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Hemoglobin, Plasma Fe^{++} and Total Protein in Olive Ridley and Hawksbill Turtles under Natural Condition in Masirah Island, Oman

A.Y.A AlKindi, I.Y. Mahmoud and H. Khan
Biology Department, College of Science, Sultan Qaboos University, P.O. Box 36,
Al-Khod Muscat, Postal Code 123, Sultanate of Oman. *Fulbright Senior Scholar, USA

Abstract: A comparative study of the hemoglobin concentrations, packed cell volumes (PCVs), mean corpuscular hemoglobin concentration (MCHC), plasma Fe^{++} and total plasma protein in olive ridley (*Lepidochelys olivacea*) and in hawksbill (*Eretmochelys inbricata*) sea turtles was carried out at Masirah Island, Arabian Sea. All the turtles examined were normal. There was no significant difference in all the hematological indices, total protein and Fe^{++} among the oviposited and non-oviposited turtles of the same specie or between the species. These similarities may signify that the two species have the same physiological response to stress under nesting condition for blood gas tensions and acid-base regulation.

Key words: Sea turtles, hemoglobin, plasma iron, protein

Introduction

Green turtles, *Chelonia mydas*, are the major sea turtles in Oman. They are also found throughout the Arabian Gulf, Yemen and Karen Island and Saudi Arabia (Ross and Barwani, 1995). The nesting grounds in Oman present one of the last protected areas in the world. The survival of the species depends on the magnitude of management and conservation. Ecological and physiological studies are important to gain better understanding of the biology of these species.

The hematological indices in sea turtles under natural conditions have not been extensively studied. These indices can be useful for future population health assessment. Unfortunately, no detailed studies have been conducted on the dynamics of the hematological parameters in marine turtles. However, extensive studies on other marine vertebrates, especially fish, have been reported (Nikinmaa *et al.*, 1984; Nikinmaa, 1986; Nikinmaa and Jensen, 1986; Milligan and Wood, 1982; Milligan and Wood, 1987).

Stress in green turtles causes a rise in catecholamine levels which triggers the series of physiological responses, such as an increase in ventilation, oxygen uptake and carbohydrate metabolism (AlKindi *et al.*, 2000a). The rise in plasma catecholamine levels can also stimulate an increase in hemoglobin, hematocrit, red cell volume and changes in erythrocyte intracellular pH and increase in blood oxygen carrying capacity (Nikinmaa *et al.*, 1984; Nikinmaa, 1986).

Stress due to handling or environmental pollution increases the hematocrit and blood hemoglobin concentrations in fish (Barton, *et al.*, 1985) and in sea turtles (George *et al.*, 1990). However, Mazur and Iwama (1993) reported a decrease in these parameters. Nutritional deficiencies can cause radical changes in the hematological parameters (Love, 1970; Adams *et al.*, 1985).

Erythrocyte swelling is caused by stress and the degree of swelling may be related to the magnitude of stress, (Milligan and Wood, 1982). Moreover, it has been reported that swelling of erythrocytes can lead to elevate the blood PCO_2, and low blood PO_2 (Milligan and Wood, 1982; Tetens and Lykkeboe, 1981).

The aim of this investigation is to measure the blood hemoglobin concentrations, hematocrit, mean corpuscular hemoglobin concentration (MCHC) and iron plasma concentrations in the olive ridley and in the hawksbill sea turtles during the nesting season.

Materials and Methods

Study area: Investigation was conducted at Masirah Island in the Sultanate of Oman, off the coast line of Arabian Sea.

Animals: Normal healthy nesting olive ridley and hawksbill sea turtles, without any physical defects or injuries, were studied during the nesting season (March-April). Blood samples (10ml) were taken during nesting exercise with a syringe and needle from the dorsal cervical sinuses according to the method of Owens and Ruiz (1980). Several trips were made to the study area for collection of data.

