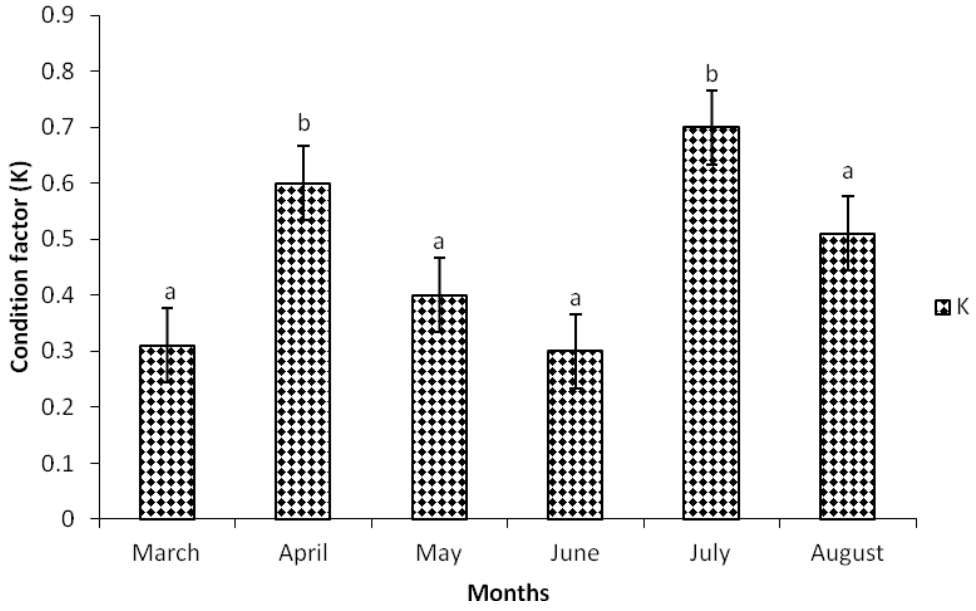

Fig. 6: Mean monthly variations in condition factors (K) of *Protopterus annectens* of Anambra River, Nigeria

3.10 Mean Monthly Variations in Hepatosomatic Index (HSI) of *Protopterus annectens* of Anambra River, Nigeria

The Mean monthly variations in hepatosomatic index (HSI) of *Protopterus annectens* of Ananbra River, is showed in figure 7, the mean value in April recorded a value significantly ($P < 0.05$) higher than the other months. Meanwhile, there were no significant ($P < 0.05$) differences among the values obtained from the other months.

