COMPARATIVE STUDY OF BLOOD CONSTITUENTS OF FOUR NILEFISH FROM DIFFERENT EVOLUTIONARY LEVELS

***Elagba H. A. Mohammed**

Natural History Museum, Faculty of Science, University of Khartoum, P.O. Box 321, Khartoum, Sudan.
*****Corresponding author: Email: elagba2000@yahoo.com

Abstract

The blood constituents of four Nilefish from different evolutionary levels: *Protopterus annectens* (Cuvier)*, Oreochromis niloticus* (Linnaeus), *Clarias lazera* (Cuvier & Valencienres) and the lungfish *Polypterus senegalus* (Owen) were determined and compared. Haemoglobin, total serum protein, urea, uric acid, sodium and potassium ions were significantly different ($p<0.05$). Alb/Glb ratio was (0.78 – 0.86), opposite to the normal ratio in higher animals, except *P. annectens* which showed same Alb/Glb ratio of higher animals. Highest level of urea was found in *P. annectens* (19 ± 3 mg/dl) and the level in aestivated was (4 - 5) folds the free-living fish. Urea was (3 ± 1 and 4 ± 2 mg/dl) in *O. niloticus* and *C. lazera*, respectively. No urea was found in *P. senegalus*. Uric acid was (4 ± 2.3 and 5 ± 1.6 mg/dl) in *O. niloticus* and *C. lazera*, respectively, (0.4 ± 0.1 mg/dl) in *P. senegalus*. No uric acid was detected in free-living *P. annectens*. Na^+ (107 ± 4.0 - 130 ± 8.0 mmol/l), K^+ (2.8 ± 0.4 - 6.4 ± 2.3 mmol/l), higher in *O. niloticus* and *C. lazera*, and least in. *P. senegalus*. In the aestivated *P. annectens*, Na^+ was (140 ± 15 mmol/l), K^+ (12.5 ± 2.3) compared to Na^+ (110 ± 0.4 mmol/l) and K^+ (4.8 ± 1.0

mmol/l) in the free-living form. High concentration of safe nontoxic urea in blood of aestivated lungfish is an adaptation to aestivation. High levels of Na^+ and K^+ resulted from respiratory evaporation during aestivation. Blood constituents and nitrogenous end products could be used to distinguish genera and species of fish. The pattern of excretion of nitrogenous wastes ageed with the evolutionary position of the four fishes along the evolutionary scale.

Keywords: Aestivation, albumin, globulin, haemoglobin, lungfish, Nilefish, potassium, sodium, urea, uric acid.

Rinning title*:* Evolution of blood constituents in four Nile fish

{**Citation:** Elagba H. A. Mohammed. Comparative study of blood constituents of four nilefish from different evolutionary levels. American Journal of Research Communication, 2014, 2(3): 43-60} www.usa-journals.com, ISSN: 2325-4076.

INTRODUCTION

The blood constituents and the end products of nitrogen metabolism differ in different genera and species of animals including fish, and could be used to distinguish them (Barrington, 1957). Haemoglobin is present in the blood of almost all teleosts. Hall and Gray (1929) found that the concentration of iron in the blood of elasmobrachs was lower than that of in the majority of teleost, and Vinogradov

(1953) concluded that in general the higher the organism rose in the evolutionary scale, the greater was the concentration of iron. The blood of active fish contains more haemoglobin than that of sluggish fish (Engel and Davis, 1964). Similarly, plasma proteins have been reported to to increase during the progress of evolution of fishes, the primitive elasmobranchs, for example, lacking albumin (Gunter et al., 1961). The literature regarding modes of nitrogen metabolism and nitrogenous waste excretion has been extensively reviewed (Wood, 1993; Korsgaard et al., 1995; Walsh, 1997; Makiko et al., 2004). These reviews have primarily focused on the metabolism, excretion and toxicity of ammonia and urea, the major end products of nitrogen metabolism. The consequence of protein oxidation which is used as a metabolic fuel by fish, is the production of nitrogenous wastes, excreted predominantly as ammonia together with a small amount of urea in teleost fish (Mommsen and Walsh, 1991; Kleiber, 1992; Wood, 2001). Bony fishes excrete ammonia as an end product of nitrogen metabolism, while mammals excrete urea and birds and reptiles convert ammonia to uric acid. The form of the nitrogenous wastes was found to depend on species, food condition and temperature (Wood, 1995; 2001). Some fishes excrete urea by the kidneys and ammonia by the gills, and marine teleosts convert their nitrogen to trimethylamine oxide or allantoic acid. Fishes have also been classified according to difference in blood urea and electrolytes (Urist and Vande Putte, 1967): Chondrichthyses with urea concentraion of 10 – 35 mmol/l, and teleosts of 1.0 mmol/l, freshwater teleost have total ions concentration between 275 – 300 mmol/l. Burovina et al. (1964) found that the concentration of intracellular sodium falls, and the potassium tend to rise

as the animal ascends the evolutionary scale. They suggested that the ratio of sodium : potassium in the muscles could be used as an additional character in biological classification.

