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Characterization of Bone Marrow and Development of a Bone Marrow Biopsy Technique in Green Turtles (*Chelonia mydas*)

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Abstract: The gross location, macroscopic and microscopic characteristics of bone marrow were described in 6 juvenile green turtles (*Chelonia mydas*) from the Hawaiian islands. Of the sites sampled, the white stromal areas of the humerus, radius, ulna, hemipelvis, femur, tibia, and fibula appeared to be the good locations for collecting bone marrow core biopsy samples for cytologic and histologic preparation. The bone marrow samples from black stromal regions of the marginal scutes of the carapace also demonstrated good cellularity and maintenance of normal cellular morphology. The 9th marginal scute of the carapace was determined to be an optimal site for non-invasive bone marrow core biopsy sampling. This study reports a successful bone marrow collection technique in live sea turtles. Characteristic features of bone marrow including lipid globules and erythrocytic and granulocytic precursors were seen in cytologic preparations of bone marrow sampled by this method. Histologic and cytologic characterization of bone marrow are pending

INTRODUCTION

Central erythropoiesis and granulopoiesis in green turtles (*Chelonia mydas*) occur in the bone marrow as in other reptiles. Although the processes of blood cell production are poorly understood in reptiles, it appears that the steps of cellular development involved are similar to mammals (Hawkey and Dennet, 1989). Normal bone marrow characterization has been reported for reptiles including terrestrial turtles (Frye, 1991; Garner et al. 1996); however it has not been performed for sea turtles.

Indications for bone marrow examination include chronic or nonregenerative anemias, leukopenia, pancytopenia (aplastic anemia), thrombocytopenia, heteropenia, blood cell dyscracia, neoplasia of the hematopoietic and reticuloendothelial systems and other atypical cellular changes in the peripheral blood (Campbell, 1996; Jenkins, 1996). No techniques have yet been developed for the non-invasive collection of bone marrow samples in live sea turtles, thus precluding elucidation of the pathology of many diseases that may lead to one of the many indications for bone marrow examination.

The objectives of this study were to examine and describe the bone marrow of green turtles at a gross anatomical, macroscopic, and microscopic level, and to identify an optimal location for the non-invasive sampling of bone marrow in live turtles for diagnostic purposes. Further studies are concurrently being conducted to characterize normal bone marrow through cytologic and histopathologic evaluation and to compare those results with those of severely anemic diseased turtles.

MATERIALS AND METHODS

Bone marrow was collected from a total of 6 juvenile green turtles (2 males, 2 females, and 2 turtles of undetermined sex) from the Hawaiian islands (Kauai, Oahu, and Maui) during the months of July and August. All the turtles were free-ranging turtles that had stranded and been retrieved by the Stranding Network of the National Marine Fisheries Service. Four of the turtles were euthanized due to

poor prognosis from either debilitating injuries or heavy infestation by fibropapillomatosis tumors, and were necropsied and sampled immediately after euthanization. Two of the turtles with more favorable prognosis were undergoing rehabilitation for head trauma injuries, and were sampled live for bone marrow core biopsies.

For the 4 euthanized turtles the long bones and shell were sectioned in order to document the gross anatomical location of bone marrow stroma, and to describe its characteristics in the various sites. The long bones (humerus, radius, ulna, hemipelvis, femur, tibia, fibula, phalanges) were sectioned sagitally, and the carapace and plastron were sectioned transversely (**Figure 1**).

Bone marrow was collected from eight different sites from the 4 euthanized turtles. These sites included the humerus, ulna, hemipelvis, femur, tibia, fibula, marginal scute of carapace, and the gular plate of the plastron, in addition to a few additional sites (radius and phalanx) for the first turtle sampled. Bone marrow biopsy cores were collected from sectioned bones for the sites listed above for cytologic and histologic preparation. An 11 gauge, 4" bone marrow biopsy needle was used to collect core pieces of bone marrow stroma.

For cytologic preparation, two different techniques were implemented to prepare the bone marrow slides: 1) a touch impression method and 2) a saline soak technique previously used to characterize bone marrow in euthanized desert tortoises (Garner et al., 1996). Duplicate bone marrow cores were taken from the sectioned bones for both techniques. The touch impression slides were prepared using forceps to gently roll and dab the bone marrow cores against a glass slide to create an in situ impression of the bone marrow, and then air dried. For the saline soak technique, a bone marrow core was soaked in 1 ml of a heparin-PBS solution (5 ml of 1X PBS into a Lithium heparin Vacutainer) in an eppendorf tube. The samples were set aside for either immediate slide preparation or for an overnight soak (~18 hours) at 4°C. The saline soaked samples were then vortexed for 30 seconds to 1 minute. The saline solution containing the marrow cells was collected in fresh tubes (to avoid large particulate matter), leaving pieces of the trabecular core behind, and centrifuged (14,000rpm) for 5 min. The supernatant was discarded and the pelleted cells were gently resuspended in 100 μl of 1X PBS. The cell mixture was then concentrated onto a slide using a manual method, following the principles of a cytofuge apparatus, and then air dried. The slides prepared for cytologic examination by both methods were then stained with a modified Wright-Giemsa stain (Fisher Diagnostics LeukoStat stain).

Bone marrow core samples were also used for histologic preparation. The cores were placed in tissue cassettes in 10% formalin and sent to a diagnostics laboratory for decalcification, sectioning, and Giemsa and H&E staining.

Using light microscopy, a subjective scoring system was implemented to evaluate the differences between bone marrow stroma color, techniques used to prepare cytology samples (touch impression, saline soak, and saline soak for 18 hours), and sample sites. Slides were given a subjective score on a scale of 1 - 3 (1 = poor/few, 2 = fair/intermediate, 3 = good/numerous) based on quality and quanitity of cells. Quality of slide preparations was determined by the degree of maintenance or alteration (distortion

and/or loss of cell membrane integrity) of the normal cell architecture and morphology. The quanitity score was determined by the degree of cellularity observed by scanning the slide with the microscope.