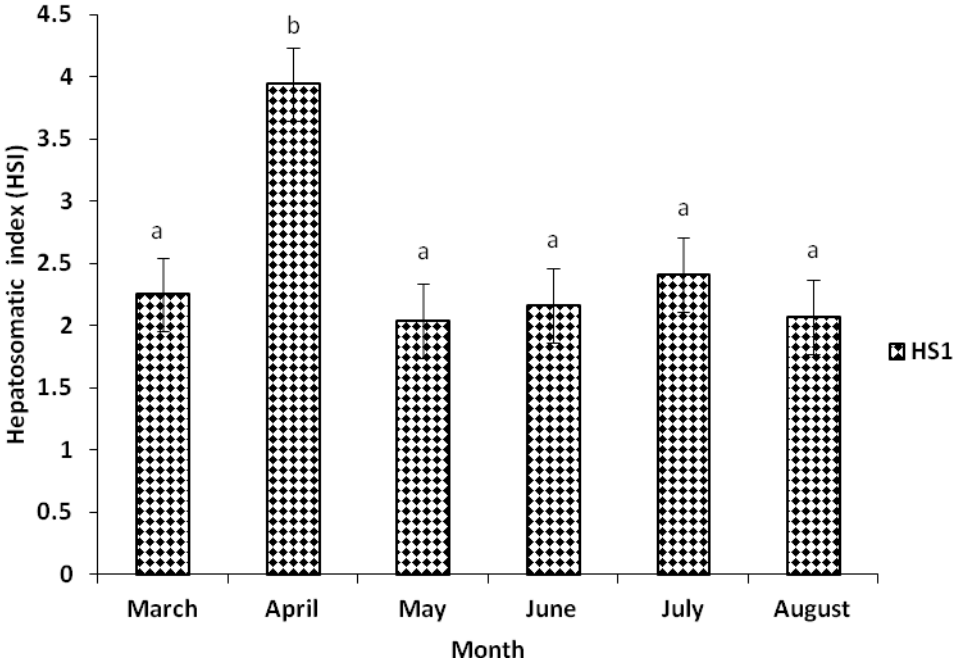

Fig. 7: Mean monthly variations in hepatosomatic index (HSI) of *Protopterus annectens* of Anambra River, Nigeria

3.11 Mean Monthly Variations in Gonadosomatic Index (GSI) of *Protopterus annectens* of Anambra River Nigeria

The mean monthly variations in gonadosomatic index (GSI) of *Protopterus annectens* of Anambra River is shown in Figure 8. The mean monthly variations in gonadosomatic index of *Protopterus annectens* showed that the month of April recorded a significant ($P < 0.05$) difference higher than the other months.There were no significant ($P < 0.05$) difference between the values obtained from the other months.s

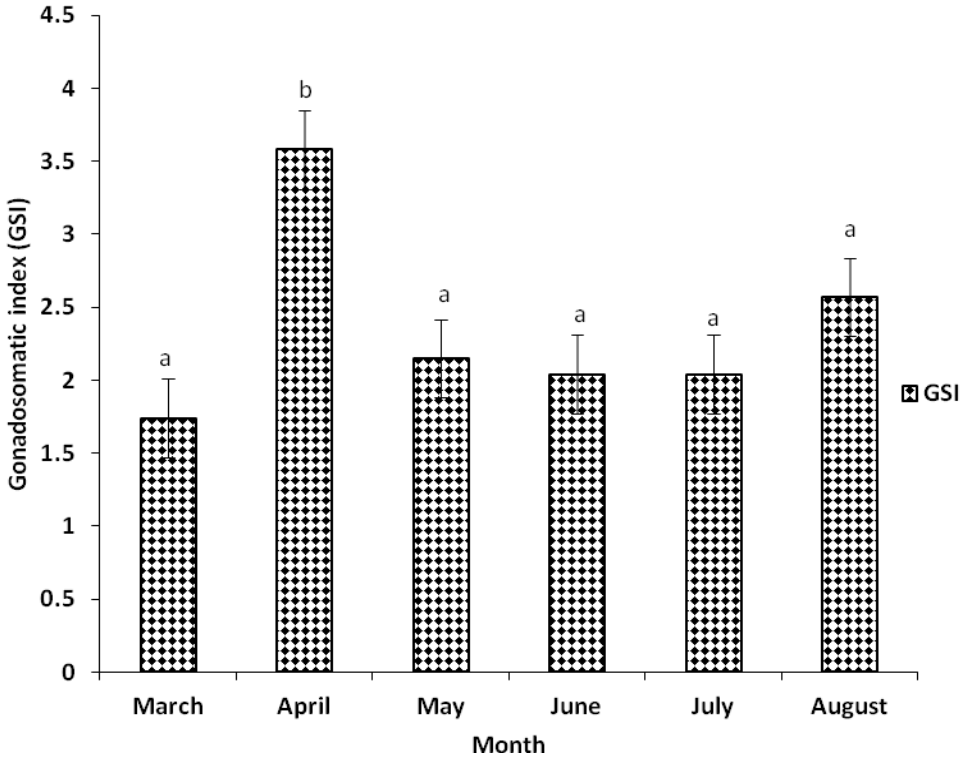

Fig. 8: Mean monthly variations in gonadosomatic index (GSI) of *Protopterus annectens* of Anambra River, Nigeria

CHAPTER FOUR

DISCUSSION

There is growing interest in the study of haematological, biochemical and biometric parameters of fish due to their importance in aquaculture. The blood is said to be a mirror in which all the vital processes taking place in the organisms are reflected, therefore blood parameters are used in understanding the biological processes taking place in fish (Gayatri and Prafulla, 2014a). Haematological, biochemical and biometric analyses of fish are important as these are linked to the health of fish.