There has been no thorough, systematic examination of the blood components and mode of nitrogenous wastes in the Nilefishes. The objective of this study was to determine some blood parameters and compared them in four species of the Nilefishes from different evolutionay levels. The importance these parameters for each species was also discussed. Another objective of the current study was to determine the form of the nitrogenous wastes for each species according to their evolutionary position. The third objective was to determine the blood constituents and their importance for the lungfish *Protopterus* during the aestivation period. The species of fish used for this study included: The lungfish *Protopterus annectens* (Owen); two teleosts, *Oreochromis niloticus* (Linnaeus) and the catfish *Clarias lazera* (Cuvier and Valencienres); the primitive chondristean fish *Polypterus senegalus* (Cuvier).

MATERIALS AND METHODS

Animals

Fifty live specimens of each of the free-living lungfish *Protpterus annectens* (Cuvier), *Oreochromis niloticus* (Linnaeus), *Clarias lazera* (Cuvier & Valencienres) and *Polypterus senegalus* (Owen), with (226 – 366 mm) total length and (263 – 349 g) body weight, were collected from Jebel Aulia area, 45 km south of

Khartoum, on the White Nile. Another 50 specimens of the aestivathed lungfish were collected from the dry bed of Khor Abu Habil stream in wester Sudan.

Blood sampling and Analysis:

Blood was collected from caudal vein of each specimen and divided in two sampling tubes: one with anticoagulent to determine hamoglobin according to Dacie and Lewis (1976). The second sample was allowed to clot and centrifuged at 1500 rpm for 10 minutes. Serum was then collected and used for analysis of other blood parameters according to Wootton (1974). Serum level of total protein, albumin, globulin, urea, uric acid, sodium and potasium was determined for each specimen. Means ± standard deviation of each blood parameter were calculated for indivoduals of each species and student T-test was perfomed to test and compare differences between the four species of fish examined using the statistical package (SPSS, 1996). For all analyses, $P<0.05$ was considered significant

RESULTS AND DISCUSSION

The concentration of haemoglobin varied between the four species of fish with a range of (5.7 – 10.9g/dl), highest in *Polypterus* and least in *Oreochromis niloticus*. An intermediate concentration was found in the aestivated form of *Protopterus* (Fig. 1). Albumin concentration (Fig. 2) varied within the range of (1.4 – 2.3 g/dl), high in *Clarias* and least i *Oreochromis niloticus*, and the range of globulin was (0.45 – 2.8 g/dl) high also in *Clarias* but least in *Protopterus*. The

Alb/Glb ratio was found to be in the range of (0.78 – 0.86). The range of urea concentration in the serum was (0-19 g/dl), high in *protopterus* and least in *Polypterus* (Fig. 3). Intermediate value was found in the two teleosts and the concentration in the aestivated form of *Protopterus* was found to be (4-5) folds that of free-living form. The range of uric acid cocentration was (0 – 5 g/dl), the lowest concentration was found in *Protopterus* and *Polypterus*, while the two teleost showed higher concentrations (Fig. 4). No urea was detected in the serum of *Polypterus* and no uric acid was detected in the aestivated *Protopterus*. Sodium concentration varied between (107 – 130 mmol/l), high in the two teleosts and least in *Polypterus* (Fig. 5). Potassium range was (2.8 -6.4 mml/l) high also in the two teleosts and low in *Polypterus* (Fig. 6). Both sodium and potassium were high in the aestivated *Protopterus*.