Blood analysis: Iron reagent is used to measure the iron concentration by a timed-endpoint method. Iron is released from transferin by acetic acid and is reduced to the ferrous state by hydroxylamine and thioglycolate. The ferrous ion is immediately complexed with the ferrozinc iron reagent. The BECKMAN SYNCHRON CX Systems were used to determine the plasma iron in this study. The system automatically proportions the appropriate samples and reagent volumes to be detected on specific absorbencies relative to plasma iron at 560nm. Blood hemoglobin was determined by cyanomethemoglobin technique using Drabkin's reagent (Sigma) that reacts with all forms of hemoglobin, except sulfhemoglobin, which occurs only in minute concentrations in the blood. The colour reaction development is measured by UV spectrophotometer at 546 nm. Hematocrit was determined using heparinized micro-capillary tubes after centrifugation in a micro-capillary centrifuge. The mean corpuscular hemoglobin concentration (MCHC) is the ratio of blood hemoglobin to the hematocrit and is calculated using the following formula:

$$\text{MCHC (g/100 ml)} = \frac{\text{Homoglobin}}{\text{hematocrit}} \times 100$$

Results

Based on the data in Table 1 and Fig. 1-5, there was no significant difference in all parameters studied between oviposited and non-oviposited turtles both intra-specifically and inter-specifically and therefore the data was pooled. These data were obtained from normal healthy individuals throughout the study period.

Discussion

The hematological indices, total plasma protein and plasma iron did not show any significant difference between the turtles when compared intra-specifically or inter-specifically. The similarities in these parameters indicated that both species might have shared certain physiological characteristics such as, blood gas tensions and acid-base regulation since, both are intermittent ventilators r elying

Table 1: Mean values (±SE) of plasma parameters during nesting season of olive ridley and hawksbill at Masirah Island.

Plasma Parameter	olive ridley	Hawksbill
Plasma Iron	9.46 ± 0.68	8.45 ± 1.0
(μM/L)	(n=12)	(n=12)
Hemoglobin	10.2 ± 0.23	11.04 + 0.38
(g/100ml)	(n=12)	(n=12)
Hematocrit	36.25±1.49	35.75± 0.60
	(n=12)	(n=12)
MCHC	28.15± 0.46	32.52 ± 2.0
(g/100ml)	(n=12)	(n=12)
Total Protein	38.58 ±1.61	37.81± 0.19
(g/L)	(n=12)	(n=12)

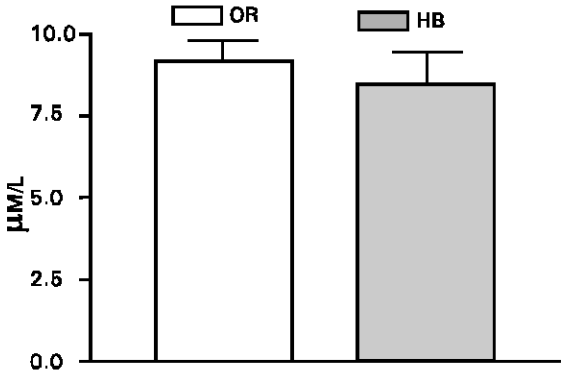

Fig. 1: Plasma iron levels (±SEM; N=12)

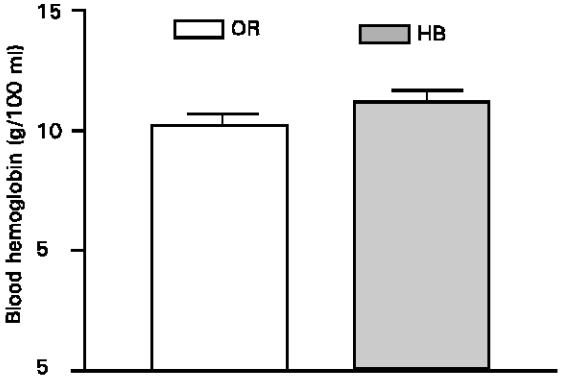

Fig. 2: Blood hemoglobin levels (±SEM; N=12)

mainly on anaerobic metabolism. Moreover, the turtles of both species at Masirah Island shared the same nesting grounds and displayed brief cyclic exercise followed by brief recovery (AlKindi and Mahmoud, 2001; AlKindi *et al.*, 2000b). Under natural conditions, hemoglobin values in the olive ridley and the hawksbill turtles remained remarkably stable during nesting season, despite the exhaustive and hard physical exercises these turtles have gone through, during different nesting phases.