Existence of an impressive baseline data regarding heamatological and biochemical normal values facilitates fish health condition assessment diagnosis in fresh water fish (Pradhan *et al.,* 2012). There are abundant excellent references used as guides in the unusual fish health, and experts need to use all available diagnostic skills to manage these cases. Findings of reference value for fish species will help to establish and identify the causes of disease in fish which presents challenges for the ichthyologist. Once reference values are determined, it may facilitate the monitoring of fish stress response, their nutritional condition, reproductive state, tissue damage due to frequent handling procedures, detection and diagnosis of metabolic disturbances or disease processes in fish population (Pradhan *et al.,* 2012) Many haematological reports are available in different species of fishes by different researchers in different approaches, but the present work basically focused on biochemical, haematological and biometric parameters of *Protopterus annectens.* It is necessary to establish normal biochemistry, heamatological and biometric characteristics of particular species of fish which would serve as reference for future comparative studies (Okafor and Chukwu, 2010).

Many publications reported that variations in blood index values can be attributed to many factors such as age, size of fish, season, spawning, sex, and genetic variation in different fish species. Discussing the findings of the present study on haematological, parameters such as packed cell volume, (PCV), Red blood cell (RBC), white blood cell (WBC), haemoglobin concentration (Hb), biochemical parameters such as glucose, total protein, albumin, globuline, urea, and biometric parameters such as condition factor (F), hepatosomatic index (HSI), and gonadosomatic index (GSI) of *Protopterus annectens* shows statistical differences in seasons and sex variations. However, this study attributes that, during dry season, because of high body metabolic rate and high ambient temperature, most of the haematological parameters like PCV, RBC, WBC, Hb, MCV, and MCH and biochemical parameters like ALP, AST, ALT, Protein, Albumin, Globuline, and Glucose showed higher values than that in wet season. The lower values, on the other hand were obtained from MCHC, cholesterol and urea during the wet season, as a result of low ambient temperature and low metabolic rate. Biometric parameters such as HSI increased with increase in length of the fish. These results are in line with the findings of (Orun *et al.,* 2003).

Reproductive activities is reduced in dry season as the female fish moved to deeper waters in order to spawn and probably prevent desiccation of the future fry (< 4.0 cm long) that might be unable to aestivate. This assumption is supported by the fact that the sex ratio of random dugout specimens was more in favour of males. (Chukwu and Kuton 2001). The authors interpreted it to be a reproductive strategy to ensure successful spawning.

Moreso, some reports demonstrated that variations in biochemical and haematological parameters between sexes and their diversity might be due to the higher

metabolic rates of male fish (Gayatri and Prafulla, 2014b). Findings in the present work support this theory, which is related to an increase in fish activity. Considering the sex variations, many studies demonstrated that the male fish has higher values in most of the haematological parameters (Orun *et al.,* 2003). These higher values in male may be attributed to their physiological activeness than female fish.