Since proper evaluation of the hematopoietic system involves careful evaluation of the peripheral blood hemogram at the same time of bone marrow biopsy (Campbell, 1996), turtles were bled by venipuncture of the dorsal postoccipital sinus (Owens and Ruiz, 1984). Duplicate blood smears from a direct unheparinized blood sample were made immediately after blood collection. Blood smear films were stained with a modified Wright-Giemsa stain. The remainder of the blood sample was placed in a Lithium-heparin Vacutainer tube for analysis of pack cell volume, total solids (protein), granulocyte count (using an eosinophil unopette #5877), and differential and absolute white blood cell count.

RESULTS

Bone Marrow Biopsy Technique

A successful non-invasive technique was developed for the collection of bone marrow in live sea turtles. The site chosen for collecting bone marrow core biopsies was the 9th marginal scute on either side of the turtle. Two core biopsies were collected for each of the two live turtles sampled, one from both the right and left side, to ensure the preparation of a touch impression slide with good cellularity and morphology. With the turtle supine, lying on its carapace, the underside of the 9th marginal scute was disinfected with alcohol. An 11 gauge, 4" bone marrow biopsy needle, with the stylet removed, was used to bore through the epidermal and cortical bone layer of the scute, entering at a point along the "line of the marrow," which lies about one-third of the distance from the lateral edge of the scute (**Figure 2.**). The biopsy needle was inserted at an angle perpendicular to the surface of the scute to allow for the shortest distance of penetration through the tough cortical layer of bone. Once through the cortical layer (usually a few millimeters, variable depending on the size of the turtle), the needle was further inserted, with a rotational motion, at a 45°angle to the scute surface proceeding toward the dorsal side, while keeping to the "line of the marrow" (**Figure 2.**). This angling of the needle allowed for the collection of more bone marrow stroma (longer core biopsy). Once greater resistance was felt when approaching the cortical layer on the dorsal surface of the marginal scute, the biopsy needle was retracted with a back-and-forth rocking motion, so as not to lose the core while pulling out the needle. The core biopsies were then used to prepare touch impression slides following the procedure described above. After making the impression onto the glass slides, the cores were placed in 10% formalin for histologic preparation and evaluation. Before returning the turtles to the water, a few drops of Nexaband surgical glue was placed into the biopsy site to avoid bleeding and infection.

Bone marrow was recognized as a meshwork of trabecular bone in the long bones and shell of the green turtles. Two types of bone marrow stroma were noted by macroscopic observation: black and white (**Figure 1**). The black stroma, which appeared as dark regions of trabecular bone, was most often observed in the diaphyses of all the long bones sampled (humerus, radius, ulna, hemipelvis, femur, tibia,

fibula, and phalanges), and comprised almost the entirety of the marrow stroma seen in the carapace and plastron. The femur and phalanx sampled showed mostly black marrow stroma with lesser quantities of white stroma. Microscopically, the black stroma showed a high quantity of small greenish-black granules (about 1μm in length) that often appeared in aggregated clusters. Also, fewer lipid droplets were noted in the black stroma. The white marrow stroma was most often observed in the proximal and/or distal metaphyseal regions of the long bones such as the humerus, radius, ulna, hemipelvis, tibia, and fibula. It was observed as white to yellowish meshwork of trabecular bone and appeared to be more "wet" than the black stroma. Touch impressions of the cores were achieved with greater ease from the white stromal regions due to the greater quantities of cellular fluid encompassing the core biopsies. The black and white marrow regions were sampled for each of the sites where both types were observed. For sites, where black stroma was predominant (carapace, plastron, and phalanx), only those samples were taken. Very minute amounts of yellow gelatinous marrow were occasionally observed when coring out the trabecular bony stroma from the metaphyseal ends of the long bones. However, the the exact location and quantity of gelatinous marrow were highly inconstant.

Upon gross observation, specific regions of the bones sampled were noted as more optimal (by exhibiting greater density of white marrow and/or greater amounts of cellular fluid surrounding core samples) for the collection of bone marrow cores to prepare touch impression slides: Humerus - lateral to the head of the humerus and medial to the greater tubercle; Ulna - distal metaphyseal region; Hemipelvis - in the ilium just lateral to the acetabulum; Femur - distal metaphyseal region; Tibia - proximal metaphyseal region; Fibula - proximal metaphyseal region; Carapace - marginal scutes 7-9; Plastron - central to lateral region of gular plate. Although the distal metaphyseal region of the radius showed good potential as a sample site, its small size, as with the phalanges, made bone marrow sample collection difficult.

The results of the subjective scoring of the bone marrow cytology samples are shown in **Table 1**. The white marrow stroma appeared to show overall better quality of cells than the black stroma, and also greater overall cellularity when compared with the black for samples collected by the touch impression technique (the saline soak technique would show great variability due to the lack of uniformity in the amount sampled and quantity of cells released through the sample preparation). In comparing techniques, the touch impression method appeared to show consistently higher quality of cells. Comparisons of quantity score between the varying techniques did not seem appropriate due to the lack of uniformity in the sample quanitity used to prepare each slide. For the evaluation of sample sites, only the touch impression slides were considered. The pelvis appeared to be a consistently optimal site for obtaining bone marrow samples showing both high cellularity and good cellular morphology. The humerus, ulna, femur, tibia, fibula, and carapace all appeared to be fairly intermediate to good sites as well. The plastron and phalanges appeared to be sites with less potential for obtaining useful bone marrow samples due to relatively low cellularity and poor cellular morphology.

DISCUSSION

Most all of the bone marrow core samples collected from the green turtles contained hard cortices of trabecular bone, interlaced with bone marrow. In contrast to the findings of desert tortoises in which no gelatinous marrow was described, very minute amounts of yellow gelatinous bone marrow was observed in the metaphyseal regions of some long bones. This finding is consistent with evidence that some reptiles of the order Chelonia have shown evidence of gelatinous marrow in their bones (Pienaar, 1962; Jenkins, 1996). However, the minuteness and inconstancy of the exact location where the gelatinous marrow can be found preclude the use of the bone marrow aspirate technique as a useful diagnostic tool in this species.

