# Haematology and serum biochemical parameters in freeranging African side neck turtle (*Pelusios sinuatus*) in Ibadan, Nigeria

A.O. Omonona<sup>1</sup>, S.G. Olukole<sup>2</sup>, F.A. Fushe<sup>1</sup>

<sup>1</sup> Department of Fisheries and Wildlife Management, Faculty of Agriculture and Forestry, University of Ibadan, Ibadan, Nigeria.

<sup>2</sup> Department of Veterinary Anatomy, Faculty of Veterinary Medicine, University of Ibadan, Nigeria. Corresponding author. E-mail: deborolukole@yahoo.com

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Abstract. The haematology and serum biochemical parameters in free-ranging African side neck turtle (Pelusios sinuatus) in Ibadan, Nigeria was carried out with the view of establishing baseline blood health indices of this species and generating data which could be useful in the comparative physiology of turtles. A total of sixty free ranging turtles comprising juveniles and adults of both sexes were used for the study. The mean values for the RBC, PCV, Hb, MCV, MCH and WBC counts observed in male juvenile were significantly higher (P < 0.05) than those of the females. Nevertheless, in adult turtles, the mean values for the RBC, PCV, Hb, MCV, MCH and WBC counts observed in females were significantly higher (P < 0.05) than those of the males. Similarly, in juvenile turtles, the absolute heterophil, lymphocyte and monocyte counts in females were relatively higher (P < 0.05) than that of male while in adult turtles there were no significant differences (P < 0.05) in these parameters between the males and females. There were no significant difference (P < 0.05) in the values for the total protein, albumin, globulin, creatinine, serum glutamic-oxalacetic transaminase (SGOT), serum glutamic-pyruvic transaminase (SGPT) and blood urea nitrogen in the males and females of both the juvenile and adult turtles. The outcome of this study presents baseline data on the haematology and serum biochemical parameters in free-ranging African side neck turtle (Pelusios sinuatus) in Ibadan, Nigeria, which could also serve as a template for the comparative physiology of fresh and sea turtles.

Keywords. Heamatological parameters, serum, Pelusios sinuatus, Reptilia.

## INTRODUCTION

The African side neck turtle (*Pelusios sinuatus*), usually found in freshwater habitats, from rivers and lakes to ephemeral ponds being widely distributed in Africa, Madagascar

and the Seychelles Islands belongs to the family *Pelomedusidae*, and is the largest species of its genus (carapace length up to 55 cm), with the females larger than the males (Anderson, 1995; Broadley and Boycott, 2009). This turtle is small to medium in size, with relatively extensive plastron that may have a hinge present between the pectoral and abdominal scutes (Olukole et al., 2010). The neural series is highly variable (four to eight), and the pleural bones almost meet the midline posterior to the neurals. A pair of mesoplastral bones is present between the hyo- and hypoplasta in contact (Boycott and Bourquin, 2000).

Blood is a special type of connective tissue composed of formed elements (erythrocytes, leukocytes and platelets) in a fluid matrix. Plasma is the fluid portion, called serum when depleted of fibrinogen (Bacha and Bacha, 2000). Reptiles had been reported to constitute a heterogeneous group among vertebrates in terms of their blood cell morphology, and demonstrated considerable variations among orders, even within the same family members. Different blood cells of reptiles had identified: erythrocytes, leukocytes (lymphocyte, monocyte, heterophile [heterophile], eosinophile and basophile) and thrombocytes (Arikan and Cicek, 2010).

Blood analysis is a useful tool for the diagnosis and health monitoring of animals, as well as to distinguish pathogenic processes from those that might be purely physiological (Christopher et al., 1999). Haematologic and biochemical parameters knowledge of freeranging freshwater turtles is important for assessing and managing their populations (Nagy and Medica, 1986). Blood is composed of cells and plasma, serving such functions as respiratory, excretory, nutritive, thermal regulation of the body, protective, and regulatory.

A number of studies had been reported hematological values in turtles, mainly marine (Bolten et al., 1992) and terrestrial turtles (Dickinson et al., 2002; Christopher et al., 1999; Diaz-Figueroa, 2005), and very few in freshwater turtles (Brenner et al., 2002). Limited research studies had been reported in the African side neck turtle; conservation, nutrition and history of migration (Broadley and Boycott, 2009); morphometry of the external body anatomy (Olukole et al., 2010). No information exists on the hematology and blood biochemistry of the African side neck turtle. This study, the first of its kind, was therefore designed to investigate into the hematological and plasma biochemical parameters in free-ranging adult and juvenile African side neck turtles in Ibadan, Nigeria, with the view of establishing baseline blood health indices of this species.