Plasma proteins have been used as a reference of hemoconcentration or hemodilation relative to the changes in plasma volume (Brown *et al.*, 1984; Haux *et al.*, 1985). There were remarkable similarities between the two populations in Masirah Island. These values remain stable, and may reflect an abundance of food source, rich in protein and iron and also indicate that the population was healthy. Based on these

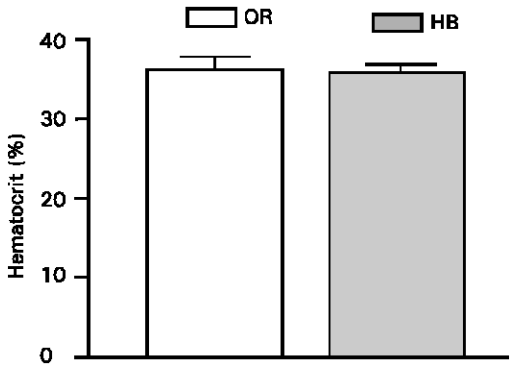

Fig. 3: Hematocrit value (±SEM; N=12)

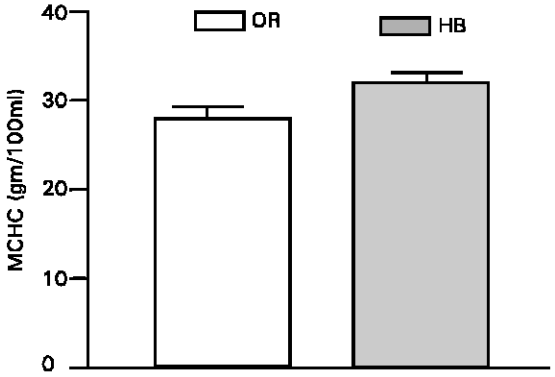

Fig. 4: MCHC values (±SEM; N=12)

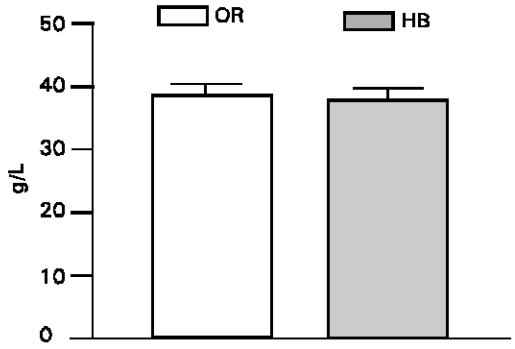

Fig. 5: Mean total protein (±SEM; N=12;)

conditions future population in the Island can make a significant recovery.

The hematocrit levels (PCVs) in olive ridley and hawksbill during nesting were not significantly different throughout sampling period. The mean corpuscular hemoglobin concentration (MCHC), an index of erythrocyte condition showed no significant difference in values in the two species. Normally, erythrocyte release into general circulation under stress increases both hematocrit and hemoglobin concentrations while, the MCHC values remain relatively unchanged (Milligan and Wood, 1982; Ttens and Lykkeboe, 1981). However, swelling of the erythrocytes during maturation is due to defective conditions such as iron

deficiency (Adams *et al.*, 1985).

It has been reported that the Kemp's ridley and loggerhead turtles have been found floating and hyperventilating due to poor oxygen-carrying capacity as their PCVs values were very low (4-10%), an anemic condition which was critically life threatening (George *et al.*, 1990). These turtles made a remarkable recovery in short time after they were fed an iron rich diet.

The hematological indices can be affected by exposure to pollution such as petroleum hydrocarbons (Barton *et al.*, 1985; Jones *et al.*, 1987; Goss and Wood, 1988; AlKindi *et al.*, 1996). Laboratory studies on juvenile loggerhead turtles with short exposures to south Louisiana crude oil affected severely the blood chemistry as well as changes in patterns of respiration and metabolism (Lutz *et al.*, 1986; Lutcavage *et al.*, 1995). There were three to six fold increase in white blood cell count as an immune response to the oil exposure, and there was also a decrease in hematocrit and hemoglobin concentration which could affect the oxygen affinity (Lutz *et al.*, 1986; Lutcavage *et al.*, 1995).

Hematocrit or hemoglobin concentration can be influenced by physical stressors such as handling and transport. Such conditions induced a decrease in hematocrit and hemoglobin levels in some fish (Beggs *et al.*, 1980; Soivio and Virtanen, 1982).

This investigation is a part of ongoing research on behaviour, physiology and population dynamics of the hawksbill and olive ridley in Masirah Island, one of their last protected nesting grounds in the world. We hope that the data obtained from this investigation can be used as a reference guide for evaluation of nesting population relative to pollution and general marine ecosystem.

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