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Comparative Study of Plasma Parameters in Olive Ridley (*Lepidochelys Olivacea*) and Hawksbill (*Eretmochelys Imbricata*) During Nesting

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دراسة مقارنة لمكونات البلازما في الزيتونية وصقرية المنقار من السلاحف البحرية خلال موسم التعشيش

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خلاصة : تمت مقارنة مكونات البلازما في سلاحف الزيتونة (*Lipodochelys oliveacea*) وصقرية المنقار (*Eretmochelys imbricata*) من السلاحف البحرية في جزيرة مصيرة بسلطنة عمان فيما يخص الأيونات البلازمية من الكالسيوم والصوديوم والبوتاسيوم وملحية البلازما وكذلك الكوليسترول وحمض اليوريك واليوريا أثناء تعرضها لظرف المعاناة الضاغط في عملية التعشيش في دراسة غير مسبوقة حسب علمنا، ولم تظهر فروق إحصائية مهمة بين النوعين, و كذلك بين السلاحف التي قامت بالتبويض والأخرى التي لم تضع البيض فيما يخص المؤشرات الفيزيولوجية المذكورة، ويستنتج من ذلك بأن هذين النوعين من السلاحف متشابهان في طريقة التعشيش و يستنبط ذلك أيضا من تقارب الفترة الزمنية المستنفذة في وضع البيض في كلا النوعين. وقد كان الهدف من هذه الدراسة هو استقصاء الدور المهم للعوامل البلازمية في عملية التعشيش وصولا إلى معرفة علمية مفيدة فيما يخص عوامل التكاثر واستجابات المعاناة والضغوط لهذين النوعين من السلاحف البحرية المهددة بالانقراض.

ABSTRACT: The aim of this study is to investigate the role of plasma level parameters during nesting activity and provide data potentially useful to future studies on the dynamics of reproductive and stress hormones in the most endangered sea turtle species in the world. Plasma parameters in the sea turtles, olive ridley *(Lipodochelys oliveacea*) and hawksbill (*Eretmochelys imbricata*) from Masirah Island, Oman, were analyzed relative to nesting stress. To date, no study has been conducted on plasma parameter levels in sea turtles during nesting. Field observations were conducted under ideal temperature conditions. At the time of sampling, there was no significant difference for cloacal, sand, air or water temperature for the two species. Electrolytes (Cl^-, Ca^{++}, K^+, Na^+ and Mg^{++}), cholesterol, urea, uric acid and osmolarity were measured during nesting. Both species were observed to spend between 1.5 and 2.00 hours on the nesting grounds. Some had successful oviposition and completed all nesting phases, while others with incomplete nesting phases failed to oviposit their eggs. Under both conditions, the turtles of both species had an exhaustive and stressful nesting exercise. Plasma parameter values, both intra-specifically and inter-specifically, were not significantly different for oviposited and non-oviposited turtles. This may indicate that both species have the same physiological adjustment relative to plasma parameters whether or not the turtles oviposited their eggs.

KEYWORDS: Nesting, Sea Turtles, Cholesterol, Uric Acid, Urea, Electrolytes, Osmolarity.

1. Introduction

Assessment of plasma chemistry in adult marine turtles has not been studied extensively. Hasbun *et al.,* (1998) studied blood chemistry in adult and sub-adult green turtles caught in trawlers. Other studies focus on juvenile green turtles (Aguirre *et al*. 1995; Bolton and Bjorndal, 1992) and captive adult green turtles (Norton *et al*. 1990). Lutz (1997) recently did an extensive review on the regulation of salt, water and pH balance in sea turtles.

The major organs, which are responsible for salt excretion in marine turtles, are two modified lachrymal glands with their main ducts opening into the posterior canthus of each eye (Abel and Ellis, 1966; Marshall and Saddlier, 1989). These glands are responsible for about 90% of the total salt excretion. The rest of the salt load is excreted through the cloacal chamber (approximately 5%), and through the kidney and the scutes of the plastron (Lutz, 1997).

Salt influx in marine turtles basically derives from two different sources: food material, such as marine algae and sea grasses, and swallowing large amounts of seawater while feeding (Lutz, 1997). This large salt load could affect normal physiological functions and must be excreted rapidly to maintain normal osmolarity and organ function.

Osmoregulation and balance between an acid and basic environment are the major physiological problems that marine turtles face. The concentration of the electrolytes in the ocean is almost three times higher than the electrolytes in body fluids of marine turtle (Lutz, 1997).