The major difference between the white and black stroma may be the presence of melanin precipitates which appear as small greenish-black granules that tend to be found in clusters within the trabeculae (Garner et al., 1996). From the results in **Table 1.** white marrow stroma generally appears to give better results for cytological preparation than does black stroma. This study demonstrates that black stroma from a few sites, such as the carapace, gives results sufficient for diagnostic purposes when made into touch impression slides (**Figures 3a. and 3b**). Hence, the characterisitic cellularity of a particular site may likely be due to its particular role in the hematopoietic system, rather than as a correlation to the color of its stroma. Histologic evaluation of both types of bone marrow stroma may help to determine whether a correlation exists between cellularity and stroma color, or sample site.

Evaluation of the touch impression and saline soak techniques demonstrated that the former was considerably more useful for cytologic studies. The touch impression method was useful in maintaining the normal cellular architecture and morphology, as well as in providing an in situ look at the bone marrow cells and particles. The photomicrographs in **Figures 3a. and 3b.** show characteristic features of bone marrow as it may appear in intact bone marrow. Lipid globules, erythrocytic and granulocytic precursor and mature cells, along with lymphoid cells are all seen in these photomicrographs. The disadvantage to the touch impression method is that it may produce on occasion, slides with low cellularity, especially when small juvenile turtles are sampled.

For sites sampled, the humerus, radius, ulna, pelvis, femur, tibia, fibula, and the hemipelvis were all good potential sites with sufficient cellularity, based on the subjective scoring system, to be useful for diagnostic purposes. Upon macroscopic and microscopic evaluation, the pelvis appeared to be a particularly optimal for collecting bone marrow samples in terms of cellularity and morphology of cells. However, access to an optimal pelvic site involves the need for general anesthesia, invasive surgery, including the incision of large muscles. The pectoral limb bones could also be good diagnostic sites for collecting bone marrow, but tend to be less attractive sites because of the heavy reliance of these sea turtles on their pectoral flippers. Any potential adverse effects from a biopsy wound may cause serious locomotive impairment for the turtles, especially if entry into the long bones involved exposure to the joint capsules.

This study reports a successful non-invasive bone marrow biopsy technique in live sea turtles. This technique may be conducted non-invasively both in captivity and in the field. The carapace was chosen as the best candidate site for sampling in live turtles. Biopsy cores may be collected on an unanesthetized animal, with no more restraint than needed for venipuncture, and minimal likelihood of causing physical trauma or localized infection. One live sampled turtle was observed two months after the initial procedure. The animal appeared to be healing normally. Since the development of this procedure, 10 more green turtles have been sampled using this technique. **Figure 3.** demonstrates the usefulness of this technique in obtaining important diagnostic information relating to normal and abnormal hematopoiesis. Further studies are currently being conducted to characterize normal bone marrow in green turtles, analyse bone marrow differential values, compare myeloid:erythroid ratios in severely anemic turtles with those of turtles with normal PCV's, and determine normal and abnormal cellularity through histologic evaluation.

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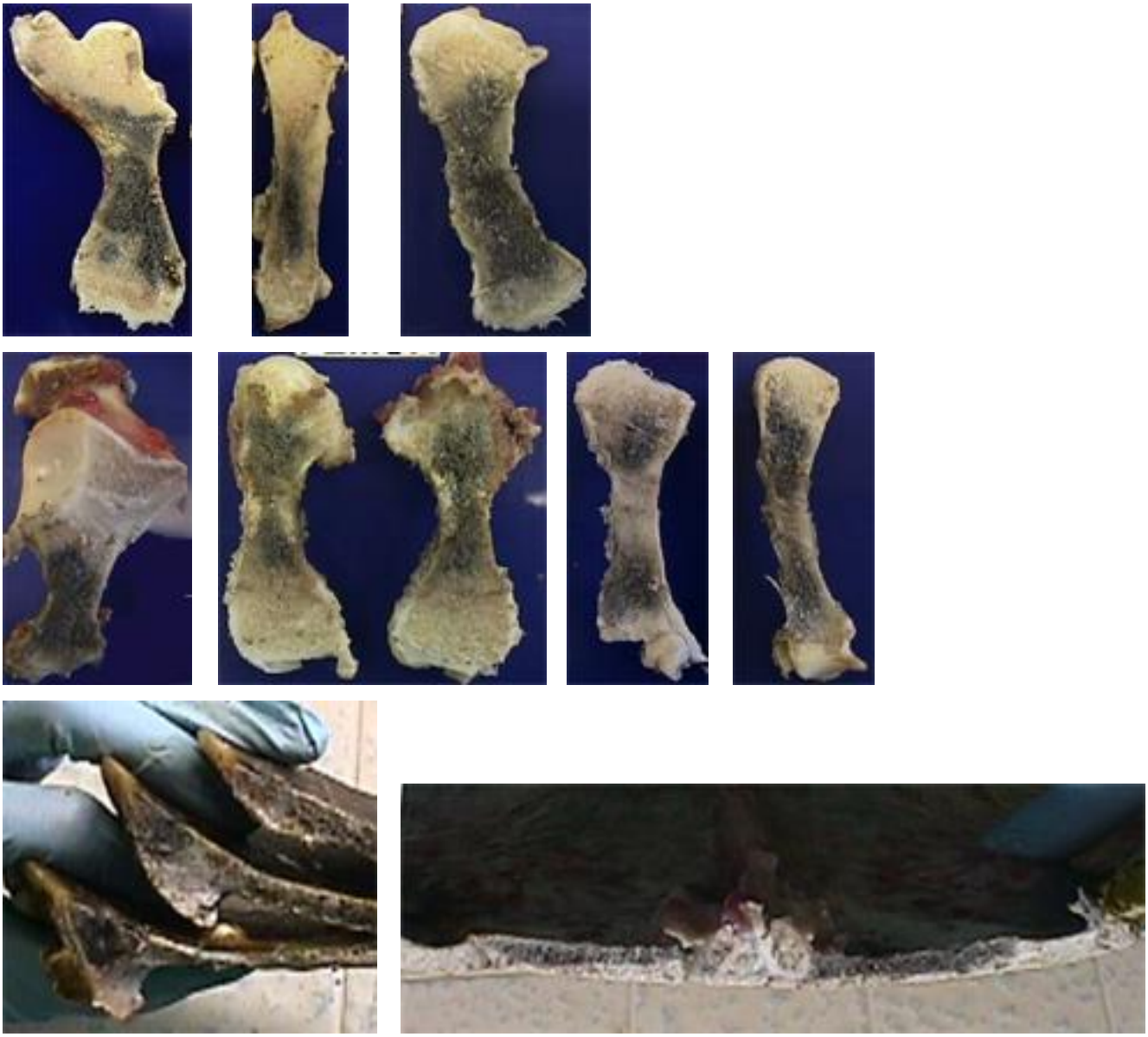

