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Hematological Survey in Two Species of Sea Turtles in the Arabian Sea During Nesting Season

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Abstract: A hematological study was conducted with two species of sea turtles, the green and the loggerhead to evaluate the blood parameters of the turtles during high and low population density periods at Ras Al-Hadd, Sultanate of Oman. Blood samples were taken from healthy individuals during nesting exercise or when they just emerge from the sea. Blood hemoglobin concentration (Hb g/100), packed cell volume (PCV), mean corpuscular hemoglobin concentration (MCHC) and plasma Fe^{++}, were analyzed. There was no significant difference both intra-specifically and inter-specifically in hemoglobin concentration. However, iron values were significantly low in the low-density green turtle while the PCV values were not significantly different when the three groups were compared. Moreover, there were no significant differences in all blood parameters of both species when the blood values were compared between the respected nesting turtles and turtles just emerging from the sea. The stable hematological indices during nesting as well as the close similarities, particularly between high density green turtle and loggerhead may signify that they may have the same physiological response to stress during nesting. In addition, significantly higher values of MCHC of low-density green turtles may indicate erythrocyte swelling.

Key words: Green turtle, loggerhead, hemoglobin, plasma iron

Introduction

Investigations on the analysis of blood parameters such as hemoglobin concentrations, plasma iron and packed cell volume have been neglected in sea turtles. Recent investigations on these parameters have generated valuable information on sea turtles of Oman (AlKindi *et al*., 2000b; AlKindi *et al*., 2001a,b). During the last three decades, extensive investigations predominately involved monitoring circulating sex and stress hormones in sea turtle populations in different geographical regions were conducted (Owens, 1995). These studies mainly involved circulating hormone levels with various aspects of reproduction such as gonadal growth, courtship, mating, migration and nesting. Stress physiology and its effects on the reproductive potential was also investigated (Owens, 1995).

Although most of these studies were conducted on turtles under natural conditions, nevertheless the status of the blood parameters was ignored. Examination of hematological indices in natural population can be useful in evaluating the overall health condition in sea turtles especially in the Gulf region where there is a rapid decline in populations due to pollution and habitat destruction.

Studies on stress indicate a significant rise in catecholamine levels in green turtles (AlKindi *et al*., 2000b) and in fish (AlKindi *et al*., 1996; AlKindi *et al*., 2000a). The release of catecholamine into the blood during stress is thought to trigger a complex series of physiological responses, which lead to an increase in oxygen uptake and an increased oxygen delivery to the tissue. The rise in catecholamine values will also stimulate an increase in hematocrit, hemoglobin, red cell volume and changes in intracellular pH of the erythrocytes. All these responses increase the blood oxygen carrying capacity (Nikinmaa *et al*., 1984; Nikinmaa, 1986). However, these changes are complicated by seasonal changes (Nikinmaa and Jensen, 1986) and species differences (Milligan and Wood, 1987). Erythrocyte swelling is a phenomenon associated with stress and the degree of swelling may be related to the magnitude of stress. Thus, the more swelling of erythrocytes, the higher is the degree of stress. It has been reported that swelling of erythrocytes can lead to elevated blood PCO_2 (Milligan and Wood, 1982) and low blood PO_2 (Tetens and Lykkeboe, 1981).

The objectives of this investigation are to examine the blood parameters during nesting season in the green turtle and loggerhead sea turtles. The results of such study will enable us to understand the inter-specific and intra-specific differences and similarities and health conditions in both species.

Materials and Methods

Study sites: This investigation was conducted at Ras Al-Hadd Turtle Reserve, Sultanate of Oman, specifically at Ras Al-Jinz, along the coastal line of the Arabian Sea for the green turtles and Masirah Island in the Arabian Sea for the loggerhead during 2000 nesting season.

Turtles: Normal healthy nesting green and loggerhead sea turtles were studied. The green turtles were studied during two periods of the nesting season: high-density nesting period June-October, 1999 (average 100-200 turtles/night) and low-density nesting period November-May, 2000 (average 10-15 turtles/night). The loggerhead turtles were studied during the high-density of their nesting season in June 2000. Blood samples (10 ml) were taken during exercise with a syringe and needle from the dorsal cervical sinuses (Owen and Ruiz, 1980). Several trips were made to the study areas during each period for blood sampling.