The purpose of this study is to examine plasma electrolyte, urea uric acid levels and cholesterol in nesting olive ridley (*Lepidochelys olivacea*) and hawksbill turtles (*Eretmochelys imbricata*). During the nesting process, marine turtles undergo a sudden and stressful change from a marine to a terrestrial environment (AlKindi *et al*. 2000b). Turtles spend several hours looking for the ideal nesting sites. During this period they have to make physiological adjustment in the chemistry of their body fluids as well as their metabolism (AlKindi *et al.* 2000b). Up to date, changes in plasma parameters in nesting turtles have not been evaluated. This investigation will monitor such changes by comparing plasma levels from turtles that have nested successfully with plasma levels from turtles that were unsuccessful in nesting.

2. Materials and Methods

2.1 Study Area

This investigation was conducted on Masirah Island, Sultanate of Oman off the east coast of the Arabian Sea, approximately 20° N, 59° E.

2.2 Animals

Olive ridley and hawksbill turtles without any physical defects or injuries were studied during the nesting season. The observations were conducted in late March and late April of 2001. Both turtle species share the same nesting grounds on Masirah Island.

Behavioral observations were recorded during different phases of nesting. Carapace length and width, and turtle weight were recorded. Additionally, air, water, soil and cloacal temperatures were measured.

A blood sample of 10 ml was taken from each female investigated during nesting process. Each sample was collected with a syringe and needle from the dorsal cervical sinuses using the method of Owens and Ruiz (1980). All samples were collected between March and April, 2001 and kept at 80° C until assay.

2.3 Blood Analysis

Beckman Synchron CX System was used to determine plasma parameters. The system automatically proportions appropriate samples and reagent volumes relative to each plasma parameter.

Osmolarity was analyzed by measuring the freezing point of plasma using an osmometer. The depression of the freezing point of plasma was compared to the depression of the freezing point of pure water.

Non-parametric test (Mann-Whitney) was used for comparing the means. $P<0.05$ is considered significant.

Table 1: Methodology of analysis for blood plasma parameters using Beckman Synchron CX System.

Methodology	Parameter	Reaction Mechanism	Detection
Colorimetric	Calcium	Ca-Arsenazo III Complex	Abs(650)
Potentiometric	Sodium	Ion Selective Electrode	Potential
Potentiometric	Potassium	Ion Selective Electrode	Potential
Potentiometric	Chloride	Ion Selective Electrode	Potential
Enzymatic	Cholesterol	Cholesterol esterase/Cholesterol oxidase with production of peroxide. Peroxidase addition to produce quinoneimine.	Abs(520)
Enzymatic	Urea	Urease addition to form ammonium ion.	ΔMho
Enzymatic	Uric Acid	Uiricase to produce allontoin and hydrogen peroxide. Hydrogen peroxide reacts with 4-aminoantipyrine (4-AAP) and 3.5-dichloro-2-hydroxybenzene sulfate (DCHBS) in presence of peroxidase to form a quinonimine.	Abs(520)

3. Results

Mean values (± SEM; sample size, n=12) of plasma parameters were measured in mmol/L. The osmolarity of plasma was expressed in mosmol/L. The comparison between intra-specific and inter-specific values showed no significant difference (P >0.10). Figures 1 and 2 show that mean values are not significant in electrolytes: Na^+ , Cl^-, Ca^{++} , K^+ and Mg^{++} levels. Cholesterol, urea and uric acid levels showed no significant mean differences between the two species (Figures 3, 4 and 6). The plasma osmolarity in both species showed a remarkable similarity between the two species.

Plasma values of turtles that had a successful oviposition compared with plasma values of turtles that did not lay eggs were not significantly different (Figures 1-6). Mean temperature values ($^{\circ}$C) for olive ridley and hawksbill turtles studies respectively were: water (25.6 ± 0.26, N=10) (26.0 ± 0.0, N=5), sand (26.4 ± 1.18, N=10) (28.2 ± 0.86, N=5), air (25.15 ± 0.72, N=10) (26.5 ± 0.31, N=5) and cloacal (27.5 ± 0.45, N=10) (27.4 ± 0.4, N=5). Respectively there was no significant difference between the cloacal temperatures, sand, air or water temperatures for both species.