## MATERIALS AND METHODS

#### *Experimental animals*

A total of sixty African side neck turtles picked up between April and June 2010 (rainy season) from various river banks in Ibadan, Nigeria, were used for the study. These comprised 30 juveniles (15 males, 15 females) and 30 adults (15 males, 15 females). The animals were housed at the animal house of the Faculty of the Veterinary Medicine, University of Ibadan and were stabilized for 72 hours prior to standard body measurement and collection of blood samples. They were fed with corn pap ad libitum, fresh water was also provided for the animals. Standard body parameters were all determined using a Draper<sup>®</sup> 115 mm vernier caliper and metric tape. The body weight of the animals was taken with the aid of a Microvar<sup>®</sup> weighing balance. The curved and straight carapace lengths of the turtles were measured after they were physically examined and found to be free from any external injury or adhesion.

#### Blood sample collection and analyses

Blood (2-4 ml) was collected from the right subclavian vein between the neck and foreflipper with the use of an 18gauge, 3.8-cm needle and a 5-ml syringe coated with sodium heparin. The blood samples collected from the animals were put into clean test tubes containing EDTA. The haematological parameters were determined as described by Zaias (2000). Drops of whole blood were used to fill some heparinised microhematocrit capillary tubes to determine packed cell volume (PCV), and hemoglobin (Hb). Whole blood was also used to make three air dried blood smears. The smears were stained with Wright's stain and examined for red blood cell (RBC), white blood cell (WBC), differential WBCs (lymphocytes, heterophil, monocytes) and platelet estimate, while Mean cell haemoglobin (MCH), Mean cell haemoglobin concentration (MCHC) and Mean cell volume (MCV) were calculated. Blood samples were also collected for biochemical analysis, centrifuged at 3000 rpm for ten minutes to isolate the serum. Total protein, albumin, globulin, creatinine, serum aspartate aminotransferase (AST), serum alanine aminotransferase (ALT) and blood urea nitrogen were determined by use of automated analysers as described by Dickson et al. (2002).

#### Data analysis

All data were expressed as means and standard error of means, comparison was by the student t test using the Graphpad Prism version 4.00 for Windows, Graphpad Software. Significance was reported at P < 0.05.

### RESULTS

The mean values for the haematological parameters of juvenile male and female African side-neck turtle (Pelusios sinuatus) are given in table 1. The average body weights for the juvenile and adult turtles used for the study were  $0.31 \pm 0.01$  kg and  $1.8 \pm 0.32$  kg, respectively. The dimensions for the curved carapace lengths adult turtle were  $18 \pm 1.43$ cm and  $21.45 \pm 1.34$  cm for male and female respectively while those of the juvenile turtles were  $10 \pm 0.93$  cm and  $12.21 \pm 0.86$  cm for male and female respectively. There was a positive correlation (r = 0.746, P < 0.01) between the weights of the turtles and their curved carapace lengths. The mean values for the RBC, PCV, Hb, MCV, MCH and WBC counts observed in male juvenile were significantly higher (P < 0.05) than their female counterparts. Nevertheless, in adult turtles, the mean values for the RBC, PCV, Hb, MCV, MCH and WBC counts observed in females were significantly higher (P < 0.05, t-test) than those of the males. Similarly, in juvenile turtles, the absolute heterophil, lymphocyte and monocyte counts in females were relatively higher (P < 0.05) than that of male while in adult turtles there were no significant difference (P < 0.05) in these parameters between the males and females (Table 1). There were no significant difference (P < 0.05) in the values for the total protein, albumin, globulin, creatinine, serum ALT, and blood urea nitrogen BUN in the males and females of both the juvenile and adult turtles (Table 2).