Biochemical analysis: Plasma iron was measured using a timed-endpoint method. In this reaction, iron is released from transferrin 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 SYNCHRON CX System automatically proportions the appropriate sample and reagent volumes into a cuvette. The ratio used is one part sample to 8 parts reagent. The system monitors the change in absorbance at 560nm. This change in absorbance is directly proportional to the concentration of iron in the sample and is used by the Beckman Synchron CX System to calculate and express the iron concentration. Hematocrit was measured using heparinized micro-capillary tubes and micro-capillary centrifuge. Blood hemoglobin was analyzed using cyanomethaglobin technique which uses Drabkin's reagent (Sigma) that reacts with all forms of hemoglobin, except sulfhemoglobin, which occurs only in minute concentrations in the blood. The colour development is measured by UV spectrophotometer (546nm). The mean corpuscular hemoglobin concentration (MCHC) is the ratio of blood hemoglobin to the hematocrit. MCHC provides an index of red blood cell status (swelling and shrinkage). MCHC is calculated using

the following formula:

$$\text{MCHC (g/100 ml)} = \frac{\text{Hemoglobin}}{\text{Hematocrit}} \times 100$$

Results

Table 1 shows mean values (± SEM) of blood parameters during nesting season for loggerhead (Masirah island) and green turtles (high-density and low-density periods at Ras Al-Hadd). Values are considered significant when $p < 0.05$.

Hematological values in the high population density green turtles and the loggerhead were similar. However, in the low population density green turtles, the iron values were significantly lower than the other two groups, while the MCHC was significantly higher in the low-density green turtle than in the other two groups. The hematocrit levels (PCV) were similar in all the three groups.

Table 1: Mean values (± SE) of plasma parameters during nesting season of loggerhead turtles at Masirah Island and green turtle at Ras Al-Hadd, Oman.

Plasma parameters	Loggerhead	Green turtles Low-density period	Green turtles High-density period	P values
Plasma Iron (mmol L^{-1})	13.62± 0.63 n= 29	14.04± 1.19 n= 23	4.29± 0.55 n= 35	P< 0.001
PCV (%)	31.78± 0.68 n= 29	33.88± 0.61 n= 23	28.87± 0.93 n= 35	P > 0.05
Hemoglobin (g/100ml)	8.87± 1.41 n= 29	9.59± 0.42 n= 23	9.92± 0.46 n= 35	P > 0.05
MCHC (%)	27.94± 0.76 n= 29	27.88± 1.05 n= 23	34.03± 1.73 n= 35	P < 0.05

Discussion

The nesting season in the green turtles occurs either during most of the year or restricted to few months of the year (Hirth and Carr, 1970; Hirth, 1971; Limpus, 1978). Nesting period in Arabian Sea, Arabian Gulf and Red Sea is nearly year-round with a peak in summer or fall depending on location (Hirth and Carr, 1970; Ross and Barwani, 1982; Miller, 1989). The year-round breeding in green turtles may be related to the warm waters of this region throughout the year (Miller, 1989).

Hemoglobin concentrations in the green and the loggerhead sea turtles remain remarkably stable during nesting season, despite the exhaustive and hard physical exercises that the sea turtles go through during different phases of nesting.

Plasma proteins have been used as reference of hemoconcentration or hemodilution relative to the changes in plasma volume (Brown *et al.*, 1984; Haux *et al.*, 1985). The low Fe^{++} concentrations in the low-density green turtles is perhaps related to periodic changes in plasma volume or to a low iron diet.

The population of the green turtles in Ras Al-Hadd (based on Government tag-return data) are known to stay near the nesting grounds for 2 to 3 months before they migrate to their regular feeding areas. During this period there is probably a gradual depletion of the rich marine algae and grasses as food source when turtles gathered in large numbers near the nesting beaches. 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 PCV values were very low (4-10%), an anemic condition which is critically life threatening (George *et al.*, 1990). These turtles made a remarkable recovery in short time after they were fed a diet rich in iron. Probably, the low-density green turtle will make a quick recovery in iron levels as soon as they leave the nesting area for nourishing feeding grounds (Mortimer, 1995).

Generally, erythrocyte release into the circulation increases both hematocrit and hemoglobin concentrations while the MCHC values remain relatively unchanged. However, swelling of the erythrocytes during maturation, due to defective conditions such as iron deficiency could affect the hematocrit and hemoglobin values. Based on our data, the low iron in the low-density green turtles may trigger the erythrocyte swelling as a physiological adjustment. Further study is needed to substantiate this suggestion.

Another factor, which can influence the hematological indices, is the exposure to pollution such as petroleum hydrocarbons. Laboratory studies on juvenile loggerhead turtles with short exposures to South Louisiana crude oil, severely affected the blood chemistry as well as changes in patterns of respiration and metabolism (Lutcavage *et al.*, 1995). There was a three to six fold increase in white blood cell count as an immune response to the oil exposure. There was also a decrease in hematocrit and hemoglobin concentration which can affect the oxygen affinity (Lutcavage *et al.*, 1995).

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

The rapid decline in sea turtle populations throughout the world is mainly attributed to the overall polluted marine environment, destruction of nesting beaches due to development, lack of protection by local authorities, excessive fishing in waters close to nesting beaches, use of turtle meat and eggs for human consumption and rapid increase in natural predators (Hillestad *et al.*, 1995).

We recommend that the turtle populations throughout the Gulf, Arabian Sea and the Red Sea regions should be examined periodically using hematological indices as a guide in evaluating the health status of turtle populations.

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