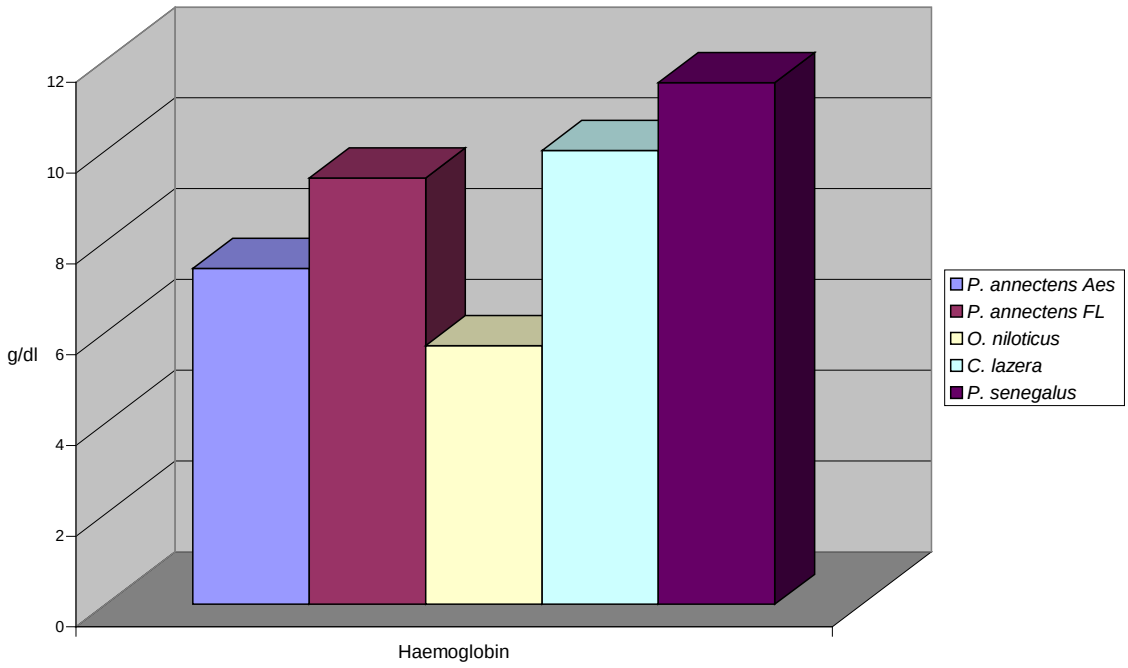

Figure 1. Haemoglobin concentration (mg/dl) in the blood of four Nile fish.

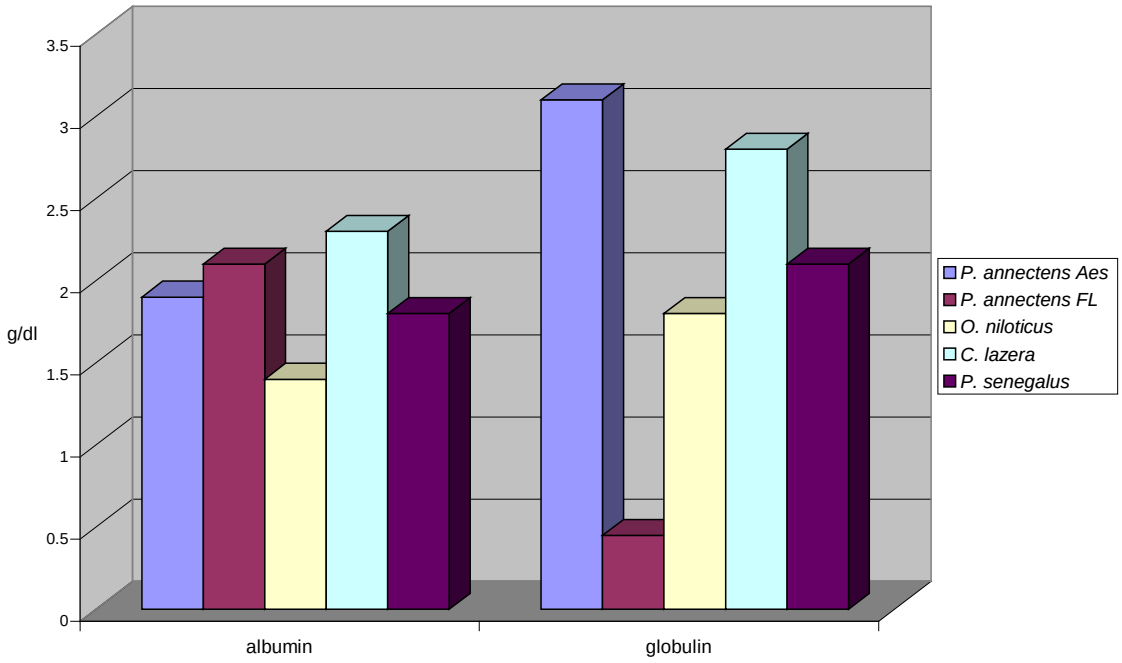

Figure 2. The concentration (mg/dl) of serum proteins (albumin and globulin) for four Nile fish.