Parameters	Juvenile Male	Juvenile Female	Adult Male	Adult Female
PCV (%)	$31.0 \pm 6.32$ <sup>a</sup>	25.5 ± 2.88 <sup>b</sup>	22.1 ± 3.93 <sup>b</sup>	32.0 ± 4.20 ª
Hb (g/L)	$13.24 \pm 2.14$ <sup>a</sup>	$8.25 \pm 0.97$ <sup>b</sup>	$7.27 \pm 1.32^{\text{ b}}$	$10.5 \pm 1.39$ <sup>a</sup>
RBC (x10 <sup>12</sup> /L))	$15.84 \pm 2.05$ <sup>a</sup>	$9.54 \pm 3.04$ <sup>b</sup>	$10.84 \pm 3.61$ <sup>b</sup>	$16.6 \pm 4.66$ <sup>a</sup>
WBC (x10 <sup>9</sup> /L)	$17.98 \pm 4.17$ <sup>a</sup>	$9.06 \pm 3.74^{\text{ b}}$	$10.38 \pm 3.01$ <sup>b</sup>	$20.5 \pm 5.34$ <sup>a</sup>
Platelets (x10 <sup>3</sup> /L)	$10.4 \pm 3.50$	$13.13 \pm 4.20$	$11.28 \pm 1.98$	9.71 ± 3.19
MCV (fL)	$25.6 \pm 5.86$ a	$19.3 \pm 4.23$ <sup>b</sup>	$32.1 \pm 4.50^{b}$	44.0 $\pm$ 5.34 $^{\rm c}$
MCH (pg)	$26.4 \pm 4.22$ <sup>a</sup>	$18.13 \pm 3.66$ <sup>b</sup>	$18.0 \pm 6.88$ <sup>b</sup>	$27.3 \pm 5.53$ <sup>a</sup>
MCHC (g/L)	$33.0\pm0.70$	$32.5\pm0.76$	$30.57\pm0.58$	$34.9\pm0.38$
Lymphocyte (%)	$68.8 \pm 8.26$ <sup>a</sup>	$57.25 \pm 7.06$ <sup>b</sup>	33.57 ± 3.58 °	$31.9 \pm 3.38$ <sup>c</sup>
Heterophil (%)	$27.4 \pm 7.47$ <sup>a</sup>	$20.5 \pm 7.01$ <sup>b</sup>	$34.30 \pm 7.32$ <sup>c</sup>	$33.6 \pm 7.74$ <sup>c</sup>
Monocyte (%)	$2.0\pm0.02$	$1.00\pm0.01$	$0.57 \pm 0.01$	$0.28\pm0.04$

Table 1. The mean and SEM values for the hematological parameters of juvenile and adult African side neck turtle (*Pelusios sinuatus*).

Means with different superscripts within rows are significantly different at P < 0.05.

**Table 2.** The mean and SEM values for the serum biochemical parameters of juvenile and adult African side neck turtle (*Pelusios sinuatus*).

Parameters	Juvenile Male (n = 15)	Juvenile Female (n = 15)	Adult Male (n = 15)	Adult Female (n = 15)
Total protein (g /dL)	$4.54 \pm 0.39$	$3.70 \pm 0.67$	3.99 ± 0 .36	$4.17 \pm 0.37$
Albumin (g/dL)	$1.18 \pm 0.13$	$1.07 \pm 0.71$	$1.13\pm0.08$	$1.10\pm0.10$
Globulin (g/dL)	$3.36\pm0.30$	$2.63 \pm 0.61$	$2.86\pm0.41$	$3.07\pm0.35$
Creatinine (u/dL)	$1.13 \pm 0.18$	$1.17 \pm 0.05$	$1.19 \pm 0.13$	$1.22 \pm 0.14$
SGOT (u/L)	$33.20 \pm 6.44$	$29.25 \pm 5.20$	$31.14 \pm 8.21$	$23.00\pm6.50$
SGPT (u/L)	$32.40 \pm 5.37$	$30.14 \pm 4.34$	$34.86 \pm 6.74$	$33.43 \pm 6.99$
BUN (g/dL)	$1.66\pm0.61$	$1.93\pm0.6$	$1.66 \pm 0.61$	$1.97\pm0.84$

### DISCUSSION

Haematological and biochemical parameters are useful tools in measuring the physiological status of turtles because they may provide information for diagnosis and prognosis of diseases (Whiting et al., 2007; Oliveira-Junior et al., 2009). Moreover, such tools have been used as physiological disturbance indicators of diseases, stress or exposition to contaminants, as well as to assess degrees of dehydration (Peterson, 2002; Christopher et al., 2003; Tavares-Dias et al., 2009). The turtles used for the study can be said to represent a normal state of physiology since their external body (carapace, plastron, head, neck, tail and limbs) were free from wounds and or adhesions. The strong positive correlation observed between the weights of the turtles and their curved carapace lengths in this study is in agreement with previous reports (Olukole et al., 2010; Boycott and Bourquin, 2000). Also, the significant differences observed between the curved carapace lengths of the male and female turtles (adult and juvenile) used for the study is in line with the existing body of literature on the family *Pelomedusidae* to which the African side neck turtle belongs (Anderson, 1995; Broadley and Boycott, 2009).

