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Normal Haematology of Free-Living Green Sea Turtles (*Chelonia mydas*) from the United Arab Emirates

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Abstract. A comprehensive health assessment was conducted in May 1997 on free-living green sea turtles (*Chelonia mydas*) occurring off the coast of Ras Al Khaima, United Arab Emirates. As an integral part of this assessment, blood samples were obtained from a total of 72 clinically normal individuals, including 25 adult females, 20 adult males, 14 sub-adults and 13 juvenile turtles to establish normal haematology reference values for free-living green sea turtles.

Introduction

The green sea turtle (*Chelonia mydas*) is a herbivorous species (Mortimer 1995) with an extensive distribution worldwide, but mostly confined between the 20°C oceanic isotherms (Gasperetti et al. 1993). In the Arabian Gulf, the green sea turtle is commonly found associated with the extensive sea grass meadows present in the shallow waters of the region (Anderson 1979, Miller 1989), feeding mainly on sea grasses of the genera *Halodule* and *Halophila* (Gasperetti et al. 1993, CR Hasbun in press). Adult individuals of this species are known to measure 80 to 120 cm curve carapace length (CCL) and weight up to 150 kg or more (Miller 1989, Gasperetti et al. 1993). The recently established Environmental Research and Wildlife Development Agency (ERWDA) initiated the Conservation of Sea Turtle Programme in early 1997. As a component of this programme, a comprehensive health assessment was conducted on the free-living green sea turtle population occurring off the coast of Ras Al-Khaima in the United Arab Emirates. As an integral part of this health assessment, blood samples were collected from individuals in order to establish normal haematology values for free-living green sea turtles.

Materials and Methods

During the month of May 1997, daily morning visits were made to selected sites along the coastline to be present at the time when local fishermen brought to the shore previously cast beach seines. Using this fishing method, sea turtles, but in particular, green sea turtles were often caught accidentally in the seines while feeding on the seagrass meadows. Turtles captured in this way were retrieved from the seines and turned on their backs until each specimen could be handled. While waiting for their turn, turtles were kept cooled by bathing them constantly with sea water. The time lapse between retrieval from the seines and blood sampling ranged between 15 to 45 min.

Each individual was turned back to its original position for blood sampling. A handler held and pulled the head forward to expose the neck for venipuncture. Volvulus or intestinal torsion can occur in large turtles when turned to their backs for restraining purposes. If turtles are returned to the original position, but from the opposite side, as to complete a 360° turn, the intestinal tract is prone to twist due to the large intestinal content mass. Special care was therefore exercised to return turtles back from their opposite side as to avoid a complete 360° turn.

Blood samples were obtained from a total of 72 individuals, including 25 adult females, 20 adult males, 14 sub-adults and 13 juvenile green sea turtles. All individuals were examined by a veterinary surgeon at the time of sampling and were declared clinically normal. A minimum volume of 0.5 ml of blood was obtained from the dorsal cervical sinus, a jugular vein or the ventral caudal sinus using 3, 5 or 10 ml disposable syringes and 19 x 1½ inch, 21 x 1 inch, 23 x 5/8 inch or 25 x 5/8 inch disposable needles depending on the size of the individual. After collection, the samples were mixed immediately with the anticoagulant agent ethylene diamine tetra-acetic acid (EDTA 1.5 mg/ml of blood) in commercially available storage tubes and placed in a cool box. Haemoglobin was estimated as oxyhaemoglobin using an Assistent MP-A 20 haemoglobinometer (Assistent, Germany). The rest of the haematological measurements were carried out as described by Hawkey and Samour (1988) and Samour et al. (1994). The differential count values were converted to relative numbers by multiplying the number of cells counted by the white blood cell count (WBC) and by dividing the resulting number by 100. Red cell and white cell morphometrics were obtained on blood films from 24 different individuals using a micrometer eyepiece under X 100 oil immersion lens. Statistical analysis of haematology values between adult males and adult female turtles and between sub-adult and juvenile turtles was conducted using a Student's unpaired t-test (Microsoft Excel Analysis Toolpack, Microsoft Corporation, USA). A value of $P<0.05$ was considered significant.