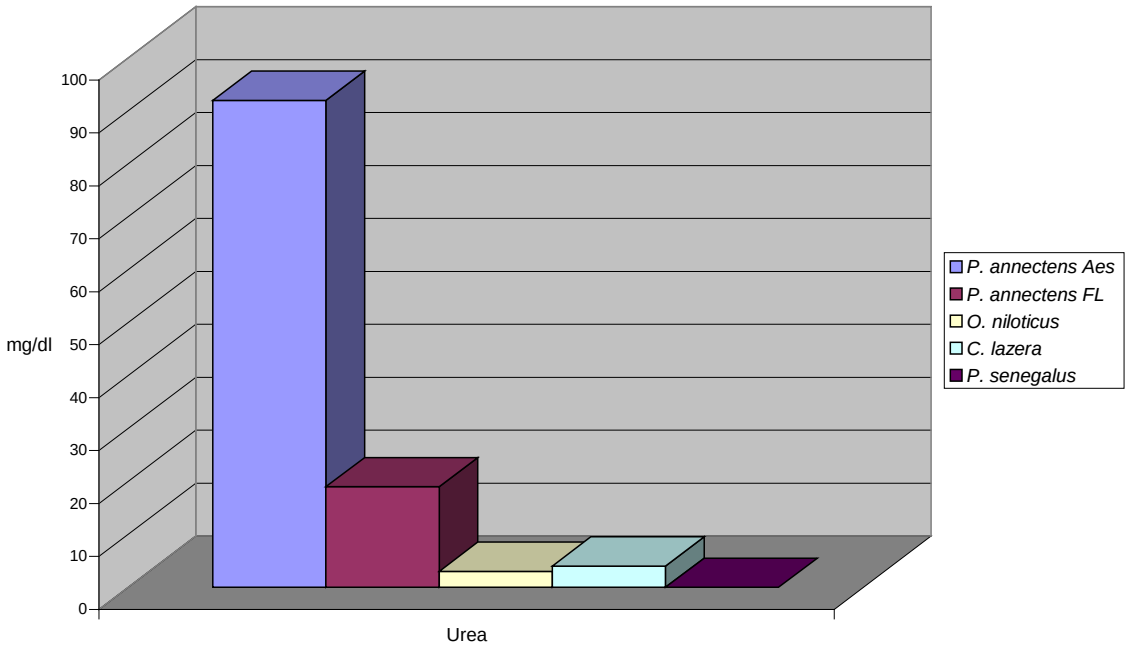

Figure 3. Urea concentration (mg/dl) in the blood of four Nile fish.

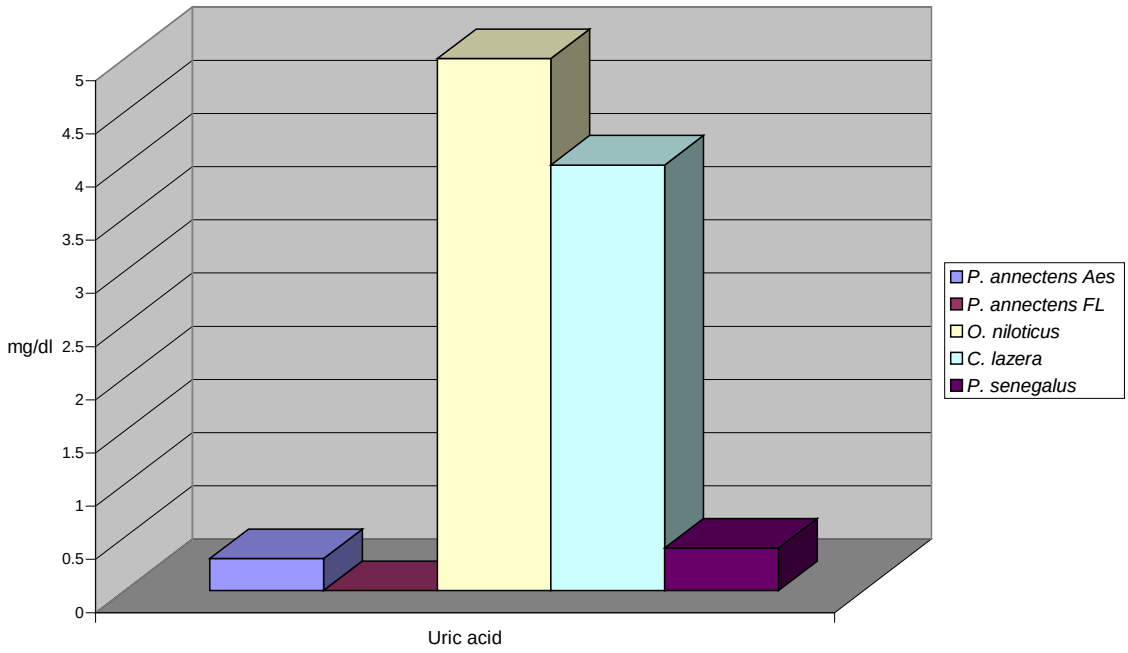

Figure 4. The concentration (mg/dl) of uric acid in the blood of four Nile fish.