The haematocrit values observed in the study were similar to those observed in freshwater turtles, such as Trachemys scripta elegans and Chrysemys picta, which were described by Moon and Foerster (2001). Sea turtles have a higher average haematocrit value, which may reflect a physiological adaptation to the environment (Moon and Foerster, 2001). Moreover, the values found for total haemoglobin were similar to those described in the literature for Chelonia mydas and Chelonoidis chilensis (Wood and Ebanks, 1984; Troiano and Silva, 1998). Nevertheless, the significantly higher values observed for the RBC, PCV, Hb, MCV, MCH and WBC counts in adult female turtles when compared to those of the males used for the study, shows a variance with previous reports on fresh water turtles Emys orbicularis and Mauremys rivulata from Turkey (Yilmaz and Tosunoglu, 2010). This difference could be species specific, but then the presence of great variation among turtle species in terms of erythrocyte count had been reported by previous researchers (Hutchison and Szarski 1965; Duguy, 1970; Yilmaz and Tosunoglu, 2010). Unlike the values for the RBC, PCV, Hb, MCV, MCH and WBC counts in the adult turtles used for the study, the male juvenile turtles showed a significantly higher values to those of the females. The absolute leukocyte count observed in the study was higher than that reported in Geoffroy's side-necked turtle (Phrynops geoffroanus Testudines) as reported by Zago et al., 2010. Nevertheless, the erythrocyte and leukocyte counts obtained in the study are similar to that reported in the in the wild and captive Central American river turtles (Dermatemys mawii). Hematological values in wild D. mawii had been reported to be different to those of other chelonians, such as the green turtle, Chelonia mydas (Bolten and Bjorndal, 1992), the California desert tortoise, Gopherus agassizii (Christopher et al., 1999). Also, in the present study the absolute heterophil, lymphocyte, monocyte counts in male juvenile African side neck turtle were higher than that of the female, though without any significant difference (P < 0.05).

Also, there were no significant differences in total protein, albumin, globulin, creatinine, serum AST, serum ALT and BUN for both sexes of juvenile and adult African side neck turtles. This corresponds to the findings of Yilmaz and Tosunoglu (2010) on fresh water turtles *Emys orbicularis* and *Mauremys rivulata*. According to Metin et al. (2008), there is no difference between females and males in terms of glucose, triglyceride, urea and total protein in *Mauremys caspica*. The mean plasma albumin concentration values of  $1.18 \pm 0.13$  g/dL,  $1.07 \pm 0.71$  g/dL ,  $1.13 \pm 0.08$  g/dL and  $1.10 \pm 0.10$  g/dL obtained in this study for juvenile male, juvenile female, adult male and adult female turtles respectively are similar to the report of Rangel-Mendoza et al., 2009, in the wild and captive Central American river turtles (*Dermatemys mawii*). Nevertheless, plasma albumin concentrations of the turtles used for this study were higher than those reported for loggerhead sea turtles ( $0.6 \pm 0.8$  g/dL) (Bolten, et al., 1992). However, the mean values observed for total protein for all the turtles used for the study are similar to the values reported in Loggerhead sea turtle (Gicking et al., 2004) and in Leatherback sea turtles *Dermochelys coriacea* (Deem et al., 2003). The serum globulin concentration (2.63-3.36) g/dL) obtained in the study is similar to the range (2.3-4.4 g/dL) reported in the loggerhead sea turtles, *Caretta caretta* (Gicking et al., 2004). Nevertheless, the absence of significant differences in serum globulin concentration of the juvenile and adult turtles of both sexes is at variance to previous report in the loggerhead sea turtles, *Caretta caretta* (Gicking et al., 2004). The serum BUN range (1.66-1.97 g/dL) obtained for the turtles used in the study is similar to that reported by Deem et al. (2003) in Leatherback sea turtles (*Dermochelys coriacea*). Findings of this study presnt baseline data on the haematology and serum biochemical parameters in free-ranging African side neck turtle (*Pelusios sinuatus*) in Ibadan, Nigeria. It also provides a template for the comparative physiology of fresh and sea turtles.

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