4. Discussion

The turtles of both *L. olivacea* and *E. imbricata* demonstrated remarkable similarities in all plasma parameters. This indicates that they share the same physiological adjustments relative to nesting conditions. There were no intra-specific or inter-specific plasma value differences between turtles with successfully completed nesting phases, and those with unsuccessful or incomplete phases without oviposition. Both groups spent between 1.5-2.00 hrs on the beach and therefore had undergone the same strenuous and exhaustive activities. Although the turtles from Masirah Island spent a long time on the nesting grounds, their electrolyte levels remained stable during the change from a marine to a terrestrial environment.

Figure 1. Mean Na^{+} and Cl^{-} lev N=12; ± SEM), OR = Olive Ridley, HB = Hawksbill

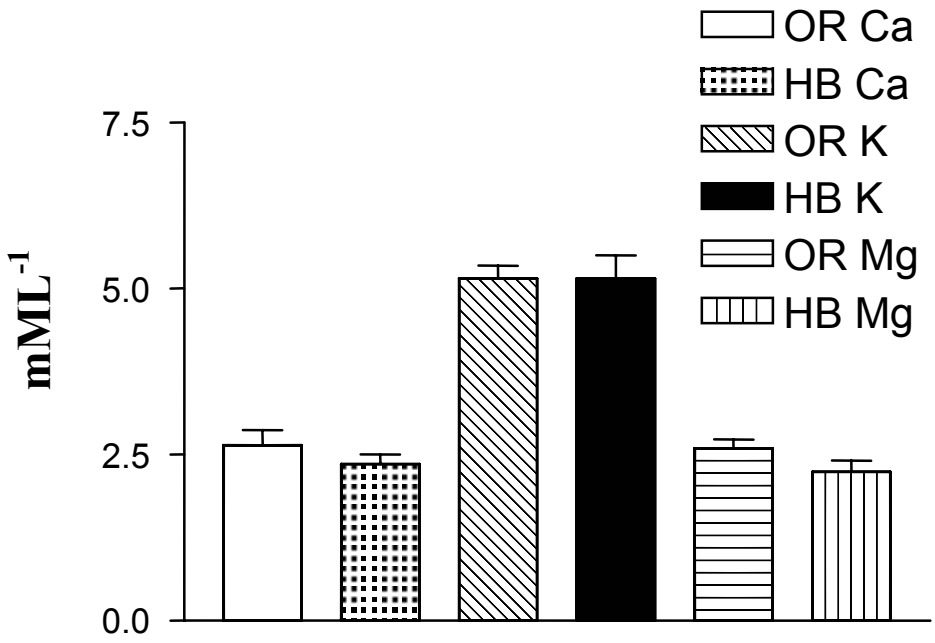


Figure 2. Mean Ca^{++}, K^{++} and Mg^{++} levels (N=12; ± SEM), OR = Olive Ridley, HB = Hawksbill