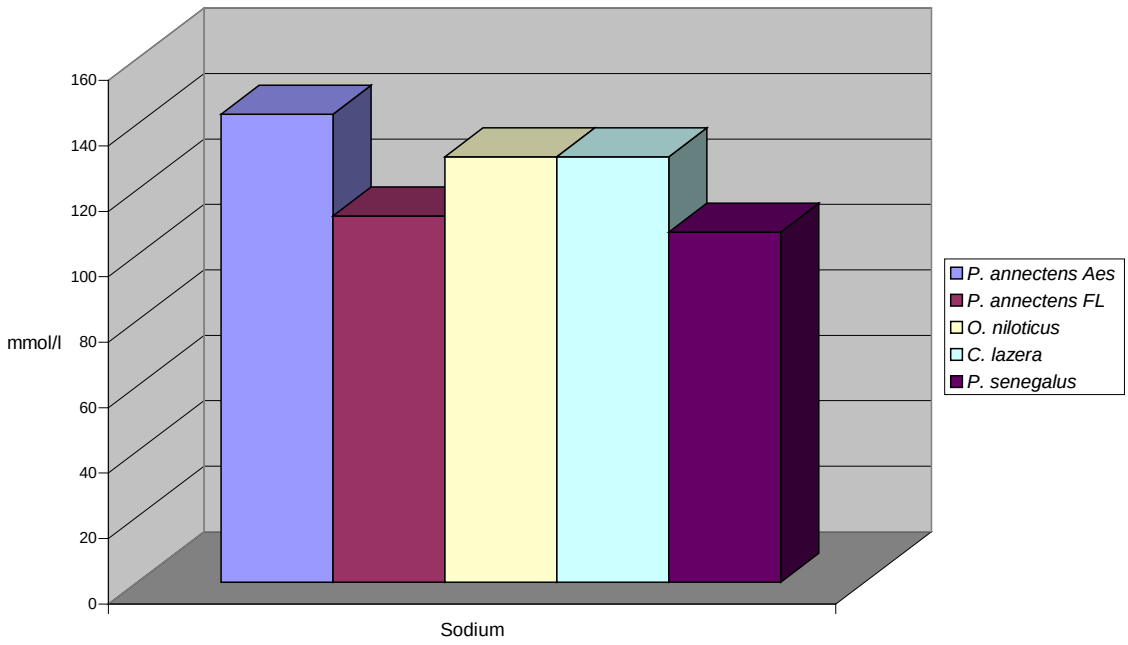

Figure 5. The concentration (mmol/l) of sodium ions (Na^+) in the blood of four Nile fish.

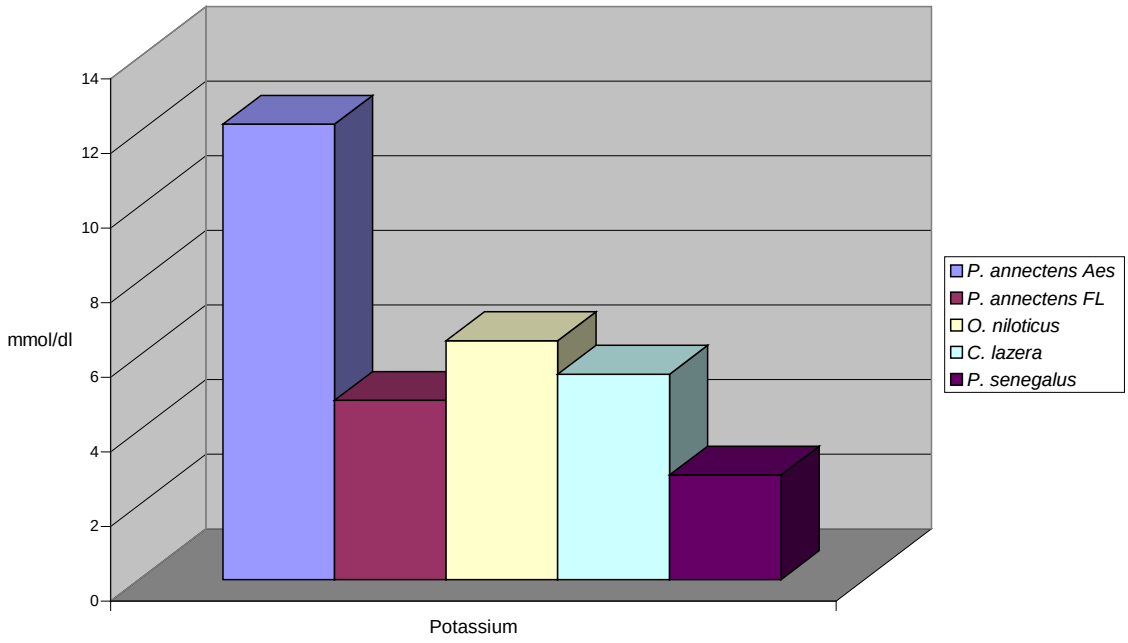

Figure 6. The concentration (mmol/l) of potassium ions (K^+) in the blood of four Nile fish.