Heamatocrit has been reported as a tool in aquaculture and fishery management for checking anaemic condition (Blaxhall and Daisely, 1973). Reported values for fish heamatocrit or PCV value are usually between 20-35% and scarcely attain values greater than 50% (Etim *et al.,* 1999). The mean haemotocrit value in this work was within the range of 24.25 ±1.02 to 35.20 ± 1.05 and this conformed with the report of Adam (2004) who reported that the rate of blood cells precipitation for *Oreochromis niloticus* is (22.71 ± 1.94 %) and also conformed with Nilza *et al.* (2003) who stated that mean value of haematocrite PCV for nile *Tilapia (Oreochromis niloticus)* cultured in semi-intensives system is (31.85 ± 1.45), Okafor and Chukwu, (2010) who reported that the mean haematocrit values for *Protopterus annectens* of all sizes fall within the range of 27.7% to 29.2%. In the present study, there was no significant ($P > 0.05$) difference showed in male and female PCV values according to season. PCV value recorded during dry season was higher than the value obtained during the wet season. This may be as a result of high ambient temperature and high body metabolic rate. In the aquatic environment, feeding is restricted to certain periods of the day and food is more abundant in dry season. This conformed with Baloggun and Fisher (1970) who stated that the peak abundance of shrimps in Nigerian Coastal waters is during the dry season. Therefore, the dry season increase in feeding intensity of *Protopterus annectens* in Anambra River could be linked to the increase abundance of one of its principal food items.

The RBC of fish determines the dissolved oxygen carrying capacity. The study revealed that mean value rate of RBC varies between $9.07 \pm 0.60 \times 10^6/mm^3$ to 11.28 - 0.81×10^6 mm^3 and no significant ($P > 0.05$) difference were observed in RBC values among the months and sex. The RBC value recorded in dry season was slightly higher than that obtained in wet season.

This RBC result obtained is higher than that recorded by Maheswaram *et al.* (2008) who recorded unsexed *Clarias batrachus* RBC value to be $2.89 \pm 0.08 \times 10^6$ mm^3 and, Onyia *et al.* (2013) also reported the *Heterobranchus bidorsalis* male and female RBC value to be $5.05 \pm 0.17 \times 10^6$ mm^3, $5.2 \pm 0.26 \times 10^6$ mm^3 respectively.

According to Lenfant and Johansen (1972), erythrocyte count greater than 1×10^6 mm^3 is considered high and indicative of high oxygen carrying capacity of the blood, which is a characteristic of fishes capable of aerial respiration and with high metabolic activity.

White Blood Cells (WBCs) are the defensive cells of the body. According to Douglass and Jane (2010), WBC levels have implications for immune responses and the ability of the animal to fight infection. Species with higher levels of WBC will be able to fight infection more effectively than other species. The present study showed that WBC counts seem to have a wide range of variation from $9057 \pm 5.10 \times 10^3$ ml to $12867 \pm 8.90 \times 10^3$ ml. The result is contrary to the report of Gayatri and prafulla (2014) who reported the WBC found in *Clarias batrachus* to be $8.59 \pm 0.27 \times 10^3$ mm and $9.71 \pm 0.43 \times 10^3$ mm respectively.

The present study showed that the mean value of Hb varies between 5.43 ± 0.23 g/dl to 11.47 ± 0.25 g/dl. This result conformed to the report of Ahmed *et al*. (2013), who showed the Hb count of *Oreochromis niloticus* to be 5.3 ± 2 ± g/dl and, Sowunmi (2003)

reported Hb value of *Clarias gariepinus* to be 8.70 gdl $^{-1.}$ In the present study, the Hb value of male fish was slightly higher than female fish in the dry season. This is in line with the findings of Gayatri and Prafulla (2014b) who reported that there was higher Hb concentration found in male *Clarias batrachus* compared to female fish due to how aggressive the male fish are.These results corraborated with the findings of Fagbeno *et al*. (2000), who reported a higher value in male *Parachanna obscura* than in female fish. The more active fishes tend to have high haemoglobin values than the more sedentary ones. Consequently, *Protopterus annectens* being relatively quiet and sedentary species (Okafor, 2006) has a slightly lower haemoglobin concentration than other more active African teleost such as *Clarias buthupugon* whose mean heamoglobin concentration is as high as 9.88 g/dl (Okafor and Chukwu, 2010).