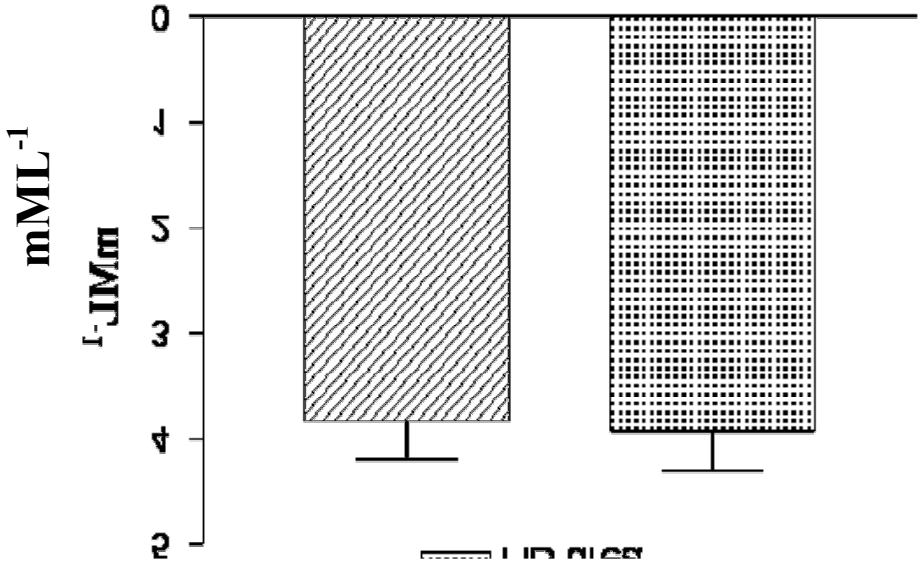


Figure 3. Mean cholesterol (N , OR = Olive Ridley, HB = Hawksbill

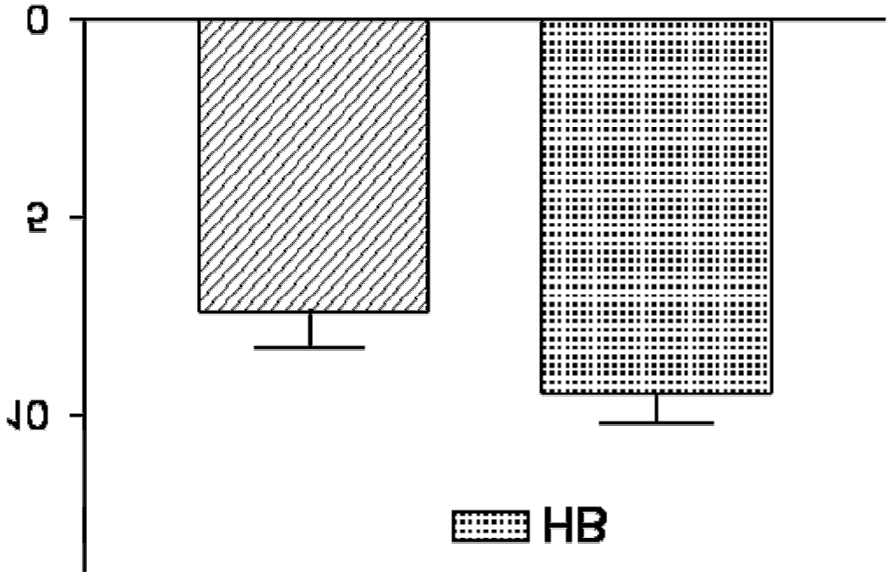


Figure 4. Mean urea levels (N , OR = Olive Ridley, HB = Hawksbill

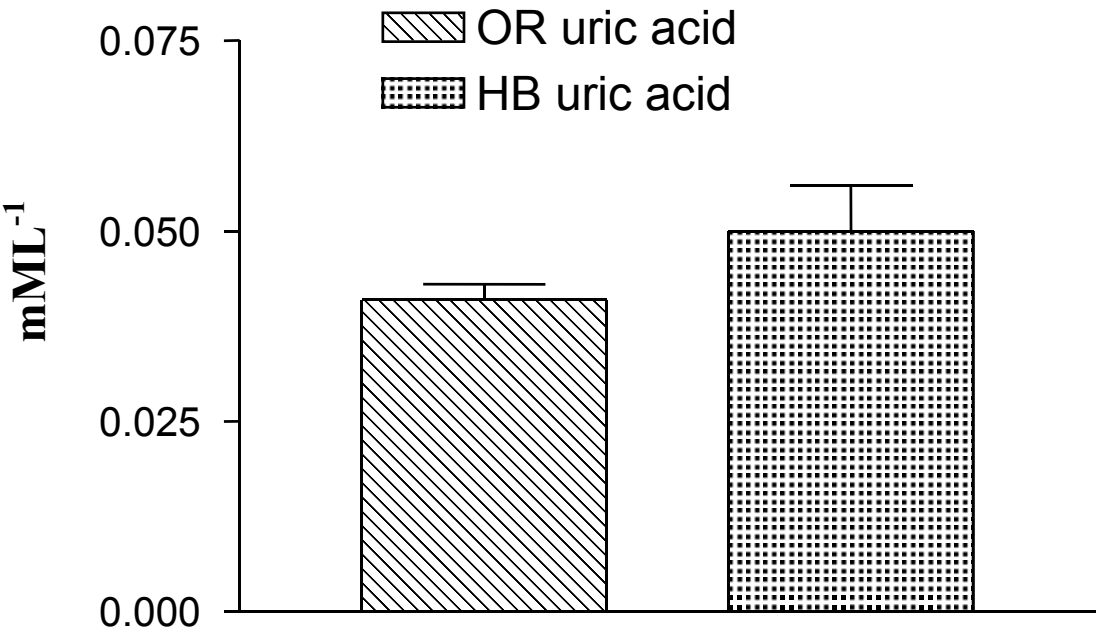
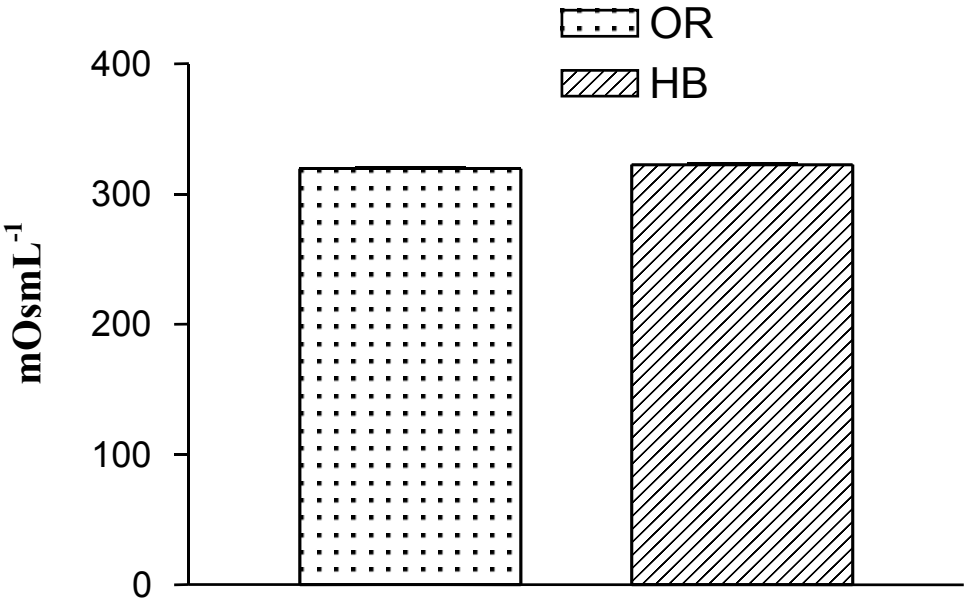