Figure 1. Longitudinal sections of long bones and transverse sections of shell of green turtle. Top row from left to right: humerus, radius, ulna. Middle row: pelvis, femur, tibia, fibula. Bottom row: carapace and marginal scutes 10, 9, 7 (top to bottom), plastron.

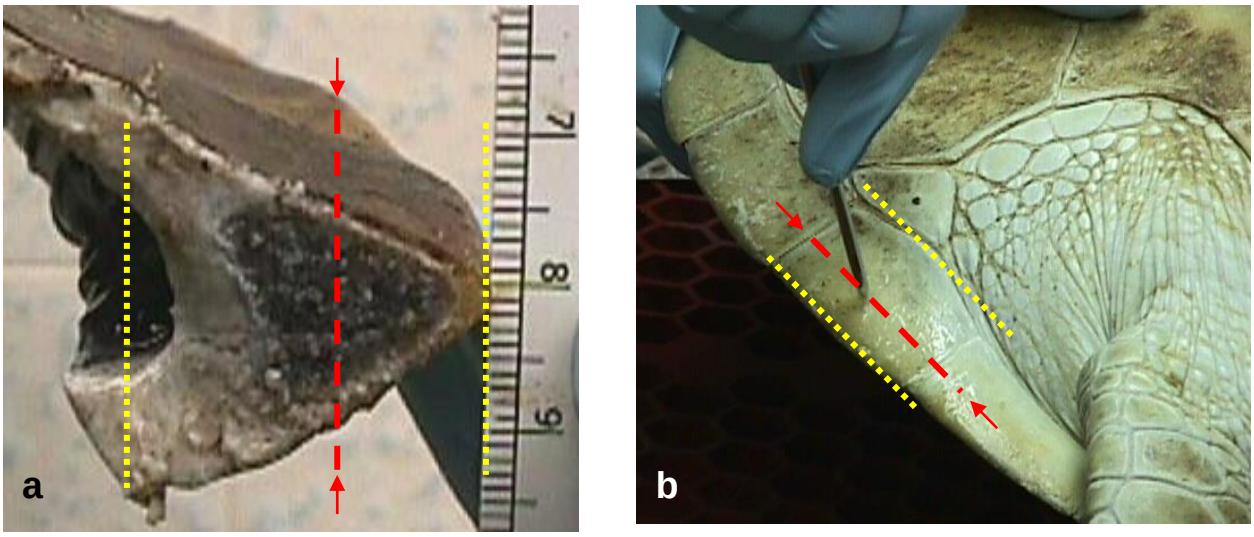

Figure 2. a) Bone marrow biopsy site (red dashed line) along line 1/3 of the way medial from outer edge of marginal scute to thickest part of scute. b) Medial and lateral margins of 9th marginal scute (yellow dotted lines); Bone marrow biopsy site - along red dashed line.