All individuals sampled were measured and classified into groups using the CCL as a primary guideline. Thus, in this study the following groups were considered: adult females (CCL >89cm), adult males (CCL >89cm), sub-adult turtles (88cm< CCL > 70cm) and juvenile turtles (CCL <69cm).

Table 1. Normal morphometrics of red blood cells in free-living adult green sea turtles

	Length (n=100)	Width (n=100)
Cytoplasm	19.08 ± 1.32 µm (15-22)	12.6 ± 11.23 µm (10-15)
Nuclei	5.87 ± 0.76 µm (4-7)	4.97 ± 0.73 µm (3-7)

Mean ± Standard Error of Mean
(Minimum-Maximum)

Table 2. Normal morphometrics of white blood cells in free-living adult green sea turtles

Heterophils (n=100)	Basophils (n=12)	Eosinophils (n=100)	Lymphocytes (n=100)	Monocytes (n=50)	Azurophils (n=12)	Thrombocytes (n=100)	
Diameter						Length	Width
15.24 ± 0.09µm (12-17)	12.14 ± 0.26µm (11-13)	20.18 ± 0.19µm (15-25)	11.51 ± 0.09µm (9-14)	14.69 ± 0.35µm (11-21)	12.90 ± 0.36µm (11-15)	10.8 ± 0.28 µm (6-14)	9.68 ± 0.30µm (6-12)

Mean ± Standard Error of Mean
(Minimum-Maximum)

Results

Blood Cell Morphology

The morphometrics of the red and white blood cells in adult free-living green sea turtle are presented in Table 1 and Table 2 respectively.

The red blood cells were large (19.08 µm x 12.6 µm) oval and elongated cells with rounded poles and a slightly basophilic cytoplasm. The nucleus (5.87 µm x 4.97 µm) was dark purple with dense chromatin clumps. The mean length and width of red cells in the present study were within the range of values published by Frair (1977).

The heterophils were large round cells (15.24 µm) with a slightly basophilic cytoplasm containing tightly packed rod-shaped pink-orange granules. The unlobed nucleus was usually round or oval in shape, pale blue in colour and located at the periphery of the cytoplasm. The basophils were small (12.14 µm) round cells with a deep purple nucleus located centrally. The cytoplasm was slightly basophilic and contained loosely-spread dark purple-reddish small granules of various sizes ranging between 0.5 µm - 2 µm. Less than 50 basophils were observed in all the blood films examined. The eosinophils were very large (20.18 µm) round cells with a colourless to slightly basophilic cytoplasm and a round to oval nucleus situated at the periphery of the cytoplasm. The cytoplasm contained several (6 - 14) relatively large pleomorphic pale-red to dark-pink granules of sizes normally between 2 µm to 4 µm. The granules were loosely disseminated in the proximity of the nucleus. The lymphocytes were small (11.51 µm) round or irregular cells and a large round nucleus situated normally at the centre. The cytoplasm was pale blue to pale purple in colour and approximately 10% of the cells observed contained small azurophilic granules. The lymphocytes of green sea turtle were commonly found compressed between two or more red cells in common with the avian lymphocyte. The nucleus to cytoplasm ratio was estimated at approximately 75:25. The monocytes were large (14.69 µm) irregular cells with a pale blue cytoplasm and a round to oval eccentric nucleus. The cytoplasm showed an irregular diffused background revealing a fine netting or lace-like appearance and very often contained small to medium size vacuoles. The nucleus to cytoplasm ratio was estimated at approximately 50:50. The azurophils were relatively small (12.90 µm) round or monocytoid cells with a highly characteristic pale blue to blue metachromic cytoplasm and an oval or round eccentric nucleus. Less than 25 azurophils were observed in all the blood films examined. The thrombocytes were rectangular to round small (10.8 x 9.68 µm) cells with an irregular cytoplasm showing numerous vacuoles. The nucleus was rectangular to round in shape and dark purple-reddish with highly dense chromatin clumps.

