Age estimation of a breeding population of olive ridley sea turtle (*Lepidochelys olivacea*) along the Odisha Coast, Eastern India using skeletochronology

Dissertation submitted to the Saurashtra University, Rajkot in partial fulfillment of Masters Degree in Wildlife Science (2011-13)

by

Anupya D. Baburam

भारतीय वन्यजीव संस्थान
Wildlife Institute of India

Supervisors

Dr. Bivash Pandav

Sh. R. Suresh Kumar

Dr. Pravati K. Mahapatra (External Supervisor)

भारतीय वन्यजीव संस्थान
Wildlife Institute of India

CERTIFICATE

This is to certify that Ms. Anupya D. Baburam has carried out original research titled "**Age estimation of a breeding population of olive ridley sea turtles (*Lepidochelys olivcea*) along the Odisha Coast, Eastern India using skeletochronology**", in partial fulfilment of Master's Degree in Wildlife Science from Saurashtra University, Rajkot. The study was carried out under our supervision from December 2012 to June 2013. We hereby certify that this work has not been submitted for any other degree to any other university.

Bivash Pandav 1/7/13

Dr. Bivash Pandav
Scientist E - WII
Supervisor

1/7/13

Sh. R. Suresh Kumar,
Scientist D - WII
Co-supervisor

P Mahapatra 1.7.2013

Dr. Pravati K. Mahapatra
Reader – P.G. Department of Zoology
Utkal University
External Supervisor

Date: 1st July, 2013
Place: Dehradun

Post Box No.18, Chandrabani, Dehradun - 248001. India
EPABX: +91-135 - 640111 to 640115, 640990; FAX: 640117; GRAM: WILDLIFE
E-Mail: wii@wii.gov.in

Table of Contents

List of Tables

List of Figures

Acknowledgements

Permission to carry out this work was granted by the Principal Chief Conservator of Forests and the Chief Wildlife Warden, Government of Odisha. I express my sincere thanks to Shri J.D. Sharma, IFS, PCCF and CWLW, Government of Odisha for granting permission to carry out this work. I am grateful to Dr. C.S. Kar, Senior Research Officer, wildlife wing of Odisha Forest Department for facilitating my work and expediting the process of obtaining necessary permission. My sincere gratitude to Shri Prasanna Behera, Bimal Acharya, Sudhansu Mishra, and Manoj Mahapatra, DFOs of Puri Wildlife Division, Chilika Wildlife Division, Berhampur Forest Division and Mangrove Forest Division (Wildlife), Rajnagar for their kind co-operation in collecting samples from their respective areas and help with the logistics. I am thankful to WWF India for funding this project.

I thank Shri P.R. Sinha, Director, WII and Dr. V.B. Mathur, Dean Faculty of Wildlife Science, WII for all their support through the past two years. I thank my supervisors Dr. Bivash Pandav, Mr. Suresh Kumar and Dr. Pravati K. Mahapatra for their guidance, help and support, the head of the zoology department of Utkal University for permitting me to conduct necessary analysis at the university laboratory. I am thankful to the director of Institute of Life Sciences (ILS) and Mr. Paritosh Nath for allowing me to use their freezing stage microtome and showing me how to operate the equipment. Dr. George Zug and Dr. Melissa Snover readily helped me with their advice and sorted out many technical problems encountered during this study; without their advice I would not have been able to complete this work. My field assistants Dambaru, Bipra, Mahendra, Magada, Vishnu, Pintu and Srikanth are thanked for all their help and fun times in the field. Nikita Mahapatra Ph.D student from Utkal University helped me tremendously with all stages of lab work. She always made herself available at times whenever I faced difficulties in the lab. I thank her for all her help and most importantly for being a friend. Madhusmita Das, Ph.D student from Utkal University helped me out with the lab work and was always there as a friend.

Dr. Karthik Shanker provided me a place to stay at Ganjam in his field station. Parabita Basu was with me from the inception of my work, offering encouragement and helping me when I was in need. Mr. and Mrs. Sudhanshu Mohanty (Nitya's parents) were of great help.

At WII, Abhishek and Moushmi helped with my statistical problems. Chongpi Tuboi, Samina Amin Charu, Prakash Mardaraj, Murali and all my classmates (Subish, Sharmila, Frank, Nishant, Satem, Anilitty, Nitya, Aditya, Vibhav, Deepak, Debabatra and Stotra) and all researchers of WII offered encouraging words and helped me when my health wasn't in the best of condition. Last but not least, the most important persons in my life, my mom and dad for their support, without which I would not be here today and to God for giving me the strength to continue when I was losing hope.

1 Abstract

A migratory population of olive ridley sea turtle (*Lepidochelys olivacea*) forms huge reproductive congregations in the coastal waters of Odisha every winter. This breeding population of olive ridley has been subjected to heavy fisheries related mortality for the past two and half decades. Although a number of studies have been carried out on olive ridleys congregating and nesting along the Odisha coast, the impact of such large scale mortality on the demography of this breeding population has been least understood. The effect of this continued mortality on the age class of olive ridleys nesting along Odisha coast is not known. In order to understand the age class of this breeding population I carried out this study from December 2012 till May 2013.

I used skeletochronological analysis to estimate the age class of this breeding olive ridley sea turtle population. Although this technique has been applied for marine turtles mostly in North Pacific, Atlantic and Gulf of Mexico coast of United States of America, no studies of this kind have been carried out on sea turtles of Eastern Pacific as well as Indian Ocean region. This study provides the information needed to bridge this gap and establish baseline for future skeletochronological studies on the breeding population of olive ridley. Humeral samples from 85 dead turtles (29 males and 56 females) washed ashore the Odisha coast was collected for skeletochronological studies. Cross sections were taken from the mid-diaphysis, just distal to the deltopectoral crest and beneath the insertion scar on the humerus were taken using first a Dremel 4000 round saw, then a freezing stage microtome. These sections were processed according to standardized histological techniques; growth rings on the stained humeral cross sections were counted to estimate age of dead turtles. Two age estimation protocols were used; the correction factor protocol and the ranking protocol; which yielded age estimates of 19.9 - 51.8 and 24 - 49 years respectively; for a size class of 56 - 74 cm (SCL). No correlation between size class and age was obtained from this analysis. This relationship was not established because the samples collected were from an adult breeding population, whereas, in the previous studies, sample collection represented individuals that greatly varied in size (hatchlings to adult). This study suggests that adult breeding population size class has no correlation with age; however, age can be correlated with size class of a younger population. This breeding population is sustaining a wide age group even though mortality rates are high in this area.

2 Introduction

2.1 Age estimation

Age estimation is an important tool and technique in wildlife and ecological studies. Information on the age of a species or population provides data on growth rate, reproductive potential and mortality rate, thus, making it one of the important biological variables (Campana 2001). For wild populations of different species, it is important to group animals into different age classes in order to assess the reproductive potential of a population. A healthy population should be able to sustain a reasonably equal amount of individuals in the different age classes such as: adults, sub-adults, juveniles and young. However, estimation of age in wild populations is often difficult to ascertain; thereby limiting and hindering conservation and management options. Information on age class serves to prevent a collapse in the population and is critical in the development of species specific conservation and management programs. This assumes all the more importance if the species concerned is subjected to severe exploitation.

For different taxa different age estimation methods have been applied. Dendrochronology (growth rings of trees) is used to estimate age of trees (Kuniholm *et al*, 1996). Statoliths (calcium carbonate structures) increment has been used to estimate the age of squids and sepioids (Dawe *et al*. 1985; Yang *et al*, 1986). Sclerochronology is another age estimation technique used to estimate the age of bivalves, mollusks, corals and other marine organisms with hard structures (Dodge and Thompson, 1974; Lutz and Rhoads, 1980; Jones 1986). For fishes, age can be estimated from several structures such as the scales (Robillard and Marsden, 1996), vertebrae (Brown and Gruber, 1988), fin rays (Cass and Beamish, 1983), cleithra (Casselman, 1974), opercula (Baker and Timmons, 1991) and the otolith (Campana 1983). Most age estimation of fishes is done using the scales and otoliths following skeletochronological methods (Campana 2001). In most mammals tooth cementum layers, tooth wear, cranial structure fusion, closure of the canine pulp cavity and tooth eruption are used for the determination of age (Chapskii, 1941; Laws, 1952; Scheffer, 1950; Klevezal & Kleinenberg, 1969; Myrick *et al*, 1984; Landon *et al*., 1998). Plumage descriptions which entail description of color patterns on the throats and retrices; the length of wings, length of culmen and the length of the retrice are used to estimate age of

birds (Stiles 1972). Skeletochronology is widely used in amphibians and reptiles for age estimation (Hagstrom, 1977; Hemelaar, 1980; Zug *et al*, 1986).

2.2 Skeletochronology

Skeletochronology is the study of growth rings from bones to estimate the age of an individual (Castanet, 1982, Zug *et al* 1986). This methodology has been widely applied across different taxa of herpetofauna (Hemelaar and van Gelder, 1980; De Buffrenil, 1980; Acker *et al.* 1986; Eden *et al.* 2007). The bones of amphibians and reptiles display growth during the warm as well as cool season; this growth creates a pattern which forms a cyclic line. In the cooler season these cyclic lines are much narrower and are called lines of arrested growth (LAG), the lines formed in the warmer season are broader and called marks of skeletal growth (MSG), (Figure 1). The fact that the growth of bone displays annual cyclic growth rings in which bone formation ceases or slows before new rapid formation of bones occurs is the basic tenet of skeletochronology (Simmons, 1992; Castanet *et al.*, 1993; Klevezal, 1996). Bones contain layers that are different in morphology and optical density, thus, making the growth rings easier to identify (Zug, *et al.* 1986). Together, both the MSG and LAG comprise one skeletal growth mark (GM), which has been interpreted as one year (Zug *et al* 1986).

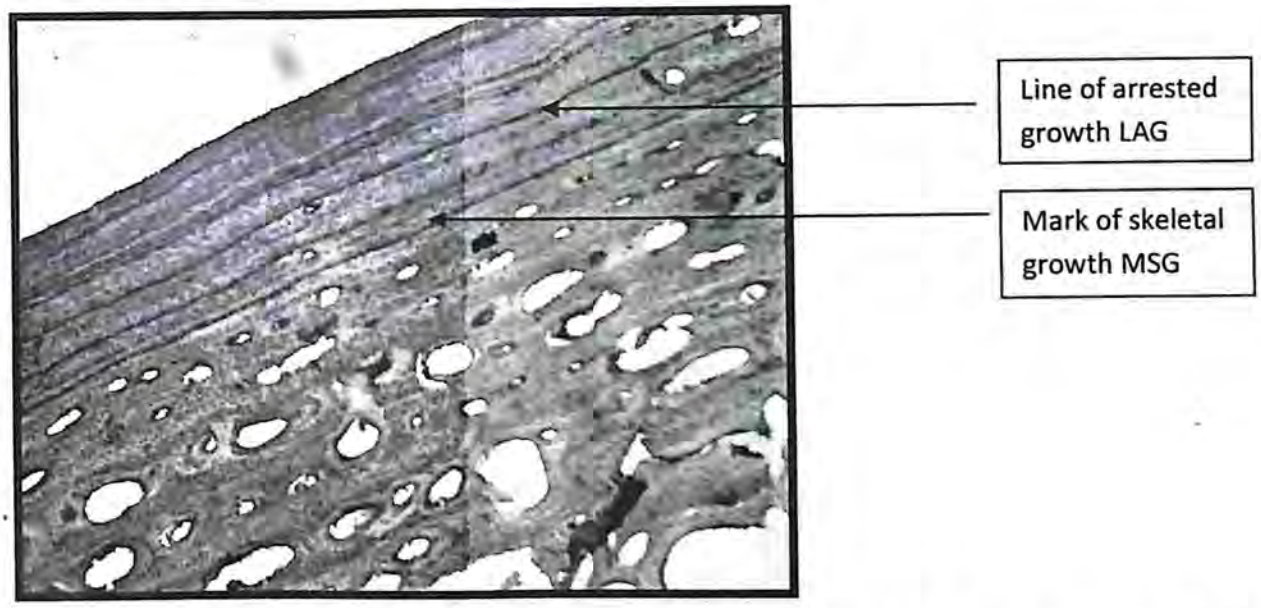

Figure 1 Growth rings (lines of arrested growth - LAG and marks of skeletal growth - MSG) in humeral bone sections

The possibility of growth rings in the bones as a means of aging turtles was first noted in Painted turtle *Chrysemys picta*, where smaller individuals had lesser numbers of growth rings than those found in larger individuals (Mattox, 1936). However, it was Peabody (1961) who promoted the idea of age being determined by these growth rings, due to his observance of the growth rings in common snapping turtle *Chelydra serpentina*, but he did not provide age in his study. Subsequently, skeletochronological studies have been extensively carried out on all sea turtle species barring Australian flatback (*Natator depressus*) and hawksbill (*Eretmochelys imbricate*) turtles (Zug *et al.* 1986, 1997, 2002, 2006 and Avens *et al.*, 2009).

2.3 The Study Setting

Marine turtle populations are declining circumglobally due to incidental capture in fishing gears, loss of nesting as well as foraging habitats and over harvesting of eggs as well as adult turtles (Tucker *et al.* 1995). Biologists across the world are trying to assess marine turtle populations that are at risk and provide necessary management strategies to minimize the risk. However, this cannot be accomplished without a thorough understanding of sea turtle population dynamics and growth. Age estimation is a major determinant in providing the necessary information needed for such studies. However, majority of the research on skeletochronology on marine turtles have been conducted in the North Pacific, Atlantic and Gulf of Mexico Coast of United States of America. Till date no such studies on sea turtles have been carried out in the Indian Ocean region.

Olive ridleys are the commonest of all marine turtles and occur in the tropics with the exception of the Gulf of Mexico. Olive ridleys are well known for their huge reproductive congregations, popularly known as arribada. The state of Odisha along the eastern coast of India is known to support some of the largest arribada sites in the world (Pandav *et al.*, 1998). The nesting population of olive ridley that migrate to Odisha coast every winter is subjected to heavy mortality due to incidental capture in fishing gears. Thousand of dead olive ridleys are found washed ashore the Odisha coast during every breeding season (Pandav *et al.*, 1997). However, the impact of such large scale mortality on the turtle population has been least understood.

Till date no information is available on the age class of turtles migrating to Odisha coast. Information regarding age at reproduction is important in understanding the population dynamics and can be used to assess the effectiveness of management strategies. Lack of and inaccurate data regarding age of sexual maturity can lead to inaccurate assumptions regarding the species resilience to a population decline and negative impacts, and would have dire consequences for management and conservation strategies (Avens, *et al.* 2009). All species of sea turtles have slow growth rates and they take years to reach sexual maturity, the amount of time for sexual maturity varies from species to species. Therefore, if turtles take decades to reach sexual maturity, the ability of the population to recovery from negative impacts will be diminished if mortality rates continue to increase. A viable population that will ensure continuity of this species should have all age class present; this age class should be hatchlings, juveniles, sub adults and adults. If only one or two age class is surviving then the population will start to decline and eventually crash. Drastic decline in this population will lead to a loss in an invaluable gene pool. Through this study I have made an attempt to estimate the age of dead olive ridley turtles that are washed ashore the Odisha coast during the breeding season, thereby providing information on age classes and developing a protocol for future work in this field.