Mean cell volume (MCV), Mean cell haemoglobin volume (MCH) and mean cell haemoglobin concentration (MCHC) were calculated indirectly with reference to RBC, Hct, and Hb, therefore, their changes are directly linked with these blood parameters. These parameters are also useful to identify and classify the type of anemia occurring in fish. For example, the MCHC reduction may indicate an anemia framework, while its increase may be related to haemolysis in the blood sample (Labarrere *et al*., 2012). MCV and MCH values did not show any marked differences between season and sexes in the result of the present study though there was a slight increase in MCV value in female fish than male fish.This is in line with the report of Gayatri and Prafula (2014b) who stated that the MCV values of female Walking Cat-fish (*Clarias batrachus*) is significantly higher than that of male due to lower PCV. In the present study, the highest MCHC value was obtained in male during wet season. A high level of MCHC indicates more Hb in a unit of RBCs. This result corraborates with the findings of Gayatri and Prafilla (2014a)

who reported higher level of MCHC value in male cat fish (*Clarias batrachus*). Meanwhile, MCHC showed a significant $P < 0.05$ difference in sex and season. This result is contrary with that of Pradhah *et al.* (2012) who reported that MCHC and MCH values of *Cirrhinus mrigala* did not show any marked difference between seasons and sexes,

Many studies consider understanding the peripheral blood leucocytes to be crucial in other to detect stress in fishes since this gives information about the immune system (Olabuenage, 2000). This study showed larger neutrophil and lymphocytes values when compared to monocytes, basophil and eosinophil. This view was corraborated by the findings of Davis *et al.* (2008), who reported a significant increase in lymphocyte count in vertebrates and suggested possible defense mechanisms of the fish against stress. This result is in line with the findings of Kori-Siakpere, (2005) who observed that there were numerous lymphocytes identified in *Synodontis membranacea* than any other differential cells and basophiles were occasionally seen in most fishes. Similarly, Samina *et al.* (2013) reported abundance of neutrophil in *Oreochromis* hybrid corresponding to the findings of the present study, In studies by Ranzani-Paiva *et al.* (2000), the highest percentage of eosinophils was found in non-parasitized *Schizodon borelli.*

Blood biochemistry is the most economical and authentic tool to assess the health state of fishes (Samina *et al.*, 2013). Thus, the results of the biochemical profile of *Protopterus annectens* can be used to asses the health status of the fish. These biochemical parameters can change with the fish species, age, and the cycle of sexual maturity and health conditions (Okafor and Chukwu, 2010).

Alanine aminotransferase (ALT) was observed to be high especially during dry season. This observation is consistent with some earlier reports which showed ALT activity in *Clarias geriepinus* to be high while total protein, albumin and globuline were

low. On contrary, Okorie *et al*. (2014) reported a lower ALP, urea, total protein, and albumin values in *Synodontis membranae*. In the present study, biochemical parameters such as ALP, AST and ALT showed higher values in female than males though the differences were not significant ($P > 0.05$).

Albumin and globuline also showed higher values in females than males with insignificant ($P > 0.05$) differences. However, protein, glucose, cholesterol and urea showed higher value in male than female but the differences were not significant ($P > 0.05$). The rise in albumin concentration in animals may result in impaired synthesis (Samina *et al*., 2013). Swain *et al.,* (2007) reported higher albumin concentration in *Labeo rohita* at the time of reproduction.. In the present study, total albumin levels for male and female *Protopterus annectens* was higher in dry season with higher value recorded among the males, though there were no significant ($P > 0.05$) difference observed among the sex and season. Similarly, Asadi *et al.,* (2006) reported a significantly higher albumin value in male Beluga (*Huso huso*) serum. Studies on Persian sturgeon revealed higher albumin values in male than female fish (Zorriehzahra, 2010). Another study on rainbow trout reported albumin value of 1.38 ± 0.05 (g/dl). The levels of plasma protein and glucose are normal. Level of glucose seems to be high because of aerial respiration experienced in lung fishes, hence the high value is indicative of air breathing character in *Protopterus annectens*