Normal haematology reference values

The results of the haematology analysis carried out on free-living clinically normal adult, sub-adult and juvenile green-sea turtles are presented in Tables 3 and 4 respectively. Minor statistical differences existed between adult female and adult male green sea turtles. Adult female turtles had a slightly higher red blood cell count (RBC) than males ($P<0.01$). Conversely, adult male turtles had a slightly higher mean cell haemoglobin (MCH) than females ($P<0.002$). There was a significant statistical difference between the eosinophil count of sub-adult ($P<0.05$) compared with juvenile turtles.

Norton et al. (1990) published haematology values from three clinically normal green sea turtles previously obtained by

Table 3. Normal haematology values in free-living adult female and male green sea turtles.

	Adult females	n	Adult males	n	p value <0.05
RBC (x 10^{12}/l)	0.40±0.095 (0.28-0.64)	25	0.34±0.01 (0.24-0.47)	20	<0.01
HB (g/dl)	9.4±0.3 (5.3-12.4)	25	9.6±0.2 (7.7-11.6)	20	ns
PCV (%)	34.5±1.25 (22-52)	25	33.2±1.3 (23.5-44)	20	ns
MCV (fl)	894.9±43.8 (601.6-1446.4)	25	974.5±37.7 (670.2-1343.8)	20	ns
MCH (pg)	242.2±10.1 (156.3-342.9)	25	284.2±8.1 (210.6-374.2)	20	<0.002
MCHC (g/dl)	27.4±0.7 (20-32.5)	25	29.7±1.1 (22.8-45.5)	20	ns
WBC (x10^9/l)	1.88±0.2 (0.2-4.3)	25	2.1±0.2 (0.6-3.6)	20	ns
Heterophils (x10^9/l)	1.09±0.12 (0.11-2.84)	25	1.38±0.16 (0.46-2.92)	20	ns
Eosinophils (x10^9/l)	0.30±0.06 (0.00-1.26)	25	0.30±0.06 (0.00-0.91)	20	ns
Basophils (x10^9/l)	0.008±0.003 (0.00-0.078)	25	0.007±0.003 (0.00-0.041)	20	ns
Lymphocytes (x10^9/l)	0.41±0.05 (0.02-0.87)	25	0.36±0.04 (0.05-0.92)	20	ns
Monocytes (x10^9/l)	0.05±0.01 (0.00-0.13)	25	0.06±0.01 (0.00-0.16)	20	ns
Azurophils (x10^9/l)	0.014±0.004 (0.00-0.060)	25	0.020±0.007 (0.00-0.125)	20	ns
Thrombocytes (x10^9/l)	1.71±0.43 (0.18-7.1)	17	1.67±0.43 (0.1-8.2)	19	ns
Fibrinogen (g/l)	2.61±0.5 (0.8-6.5)	11	1.68±0.26 (0.5-4.45)	15	ns

Mean ± Standard Error of Mean
(Minimum-Maximum)
ns: not significant

Dr. ER Jacobson at the College of Veterinary Medicine, University of Florida. Unfortunately, the age of these three specimens was not indicated, making difficult the comparison of those values with the results obtained in the present study. Nevertheless, the mean values published by Norton et al. (1990) for WBC of 1.2 x 10^9/l, haemoglobin of 10.1 g/dl, packed cell volume (PCV) of 36 %, mean cell haemoglobin concentration (MCHC) of 28.06 g/dl, heterophil count of 1.05 x 10^9/l, lymphocyte count of 0.08 x 10^9/l and monocyte count of 0.06 x 10^9/l were within the range of values obtained in this study. The mean RBC value published by Norton et al. (1990) of 0.61 x 10^{12}/l was within the range of values obtained for juvenile and adult female turtles in the present study. However, this was slightly higher than the upper value obtained for sub-adult turtles and considerably higher than the upper value obtained for adult male turtles in this study. The mean RBC value of 0.53 x 10^{12}/l obtained by Frair (1977) was within the range of values obtained for juvenile, sub adult and adult female turtles, but higher than the upper limit value in male green sea turtles in the present study. The mean cell volume (MCV) published by Norton et al. (1990) of 590 fl was within the range of values obtained for juvenile turtles, but lower than the minimum values obtained for sub-adult and adult green sea turtles in this study.