2.4 Literature Review

2.4.1 Skeletochronological studies on amphibians and reptiles

Skeletochronological studies have been extensively conducted on a number of amphibian and reptile species, dating back from as early as 1936 to presently. These studies have shown that the age of these species can be determined by the number of growth layers present in the bones. In studies conducted on anurans, Hemelaar and van Gelder (1980) study on mark and recapture of the *Bufo bufo* toads showed that more than 90 % of the these toads that were recaptured showed an increase in the number of marks of skeletal growth (MSG), the increase in MSG was equivalent to the number of years between mark and recapture. Acker *et al.* (1986) conducted a study on the *Bufo americanus* to estimate the age of the males using skeletochronology. In their study they found similar results to that of Hemelaar and van Gelder (1980). However, in their study they concluded that larger toads are not necessarily older. Halliday and Verrell (1988)

conducted a survey on four methods that has been used to estimate the age of amphibians and reptiles in the past. Of the four methods (mark - recapture, extrapolation from size - frequency data, skeletochronology and testis lobation), only mark - recapture and skeletochronology provided the most accurate data. Francillon - Vieillot *et al.* (1990) found that skeletochronology provides age estimation; however, body size is a poor predictor of age in the *Triturus cristatus* and *Triturus marmoratus* newts. Studies by Kellner and Green (1995) and Platz and Lathrop (1993) found the same results in their study on the Fowler's toad *Bufo woodhousii fowleri* and the male chorus frog - body size is a poor predictor of age in these anuran species. Other studies over the years have reinforced that skeletochronology can be used to estimate the age of an amphibians along with the theory of growth rings being annual and one growth ring is equivalent to one year of the individual's life (Bastein and Leclair, 1992; Kusano *et al.* 1995; Kuzmin and Ischenko, 1997; Matthews and Miaud, 2007). Most of these studies have used temperate species and have associated the growth rings to periods of hibernation and environmental factors. Kellner and Green (1995) and Guarino *et al.* (1998) proved that tropical species also have LAGs present, and can be aged by skeletochronology.

Italian viper, *Vipera aspis* born and raised in captivity matched aged with the number of growth rings present in the bones (Castanet and Naulleau, 1974). The deposition of a single zone (growth period) was demonstrated in the himehabu pit viper *Trimeresurus okinavensis,* where the growth period was between June to September (Minakami, 1979). Study on a captive Siamese crocodile *Crocodylus siamensis* which was four years old, showed the presence of three complete growth rings and had an incomplete fourth ring at the time of death (De Buffrenil 1980). With the use of fluorescent labeling Castanet (1982) was able to show the deposition of one layer each year in European green lizard *Lacerta viridis.* A study by Zug *et al.* (1983), showed the number of growth rings matched closely with the known age of marked-recaptured common green iguana *Iguana iguana.*

Capture-Mark-Recapture is one of the most precise methods used to arrive at age of turtles. However, this method is time consuming, labor intensive, logistically difficult, expensive and not suitable for short term studies. An alternative technique was suggested by the research activities of the Universite de Paris "Equipe de Recherche 'Formation squelettiques, lead by Dr. Castanet.

In these research activities, dead turtle's stranding was used as specimens for skeletochronological studies. Studies conducted by Castanet (1970) and Castanet *et al* (1977), provided the basis for their argument that the bones of ectothermic vertebrates have cyclic growth rings which were annual. A study conducted on Red-eared slider *Trachemys scripta elegans,* found the presence of annual growth layers in long bones, however, due to extensive resorption and remodeling throughout the growth, the researcher rejected the use of these bone growth for age estimation (Suzuki, 1963). Hammer (1969), conducted a study which contrasted the findings of Suzuki (1963). In his study Hammer found a high correlation (r = 0.94) to the number of growth rings to the length of the carapace. Hammer used a number of skeletal materials, but found that growth rings were more evident and pronounced in the long bones. However, Dobie (1971) could not find the same correlation in his studies on alligator snapping turtle *Macroclemys temminckii* as did Hammer in his study. Dobie (1971) used sections of the mandible and the vertebrae. High correlation (r = 0.89) was found between the number of rings and the carapace length of *Testudo hermanni*, along with concurrence between the number of growth rings present and known age of the said species in a study conducted by Castanet and Cheylan (1979).

2.4.2 Skeletochronological studies on sea turtles

Although a number of studies have been conducted on skeletochronological analysis of age estimation of herpetofauna; Castanet (1982), advised against the use of skeletochronology for sea turtle species, due to the amount of resorption and remodeling in their bones. However, since 1986 till date a number of studies on skeletochronological analysis of age estimation on sea turtles have been conducted. These studies have been summarized below.

On a global perspective, skeletochronological studies have been carried out on all species of sea turtles with the exception of Australian flat-back and hawksbill sea turtles. Skeletochronology has been used to estimate the age of sea turtles in the North Pacific, Atlantic and Gulf of Mexico Coast of United States of America. Protocols have been developed for the age estimation of sea turtles in that part of the world. Zug *et al.* (1986) were amongst the first to conduct skeletochronological on the loggerhead (*Caretta caretta*) turtles, in the Cumberland islands and

Camden County, Georgia, U.S.A. From this study the first protocol for age estimation for marine turtles was developed. The results from this study highlighted the importance of skeletochronology as a tool for age estimation along with which bones and part of the bone is most useful for this study. The bones selected for use were "the third peripheral bone from the carapace, the dentary - a section which is adjacent to the symphysis and one in the middle ramus - a centrum of a cervical vertebrae, penultimate phalanx of the forefoot, an ulna and humerus" (Zug, *et al.* 1986). Growth layers were present in the dentary, but due to the spongy nature of the bone, the layers were irregularly arranged for accuracy in counts and measurements; there was no discernible pattern in the phalanx, centrum and peripheral bone; the ulna had a weak pattern; while the shaft of the humerus showed a clear pattern of growth layers and was thus selected for the histological selections. The final results showed that the average age estimation of loggerheads nesting at Cumberland Island was between 13 - 15 years and they had a curved carapace length (CCL) of approximately 800 - 900 mm. There have been many other studies of similar nature on the other species of sea turtles. The results from those studies showed age estimation of: Kemp's ridley from the Atlantic and Gulf of Mexico coasts of USA was between 2 - 15 years with a straight carapace length (SCL) of 188 - 720 mm (Zug *et al.* 1997); the Hawaiian green sea turtles 4.1 - 34.6 years using correction factor protocol and 3.3 - 49.4 years using spline integration protocol with a straight carapace length of 28.7 - 96.0 cm (Zug *et al.* 2002). In north Pacific, age of olive ridley (*Lepidochelys olivacea*) using rank protocol and correction factor was found to be 5 - 38 and 7 - 24 years respectively with a SCL of 4 - 65 cm (Zug *et al.* 2006). Leatherbacks (*Dermochelys coriacea*) from the western North Atlantic showed age at maturation to be between 24.5 - 29 years with a curved carapace length (CCL) from 122 - 173 cm (Avens *et al.* 2009).

The age estimation of *Chelonia mydas* green sea turtles has been carried out in Florida, Hawaii, U.S.A Atlantic Coast, Bahamas and Southeastern U.S Atlantic (Zug & Glor, 1998; Zug *et al.*, 2002; Goshe *et al.*, 2010; Bjorndal *et al.*, 1998; Goshe 2009); the leatherback in the British Virgin Islands (Avens *et al.*, 2009), Kemp's ridley, *Lepidochelys kempii*, in Southeastern United States, Long Island, Padre Island, New York (Zug *et al.*, 1997; Schmid and Witzell, 1997; Goshe *et al.*, 2009; Snover, 2002; Snover & Hohn, 2004); loggerheads, *Caretta caretta,* in Atlantic and Gulf of Mexico coast in U.S.A, Cumberland Island, Camden County, North Pacific and the

Mediterranean Sea (Braun-McNeil *et al.* 2008; Guarino *et al.* 2004; Parham and Zug, 1997; Snover, 2002; Snover & Hohn, 2004;; Snover and Hohn, 2007; Zug *et al.*,1 995; Zug *et al.*, 1986) and the olive ridley has been studied from the North Pacific Ocean (Zug *et al.*, 2006).

Zug *et al* (1986) conducted a study on loggerheads *Caretta caretta,* on the Cumberland Islands to estimate the age. In this study they used several skeletal materials such as the femur, third peripheral bone from the carapace, the dentary, and centrum of the cervical vertebrae, penultimate phalanx of the forelimb, humerus and ulna to test for growth rings. From these samples, they found the humerus bones to be the most suitable sample because it had the least amount of resorption and remodeling. A region just distal to the deltopectoral crest was selected for the cross sections. From the histological preparations, cyclic marks could be observed from these samples under the microscope that were counted to estimate age for different size classes.

Zug (1990) developed the Ranking Protocol technique which is a modification of Hemelaar's (1980) analytical technique that was used to age Common Toad *Bufo bufo.* Zug modified this technique specifically to account for the resorbed periosteal growth layers in the humeri of turtles. The steps in the protocol are as follows, according to Zug (1990):

1) Rank the bone sections in order of increasing resorption core diameters.
2) Starting with the section with the smallest core, assign the innermost (smallest) periosteal diameter to the lowest growth class possessing an appropriate range of MSGs' diameters.
3) The succeeding (outer) MSGs' diameters are placed in successive classes. No classes are skipped.
4) The class containing the outermost diameter of the bone is the estimate of the number of growth cycles.
5) Each growth cycle presumably equals one year.

Zug *et al.* (1995) conducted a study on juvenile loggerheads (*Caretta caretta*), which was salvaged from drift net fishing in the North Pacific Ocean. They used the ranking protocol and regression growth model to estimate the age of these turtles. Regression growth model assumes that the thickness (width) of each growth layer is a function of its distance from the initial diaphysis (periosteal surface at hatching), by which age can be estimated by integrating this function over the entire radius of the humerus. Regression analysis determines the relationship

between layer width and the radius of the humerus at the end of each growth. The results from both the protocols yielded similar results which was reliable. Zug *et al.*, al. (1997), conducted a survey on the Kemp's Ridley (*Lepidochelys kempii*) in the Atlantic and Gulf coasts of USA and predicted that sexual maturity is attained between 11 - 16 years and the regression growth model predicted maturity at a bit older age: 13 - 19 years. Their data also showed the kemp's ridley from the Gulf of Mexico had a faster growth rate than those from the Atlantic Ocean; however, the data set was too limited for a more reliable interpretation. From the samples collected and after age estimation was done, they found that the Atlantic samples lacked adult turtles.

Parham and Zug (1997) developed a new protocol called the correction factor. This protocol was developed to aid in accuracy for estimating age. The bones of sea turtles are under constant resorption and remodeling, which makes reading of growth rings difficult. Resorption can be seen within the core of the shaft (diaphysis) of the humerus and femur, but the periosteal layer on the outer periphery is retained. Using this information, Parham and Zug developed the correction factor protocol. This protocol is dependent on the presence and size of growth layers in sample consisting of juvenile specimens. "A growth trajectory (X, number of growth layers; Y, radius of the humerus) is determined for each individual by connecting the point (Xi, Yi) at smallest size to point at death (Xi + n, Yi + n). The "average" slope of the trajectories of the smallest individuals is determined by regression analysis (least square) and the Y-intercept is set at the radius of the hatchling's humerus. The number of lost layers is determined by substituting the radius of the resorption core in the regression equation: Y = A + BX, or radius = hatchling radius + [(slope)*(number of lost layers)]; number of lost layers = (radius - hatchling radius)/slope" (Parham & Zug, 1998). The correction factor (the number of lost layers) would be added to the number of layers observed and this gives the estimate of the total number of layers for an individual, indicating the age.

Schmid and Witzel (1997) used mark recapture and the Bertalanffy method to estimate the age of Kemp's ridley at two locations in Florida. During 1986 - 1991 they tagged 251 turtles in Cedar key (western Florida; Atlantic Ocean) and 113 at Canaveral (eastern Florida; Gulf of Mexico) and recaptured 24 and 12 respectively. The mean carapace length of the turtles from these two locations was different; there is evidence which suggest that the turtles from Gulf of Mexico have a faster growth rate than those of the Atlantic Ocean. Seasonality also influences growth

rates; they found that growth rates during the summer were greater as compared to the growth rates of turtles captured after winter (Schmid and Witzel, 1997).

Green sea turtles (*Chelonia mydas*), were collected during the cold stunning event by Zug and Glor (1998). These turtles had a straight carapace length between 28 - 74 cm, with an estimated age between 3 - 14 years and growth rate was between 30 - 52 mm per annum. The age estimates also showed that green turtles from the western Atlantic changed from a pelagic life to a neritic life as they became adults. On the other hand, a study of age estimation via skeletochronology on the Hawaiian Green Turtles (*Chelonia mydas*) was carried out by Zug, *et al.* 2002. They used correction factor (CF) and spline integration to determine age. For correction factor the age estimation was 4.1 - 34.6 years, while spline integration age estimation was 3.3 - 49.4 years. The growth rate was found to be 4 - 5 cm per year in juveniles, then it starting decreasing to a constant rate of 2 cm per year at age 10; and began declining to 1 cm per annum as the turtles reached 30 yrs. Spline integration method uses the curvilinear relationship between humerus diameter and the width of periosteal growth increments within the humerus.

Guarino *et al.* (2004) performed skeletochronology on the digits of loggerheads from the Italian Adriatic and Mediterranean coasts. The second and third intermediate and proximal phalanges were collected from the forelimbs for skeletochronological analysis. Cross sections were taken from the mid-diaphyseal level after which histological process was followed. This study showed the phalanges are just as useful in age determination as the humerus. Guarino *et al.* (2004) found the phalanges to be advantageous over the humerus due to faster skeletochronological preparations of the cross sections. However, numerous cross sections have to be taken to account for the resorption of the LAGs. Never-the-less, they were able to estimate the age of forty-three (43) specimens and they found the age to be between 9 - 18 years for specimens between curve carapace length (CCL) of 28 - 49 cm, individuals over 50 cm were more than 20 years of age, while their largest specimen was 83 cm and was aged between 32 - 37 years old.

Zug, *et al.* 2006, conducted a survey on the olive ridley (*Lepidochelys olivacea*) sea turtle population in the North-Central Pacific Ocean. Specimens where collected from long-line incidental fishery catch and from dead turtles washed ashore. They used the ranking and correction factor protocols and yielded results of age estimation of 5 - 38 from the ranking method and the correction factor age estimation was 7 - 24 years. The rank estimates where

highly correlated to straight carapace length while the correction factor (CF) was not; thus CF age estimates was considered more biologically accurate due to its dissociation with age and size. Another means of estimating the age of sea turtles was applied by Snover, *et al.* 2007. They used back - calculation to calculate the growth rate by using the diameter of the skeletal growth mark which represented the time of capture as a predictor. Results showed the mean difference between the carapace length at time of capture and the estimated carapace length which was obtained from the back - calculation was 0.6 cm ± 0.2 SE. The estimates for annual growth rate mean error was 0.2 cm yr–1 ± 0.05 SE. Back - calculation combined with skeletochronology provides a powerful tool for studying growth dynamics of sea turtles (Snover, *et al.* 2007).

Braun-McNeil, *et al.* 2008, suggested from their study that there is no clear relationship between carapace length and the age of sea turtles. They however, suggested that to understand the growth rates and life stages better, a fair understanding of environmental factors such as prey selection, availability of food, and the individual's behavior such as foraging and migration should be investigated more thoroughly to understand growth patterns. Chinsamy and Valenzuela (2008) conducted a study on the endangered side neck turtle (*Podocnemis expansa*). This species of turtle is found in Amazon. Bone specimens were collected from the leftovers from natural predators; and even though these bone specimens were subjected to harsh environmental conditions, histological details could still be derived from them. They were able to determine that there is a positive correlation with the annual growth rings and the carapace length. The study was a pilot study and it concluded skeletochronology is an effective measure to estimate the age of turtles, which gives better insight into the conservation and management practices (Chinsamy and Valenzuela, 2008).

Goshe, *et al.* 2009, evaluated histological techniques used for aging turtles. Their study focused on comparing stained and unstained cross sections of the humeri from kemp's ridley and loggerhead sea turtles to determine whether both methods resulted in equal numbers of visible lines of arrested growths (LAGs). They found stained sections when viewed at a higher magnification resulted in more closely spaced LAGs; while the unstained sections had less distinct lines. They recommended stained sections to be used for skeletochronological studies. A more recent study by Casale *et al.* (2011) reported from their study that loggerhead from the Mediterranean Sea take between 14.9 - 28.5 years to reach a curve carapace length (CCL) of

66.5 - 84.7 cm. They compared these results to the average size of nesting females found in important rookeries in the Mediterranean and confirmed that their results corresponded to the size of those nesting females (Casale, *et al.* 2011).