Figure 5. Mean uric acid (N=12, ± SEM) , OR = Olive Ridley, HB = Hawksbill



The present investigation reveals that the natural populations of olive ridley and hawksbill turtles on Masirah Island maintain constant plasma osmolarity while they are on the nesting grounds and throughout the whole nesting season. This indicates that a short term terrestrial environment during nesting has no effect on changes in plasma osmolarity. Our field observations indicate that the lachrymal glands are always active in secreting salt at the time of nesting. Holmes and McBean (1964) described that sea turtles, in general, can tolerate prolonged osmolarity changes from seawater to freshwater without encountering any osmotic problems. Kooistra and Evans (1976) transferred marine turtles from seawater to freshwater. After 25 hrs, the turtle's orbital salt glands stopped secreting salt and after 17 days in freshwater, the efflux of Na^+ had

declined 97% and the cloaca became the major organ of salt secretion. These experiments provide us with some evidence that marine turtles can adjust to a wide osmotic condition.

Plasma chemistry during nesting has not been investigated. Most previous investigations have either dealt with turtles in their marine environment or with turtles that were subject to experimental conditions (Holmes and McBean, 1964; Kooistra and Evans 1975; Bolton and Bjorndal 1992; Lutz, 1997). Hasbun *et al.* (1998) investigated the blood chemistry of male and female adult and sub-adult green turtles in Ras Al-Khaimah (United Arab Emirates) during the month of May, prior to the nesting season. Blood samples were collected within an hour of capture. Although a few of these samples were collected from sub-adults and from males, there were some similarities with our data. Comparing the present electrolyte data, no significant differences of Na^{+}, K^{+}, Ca^{++} and Mg^{++} levels were detected in both species. Plasma electrolyte levels reported by Lutz and Dunbar-Cooper (1987) and Lutz *et al.* (1986) were similar to our data for Na^{+}, Cl^{-} and K^{+}. However Lutz (1997) reported Ca^{++} and Mg^{++} levels that were lower than the values obtained here for olive ridley and hawksbill turtles. The high plasma Ca^{++} levels (mmole/L) in the present study were collected from successfully nesting turtles. Blood samples taken from non-nesting males, or sub-adult turtles, showed lower levels. Moreover olive ridley and hawksbill turtles show a similar range for Na^{+}, Cl^{-}, Ca^{++} and Mg^{++} levels compared to green turtles at Ras Al Hadd, Oman (AlKindi *et al*. 2001).