The difference in the haemoglobin level of the the fish studied may be related to the different oxygen capacity in the blood of each species. It was reported that fish species which have high haemoglobin level are generally either active or live in poorly oxygenated waters (Love, 1970). Therefore, since *P. senegalus* is a slow-swimming fish that lives in the bottom where the level of dissolved oxygen is very low, it contains more haemoglobin in order extract large quantity of oxygen required for its metabolic activity. On the other hand, the high concentration of haemoglobin in *C. lazera* may be related to its high metabolic rate. This fish inhabits pools and shallow waters, but overcomes the shortage of oxygen by

breathing the atmospheric air by means of accessory air-breathing organs to satisfy its respiratory needs.

The globulin level was found to be higher than albumin and the Alb/Glb ratio was (0.78 – 0.86), opposite to the normal ratio in higher animals where the concentration of albumin exceeds 55% of the total serum proteins (Wootton, 1974). *Protopterus annectens* showed same Alb/Glb ratio of higher animals, where albumin formed 63% of the total serum proteins. This could be explained on evolutionary basis, since *P. annectens* occupies a phylogenetic position between the aquatic and land animals (Young, 1981). The level of globulin also varied dramatically between the free- and aestivated *Protopterus*. High albimin concentration found in the serum of the aestivated fish might be required to maintain the oncotic pressure during the aestivation period.

The most interesting variation between the four species was observed in the level of urea and uric acid. The concentration urea and uric acid was high in the lungfish and intermediate in the two teleosts, but an interesting finding was the absence of urea and the extremely low detectable quantities of uric acid in *Polypterus*. This could be explained from evolutionary point of view. According to Barrington (1957); Mommsen and Walsh (1991), the function of excretion and osmoregulation is usually related to the systematic position of the animal species and could be also explained from evolutionary point of view. This could also indicate that *Polypterus* fish has other means for excreting its nitrogenous end products (Smith, 1929; Walsh et al., 2001). The consequence of protein oxidation is the production of nitrogenous waste, which is excreted predominantly as

ammonia together with a small amount of urea in teleost fish (Mommsen and Walsh, 1991; 1992; Wood, 1993). The intermediate concentration of uric acid (4 - 5 mg/dl) and urea (3 – 4 mg/dl) in the two teleosts *O.niloticus* and *C. lazera* supported other findings that freshwater fishes excrete their nitrogenous end products in different forms as uric acid, creatinine and urea by gills and kidneys (Smith, 1929; Hurkat and Mathar, 1976) and the total nitrogen excretion dependent on species, food condition and temperature (Wood, 1995; 2001).

Another significant finding was the high concentration of urea in the blood of the lungfish. This could also be explained by its evolutionary position between fishes and amphibians, where it has several features common with both fish and terrestrial animals (Lehninger, 1975). It is well known that adult amphibians excrete their nitrogenous end products in form of urea, while their aquatic tadpoles excrete ammonia. Although it was observed that lungfishes excrete part of their nitrogenous wastes as ammonia (Smith, 1930), the presence of high urea found in the blood of the lungfish indicated that urea could also be an end product of this fish (Janssens and Cohen, 1968). Both lungfish and amphibia possess the ability to synthesize this compound (Sawyer, 1966). The fish also preserve its osmotic balance by means of elevated urea concentrations. It could also be assumed that the fish converted most of the nitrogen resulting from the breakdown of the nucleic acids to ammonia and urea only, since there was an extremely very low level of uric acid in its blood.

The presence of a large amount of urea in the blood of aestivated (4 - 5 folds) of tha free-living lungfish is, probably, an adaptation to maintain life during the aestivation period out of water. Babiker and El-Hakeem (1979) reported a (35 – 40 folds) increase of urea in aestivated lungfish compared to free-living form. Smith (1930) also observed the development of marked amount of urea in the blood of the lungfish during the aestivation period. Janssens and Cohen (1968) observed high activity of enzymes of urea cycle in the aestivated fish and that ammonia which is formed during the aestivation period was converted to urea which is safer substance to store. The literature regarding modes of nitrogen metabolism and nitrogenous waste excretion has been extensively reviewed in a number papers (Korsgaard et al., 1995; Walsh, 1997). These reviews have primarily focused on the metabolism, excretion and toxicity of ammonia and urea, the major end products of nitrogen metabolism. Other end products such as creatine, creatinine, uric acid and ammonia were not accumulated since they could be very toxic. High urea level in the aestivated fish might also indicate that the fish depends on the stored tissue proteins to provide free glucose through gluconeogenesis. On this basis, protein has been estimated to contribute 14–36% in fasted salmonids (Brett and Zala, 1975; Wiggs et al., 1989; Lauff and Wood, 1996a; b; Alsop and Wood, 1997; Kieffer et al., 1998), with similar values usually determined in other species (Kutty and Peer Mohamed, 1975; Jobling, 1980; Alsop et al., 1999). In the present case I assume that stored protein (and amino acid) fuels were converted by oxidation to nitrogenous waste products in form of safe urea.