2.5 Objectives

The objectives of this project were to:

- Estimate the age of dead sea turtles washed ashore, using skeletochronology
- Assess the relationship between estimated age and morphometric characteristics
- Establish a base line age distribution of the population of olive ridley using this protocol

2.6 Study Site

Odisha is found within the geographical limits of 17° 31' to 20° 31' N and from 81° 31' to 87° 30' E, which covers approximately 1,56,000 km^2. The coastline of Odisha is 480 km which stretches from Subarnarekha River mouth in the west next to Udyapur village on the borderline of West Bengal, to Ichhapuram in Andhra Pradesh. Balasore, Bhadrakh, Kendrapara, Jagatsinghpur, Puri and Ganjam are the districts which traverses the coastline. Major rivers in Odisha are Subarnarekha, Budhabalanga, Brahmani, Baitarani, Mahanadi, Devi and Rushikulya. This study was conducted along the Chilika coast, Gahirmatha, Rushilya, and Devi rookeries, covering a total of 90 km.

2.6.1 The intensive study areas

This study focuses on olive ridleys which were collected from Gahirmatha, Rushikulya rookeries, Devi River mouth and Chilika coast (Figure 2).

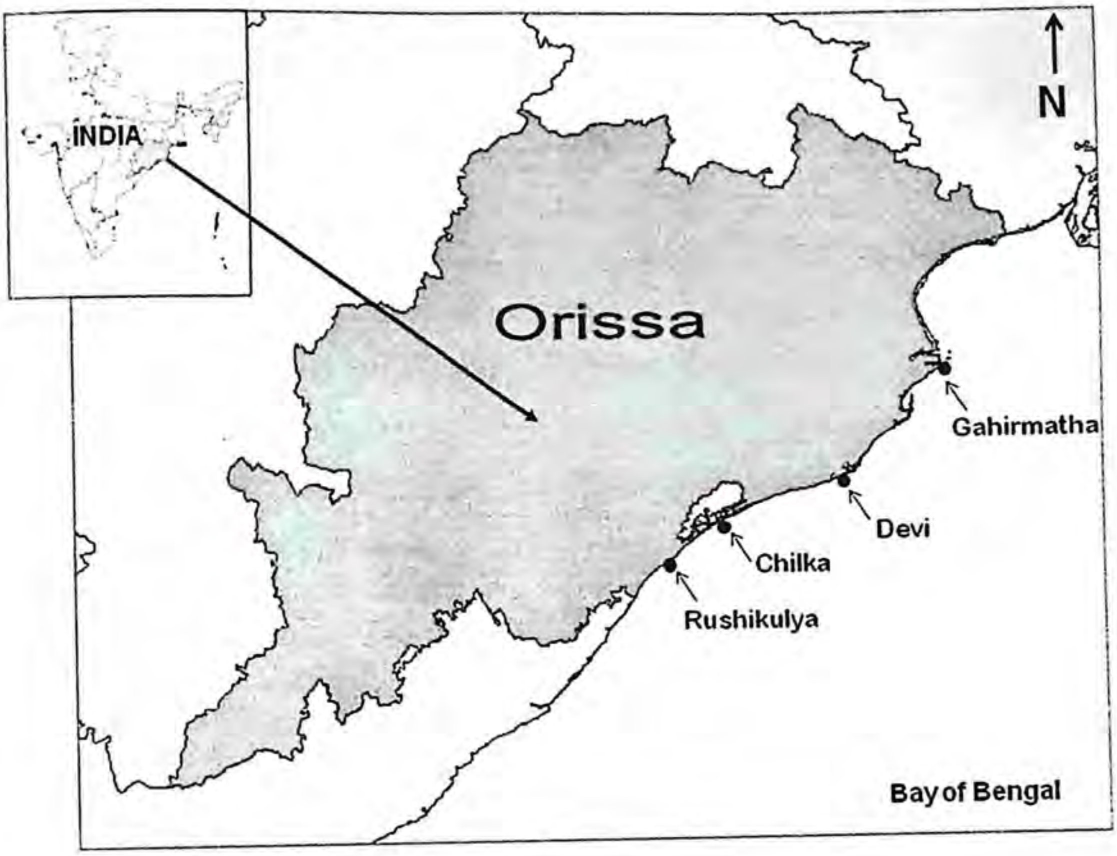

Figure 2 Map of Odisha showing study sites

2.6.1.1 *Gahirmatha rookery*

Gahirmatha rookery was included as part of Bhitarkanika Wildlife Sanctuary in April 1975, after a recommendation by Dr. H. R. Bustard, whom was conducting a survey on crocodiles for FAO-UNDP project, where he observed olive ridley nesting en masse. After these observations he made aware the importance of this rookery for olive ridleys and said it may be the largest rookery worldwide for olive ridleys arribada. Gahirmatha has a total coastline of 35 km between the mouth of river Maipura and Barunei.

Coastal waters off Gahirmatha were declared as a wildlife sanctuary in September 1997, for the protection of olive ridleys nesting in the area. Total area of Gahirmatha Marine Wildlife

Sanctuary is 1435 km^2 with a core area of 725 km^2 and a buffer area of 709 km^2. Mating and courtship takes place 4 km south of the Nasi group of Islands.

2.6.1.2 Devi River mouth rookery

More than ten years after the first report of mass nesting in Devi by Kar (1982), Pandav *et al* (1994) confirmed the continuance of mass nesting at this rookery. Mass nesting takes place on an island located between Devi and Petaphutei river mouths. Due to Casurina plantation majority of the nesting ground at this rookery has been lost. After the super cyclone in 1999, mass nesting activities have neither been monitored nor documented. This area receives large number of dead turtles during the breeding season.

2.6.1.3 Rushikulya rookery

Rushikulya rookery is located 320 km south of Gahirmatha on the southern Odisha coast. Mass nesting area is located on a sand spit on the northern side of the river. This river opens to the Bay of Bengal on the northern side near Ganjam town. The estuary is connected with Chilika Lake by a man made canal known as the Palur Canal.

2.6.1.4 Chilika

Chilika Lake is a brackish water lagoon which is spread through the districts of Puri, Khurda and Ganjam at the mouth of Daya River, flowing into the Bay of Bengal. It is the second largest lagoon in the world. It is home to about 160 species of birds during the migratory season, the endangered Irrawaddy Dolphin and has high fish diversity. Ramsar Convention designated Chilika Lake as a wetland of international importance in 1981. The coastline of Chilika Lake is approximately 64 km.

3 Methodology

3.1 Sample collection

The intensive study areas are the Gahirmatha and Rushikulya rookeries, Devi River Mouth and Chilika coast. Gahirmatha rookery and Devi river mouth were visited for two weeks each and they were monitored every day, while Rushikulya rookery and Chilika coast were visited for 5 weeks during January-February 2013. Several external morphometric measurements were taken from the dead olive ridleys washed ashore. These measurements were: straight carapace length (SCL), straight carapace width (SCW), curved carapace length (CCL), curve carapace width (CCW), plastron length (PL), plastron width (PW), head length (HdL) and head width (HdW). Measurements were taken using a tree caliper to the nearest 0.1 cm. Humerus bones were removed from the fore flippers for analysis of growth rings through skeletochronological processes in the lab.

3.2 Measurements and counts

After the humerus was air dried the following measurements were taken (Figureure 3 & 4):

1) Maximum length (ML), distance from proximal-most tip of ulnar process to distal articular surface
2) Longitudinal length (LL), distance from proximal surface of head to distal articular surface, parallel to longitudinal axis of humerus;
3) Ulnar process length (UPL), distance from proximal tip of ulnar process to juncture of head and process;
4) Proximal length (PL), distance from proximal surface of head to distal edge of radial process, parallel to longitudinal axis;
5) Proximal width (PW), distance from preaxial surface of head to postaxial surface of ulnar process, perpendicular to longitudinal axis;
6) Radial process length (RPL), distance from pre- to postaxial edges of process, diagonal to longitudinal axis;

7) Width at deltopectoral crest (DpCW), transverse distance of shaft from pre- to postaxial surfaces at deltopectoral crest;
8) Distal width (DW), transverse distance from pre- to postaxial surfaces at juncture of articular condoyle with shaft;
9) Maximum head diameter (MaxHD) of the shaft;
10) Minimum head diameter (MinHD) of the shaft.

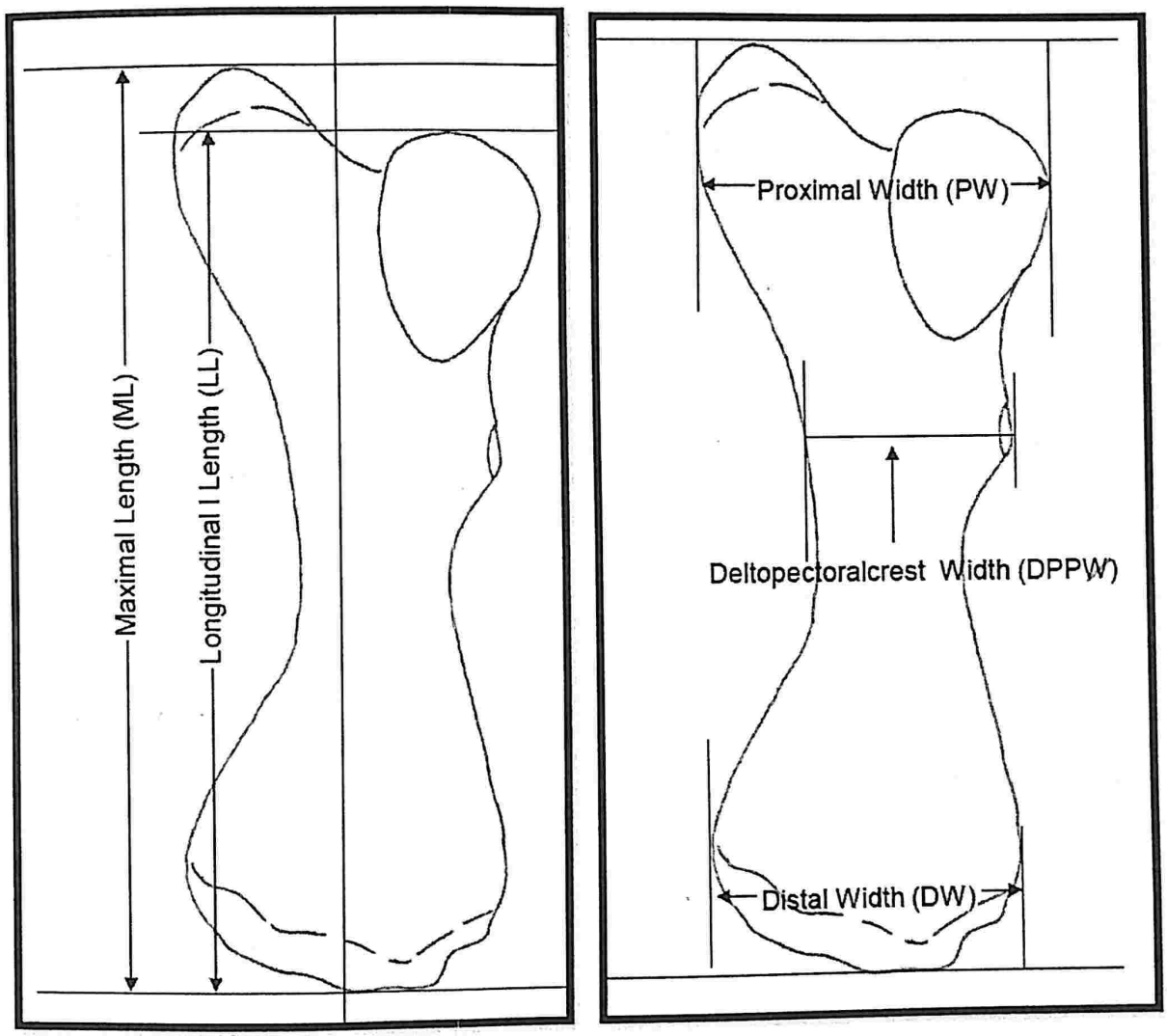

Figure 3. Morphometric measurements of the humerus bone

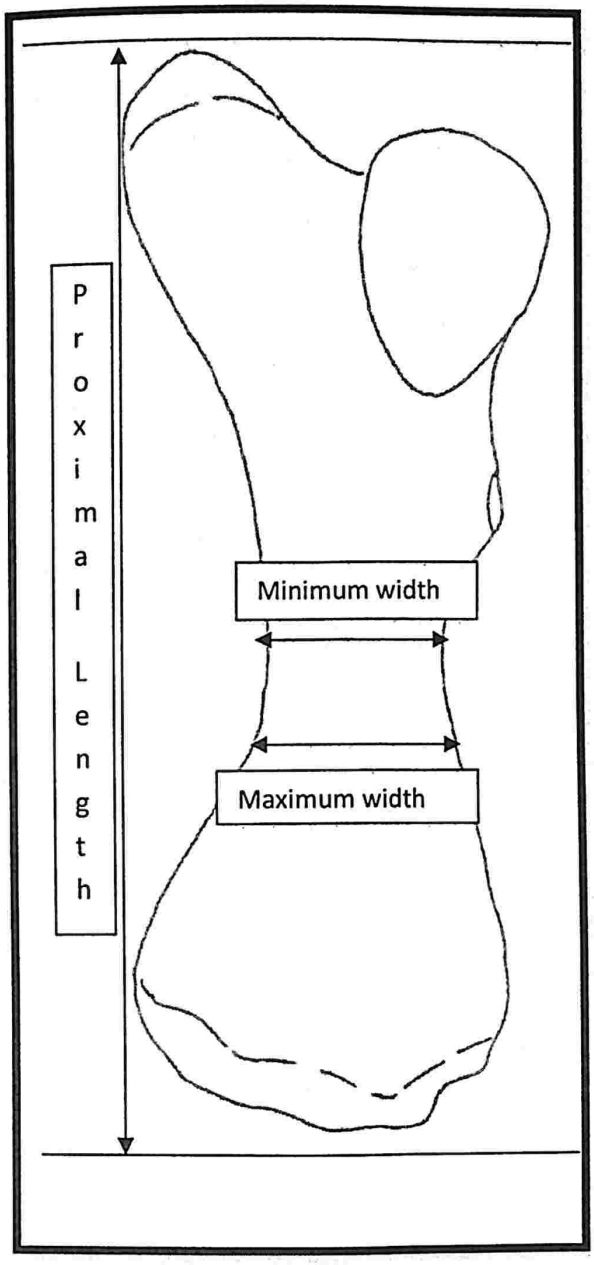

Figure 4. Humerus bone showing the different measurements taken

3.3 Preparation of bone

The humerus bones were removed from the forelimbs using a knife. These bones were flensed of tissue, boiled and finally left to be dried for approximately three weeks. Using a Dremel 4000 circular saw at an rpm of 5000, 3 - 5 mm cross section of the humerus, just distal to the

deltopectoral muscle insertion scar (Figure 5 & 6), was removed for histological studies. These cross sections were fixed in 4 % formalin for 24 hours, rinsed with tap water for 30 minutes and stored in a 7% nitric acid for 26 - 32 hours depending on the size of the bone for decalcification (Figure 7). After decalcification (Figure 8), the sections were rinsed in tap water for 1 hour each then stored over night in clean tap water to remove any remnant decalcifying agent. From the decalcified bones a 25 µm thin cross section was removed by placing the sections onto a template covering it with Jung Tissue Freezing Media then using a Leica CM 1850 freezing stage microtome (Figure 9) at a temperature of 18 - 20 °C the cross sections were cut into 25 µm thin sections and placed onto a slide (Figure 10). To prevent the tissues from dehydration, 3 drops of a glycerol-ethanol mixture (40 ml glycerol: 60 ml 70% ethanol) was placed on the tissues and stored in a refrigerator until ready for staining (Figure 11).