The mean PCV value of 35.2 % published by Bolten and Bjorndal (1992) for free-living juvenile green sea turtles in the Southern Bahamas was slightly higher than the mean value of 32.3 % obtained for juvenile specimens in the present study. Conversely, the mean PCV value of 29.2 % obtained by Frair (1977) was considerable lower than the value published by Bolten and Bjorndal (1992) and the value obtained in the present study for juvenile, sub-adult and adult green sea turtles.

Wood and Ebanks (1984) studied age-related haematological changes in farm-reared green sea turtles in the Grand Cayman island, British West Indies. In this study, all individuals were classified according to age and mean body weight. It is difficult to compare the haematology values obtained by Wood and Ebanks (1984) and those obtained in the present study since different criteria were used to group the specimens sampled. However, the mean PCV values for all groups of

Table 4. Normal haematology values of free-living sub-adult and juvenile green sea turtles.

	Sub-adult	n	Juvenile	n	p value <0.05
RBC (x 10^{12}/l)	0.38±0.02 (0.25-0.59)	14	0.42±0.03 (0.25-0.68)	13	ns
HB (g/dl)	9.08±0.40 (6.2-11.4)	14	8.8±0.3 (7.1-10.7)	13	ns
PCV (%)	33.5±1.6 (24.5-41.5)	14	32.3±1.5 (26.5-44)	13	ns
MCV (fl)	905.5±41.1 (627.1-1187.5)	14	827.6±85.9 (561.2-1760)	13	ns
MCH (pg)	248.5±15.3 (159-332)	14	225.7±21 (142.6-428)	13	ns
MCHC (g/dl)	27.4±1.0 (21-34.5)	14	27.4±0.6 (24.3-30.3)	13	ns
WBC (x10^9/l)	2.08±0.27 (0.95-4.5)	14	1.84±0.24 (0.47-3.2)	13	ns
Heterophils (x10^9/l)	1.43±0.27 (0.43-3.92)	13	1.29±0.17 (0.27-2.18)	12	ns
Eosinophils (x10^9/l)	0.25±0.06 (0.00-0.58)	13	0.11±0.03 (0.00-0.34)	12	<0.05
Basophils (x10^9/l)	0.006±0.003 (0.00-0.032)	13	0.008±0.004 (0.00-0.04)	12	ns
Lymphocytes (x10^9/l)	0.33±0.06 (0.10-0.93)	13	0.40±0.08 (0.04-0.98)	12	ns
Monocytes (x10^9/l)	0.07±0.02 (0.00-0.25)	13	0.12±0.02 (0.00-0.30)	12	ns
Azurophils (x10^9/l)	0.013±0.005 (0.00-0.042)	13	0.028±0.011 (0.00-0.096)	12	ns
Thrombocytes (x10^9/l)	0.95±0.19 (0.23-2.4)	12	0.71±0.14 (0.1-1.2)	8	ns
Fibrinogen (g/l)	2.7±0.63 (1.0-5.63)	8	2.7±0.8 (0.5-5.7)	6	ns

Mean ± Standard Error of Mean
(Minimum-Maximum)
ns: not significant

green sea turtles in the present study were within the range of values published by Wood and Ebanks (1984) for similar specimens. However, the upper value for PCV obtained by Wood and Ebanks (1984) for all the groups were generally higher than the upper values estimated for similar groups of individuals in the present study. The mean haemoglobin (Hb), RBC and WBC values obtained in this study for juvenile, sub-adult and adults green sea turtles were within the range of values published by Wood and Ebanks (1984).

Wood and Ebanks (1984) described five different types of white cells in a haematology study carried out on farm-reared green sea turtles. The nomenclature and description of white blood cells used by these authors was based on information published by Saint Giron (1970). It is difficult to evaluate the data obtained by Wood and Ebanks (1984) since some of the white blood cells appeared to have been misidentified. Thus, the differential count and the description and measurements of the white blood cells obtained by Wood and Ebanks (1984) were very different to the results obtained in the present study. The classification of the white blood cells of green sea turtles in the present study was based on current haematology nomenclature and description as described by Hawkey and Dennett (1989).