Nicholson and Lutz (1989) showed that the salt glands in the green turtles maintain the same salt concentration whether the turtles were treated with hypertonic NaCl solutions or hypertonic sucrose solutions. They postulated that the glands regulate salt levels by maintaining relatively constant electrolyte levels. This was attributed to their adjustment to the marine condition. Moreover, Holmes and McBean (1964) found when green turtles transferred from seawater to freshwater, the orbital salt gland is turned off within 25 hrs. after transfer but when the green turtles transferred from freshwater to seawater, the gland became functional 400 hrs. after transfer.

The contents of inactive salt glands in the green turtle are similar to the plasma ionic components that we have measured in olive ridley and hawksbill turtles. However, if salt glands of green, olive ridley and hawksbill turtles are active, the ionic concentration of their glands rise rapidly to an average of 1900 mOsm/l, which is almost six-fold higher than the turtle's plasma level and twice of that of seawater (Lutz, 1997).

In sea turtles, salt efflux is accomplished by the esophagus and the lachrymal salt glands (Lutz, 1997). Sources of salt load are derived from food intake such as sea grasses and algae. Swallowed seawater during feeding (incidental drinking) adds a significant amount of salt load to the body. The response of the animal is an immediate ejection by a powerful esophageal reverse peristalsis to force the water through the nostrils while the food material is held in the esophagus by closely packed conical papillae (Bjorndal, 1985). About 90% of salt secretion from food material occurs in the lachrymal glands. These two methods maintain normal osmolarity in marine turtles and are vital for their survival in a hostile environment.

Our data indicate that chloride and sodium are the major plasma electrolytes in olive ridley and hawksbill turtles during nesting season. Our findings showed high cholesterol levels in olive ridley and hawksbill turtles which were comparable to that of green turtles (AlKindi *et al.* 2001). This can be explained by the blood sampling period, which was conducted during the nesting season, when higher cholesterol levels are vital for the synthesis of steroids and for vitellogenesis. The cloacal temperature in olive ridley and hawksbill turtles showed no significant difference with sand, air and water temperatures.

Plasma urea levels in olive ridley and hawksbill turtles were not significantly different. However, the plasma urea levels values were about seven times higher than plasma urea levels in green turtles that were measured at Ras Al-Hadd, Oman (Al-Kindi *et al*. 2001). This difference may be related to an excess in nitrogenous excretory product at the time of sampling. Urea appears to be a major secretory nitrogenous product in sea turtles. In both studies uric acid levels were very low, which indicates that uric acid is a minor excretory product. Bjorndal (1979) and Nicholson & Lutz (1989) reported urea concentrations from urine or salt glands that are about two to three times

higher than plasma urea in olive ridley and hawksbill turtles. This is not surprising since plasma urea levels are generally much lower than the urea concentrations measured in kidneys or salt glands.

Bolton and Bjorndal (1992) reported mean values for urea (7.0 mg/dl) and uric acid (1.5 mg/dl) which are much higher than the values from the present study. The population of juvenile green turtles, that were used, had higher metabolic rates and therefore a higher output of nitrogenous end products.

The drastic changes in plasma lactate levels have also been observed in nesting green turtles at Ras Al Hadd (AlKindi *et al.* 2000b). When the turtles emerge from the sea they generally go through exhaustive physical exercises during nesting phases. Before a turtle chooses a final site to lay eggs, the female frequently makes several digging attempts at different sites. The abandonment of a site is related to unfavorable conditions such as a lack of soil moisture and soil obstacles. Nesting usually takes between 2.5-4.5hr, sometimes longer. During the nesting process olive ridley and hawksbill turtles show high plasma catecholamine levels which are stress related. AlKindi *et al.* (2000b) measured catecholamine levels in green turtles that first emerged from the sea. They undergo bursts of exhaustive exercise which usually last for less than one minute followed by a recovery period lasting 10-30 seconds for deep breathing (AlKindi, personal communication).

Plasma profiles from natural populations of sea turtles are urgently needed. They can be used to assess the health and normal physiological condition of a population (Rosskopt and Woerpel, 1982; Jacobson *et al.* 1991; Bolton and Bjorndal, 1992). There is a steady decline in sea turtles populations all over the world due to pollution of marine habitats and the destruction of feeding and nesting habitats. As a result, marine turtles are prone to diseases such as e.g., fibropapilloma which was reported in many geographical populations (Jacobson *et al.* 1989; Balazs and Pooley, 1991). Data from this study can be used as a reference for future assessment of nesting populations relative to health condition, reproductive potential, and growth.

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