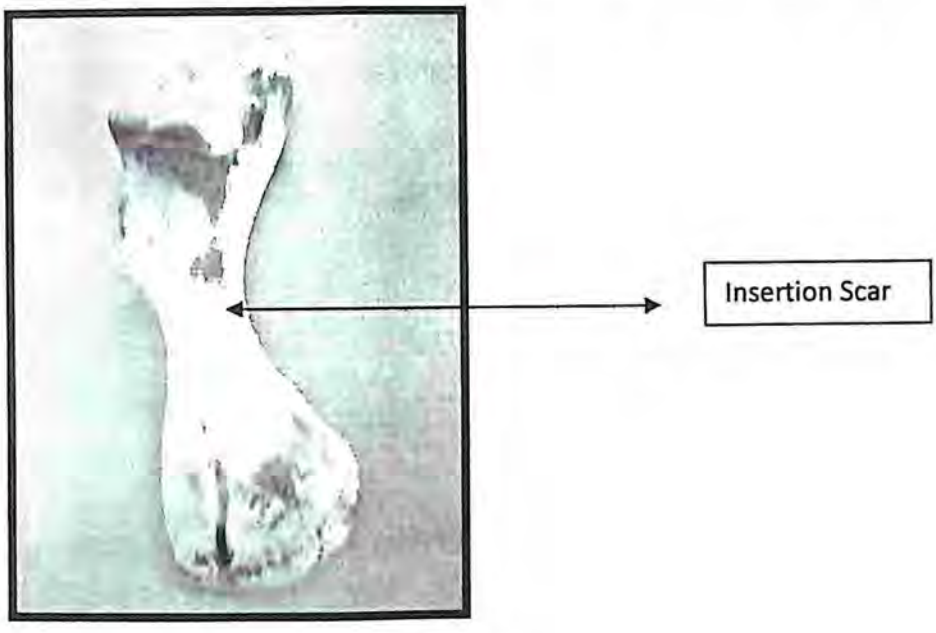

Figure 5. Humerus bone showing insertion scar

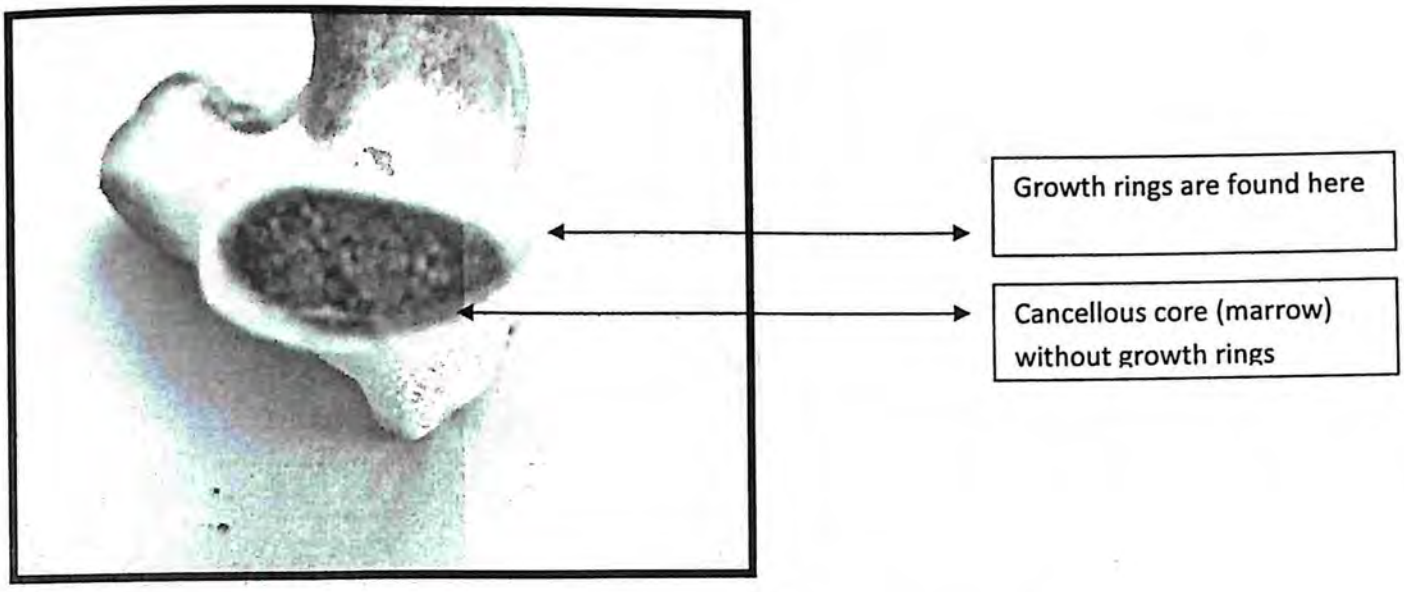

Figure 6. Humerus bone showing the point where the cross section was removed.

Figure 7. Cross section (3-5 mm) after fixing in formalin overnight

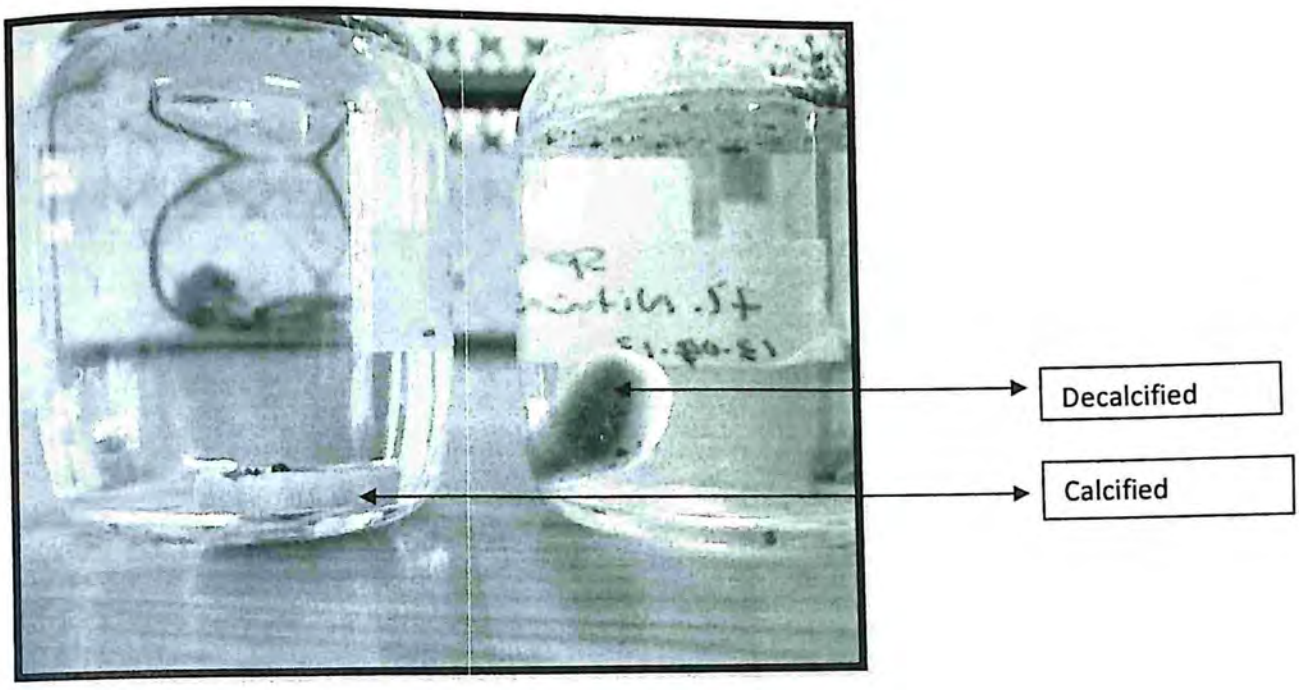

Figure 8. Showing decalcified and calcified bones

The decalcified bone can be seen floating in the nitric acid, while the calcified bone is still at the bottom of the beaker.

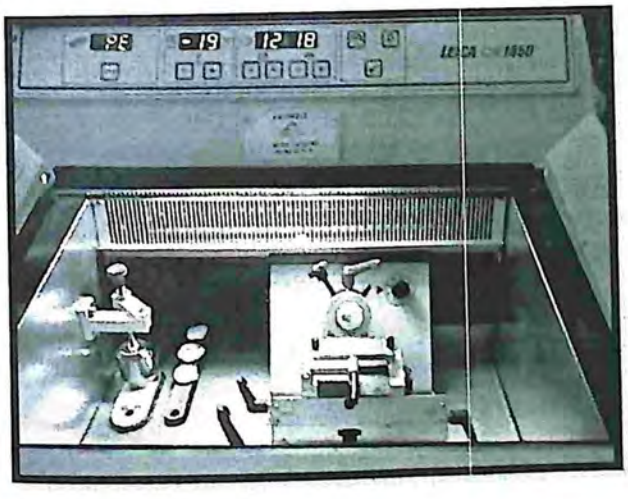

Figure 9. Leica CM 1850 freezing stage microtome

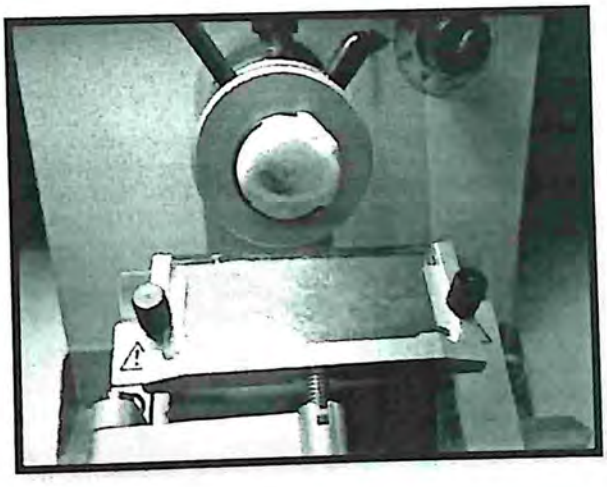

Figure 10. Freezing stage microtome showing the placement of tissue

Figure 11. Cross sections of 25 µm thin after cryosectioning

3.4 Staining of tissues

Using a slide marker, each slide was numbered according to the specimen it contained. The glycerol-ethanol solution was washed off from the tissues with distilled water. A 1:2 hematoxylin solution was prepared by mixing 1 part hematoxylin: 2 part distilled water. To the slides 5 - 6 drops of the hematoxylin solution was added for 30 seconds (or until the tissue sample became a violet/blue color). The slides were again washed with distilled water to remove the excess hematoxylin. 100 µm of 90% ethanol was added to the slides for 1 minute, the slides were covered to prevent the ethanol from evaporating. After one minute, the excess 90 % ethanol was removed then 100 µm of 100% ethanol was added to the slides and covered for one minute. The excess ethanol was removed and the slides were allowed to dry. The time for drying varies between 5 - 10 minutes. Ethanol was added to the slides to hydrate the water from the tissue,

since the slides were being prepared for permanent staining. After the slides were dry, 100 µm of xylene was added to the slide ensuring it covered the entire tissue and the slide properly, the xylene was drained from the slides and few drops DPX; a sealant agent was added to slides then a cover slip was applied to complete the permanent slides and the sealing process (Figure 12 & 13). The slides were left to dry overnight then viewed under a stereo microscope and a light microscope. Photographs were taken using a Cat Cam at the lowest magnification on both microscopes.

Figure 12. Staining process

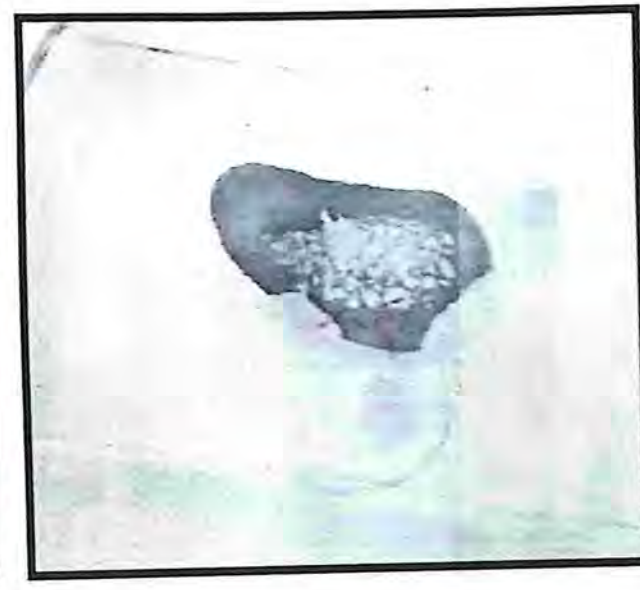

Figure 13. Stained section

3.5 Measurement of humerus bone cross section

The histological sections were measured grossly and microscopically. The length and width of bone sections were measured with a digital caliper to 0.1 mm. The periosteal layers were measured to 1.0 fxm with a micrometer and counted at 4 X magnification under a light microscope. The counts and measurements of the layers were made along two perpendicular axes, the long (pre-postaxial) and short (dorsoventral) axes, of the bone section.

3.6 Data analysis

3.6.1 Counting of growth Marks

After staining, the cross sections were photographed using Cat Cam on a compound microscope at 4X magnification. Photographs enabled permanent storage of the cross sections allowing for counting the growth rings in a magnified view. Growth rings were counted on both the ventral and dorsal surface of the cross section, first from the outer periosteal layer towards the inner periosteal layer and then in the reverse manner. For a full view of the cross sections photographs were taken using a stereomicroscope at a low magnification (0.63 X).

3.6.2 Data from dead turtles

Dead turtles from which bones were collected were classified as males and females from the different field sites. To analyze the data collected from internal and external morphometric characteristics, summary statistics, and regression and correlation analysis were performed. Regression and correlation analysis were conducted to determine the relationship between these two morphological characteristics and age estimates from the cross section analysis.

3.6.3 Age estimation

3.6.3.1 Ranking protocol

Ranking protocol has been used in previous studies to estimate the age of turtles. This protocol was developed by Zug (1990), where he modified Hemelaar (1985) analytical technique to account for calculation of resorbed growth layers. The data set required to arrive at age estimates using this protocol include: the diameter of the individual cross sections, the diameter of the resorption core and the diameter of each growth layer. The protocol estimates the age by the following steps:

I. Using the resorption core diameters the bones are ranked from smallest to largest

II. The bone with the smallest core diameter has its innermost growth layer assigned to the smallest growth class, while the subsequent layers are assigned to growth classes accordingly.

III. The remaining bone sections are ranked and assigned growth classes from the previously ranked bone

IV. The growth class containing the outermost growth layer indicates the age of the individual

3.6.3.2 Correction Factor Protocol

Correction factor protocol was developed by Parham and Zug (1997) to determine the age of the individual. The correction factor protocol is developed from the smallest individual (by size) from the sample collected. This protocol accounts for the growth layers lost to resorption, by using the data on growth layers from the smallest individuals in the sample. The protocol is based on an equation:

Age = # of LAG + CF

To calculate the correction factor an average slope value is used. The average slope value is calculated from a growth trajectory of the smallest individual. X = number of growth layers and Y = radius of the humerus. The slope is determined by connecting (Xi, Yi) from the smallest point to the point at death (Xi + n, Yi + n) (Parham and Zug, 1997). The value of the average slope is then used to determine the number of lost layers (correction factor) by this equation:

CF = (radius of the cross section - radius of the hatchling's humerus) / slope

Correction factor values are found for all the individuals, which are then added to the number of observed LAG to determine the estimated age of the individuals.

3.6.4 Relationship between age and size class

Regression analyses were used to determine whether a relationship exist between the external and/or internal morphometric characteristics with age estimates from the two protocols mentioned above. This analysis helped to determine whether any of the morphometric characteristics can be used as a predictor of age.

3.6.5 Aging arribada population

External morphometric characteristics of live turtles were taken during the arribada at Rushikulya rookery. Summary statistics was done to describe the data set. From the data generated from the skeletochronological analysis of the dead turtles, age was assigned to the different size classes obtained from the nesting population. The age estimates assigned to size class can be used as a baseline database to age future breeding population in coming arribadas.