The level of sodium and potassium was significantly high ($P<0.05$) in the blood of the two teleosts and least in *P. senegalus*. The level of the two ions was also observed to be very high in the aestivated lungfish. This might also be associated with aestivation due to reduction of water in the body of the fish (Babiker and El-Hakeem (1979). The aestivated fish might lose water from its body by respiratory evaporation and imbibition into the mud surrounding the cocoon.

The present results support the assumption that the blood constituents and the nitrogenous excretion in fish appears in different end products which characterized different species and could be used to distinguish them (Walsh et al., 2001). The Alb/Glb ratio and the pattern of excretion of nitrogenous wastes ageed with the position of the four fishes along the evolutionary scale. The observed difference in urea concentratios could be explained from an evolutionary point of view. The different levels of urea, Na^+ and K^+ between the free-living and aestivated lungfish indicate an adaptation technique to maintain life during the aestivation period out of water. Thorough investigation on molecular basis is needed to verify the relationship between the four species along the evolutionary scale.

REFERENCES

Alsop, D. H., Kieffer, J. D. and Wood, C. M. (1999). The effect of temperature and swimming speed on instantaneous fuel use and nitrogenous waste excretion of Nile tilapia. Physiol. Biochem. Zool. 72: 474 - 483.

Alsop, D. H. and Wood, C. M. (1997). The interactive effects of feeding and exercise on oxygen consumption, swimming performance and protein usage in juvenile rainbow trout. J. Biol. Chem. 200: 2337 - 2346.

Babiker, M. M. and El-Hakeem, O. H. (1979). Changes in blood characteristics and constituents associated with aestivation in the African lungfish *Protopterus annectens* (Owen). Zool. Anal. Jeneva. 202: 9 - 16.

Barrington, E. J. W. (1957). Metabolism. In: Brown, M. (ed.): The Physiology of Fishes. Vol. 1. Academic Press, Inc. Publisher, New York.

Brett, J. R. and Zala, C. A. (1975). Daily pattern of nitrogen excretion and oxygen consumption of sockeye salmon (*Oncorhynchus nerka*) under controlled conditions. J. Fish. Res. Bd. Canada. 32: 2479 - 2486.

Burovina, I. V., Glazunov, V. V., Leontyev, V. P., Nesterov, V. P and Skul´skii, I. A. (1964). Alkaline elements in the evolution of sea organisms. Biologia. 25: 115 - 23.

Dacie, A. S. and Lewis, S .M. (1976). Practical Haematology. (5th ed). Churchill Livingstone, London.

Engel, D. W. and Davis, E. M. (1964). Relationship between activity and blood composition in certain marine teleosts. Copeia. 3: 586 - 7.

Gunther, G., Sulya, L. L. and Box, B. (1961). Some evolutionary patterns in fishes´ blood. Biol. Bull. Marine Biolog. Labrot. 121: 302 - 6.

Hall, F. G. and Gray, I. E. (1929). The haemoglobin concentration of the blood of marine fishes. J. Biolog. Chem. 81: 589 - 94.

Hurkat, P. C. and Mathar, P. N. (1976). A Textbook of Animal Physiology. (1st ed). S. Chand & Co. LMT. Ram Nagar, New Delhi.

Janssens, P. A and Cohen, P. P. (1968). Biosynthesis of urea in the aestivating African lungfish and in *Xenopus caevis* under conditions of water shortage. *Comp. Biochem. Physiol.* 24: 887 - 898.

Jobling, M. (1980). Effects of starvation on proximate chemical composition and energy utilization of plaice, *Pleuronectes platessa.* J. Fish. Biol. 17: 325 - 334.

Kleiber, M. (1992). Respiratory exchange and metabolic rate. In: Geiser, S. R. (ed.): Handbook of Physiology. Bethesda: Am. Physiol. Soc.

Kieffer, J. D., Alsop, D. and Wood, C. M. (1998). A respirometric analysis of fuel use during aerobic swimming at different temperatures in rainbow trout (*Oncorhynchus mykiss*). J. Exp. Biol. 201: 3123 - 3133.