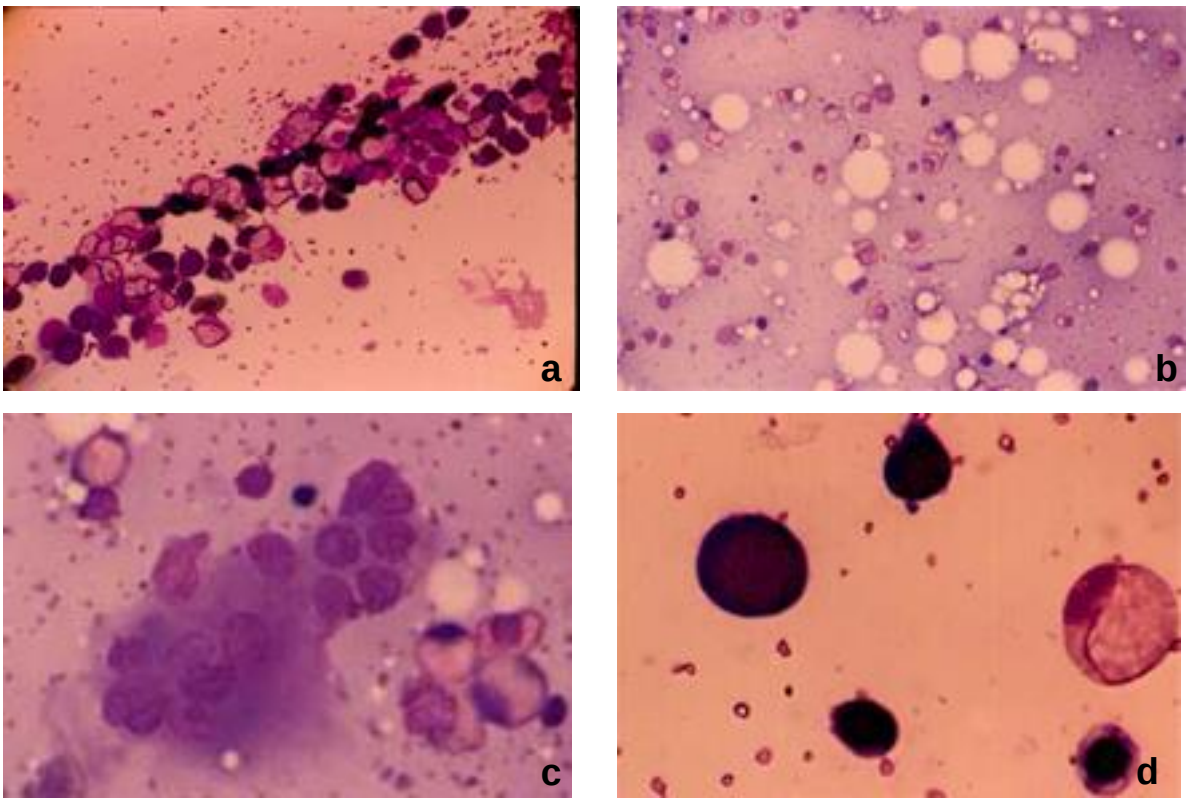

Figure 3. Photomicrographs of bone marrow touch impression slides sampled from carapace of green turtles stained with modified Wright-Giemsa stain. a) Erythrocytic and granulocytic precursors and mature cells, and lymphoid cells. b) Lipid droplets characteristic of bone marrow. c) Osteoclast. d) Rubriblast and other erythrocytic precursor cells, and mature heterophil.

Table 1. Subjective scoring system used to evaluate differences in cytologic preparations of bone marrow by stromal color, technique used for cytologic preparation, and sample site. Scored on scale of 1 - 3 (1 = poor/few, 2 = fair/intermediate, 3 = good/numerous) based on quality and quanitity of cells.

	Quality Score - mean	n =	**Quantity Score** - mean	n =
Marrow stroma color:				
Black stroma	1.55	33	2.00	12
White stroma	2.06	18	2.83	6
Technique:				
Touch impression	2.26	53	2.38	53
Saline soak - fresh	1.60	52	2.12	52
Saline soak - 18 hrs	1.47	17	1.88	17
Sample sites:				
Humerus	2.20	5	2.60	5
Ulna	2.40	5	2.20	5
Radius	3.00	3	2.50	3
Hemipelvis	2.80	5	2.60	5
Femur	2.20	5	2.4	5
Tibia	2.67	6	2.33	6
Fibula	2.60	5	2.60	5
Carapace - marginal scute	2.43	7	2.43	7
Plastron - gular plate	2.00	7	2.29	7

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Turtle Bone Marrow - Histology Evaluation

1. **Sample quality** - based on quality of cells on slide preparation and quantity of cells present.
    - Excellent
    - Good
    - Moderate
    - Poor
2. **Myeloid : Erythroid Ratio** - 200 cell count; noting absolute numbers as well as ratio
    - myeloid cells - granulocytes, monocytes (non-erythroid, non-lymphoid)
    - erythroid cells
3. **Erythropoiesis evaluation** - describe based on maturation pyramid (generally fewer immature & more mature)
    a. **orderly** (follows maturation pyramid) or **bulge** (where - top (very immature), middle, bottom (mature))
    b. **completion** (see mature cells) or **maturation arrest**
4. **Granulopoiesis evaluation -** same as above
    a. **orderly** or **bulge**
    b. **completion** or **maturation arrest**
5. **Other cell types -** note
    - lymphocytes- note as percent; # seen during 200 cell count for M:E ration
    - osteoclasts
    - spindle cells
    - etc.
6. **Melanin granules**
    - present
    - absent
7. **Notes**

Cytology Sample Scoring

Turtle ID #	Sample Site	Stroma color	Technique	Approach	Quantity	Quality	notes	tumor status
15216	humerus	black	tp	inside	1	1		non-tumored
15216	humerus	white	tp	inside	2	3		non-tumored
15216	humerus	black	ss	inside	1	1	black granules	non-tumored
15216	humerus	white	ss	inside	2	2		non-tumored
15216	humerus	black	ss-18	inside	1	1	black granules	non-tumored
15216	humerus	white	ss-18	inside	3	2		non-tumored
15216	femur	black	tp	inside	3	3	mostly black; black granules	non-tumored
15216	femur	black	ss	inside	2	2	mostly black	non-tumored
15216	femur	black	ss-18	inside	2	2	mostly black; black granules	non-tumored
15216	pelvis	black	tp	inside	1	1		non-tumored
15216	pelvis	white	tp	inside	3	3		non-tumored
15216	pelvis	black	ss	inside	1	1	black granules	non-tumored
15216	pelvis	white	ss	inside	3	2		non-tumored
15216	pelvis	black	ss-18	inside	1	1	black granules	non-tumored
15216	pelvis	white	ss-18	inside	2	2		non-tumored
15216	radius	black	tp	inside	3	2	black granules	non-tumored
15216	radius	white	tp	inside	3	3		non-tumored
15216	radius	black	ss	inside	1	1	black granules	non-tumored
15216	radius	white	ss	inside	2	1	few black granules	non-tumored
15216	radius	black	ss-18	inside	2	2	black granules	non-tumored
15216	radius	white	ss-18	inside	1	1		non-tumored
15216	ulna	black	tp	inside	1	3	black granules	non-tumored
15216	ulna	white	tp	inside	3	3		non-tumored
15216	ulna	black	ss	inside	2	1	black granules	non-tumored
15216	ulna	white	ss	inside	1	2		non-tumored
15216	ulna	black	ss-18	inside	3	1	black granules	non-tumored
15216	ulna	white	ss-18	inside	2	2		non-tumored
15216	tibia	black	tp	inside	3	2	black granules	non-tumored
15216	tibia	white	tp	inside	3	3		non-tumored
15216	tibia	black	ss	inside	1	1		non-tumored
15216	tibia	white	ss	inside	1	1		non-tumored
15216	tibia	black	ss-18	inside	2	2	black granules	non-tumored
15216	tibia	white	ss-18	inside	2	1		non-tumored
15216	fibula	black	tp	inside	3	3	high # fat globules	non-tumored
15216	fibula	white	tp	inside	3	3		non-tumored
15216	fibula	black	ss	inside	1	1	black granules	non-tumored
15216	fibula	white	ss	inside	1	1	black granules	non-tumored
15216	fibula	black	ss-18	inside	2	1	black granules	non-tumored