4 Results

A total of 78 days (3260 man hours) were spent surveying a total of 90 km beach stretch along the Odisha Coast to locate dead turtles. During the beach surveys 215 dead turtles were encountered, however humerus bones could be collected from only 85 individuals (Table 1), as in most cases humerus bones were missing due to predation. In some other cases the humerus bones were intact while the carapace was decayed or broken thus the external morphological characteristics could not be properly recorded, and therefore these were skipped. During arribada 4 days (70 man hours) were spend in the field taking the carapace lengths and widths of 1212 nesting turtles at Rushikulya rookery. At hatchling emergence 1 day (4 man hours) was spent collecting 4 hatchlings.

Table 1. Summary of dead male and female turtles collected from the four sites.

Location	**Male**	**Female**	**Total**
Gahirmatha	2	23	25
Rushikulya	8	3	11
Chilika	19	20	39
Devi	-	10	10
	29	56	85

4.1 Humerus bone section

Examination of the humerus bone cross section just below the deltopectoral crest, showed the cross section to be oblong in shape along the anterior - posterior axis. Approximately two-thirds of the cross section is made up of spongy material, the marrow which is cancellous bone tissues and is the site where resorption and redeposition takes place. The outer periphery of the bone is compact and solid and is the area where growth layers are observed (Figure. 14).

Figure 14. A stained cross section of a humerus bone showing the core and hard outer layer that contains the growth layers.

4.2 Morphological characteristics of the dead olive ridley turtles

Female olive ridley turtles were found to have a larger straight carapace length (SCL) than males, though not statistically significant (Mann-Whitney $U = 653.5$, $p = 0.142$). The Straight Carapace Length (SCL) of the male dead turtles averaged 63.7 cm ± 2.4 (59.6-68.3), while females were 64.6 cm ± 3.2 (56.4-72.2). In case of males the maximum number of turtles observed was in the size category of 62 cm, while in females it was at 66 cm. The size class 70 - 74 cm, only females were collected and within the size class 58 cm, there were more males than females(Figureure 15)..Only head length (HdL) was found to be significantly different between the sexes (Mann-Whitney $U = 334.5$, $p = 0.001$).Details of the external morphometric characters and the humerus bone characters of the male and female turtles examined are given in Table 2 & 3 and the frequency distribution is shown in Figure 15.

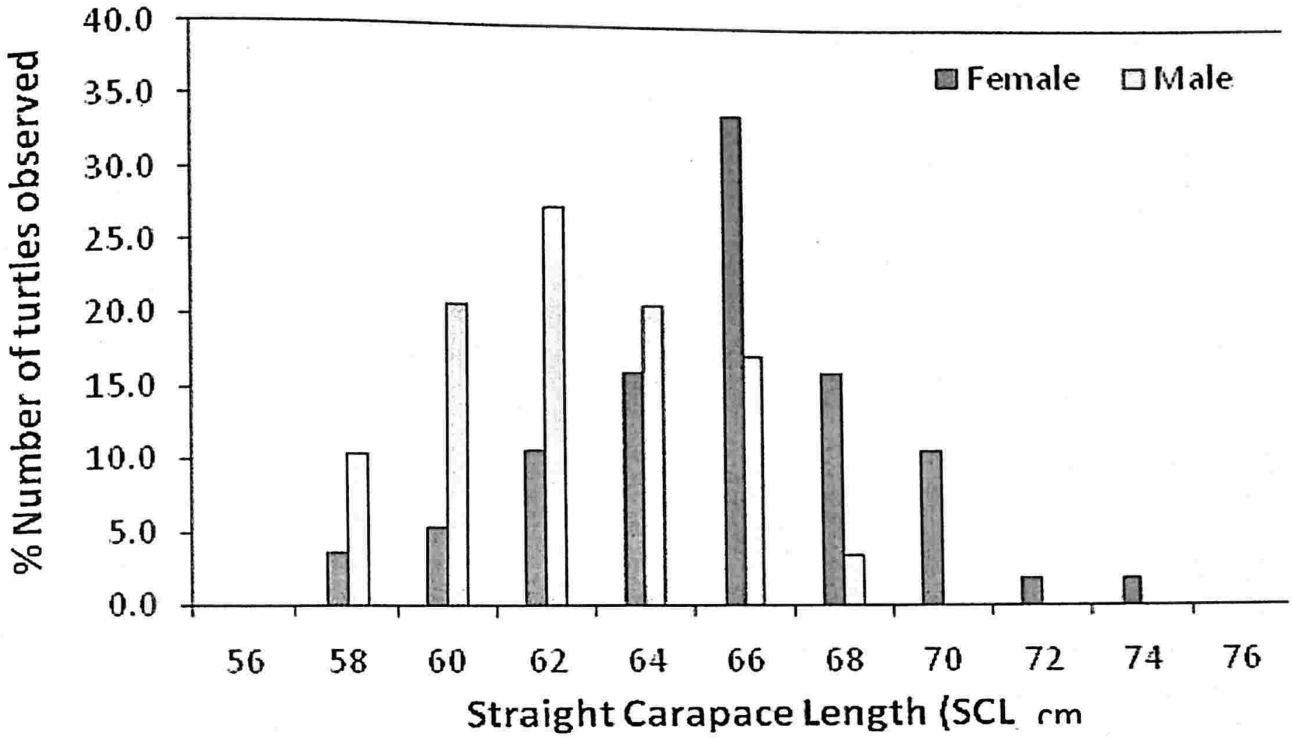

Figure 15. A comparison of the frequency distribution of carapace size (SCL) between 29 male and 56 female dead turtles recorded during the study.

Table 2. A comparison of the external morphology between 29 male and 56 female dead turtles recorded during the study.

Morphometric Characteristics	Male	Female
Straight Carapace Length (SCL)	63.7 ± 2.4 (59.6 – 68.3, 29)	64.6 ± 3.2 (56.4 – 72.2, 56)
Straight Carapace Width (SCW)	57.1 ± 1.9 (53.3 – 60.5, 29)	57.2 ± 2.4 (52.4 – 63.1, 56)
Curved Carapace Length (CCL)	67.4 ± 2.7 (62.5 – 73.0, 29)	69.1 ± 3.2 (61.7 – 77.3, 56)
Curved Carapace Width (CCW)	65.8 ± 2.4 (61.4 – 71.2, 29)	67.4 ± 3.1 (60.9 – 74.5, 56)
Head Length (HdL)**	16.8 ± 0.7 (15.2 – 18.2, 25)	17.6 ± 1.0 (15.7 – 19.7, 51)
Head Width (HdW)	10.7 ± 1.0 (7.0 – 12.0, 25)	11.0 ± 0.6 (9.7 – 12.2, 51)

Table 3. Comparison of humerus bone morphology between the 29 male and 56 female dead turtles collected during the study and was not significantly different between the sexes.

Morphometric Characteristics	Male (n = 29)	Female (n = 56)
Maximum length (ML)	148.7 ± 7.2 (135.1-163.8)	150.5 ± 10.3 (125.4 -171.3)
Longitudinal length (LL)	141.8 ± 6.1 (130.6 – 151.5)	141.0 ± 7.9 (120.4 – 159.0)
Ulnar process length (UPL)	60.8 ± 4.6 (52.9 - 68.2)	60.2 ± 6.9 (42.1 – 73.1)
Proximal length (PL)	126.2 ± 6.4 (113.8 – 144.6)	124.7 ± 7.7 (104.5 – 139.3)
Proximal width (PW)	58.5 ± 2.9 (53.5 – 65.2)	58.7 ± 4.3 (49.0 – 69.2)
Radial process length (RPL)	29.0 ± 1.9 (25.8 – 33.7)	28.9 ± 2.5 (24.1 – 34.9)
Width of Delto-pectoral crest (DpCW)	36.5 ± 1.6 (34.0 – 40.8)	36.4 ± 1.8 (31.8 – 40.2)
Distal width (DW)	51.7 ± 2.6 (46.4 – 56.3)	51.1 ± 3.5 (41.1 – 56.7)
Maximum head diameter (MaxHD)	27.0 ± 1.9 (23.5 – 31.9)	27.1 ± 3.0 (22.9 – 38.9)
Minimum head diameter (MinHD)	22.7 ± 1.1 (20.6 – 24.8)	22.9 ± 1.3 (19.8 – 25.8)

A comparison of the straight carapace length (SCL), with the maximal length (ML) of the humerus bone showed a strong correlation (R = 0.79 for males, R = 0.77 for females; Figure 16). A positive correlation between the SCL and the humerus longitudinal length (LL) was also found (R = 0.78 for males, R = 0.71 for females).

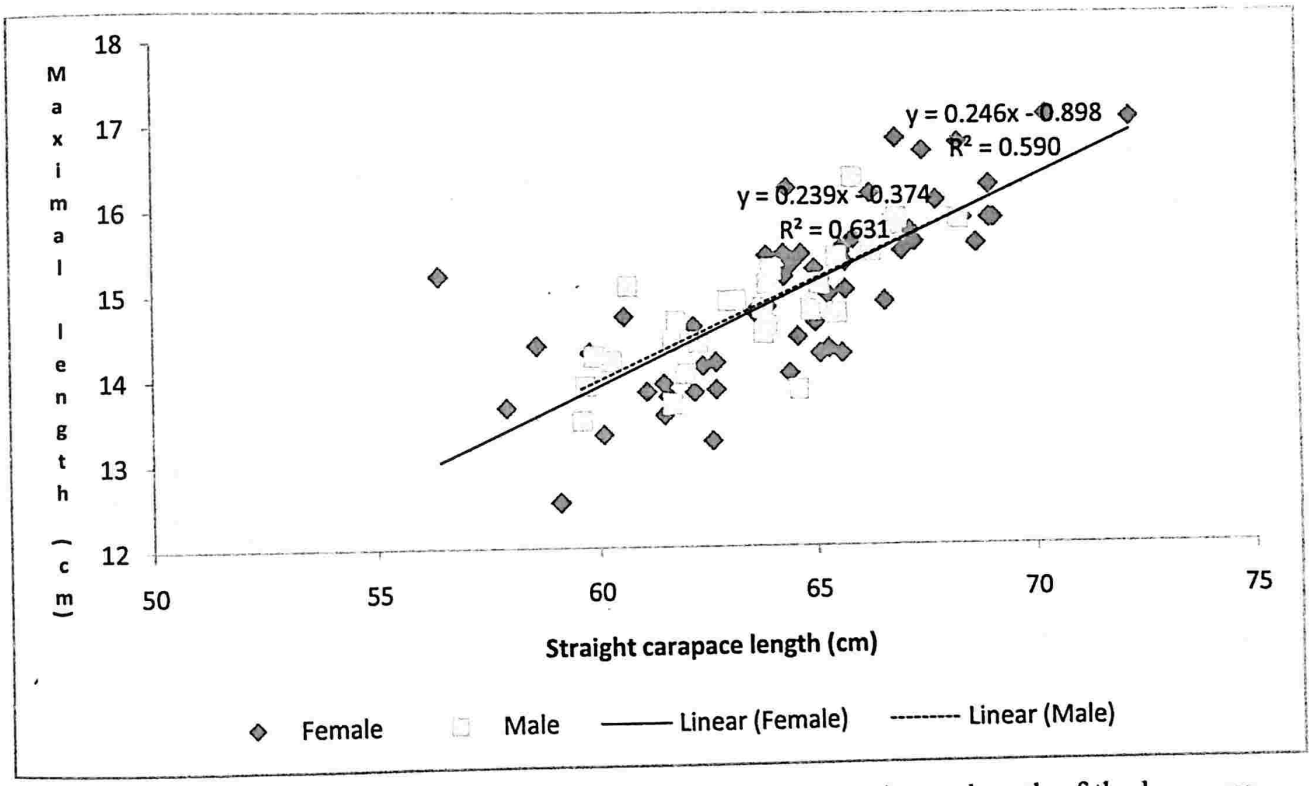

Figure 16 Relationship between straight carapace length and maximum length of the humerus

4.3 Age Estimation: Ranking and Correction Factor Protocol

Of the 85 humerus bone cross sections only 74 sections have been used for the age estimation, as the data contained outliers that could not be accounted for. Due to time constraints it was not possible to re-run test on these individuals to account for the problems encountered while estimating their ages. The smallest and largest resorption core width in the samples ranged from

9.20 mm to 19.48 mm respectively. Further, the resorption core width was not correlated with the diameter of the cross section (R = 0.24). Table 5 has a summarization of the age estimates by both protocols for female turtles.

Table 5 Age estimates for females from the Ranking and Correction Factor Protocols

S. No	Tag #	Sex	SCL (cm)	CCL (cm)	Diameter of RC	Observed LAG	RP Age	CF age
1	079 - F	F	65.3	68.8	9.20	36	36	39.2
2	080 - F	F	67.5	70.0	9.50	34	37	37.3
4	081 - F	F	63.6	68.3	9.70	35	37	38.4
5	077 - F	F	62.2	66.4	10.10	39	40	42.6
6	083 - F	F	62.7	67.1	10.20	31	35	34.6
8	019 - F	F	69.0	74.2	10.30	35	38	38.7
10	011 - F	F	72.2	77.3	10.51	31	43	34.8
11	020 - F	F	66.3	71.0	10.77	16	37	19.9
12	082 - F	F	65.9	69.9	10.80	34	35	37.9
13	076 - F	F	61.5	65.9	10.90	30	30	34.0
14	085 - F	F	64.6	68.2	10.90	30	33	34.0
17	062 - F	F	65.1	69.6	11.20	21	28	25.1
18	074 - F	F	66.3	70.0	11.20	30	37	34.1
20	003 - F	F	63.9	67.2	11.54	23	35	27.2
21	010 - F	F	60.6	66.4	11.66	18	26	22.3
23	034 - F	F	61.6	66.7	11.80	16	24	20.4
26	021 - F	F	64.3	68.2	11.83	29	39	33.4
28	017 - F	F	67.8	72.5	12.11	28	38	32.5
29	052 - F	F	60.1	63.8	12.20	21	25	25.5
31	057 - F	F	62.6	66.0	12.40	27	27	31.6
32	071 - F	F	65.7	69.5	12.80	31	34	35.8
35	008 - F	F	68.3	72.3	13.02	23	35	27.9
37	054 - F	F	62.4	66.4	13.14	33	41	38.0

40	051 - F	F	62.2	67.1	13.20	17	24	22.0
41	015 - F	F	68.4	73.0	13.24	23	29	28.0
43	016 - F	F	69.1	73.9	13.53	36	37	41.1
44	078 - F	F	61.1	65.3	14.00	31	35	36.4
46	070 - F	F	65.6	71.3	14.00	39	42	44.4
47	047 - F	F	66.6	71.9	14.00	21	26	26.4
49	046 - F	F	57.9	61.7	14.10	23	27	28.4
50	002 - F	F	63.9	68.0	14.29	22	37	27.5
51	055 - F	F	64.7	69.2	14.30	31	35	36.5
52	039 - F	F	61.5	65.8	14.40	25	35	30.5
53	075 - F	F	65.0	68.9	14.50	27	30	32.6
54	013 - F	F	67.2	72.0	14.80	21	31	26.7
57	058 - F	F	64.3	69.1	15.00	26	29	31.8
56	048 - F	F	64.4	68.9	15.00	17	29	22.8
60	007 - F	F	64.5	68.8	15.54	24	38	30.1
62	037 - F	F	64.5	69.3	16.10	27	36	33.3
63	060 - F	F	65.6	69.8	16.10	29	34	35.3
65	053 - F	F	59.8	64.5	16.40	24	31	30.5
67	028 - F	F	65.3	69.0	16.60	17	25	23.5
68	044 - F	F	56.4	70.0	16.70	23	36	29.6
72	018 - F	F	65.7	69.3	17.90	24	33	31.1
73	006 - F	F	65.0	68.0	17.95	27	38	34.2
74	009 - F	F	65.6	72.8	19.46	44	49	51.8
75	024 - F	F	70.3	71.5	19.48	21	35	28.9

Meaning of the abbreviations in the above table:

SCL - Straight carapace length

CCL - Curved carapace length

Diameter of RC - Diameter of resorption core

Observed LAG - Observed lines of arrested growth

RP Age - ranking protocol age

CF Age - correction factor age

Age estimation was conducted on forty-eight (48) female individuals. The number of observed LAGs ranged from 16 - 44 and a median of 27. The sample size class of the female population ranged from straight carapace length of 56 - 72 cm, with majority of the sample being within the SCL range of 64 - 66 cm. According to the ranking protocol, the minimum age is 24, maximum 49 and the median age is 35 years. However, within size classes there is also a variation in the age; for instance, size class 66 cm has age variations from 26 - 37 years. There is no pattern between the size class and the estimated age according to the ranking protocol. For the correction factor protocol, the slope value is determined from the youngest individuals, however, in this study all the samples came from the breeding population, thus the slope value used here was borrowed from Zug *et al* (2006) for smaller individuals.