Discussion

Green sea turtles retrieved from the beach seines appeared in good health and were not injured during the fishing procedure. It was estimated that the specimens remained within the seines for up to 4 to 6 hours. The turtles were not entangled in the seines *per se*, but remained swimming within the area encircled by the seine. Although the turtles were "caught" and remained within the seines for a relatively long period of time, it was not considered that this could have affected the haematology values obtained.

Blood samples were successfully collected from the turtles in this study using the jugular vein, the dorsal cervical sinus or the ventral caudal sinus, although approximately 85% of the samples were obtained from the jugular vein. Usually, blood collection was attempted using the jugular vein as the route of choice, but in some cases sampling was only successful after several attempts. This occurred mainly with adult individuals and it was attributed to the difficulty in restraining the neck of these rather large and powerful turtles. The dorsal cervical sinus and the ventral caudal sinus were used only as alternative venipuncture sites.

A similar phenomenon as that described by Hart et al. (1991), when collecting blood samples from Aldabra giant tortoises (*Geochelone gigantea*), was also encountered with some specimens of green sea turtles in this study. After positioning the needle and suction was initiated, a clear fluid, instead of venous blood, flowed into the syringe. This phenomenon was observed when collecting samples mainly from the jugular vein and never from the dorsal cervical sinus. This was not observed when sampling from the ventral caudal sinus since blood samples were obtained from three individuals only using this route. The possibility that the clear fluid could have been extracellular in origin was discarded on anatomical grounds in agreement with Hart et al. (1991). It was concluded that sludging of circulating blood cells in the jugular vein may have been the reason for this observation, although there is no hard evidence to support this. However, it is interesting to note that the clear fluid has been observed only during venipuncture of the dorsal coccygeal vein in Aldabra giant tortoises by Hart et al. (1991) and the jugular vein in green sea turtle during the present study. To the knowledge of the authors the clear fluid has never been reported during cardiac puncture or venipuncture of the dorsal cervical sinus, which are the most common ways of collecting blood samples from sea turtles (Frair 1977, Bolten and Bjorndal 1992, Wood and Ebanks 1984). It is beyond the scope of this report to discuss this particular issue, but it cannot longer be ignored and it should be the subject of further studies. In this respect, two samples of pure clear fluid obtained from two different green sea turtles were sent to the Central Veterinary Research Laboratory in Dubai, UAE for biochemical analysis. The results obtained were very similar to those values of blood plasma for the same species. Clearly, further work is needed around this topic. Nonetheless, the blood samples analysed in this study were of venous origin and samples containing this clear fluid were discarded and a fresh sample was obtained for analysis.

Considering the duration of dives of sea turtles and their need to store oxygen, it is likely that the RBC's have such a large size in order to carry more oxygen. Sea turtles are known to dive for extended periods of time in search for food. Gitschlag (1996) reported an average submergence time of 63.2 min from a Kemp's ridley turtle (*Lepidochelys kempii*) in the Gulf of Mexico. He also recorded the submergence at one time of 217 min. Byles (1989) reported a maximum duration of dive of 100 min for the same species. Sea turtles are also known to dive at great depths. Kooyman (1972) reported an adult green turtle feeding as low as 290 m. Leatherback turtles (*Dermochelys coriacea*) are known to dive deeper and have been recorded at 475 m (Eckert et al. 1984). Vandam and Diez (1996) studied the diving behaviour of the hawksbill (*Eretmochelys imbricata*) sea turtle and inferred that dives were aerobic with turtles making use of oxygen stores in addition to that of the lungs.

The results presented in this report represent haematology reference values of a group of adult, sub-adult and juvenile green sea turtles from a particular population and during a specific time of the year. These results form the first set of data on normal reference values for this species in the United Arab Emirates. The results of the other aspects of the health assessment such as clinical and pathological findings, blood chemistry and microbiological analysis, including protozoology, bacteriology and mycology investigations will be published elsewhere.

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