Cytology Sample Scoring

Turtle ID #	Sample Site	Stroma color	Technique	Approach	Quantity	Quality	notes	tumor status
15216	fibula	white	ss-18	inside	2	2		non-tumored
15216	phalanx 2; digit 3	black	tp	inside	1	1	mostly black	non-tumored
15216	phalanx 1	black	ss	inside	1	1	mostly black	non-tumored
15216	phalanx 1	black	ss-18	inside	1	1	mostly black; black granules	non-tumored
15216	carapace 4L	black	tp	inside	2	2		non-tumored
15216	carapace 4L	black	ss	inside	1	1		non-tumored
15216	carapace 4L	black	ss-18	inside	1	1		non-tumored
15216	carapace 6L	black	tp	inside	3	3		non-tumored
15216	carapace 6L	black	ss	inside	2	1		non-tumored
15216	carapace 6L	black	ss-18	inside	3	2		non-tumored
15216	plastron	black	tp	inside	3	3		non-tumored
15216	plastron	black	ss	inside	2	1		non-tumored
15216	plastron	black	ss-18	inside	2	1	black granules	non-tumored
15222	carapace		tp	inside	3	3		tumored
15222	carapace		ss	inside	3	3		tumored
15222	carapace		tp	external	2	2	too thick to coverslip	tumored
15222	carapace		ss	external	3	2		tumored
15222	plastron - gular		tp	inside	3	3	***good slide	tumored
15222	plastron - gular		ss	inside	3	2	cells - too dense on slide	tumored
15222	plastron - gular		tp	external	1	1		tumored
15222	plastron - gular		ss	external	1	1		tumored
15222	plastron - gular		tp	external	1	3		tumored
15222	plastron - anal		tp	external	2	1		tumored
15222	plastron - anal		ss	external	1	1		tumored
15222	humerus		tp	inside	3	3	*** good slide	tumored
15222	humerus		ss	inside	3	2	too thick to coverslip	tumored
15222	humerus		tp	external	1	2		tumored
15222	humerus		ss	external	1	2		tumored
15222	ulna		tp	inside	3	2		tumored
15222	ulna		ss	inside	3	1		tumored
15222	ulna		tp	external	3	3		tumored
15222	ulna		ss	external	3	3		tumored
15222	radius		tp	inside	3	2	too thick to coverslip	tumored
15222	radius		ss	inside	3	2		tumored
15222	phalange		tp	inside	2	1		tumored
15222	phalange		ss	inside	3	2		tumored
15222	pelvis		tp	inside	3	3	*** good slide	tumored
15222	pelvis		ss	inside	3	2	cells - too dense on slide	tumored

Cytology Sample Scoring

Turtle ID #	Sample Site	Stroma color	Technique	Approach	Quantity	Quality	notes	tumor status
15222	pelvis		tp	external	2	3	*** good slide	tumored
15222	pelvis		ss	external	2	2		tumored
15222	femur		tp	inside	1	2		tumored
15222	femur		ss	inside	2	2		tumored
15222	femur		tp	external	3	2		tumored
15222	femur		ss	external	3	2		tumored
15222	tibia		tp	inside	2	2		tumored
15222	tibia		ss	inside	3	2		tumored
15222	tibia		tp	external	2	2		tumored
15222	tibia		ss	external	3	2		tumored
15222	fibula		tp	inside	3	2		tumored
15222	fibula		ss	inside	3	2		tumored
15226	carapace - top		tp	external	3	2	osteoclasts - black pigment granules inside	tumored
15226	carapace - under		tp	external	1	2		tumored
15226	plastron - gular		tp	external	1	2		tumored
15226	humerus		tp	external	3	2		tumored
15226	ulna		tp	external	2	1		tumored
15226	pelvis		tp	external	3	2		tumored
15226	femur		tp	external	2	1		tumored
15226	tibia		tp	external	3	2		tumored
15226	fibula		tp	external	2	2		tumored
15226	carapace		ss	external	1	1		tumored
15226	plastron - gular		ss	external	2	1		tumored
15226	humerus		ss	external	3	2		tumored
15226	ulna		ss	external	2	1		tumored
15226	pelvis		ss	external	3	2		tumored
15226	femur		ss	external	3	1		tumored
15226	tibia		ss	external	3	2		tumored
15226	fibula		ss	external	2	2		tumored
15227	carapace		tp	external	3	3	good slide	tumored
15227	plastron		tp	external	3	3		tumored
15227	humerus		tp	external	3	2		tumored
15227	ulna		tp	external	1	2		tumored
15227	pelvis		tp	external	3	2	slide - upside down	tumored
15227	femur		tp	external	3	3	good slide	tumored
15227	tibia		tp	external	3	3	good slide	tumored
15227	fibula		tp	external	3	2		tumored
15227	carapace		ss	external	3	2		tumored

Cytology Sample Scoring

Turtle ID #	Sample Site	Stroma color	Technique	Approach	Quantity	Quality	notes	tumor status
15227	plastron		ss	external	2	2	suspicious - blood???	tumored
15227	humerus		ss	external	3	2		tumored
15227	ulna		ss	external	3	2		tumored
15227	pelvis		ss	external	1	1		tumored
15227	femur		ss	external	3	2		tumored
15227	tibia		ss	external	2	2		tumored
15227	fibula		ss	external	3	2		tumored
5	carapace		tp	external	1	2	*** live turte sampling	non-tumored
5	carapace		tp	external	1	2	*** live turte sampling	non-tumored
6	carapace		tp	external	2	3	*** live turte sampling; 1eye tumor - size #1	tumored