In the correction factor protocol the minimum age estimate is 19.9 years, maximum; 51.8 and the median 32.5 years. In this case, the size class that has the minimum year is 66.3 cm, while the maximum estimate year is for an individual of 65.6 cm. In this case, the smallest individual (56.4 cm) is 29.6 years, while the biggest individual (72.2 cm) is 34.8 years old. The age estimated by the correction factor protocol also shows that there is no definite relationship between size class and estimated age. Figure 17 shows the comparison of the age estimated by both protocols.

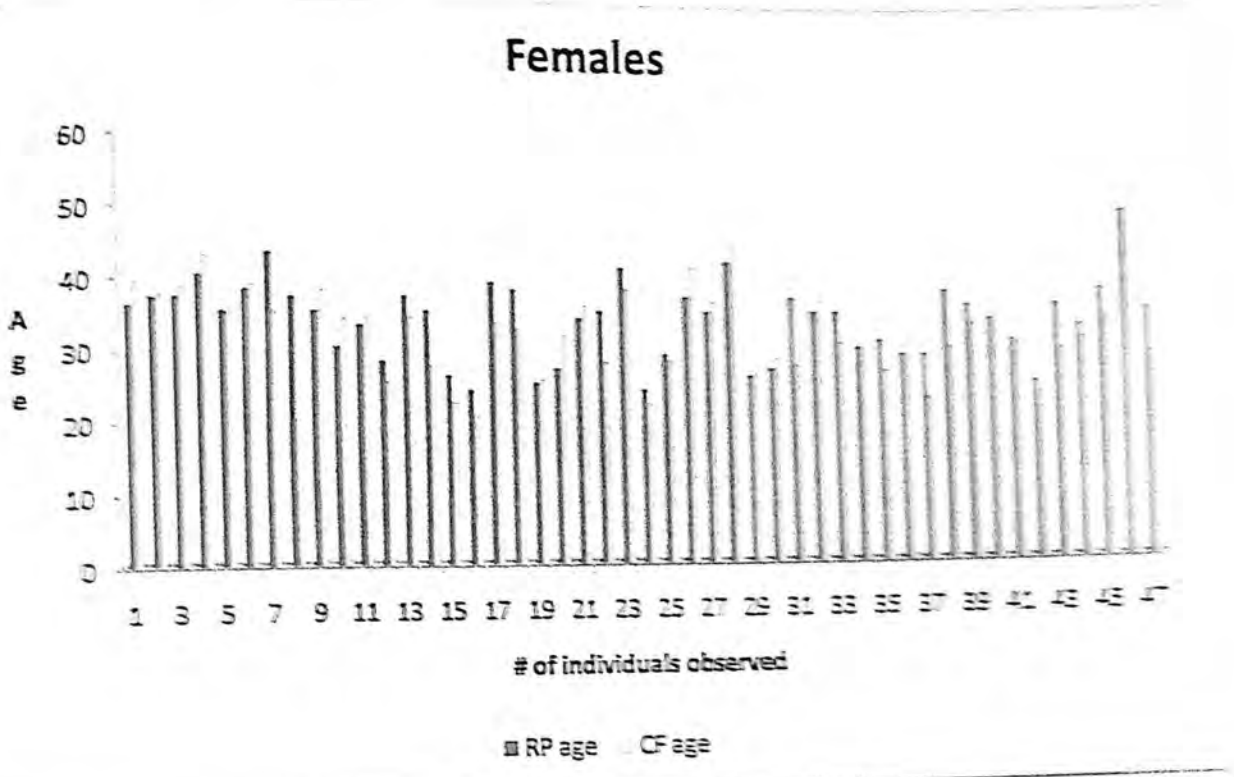

Figure 17 Age estimations by both protocols against number of individuals observed for females. There is a difference for age estimation by the two protocols.

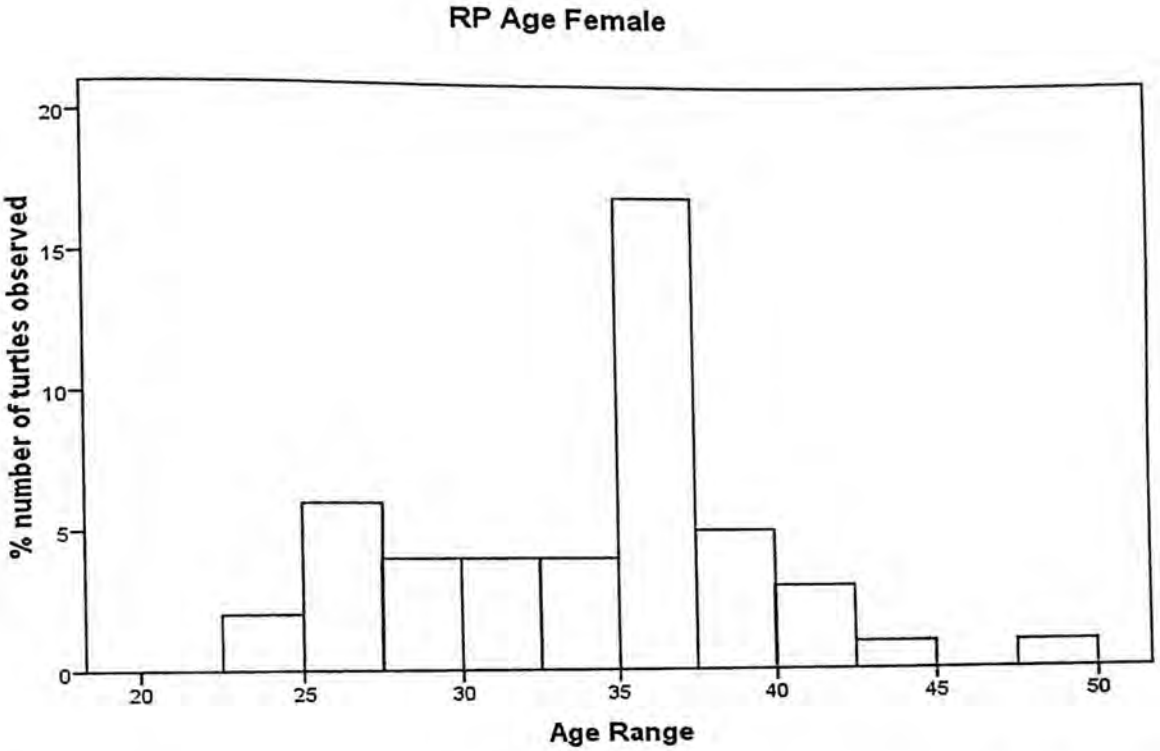

Figure 18 Frequency distribution of age ranges from the ranking protocol of females against the number of turtles observed.

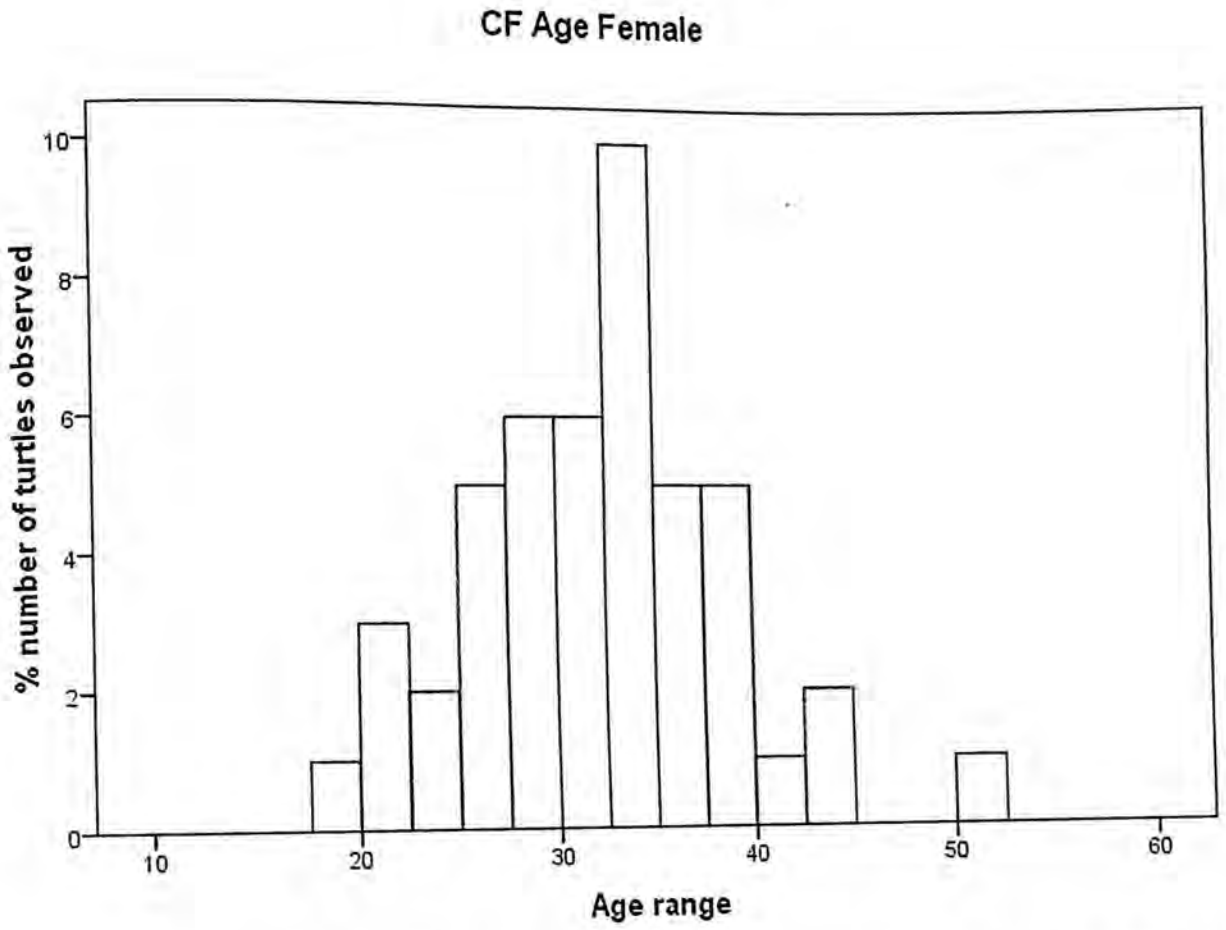

Figure 19 Frequency distribution of age ranges from the correction factor protocol of females against the number of turtles observed.

The ranking protocol shows the frequency distribution of age range for the size class 56 - 74 cm for females to be from 24 - 49 years (Figure 18), with the majority of individuals being within the age range 35 years, while in the correction factor protocol the age range is 19.8 - 51.2 years and majority of the individuals falling into the age group of 33 years (Figure 19).

Table 6 Age estimates for males from the Ranking and Correction Factor Protocols

S. No	Tag #	Sex	SCL (cm)	CCL (cm)	Diameter of RC	Observed LAG	RP Age	CF age
3	033-M	M	59.9	64.9	9.60	23	27	26.4
7	066 - M	M	64.9	67.8	10.20	30	33	33.6
9	065 - M	M	59.7	63.9	10.40	32	30	35.7
15	064 - M	M	65.1	69.2	10.90	27	28	31.0
16	069 - M	M	65.5	70.7	11.10	28	30	32.0
19	005 - M	M	65.9	62.5	11.51	27	41	31.2
22	068 - M	M	60.3	64.2	11.70	34	35	38.3
24	056 - M	M	64.0	67.2	11.80	20	27	24.4
25	067 - M	M	66.9	71.2	11.80	23	30	27.4
27	026 - M	M	62.3	67.7	11.84	17	28	21.4
30	061 - M	M	63.0	68.6	12.20	41	41	45.5
33	030 - M	M	59.6	63.7	12.90	19	27	23.9
36	027 - M	M	63.2	67.6	13.03	18	32	22.9
38	001 - M	M	68.3	73.0	13.19	32	38	37.0
39	072 - M	M	61.7	65.2	13.20	28	31	33.0
42	029 - M	M	65.5	69.5	13.50	20	30	25.1
45	038 -M	M	66.8	70.3	14.00	26	37	31.4
48	032 - M	M	61.8	66.1	14.02	20	31	25.4
55	045 - M	M	66.3	71.0	14.90	27	32	32.8
58	049 - M	M	63.9	68.1	15.30	35	37	41.0
59	050 - M	M	62.0	65.2	15.40	24	27	30.0
61	035 - M	M	63.8	68.7	15.90	19	31	25.2
64	042 - M	M	64.6	67.9	16.30	28	37	34.4
66	031 - M	M	63.8	66.4	16.60	26	37	32.5
69	040 - M	M	61.7	63.0	16.80	18	33	24.6
70	041 - M	M	63.9	67.1	16.90	22	35	28.7
71	043 - M	M	66.3	69.6	16.90	24	36	30.7

There are twenty-four (24) males in this sample with a size class ranging from 59 - 68 cm and a median value of 63.9 cm (Table 6). Age estimation from the ranking protocol ages these individuals from 27 - 41 years. Here the median age is 32 and as with the female age estimation, age is not dependent on size class. The correction factor protocol ages these individuals from 21.4 - 41 years, median age 31. The largest individual is 68.3 cm and is aged at 38 and 37 years from the ranking and correction factor protocol respectively, while the smallest individual is 59.6 cm and is aged at 27 and 23.9 years by the ranking and correction factor protocol respectively. Interestingly another individual (59.7 cm) has been aged as 30 years (ranking protocol) and 35.7 years (correction factor protocol). Between these two individuals, the size class differs by 0.1, yet however, there is a difference of 3 years by the ranking protocol and 12 years by the correction factor protocol of these individuals (Figure 20).