Korsgaard, B., Mommsen, T. P. and Wright, P. A. (1995). Nitrogen excretion in teleostean fish: adaptive relationships to environment, ontogenesis, and viviparity. In: Walsh, P. J. and Wright, P. A. (eds): Nitrogen Metabolism and Excretion. Pp. 259 - 288.

Kutty, M. N. and Peer Mohamed, M. (1975). Metabolic adaptations of mullet *Rhinomucil corsula* (Hamilton) with special reference to energy utilization. *Aquaculture* 5, 253-270.

Lauff, R. F. and Wood, C. M. (1996a). Respiratory gas exchange, nitrogenous waste excretion, and fuel usage during starvation in juvenile rainbow trout. J. Comp. Physiol. B. 165: 542 - 551.

Lauff, R. F. and Wood, C. M. (1996b). Respiratory gas exchange, nitrogenous waste excretion, and fuel usage during aerobic swimming in juvenile rainbow trout. J. Comp. Physiol. B. 166: 501 - 509.

Lehninger, A. L. (1975). Biochemistry. (2nd ed). Worth Publishers Inc. New York.

Love, R. M. (197). Differences between and within species. In: The Chemical Biology of Fishes. Academic Press, London.

Makiko K., Sara J. C., Chris N. G. and Chris M. W. (2004). The effect of feeding and fasting on the excretion of ammonia, urea and other nitrogenous waste products in rainbow trout. *J. Exp. Biol.* 207: 1993 - 2002. doi: 10.1242/jeb.00901

Mommsen,T. P. and Walsh, P. J. (1991). Urea synthesis in fishes: Evolutionary and biochemical perspectives. In: Hochachka, P. W. and Mommsen, T. P. (eds): Biochemistry and Molecular Biology of Fishes. Vol. 1: 137 – 163. New York: Elsevier, 1991.

Mommsen, T. P. and Walsh, P. J. (1992). Biochemical and environmental perspectives on nitrogen metabolism in fishes. Experientia. 48: 583 - 592.

Sawyer, W. H. (1966). Diuretic and natriuretic responses of lungfish *Protopterus aethiopicus* to arginine vasotocin. Am. J. Physiol. 210: 191 - 7.

Smith, H. W. (1929). The excretion of ammonia and urea by the gills of fish. J. Biol. Chem. 81: 727 - 742.

Smith, H. W. (1930). Metabolism of the lungfish *Protopterus aethiopicus*. J. Biol. Chem. 8: 97 - 130.

SPSS/ PC. (1996). Statistical Package for Social Science. Version 10. Marija J. N. SPSS. Inc. Chicago Illinois.

Urist, M. R. and Vande Putte, K. A. (1967). Comparative biochemistry of the blood of fishes. Identification of fishes by the chemical Composition of serum. In: Gilbert, P. W., Mathewson, R. F. and Rall, D. F. (eds): Sharks, Skates and Rays. John Hopkinson Press, Baltimore.

Vinogradov, A. P. (1953). The Elementary Chemical Composition of Marine Organisms. Sears Foundation, New Haven.

Walsh, P. J. (1997). Nitrogen metabolism and excretion. In: Evans, D. H. (ed): The Physiology of Fishes. (2nd ed). Boca Raton: CRC Press. Pp. 199 - 214.

Walsh, P. J., Wang, Y., Campbell, C. E., DE Boeck, G. and Wood, C. M. (2001). Patterns of nitrogenous waste excretion and gill urea transporter mRNA expression in several species of marine fish. Marine Biol. 139: 839 - 844.

Wiggs, A. J., Henderson, E. B., Saunders, R. L. and Kutty, M. N. (1989). Activity, respiration, and excretion of ammonia by Atlantic salmon (*Salmo salar*) smolt and postmolt. *Canad. J. Fish. Aqua. Sci.* 46: 790 - 795.

Wood, C. M. (2001). The influence of feeding, exercise, and temperature on nitrogen metabolism and excretion. In: Anderson P. A. and Wright, P. A. (eds): Fish Physiology. Vol. 20, Academic Press, Orlando.

Wood, C. M. (1993). Ammonia and urea metabolism and excretion. In: Evans, D. (ed.): The Physiology of Fishes. CRC Press, Boca Raton.

Wood, C. M., Excretion. (1995). In: Groot, C., Margolis, L and Clarke, W.C. (eds): Physiological Ecology of the Pacific Salmon. Vancouver: Government of Canada Special Publications Branch; UBC Press.

Wootton, I. D. P. (1974). Macroanalysis in Medical Biochemistry. (5th ed). Churchill Livingstone, London.

Young, J. Z. (1981). The Life of Vertebrates. (3rd ed). Clarendon Press, Oxford.