Plasma protein gives an index of the health status of the fish and an indicator of nutritional status. The present study revealed no significant ($P > 0.05$) differences in serum protein levels in male and female *Protopterus annectens.* Plasma protein is the protein component of the blood and is vulnerable to increase with starvation or any other

stress (Knowles, 2006). In the present study, plasma protein concentration in *Protopterus annectens* ranged from 5.18 ± 0.50 (g/l) to 6.75 ± 0.60 (g/l)

Generally, glucose is continuously required as an energy source by all body cells and must be maintained at adequate levels in the plasma (Percin, 2008). In this study, the glucose concentration was much lower than that obtained for some fresh water fish such as, Lake trout (Edsal, 1999), walking cat fish (*Clarias batrachus*), (Gayatri and Prafulla, 2014). In the present study, total protein and glucose in male were higher than female fish. The higher concentration of glucose and total protein in males than females *Protopterus annectens* may be attributed to higher growth rate and higher food conversion efficiency.

Cholesterol concentration varies both among and within fish species because of variations in diet activity and sexual development. In this study, male *Protopterus annectens* recorded a higher cholesterol value than the female fish, without significant differences. The total cholesterol level was high. This may be due to high proportion of fat in the chemical composition of their food.

The length frequency histogram (Fig. 4) showed distinct seasonal mean length frequency variations, likewise, weight frequency histogram (Fig. 5) which showed distinct seasonal mean weight frequency variations. The present study showed that the length of the fish varied significantly with the season. This observation is consistent with several reports such as Baghizadeh and Khara (2015), who reported that the size of the fish determines its hematocrit. This report corroborated by the findings of (Olufemi, 2011) who suggested that the number of red blood cells and hemoglobin concentration tend to increase with length and age of the fish species. The length weight relationship of *Protopterus annectens* of Anambra River showed a negative allometric growth as the

value of ób ô (slope), obtained for the fish was less than 3. This is in line with findings of Onyekachi and Edah (2014) who reported that the length-weight relationship of *Callinectes amnicola* from Lagos Lagoon showed negative allometric growth. Their report corresponded to the finding of Lawal and Kusemiju (2006), who reported that the value of ób ô obtained for the blue crab (*Callinectes amnicola*) was less than 3.

Condition factor estimates the general well being of the individual fish. The condition factor (K) which was used to determine the condition of the habitat and overall well being of fish varied by size for *Protopterus annectens.* From the data obtained in the present study, the highest óK ô value was recorded in the month of July (Fig. 6), which was approximately 1, showing that the fish were in normal condition in the months of April and July.

Hepatosomatic index (HSI) provides an indication on the status of energy reserve in an organism and associated with the liver energetic reserves and metabolic activity. From Fig. 7, the mean monthly HSI recorded the highest value in the month of April and was significantly different from other months ($P < 0.05$). This implies that food was available in large amount during the month of April making the condition more favourable during the month. From the present study, HSI increased as the length increases. This is in agreement with Shalaka and Pragna (2010), who stated that increase in the daily weight of the fish body is related to the increase in the hepatosomatic index value of the fish and it is also observed that hapetasomatic index depends upon seasonal cycle.

The mean monthly gonadosomatic index (GSI) of the fish varied from 1.74 to 3.58%. Protopterus annectens generally remains inactive in the dry season in a formof

aestivation. This is contrary to the study of Odo et al. (2012) who stated that there were only slight variations in monthly GSI of *Parachanna obscura.*

Conclusion

This study has provided valuable data on the haematology and plasma biochemistry of *Protopterus annectens,* which may be useful in the development of diagnostic tools for monitoring fish health and any changes in the quality of water and related soil. The study revealed significant differences in blood profile of *Protopterus annectens* owing to season and sexuality of fish. The findings of the present research will constitute a baseline data for further research in fish heamatology, biochemistry and biometrics of other species of fish.

Recommendation

It is recommended that pollution status and heavy metal profile of Anambra River should be investigated so as to know if there will be any change in these haematological and biochemistry profile of *Protopterus annectens* already established in this study.

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