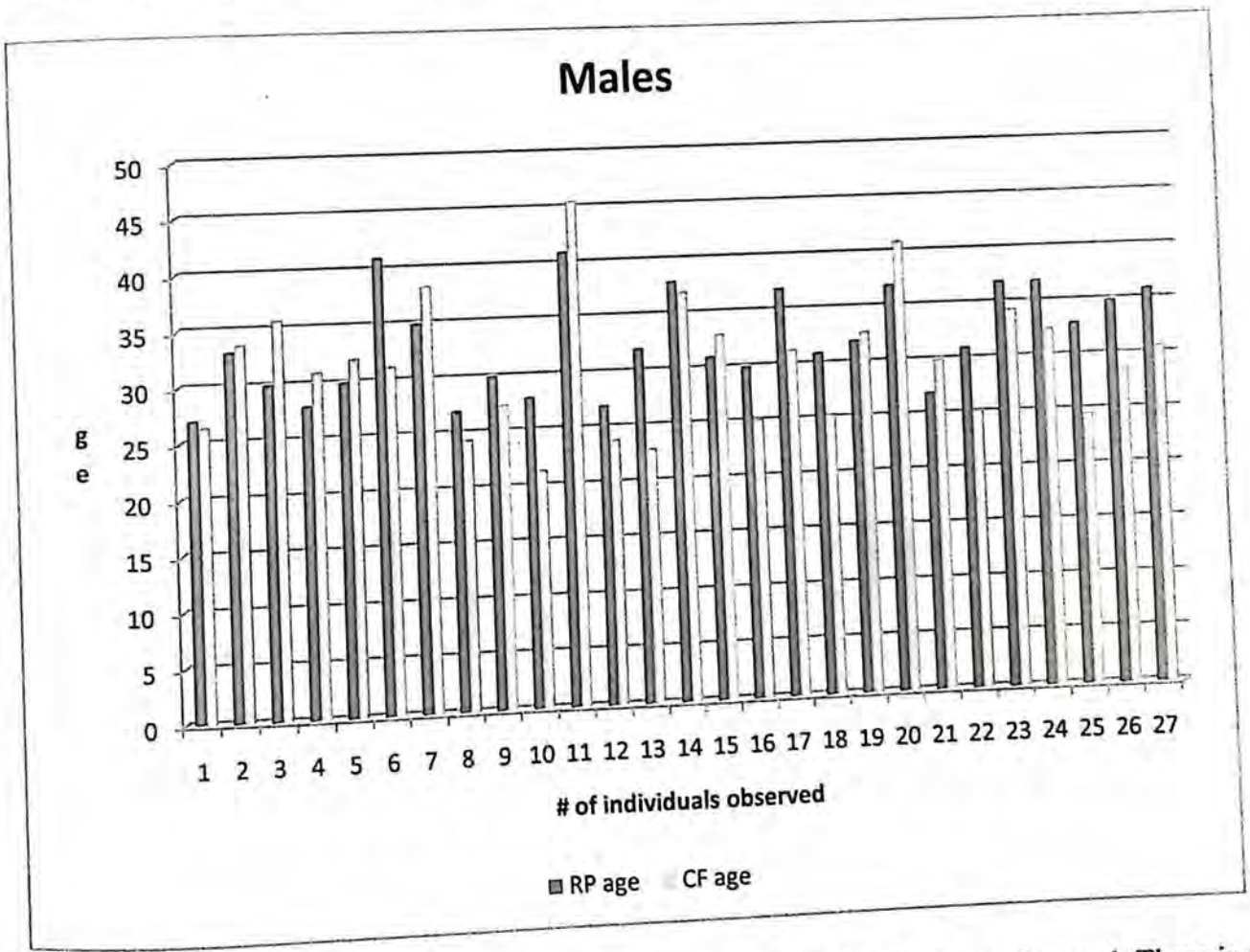

Figure 20 Age estimations by both protocols against number of individuals observed. There is a difference for age estimation by the two protocols.

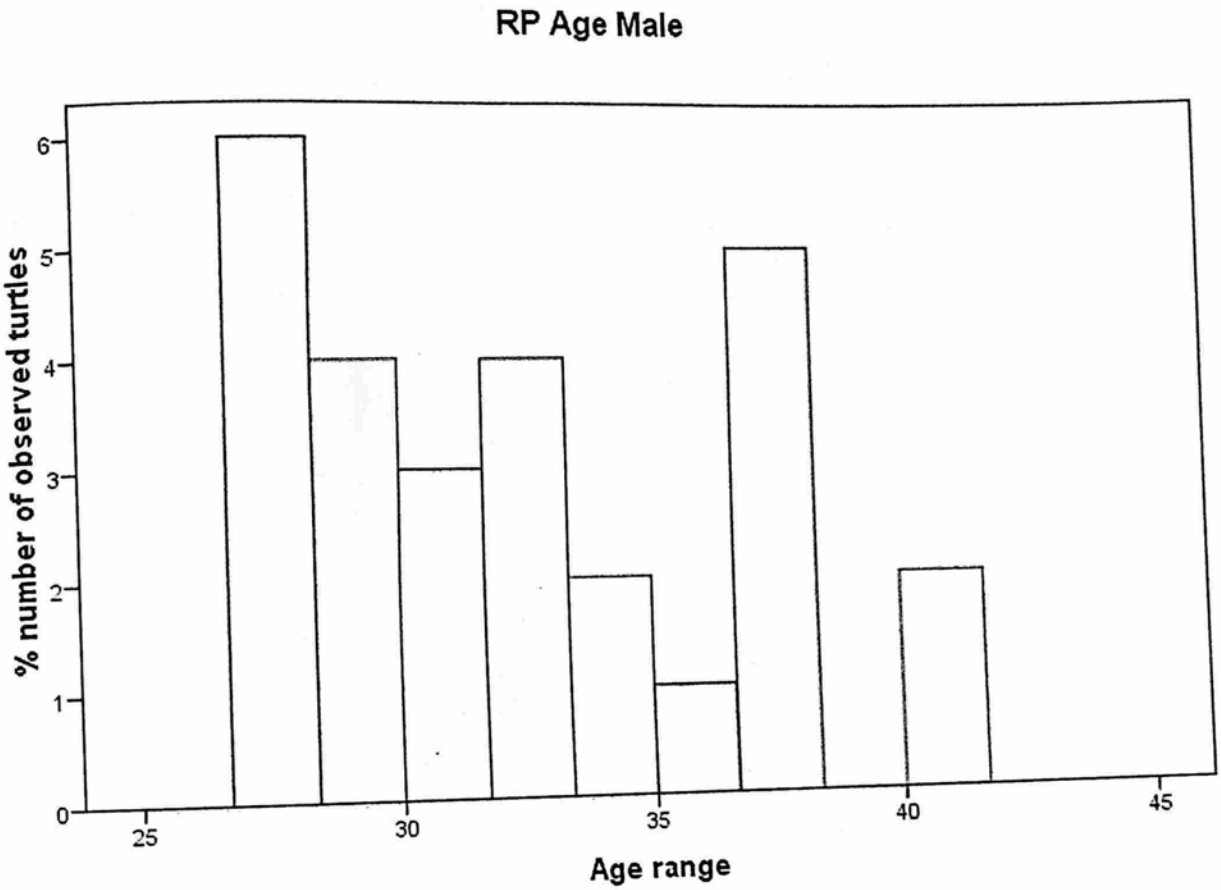

Figure 21 Frequency distribution of age ranges from the ranking protocol of males against the number of turtles observed.

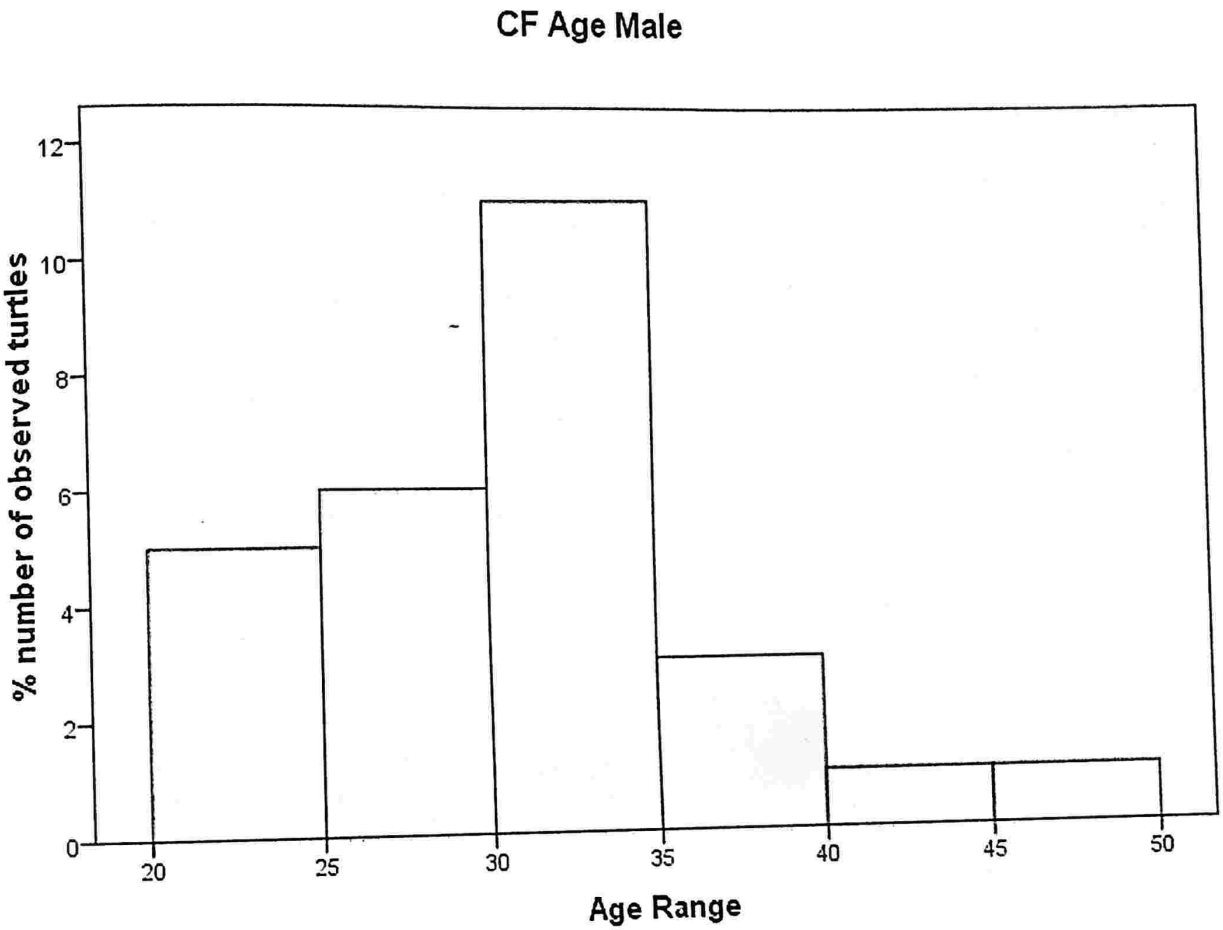

Figure 22 Frequency distribution of age ranges from the correction factor protocol of males against the number of turtles observed.

The ranking protocol shows the frequency distribution of age range for the size class 56 - 74 cm for females to be from 26 - 41 years (Figure 21), with the majority of individuals being within the age range 32 years, while in the correction factor protocol the age range is 20 - 46 year and majority of the individuals falling into the age group of 31 years (Figure 22).

Examination of a femur bone cross section for a turtle (Tag # 032 – M) showed the presence of growth layers and 19 LAGs were observed. For the same individual the number of LAGs observed in the humerus bone section was 20. Examination of a phalanges bone cross section for a turtle (Tag # 002 – F) showed the presence of growth layers, 19 LAGs were observed. For the same individual the humerus bone section showed 22 LAGs.

4.4 Size and age relationship

The cross sections of humerus bones showed the presence of lines of arrested growth (LAG) when histological lab preparations were followed. These cross sections when stained showed two growth rings, a broad zone which is usually stained light called the marks of skeletal growth (MSG) and a thin zone stained darker called lines of arrested growth (LAG) (Zug *et al.* 1986). Observations during lab preparation and viewing under the microscope showed in older individuals, the LAGs become harder to distinguish and the osteoblast cells were larger in quantity than the smaller individuals (Figure 23 & 24).

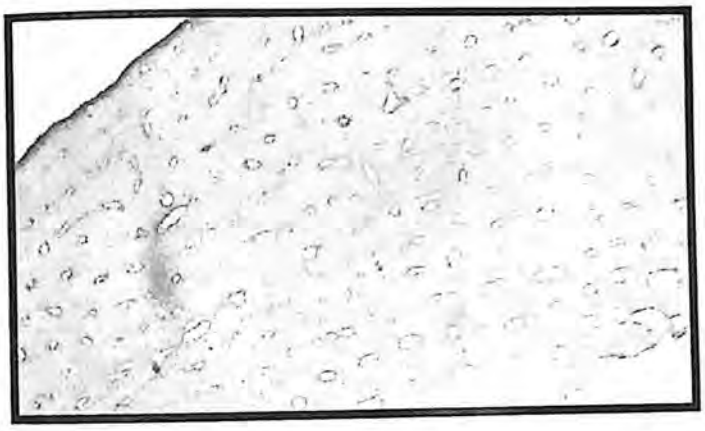

Figure 23 LAGs becoming fainter because of the presence of more osteoblast cells

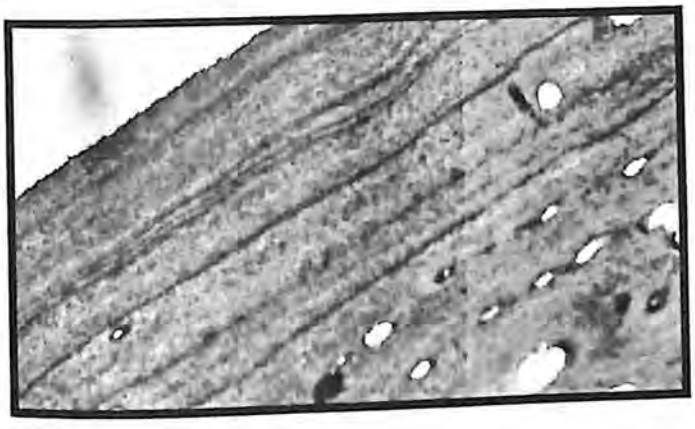

Figure 24 Well defined LAGs with fewer osteoblast cells.

No correlation exists between SCL and the age estimated from the Ranking and Correction Factor protocols (Figure 25 & 26). Age estimated highly varied with body size (SCL).

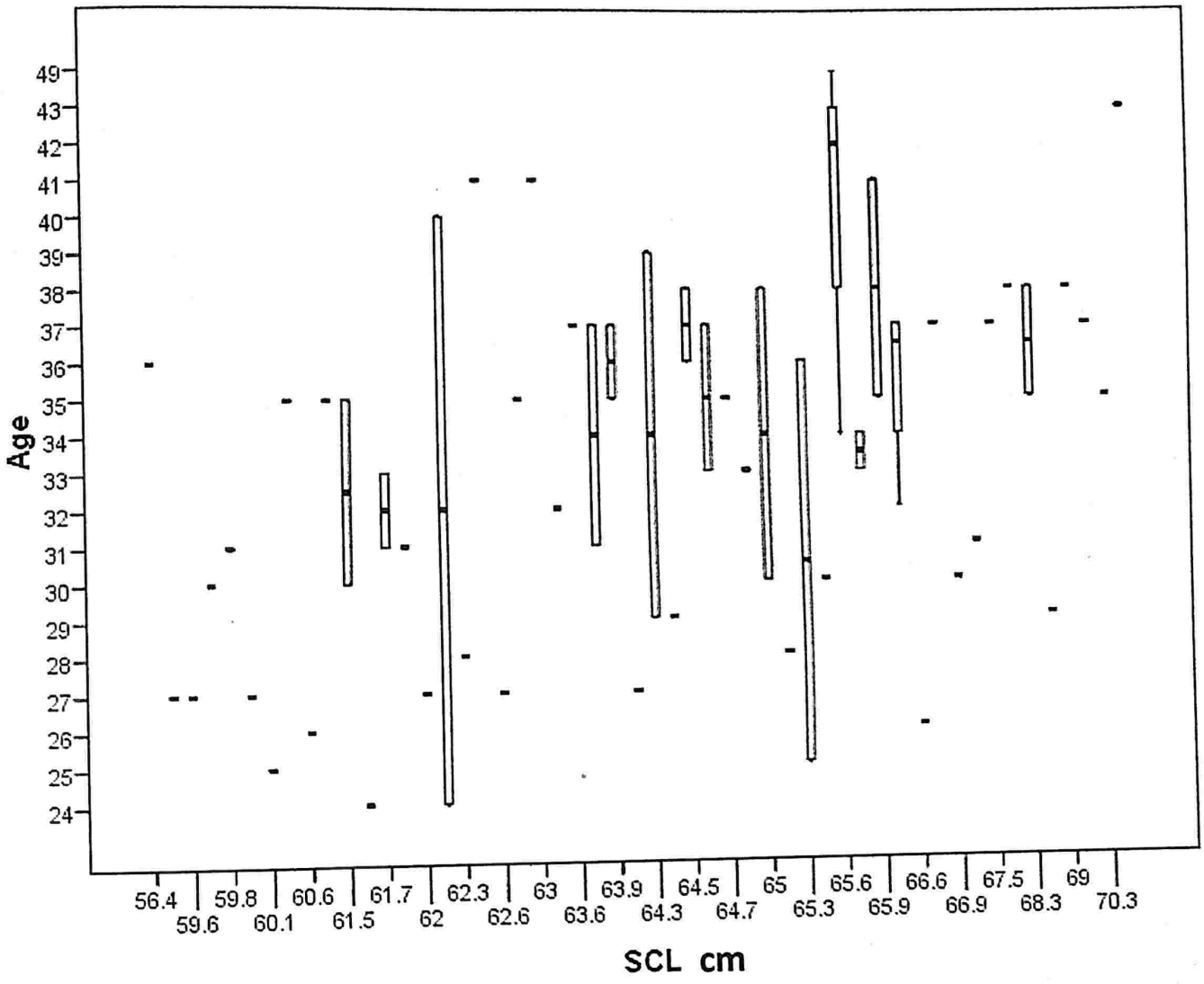

Figure 25 Relationship between straight carapace length (SCL) and age from the ranking protocol