Cytology Sample Scoring

Turtle ID #	Sample Site	Stroma color	Technique	Approach	Quantity	Quality	notes	tumor status
15216	humerus	black	ss	inside	1	1	black granules	non-tumored
15216	humerus	white	ss	inside	2	2		non-tumored
15216	femur	black	ss	inside	2	2	mostly black	non-tumored
15216	pelvis	black	ss	inside	1	1	black granules	non-tumored
15216	pelvis	white	ss	inside	3	2		non-tumored
15216	radius	black	ss	inside	1	1	black granules	non-tumored
15216	radius	white	ss	inside	2	1	few black granules	non-tumored
15216	ulna	black	ss	inside	2	1	black granules	non-tumored
15216	ulna	white	ss	inside	1	2		non-tumored
15216	tibia	black	ss	inside	1	1		non-tumored
15216	tibia	white	ss	inside	1	1		non-tumored
15216	fibula	black	ss	inside	1	1	black granules	non-tumored
15216	fibula	white	ss	inside	1	1	black granules	non-tumored
15216	phalanx 1	black	ss	inside	1	1	mostly black	non-tumored
15216	carapace 4L	black	ss	inside	1	1		non-tumored
15216	carapace 6L	black	ss	inside	2	1		non-tumored
15216	plastron	black	ss	inside	2	1		non-tumored
15222	carapace		ss	inside	3	3		tumored
15222	carapace		ss	external	3	2		tumored
15222	plastron - gular		ss	inside	3	2	cells - too dense on slide	tumored
15222	plastron - gular		ss	external	1	1		tumored
15222	plastron - anal		ss	external	1	1		tumored
15222	humerus		ss	inside	3	2	too thick to coverslip	tumored
15222	humerus		ss	external	1	2		tumored
15222	ulna		ss	inside	3	1		tumored
15222	ulna		ss	external	3	3		tumored
15222	radius		ss	inside	3	2		tumored
15222	pelvis		ss	inside	3	2	cells - too dense on slide	tumored
15222	pelvis		ss	external	2	2		tumored
15222	femur		ss	inside	2	2		tumored
15222	femur		ss	external	3	2		tumored
15222	tibia		ss	inside	3	2		tumored
15222	tibia		ss	external	3	2		tumored
15222	fibula		ss	inside	3	2		tumored
15226	carapace		ss	external	1	1		tumored
15226	plastron - gular		ss	external	2	1		tumored
15226	humerus		ss	external	3	2		tumored
15226	ulna		ss	external	2	1		tumored

Cytology Sample Scoring

Turtle ID #	Sample Site	Stroma color	Technique	Approach	Quantity	Quality	notes	tumor status
15226	pelvis		ss	external	3	2		tumored
15226	femur		ss	external	3	1		tumored
15226	tibia		ss	external	3	2		tumored
15226	fibula		ss	external	2	2		tumored
15227	carapace		ss	external	3	2		tumored
15227	plastron		ss	external	2	2	suspicious - blood???	tumored
15227	humerus		ss	external	3	2		tumored
15227	ulna		ss	external	3	2		tumored
15227	pelvis		ss	external	1	1		tumored
15227	femur		ss	external	3	2		tumored
15227	tibia		ss	external	2	2		tumored
15227	fibula		ss	external	3	2		tumored
15222	phalange		ss	inside	3	2		tumored
15216	humerus	black	ss-18	inside	1	1	black granules	non-tumored
15216	humerus	white	ss-18	inside	3	2		non-tumored
15216	femur	black	ss-18	inside	2	2	mostly black; black granules	non-tumored
15216	pelvis	black	ss-18	inside	1	1	black granules	non-tumored
15216	pelvis	white	ss-18	inside	2	2		non-tumored
15216	radius	black	ss-18	inside	2	2	black granules	non-tumored
15216	radius	white	ss-18	inside	1	1		non-tumored
15216	ulna	black	ss-18	inside	3	1	black granules	non-tumored
15216	ulna	white	ss-18	inside	2	2		non-tumored
15216	tibia	black	ss-18	inside	2	2	black granules	non-tumored
15216	tibia	white	ss-18	inside	2	1		non-tumored
15216	fibula	black	ss-18	inside	2	1	black granules	non-tumored
15216	fibula	white	ss-18	inside	2	2		non-tumored
15216	phalanx 1	black	ss-18	inside	1	1	mostly black; black granules	non-tumored
15216	carapace 4L	black	ss-18	inside	1	1		non-tumored
15216	carapace 6L	black	ss-18	inside	3	2		non-tumored
15216	plastron	black	ss-18	inside	2	1	black granules	non-tumored
15216	humerus	black	tp	inside	1	1		non-tumored
15216	humerus	white	tp	inside	2	3		non-tumored
15216	femur	black	tp	inside	3	3	mostly black; black granules	non-tumored
15216	pelvis	black	tp	inside	1	1		non-tumored
15216	pelvis	white	tp	inside	3	3		non-tumored
15216	radius	white	tp	inside	3	3		non-tumored
15216	ulna	black	tp	inside	1	3	black granules	non-tumored
15216	ulna	white	tp	inside	3	3		non-tumored

Cytology Sample Scoring

Turtle ID #	Sample Site	Stroma color	Technique	Approach	Quantity	Quality	notes	tumor status
15216	tibia	black	tp	inside	3	2	black granules	non-tumored
15216	tibia	white	tp	inside	3	3		non-tumored
15216	fibula	black	tp	inside	3	3	high # fat globules	non-tumored
15216	fibula	white	tp	inside	3	3		non-tumored
15216	phalanx 2; digit 3	black	tp	inside	1	1	mostly black	non-tumored
15216	carapace 4L	black	tp	inside	2	2		non-tumored
15216	carapace 6L	black	tp	inside	3	3		non-tumored
15216	plastron	black	tp	inside	3	3		non-tumored
15222	carapace		tp	inside	3	3		tumored
15222	carapace		tp	external	2	2	too thick to coverslip	tumored
15222	plastron - gular		tp	inside	3	3	***good slide	tumored
15222	plastron - gular		tp	external	1	1		tumored
15222	humerus		tp	inside	3	3	*** good slide	tumored
15222	ulna		tp	inside	3	2		tumored
15222	ulna		tp	external	3	3		tumored
15222	radius		tp	inside	3	2	too thick to coverslip	tumored
15222	pelvis		tp	inside	3	3	*** good slide	tumored
15222	pelvis		tp	external	2	3	*** good slide	tumored
15222	femur		tp	inside	1	2		tumored
15222	femur		tp	external	3	2		tumored
15222	tibia		tp	inside	2	2		tumored
15222	fibula		tp	inside	3	2		tumored
15226	carapace - top		tp	external	3	2	osteoclasts - black pigment granules inside	tumored
15226	carapace - under		tp	external	1	2		tumored
15226	plastron - gular		tp	external	1	2		tumored
15226	humerus		tp	external	3	2		tumored
15226	ulna		tp	external	2	1		tumored
15226	pelvis		tp	external	3	2		tumored
15226	femur		tp	external	2	1		tumored
15226	tibia		tp	external	3	2		tumored
15226	fibula		tp	external	2	2		tumored
15227	carapace		tp	external	3	3	good slide	tumored
15227	plastron		tp	external	3	3		tumored
15227	humerus		tp	external	3	2		tumored
15227	ulna		tp	external	1	2		tumored
15227	pelvis		tp	external	3	2	slide - upside down	tumored
15227	femur		tp	external	3	3	good slide	tumored
15227	tibia		tp	external	3	3	good slide	tumored

Cytology Sample Scoring

Turtle ID #	Sample Site	Stroma color	Technique	Approach	Quantity	Quality	notes	tumor status
15216	radius	black	tp	inside	3	2	black granules	non-tumored
15222	plastron - gular		tp	external	1	3		tumored
15222	plastron - anal		tp	external	2	1		tumored
15222	humerus		tp	external	1	2		tumored
15222	phalange		tp	inside	2	1		tumored
15222	tibia		tp	external	2	2		tumored
15227	fibula		tp	external	3	2		tumored

Bone Marrow Histology Samples

ID #	sample site	bone stroma type	notes	date	
15216-1a	radius	black	non-tumored turtle	7/12/1999	
15216-1b	tibia	white	non-tumored turtle	7/12/1999	
15216-1c	phalanx - 1	black only	front flipper	7/12/1999	
15216-1d	phalanx - 2	black only	front flipper	7/12/1999	
15216-1e	humerus	black	non-tumored turtle	7/12/1999	
15216-1f	ulna	black	non-tumored turtle	7/12/1999	
15216-1g	humerus	white	non-tumored turtle	7/12/1999	
15216-1h	claw	black only	non-tumored turtle	7/12/1999	
15216-1i	femur	black only	non-tumored turtle	7/12/1999	
15216-1j	pelvis	white	non-tumored turtle	7/12/1999	
15216-1k	radius	white	non-tumored turtle	7/12/1999	
15216-1l	plastron	black only	non-tumored turtle	7/12/1999	
15216-1m	pelvis	white	non-tumored turtle	7/12/1999	
15216-1n	pelvis	black	non-tumored turtle	7/12/1999	
15216-1o	fibula	white	non-tumored turtle	7/12/1999	
15216-1p	tibia	black	non-tumored turtle	7/12/1999	
15216-1q	carapace 6L	black only	6th lateral marginal scute	7/12/1999	
15216-1r	carapace 4L	black only	4th lateral marginal scute	7/12/1999	
15216-1s	ulna	white	non-tumored turtle	7/12/1999	
15216-1t	fibula	black	non-tumored turtle	7/12/1999	
15222-1b	humerus		tumored turtle		
15222-1d	ulna		tumored turtle		
15222-1e	radius		tumored turtle		not processed
15222-1f	radius		tumored turtle		not processed
15222-1g	phalange		tumored turtle		not processed
15222-1h	phalange		tumored turtle		not processed
15222-1j	pelvis		tumored turtle		
15222-1l	femur		tumored turtle		
15222-1n	tibia		tumored turtle		
15222-1p	fibula		tumored turtle		
15222-1r	carapace		tumored turtle		
15222-1t	plastron-gular		tumored turtle		
15222-1u	plastron-anal		tumored turtle		not processed
15222-1v	plastron-anal		tumored turtle		not processed

Bone Marrow Histology Samples

ID #	sample site	bone stroma type	notes	date
15226-1a	carapace-under		tumored turtle	8/2/1999
15226-1b	plastron-gular		tumored turtle	8/2/1999
15226-1c	ulna		tumored turtle	8/2/1999
15226-1d	humerus		tumored turtle	8/2/1999
15226-1e	pelvis		tumored turtle	8/2/1999
15226-1f	femur		tumored turtle	8/2/1999
15226-1g	tibia		tumored turtle	8/2/1999
15226-1h	fibula		tumored turtle	8/2/1999
15227-1a	carapace-under		tumored turtle	8/3/1999
15227-1b	plastron-gular		tumored turtle	8/3/1999
15227-1c	humerus		tumored turtle	8/3/1999
15227-1d	ulna		tumored turtle	8/3/1999
15227-1e	pelvis		tumored turtle	8/3/1999
15227-1f	femur		tumored turtle	8/3/1999
15227-1g	tibia		tumored turtle	8/3/1999
15227-1h	fibula		tumored turtle	8/3/1999
T #5	Carapace			
T#6	Carapace			
K 5	carapace			9/10/2013
K 6	carapace			9/10/2013
K 8	carapace			9/10/2013
K 9	carapace			9/10/2013
K 10	carapace			9/10/2013
K 11	carapace			9/10/2013
K 12	carapace			9/10/2013
K 13	carapace			9/10/2013
K 14	carapace			9/10/2013