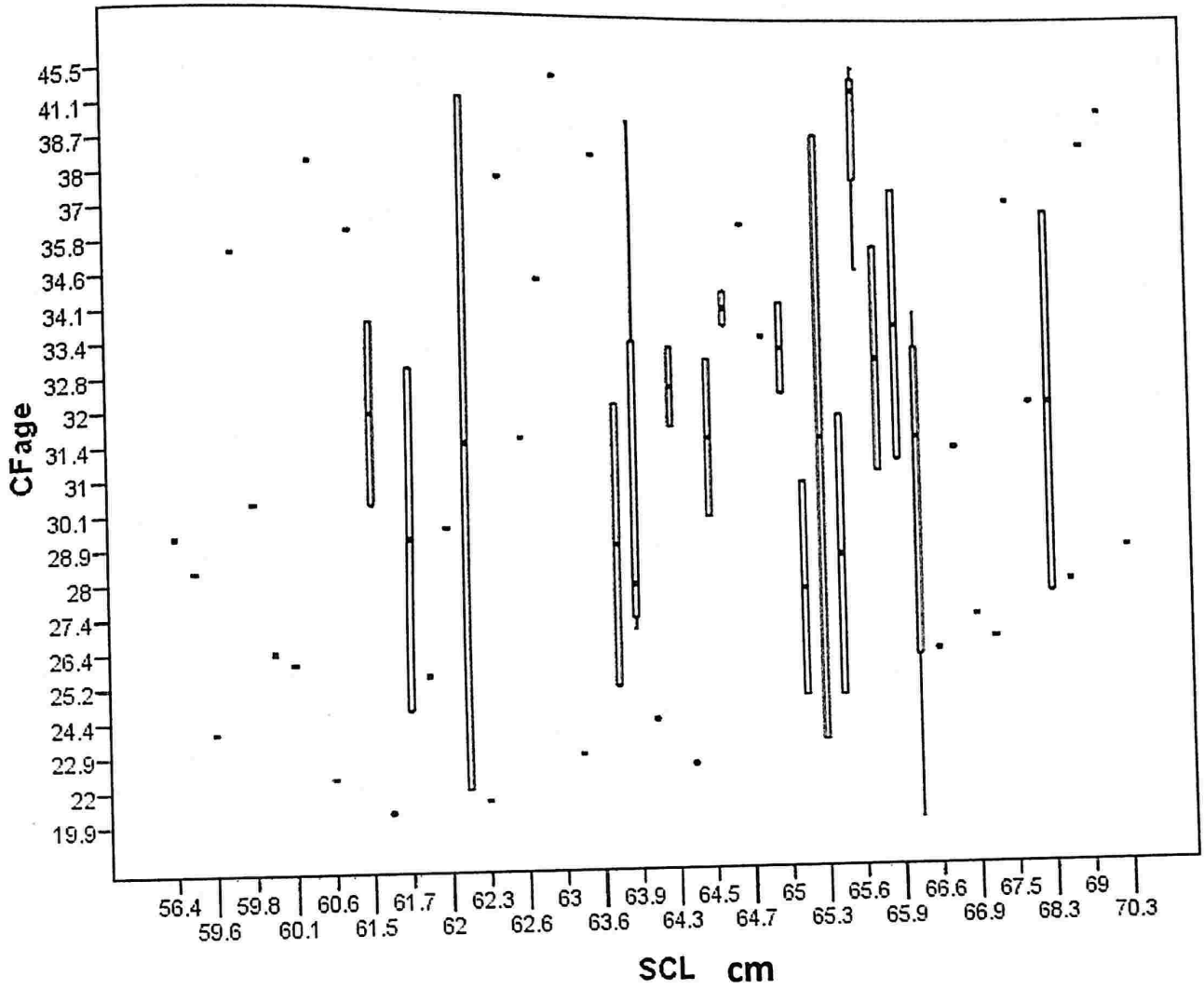

Figure 26 Relationship between straight carapace length (SCL) and age from the correction factor protocol

Table 7 Comparison of age between the North Pacific and Indian Ocean olive ridley

Serial #	**Carapace length (SCL, cm)**	**North Pacific (Zug *et al.* 2006)**		**Indian Ocean (current study)**	
		Ranking protocol	**Correction factor**	**Ranking protocol**	**Correction factor**
1	20.5	5	7.3		

2	33.0	12	9.4		
3	38.2	18	12.0		
4	43.7	18	17.0		
5	46.6	24	13.6		
6	47.9	23	13.1		
7	52.7	26	16.7		
8	54.0	27	16.9		
9	55.3	27	16.9		
10	56.4			36	29.6
11	56.5	28	17.9		
12	57.5	34	17.7		
13	57.7	34	18.1		
14	57.7	34	18.6		
15	57.8	33	19.6		
16	57.9			27	28.4
17	59.6			27	23.9
18	59.7			30	35.7
19	59.8			31	30.5
20	59.9			27	26.4
21	60.0	36	24.1		
22	60.1			25	25.5
23	60.2	34	15.7		
24	60.3	37	18.0	35	38.3
25	60.4	32	23.4		
26	60.6			26	22.3
27	61.1			35	36.4
28	61.5			30	34.0
29	61.5			35	30.5
30	61.6	33	14.8	24	20.4
31	61.7			31	33.0

32	61.7			33	24.6
33	61.8	31	16.3	31	25.4
34	62.0			27	30.0
35	62.1	32	13.7		
36	62.1	34	18.1		
37	62.2			40	42.6
38	62.2			24	22.0
39	62.3			28	21.4
40	62.4	37	14.7	41	38.0
41	62.5	38	12.8		
42	62.6			27	31.6
43	62.7			35	34.6
44	63.0	34	19.3	41	45.5
45	63.2			32	22.9
46	63.6			37	38.4
47	63.8			31	25.2
48	63.8			37	32.5
49	63.9			35	27.2
50	63.9			37	27.5
51	63.9			37	41.0
52	64.0			27	24.4
53	64.3			39	33.4
54	64.3			29	31.8
55	64.4	38	12.9	29	22.8
56	64.5			38	30.1
57	64.5			36	33.3
58	64.6			33	34.0
59	64.6			37	34.4
60	64.7			35	36.5
61	64.9			33	33.6

62	65.0			30	32.6
63	65.0			38	34.2
64	65.1			28	25.1
65	65.1			28	31.0
66	65.3			36	39.2
67	65.3			25	23.5
68	65.5			30	32.0
69	65.5			30	25.1
70	65.6			42	44.4
71	65.6			34	35.3
72	65.6			49	51.8
73	65.7			34	35.8
74	65.7			33	31.1
75	65.9			35	37.9
76	65.9			41	31.2
77	66.3			37	19.9
78	66.3			37	34.1
79	66.3			32	32.8
80	66.3			36	30.7
81	66.6			26	26.4
82	66.8			37	31.4
83	66.9			30	27.4
84	67.2			31	26.7
85	67.5			37	37.3
86	67.8			38	32.5
87	68.3			35	27.9
88	68.3			38	37.0
89	68.4			29	28.0
90	69.0			38	38.7
91	69.1			37	41.1

92	70.3			35	28.9
93	72.2			43	34.8

From the above table it is shown that age was estimated for 27 individuals from the North Pacific ranging in SCL from 20.5 - 64.5 cm. From the ranking protocol the age ranged from 5 - 38 years (Table 7). From size classes 20.5 - 57.5 cm, the age estimation increases as the size classes increases, however, the years fluctuate from size class 57.7 - 64.5 cm, there is no definite pattern of age estimation as the size classes increases from 57.7 according to the ranking protocol. From the correction factor protocol, as size class increases so does the years from size class 20.5 - 60.0 cm, with the exception of size class 43.7 cm which is aged at 17 years, otherwise all others up to 60.0 cm follow a pattern. Beyond this size class there is no definite pattern with age and size class from the North Pacific olive ridleys. As mentioned earlier, the Indian Ocean olive ridleys do not have a positive relationship between size class and age estimation from either of the two protocols. Comparison of age estimation of the Indian Ocean olive ridleys with the North Pacific olive ridleys showed for those individuals with the same size class, the ranking protocol from both population vary from 2 - 11 years. The correction factor protocol has no similarities between the two populations. The age estimation from the Indian Ocean population is older than the North Pacific population.

4.5 Aging arribada population

The straight carapace length measurements from 1212 nesting turtles during the arribada in 2013 ranged from 56.1 to 72.2 cm, and the majority falling in the 66 - 68 cm class interval (Figure 26). Carapace morphology has been summarized in Table 8. Since there was no correlation between body size and age estimated it was not possible to age individual nesting turtles of the arribada. A range of age estimates could only be assigned to the turtles (Table 9).

Table 8 Carapace morphology (in cm) of 1212 females during arribada March 2013

Morphometric Characteristics	Mean ± SD (Range)
Straight Carapace Length (SCL)	64.8 ± 2.5 (56.1 – 72.2)
Straight Carapace Width (SCW)	57.6 ± 2.3 (50.2 – 68.4)
Curved Carapace Length (CCL)	68.8 ± 2.7 (54.9 – 78.9)
Curved Carapace Width (CCW)	67.8 ± 2.7 (53.2 – 78.7)

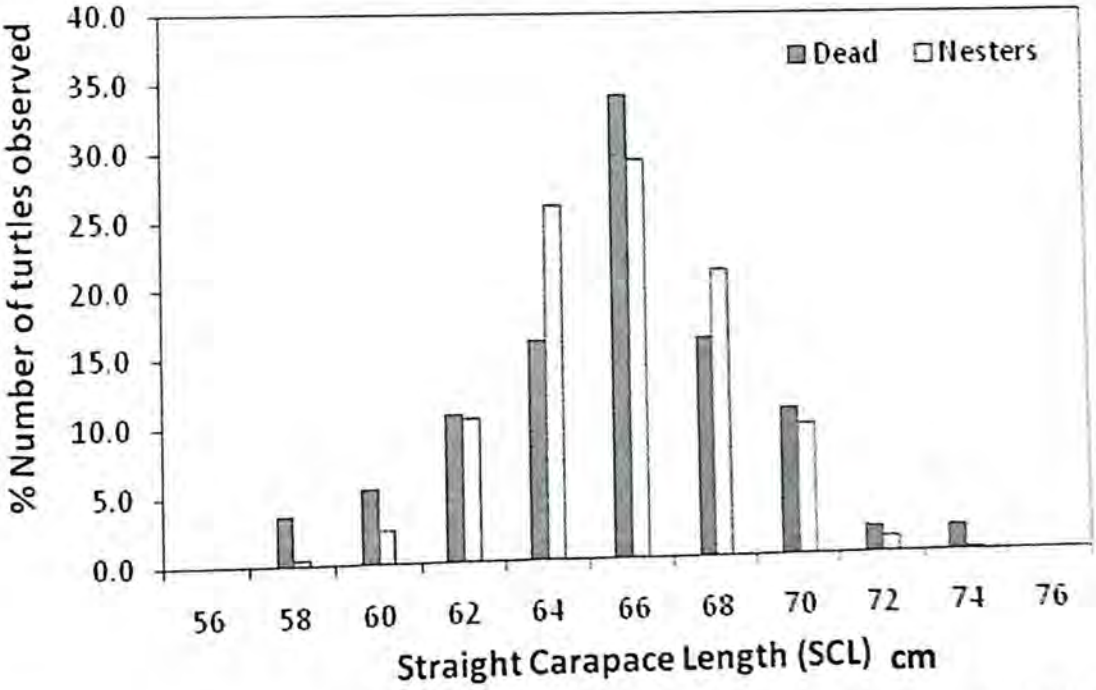

Figure 27 A comparison of the frequency distribution of carapace sizes (SCL) between the 56 dead females and the 1212 nesting females from Rushikulya rookery.

From the arribada population majority of the individuals were from the size class 64 - 68 cm. The youngest arribada individual was from the size range 58 - 59 cm. Table 9 provides a baseline data for age estimation of the arribada population based on size class. Individuals as small as 56 cm can be as young as 27 years and as old as 31.5 years; while large individuals from the size range 72 - 73 cm can be as old as 34 - 43 years. The size class from 64 - 68 cm has an age estimate of 22 - 49 years.

Table 9 Age assignment from both protocols to size class of the arribada females

SCL range (cm)	**Ranking Protocol Age (Median; Max; Min)**	**Correction Factor Protocol Age (Median; Max; Min)**
56 - 57	31.5 (36; 27)	29 (29.6; 28.4)
58 - 59	30 (31; 27)	28.5 (35.7; 23.9)
60 - 61	30 (35; 24)	30.5 (38.3; 20.4)
62 - 63	32 (41; 24)	30.8 (45.5; 21.4)
64 - 65	35.5 (49; 25)	33.3 (51.8; 22.8)
66 - 67	32 (38; 26)	31.4 (37.3; 19.9)
68 - 69	36 (38; 29)	36.9 (41.2; 27.9)
70 - 71	35	28.9
72 - 73	43	34.8

5 Discussion

Demographic studies on olive ridley are not well studied. Being a marine species and having a migratory behavior makes studies on population difficult (Reichart 1993). With the exception of arribada studies and other localized studies such as telemetry tracking, tagging studies very little information is available on this species. Data to date on population dynamics are based on studies from females, which only represents a part of their entire life. In the past, few studies have been conducted to account for different aspects of population dynamic studies. Methods and the shortcomings for estimation of population size has been discussed by Meylan (1981), population model was developed by Marquez *et al* (1976) for olive ridleys of the Eastern Pacific Ocean, and the assessment of the ways available for females arrival for arribada on shore has been done by Marquez and Van Dissel (1982). Understanding population dynamics for effective management entails studying the natality, morality, sexual maturity, sex ratio and longevity (Reichart 1993). In any sea turtle conservation plan and management, the overall goal is to promote long term survival of the population, which includes: safe guarding critical habitats, sustained recovery of depleting stocks and the well being of the species and the needs of communities and humans. To fully and effectively carry out conservation and management plans, data on population dynamics is necessary, otherwise decisions on effective management becomes a matter of making educated guesses.

Skeletochronological studies provide data for the estimation of age for sea turtles. The ideal technique for age estimation would be mark and recapture, however due to migratory and marine lifestyles this method is time demanding in short term studies, labor intensive, logistically difficult and expensive. Age estimation is important for population dynamics studies and also for effective management and conservation of a species. Skeletochronology is analogous to dendrochronology (study of growth rings in trees), thus skeletochronological studies assumes that the growth rings present within the cross sections of long bones (humerus, femur or phalanges) are representative a one year of the individuals life. Zug *et al* (1986) used humeral bones to estimate the age of the North-western Atlantic population of loggerheads (*Caretta caretta*) where they found these turtles attained sexual maturity between 13 - 15 years ranging from size classes between 800 - 900 mm CCL. Skeletochronological studies are means by which

age estimations of populations can be estimated. Klinger and Musick (1992) validated the assumption of one growth layer being equivalent to one year from skeletochronological studies by injecting oxytetracycline (OTC) into marked loggerheads. Several of these turtles were recaptured by fishermen during a 4 year period or found dead stranded along the beach. Biopsies were taken from the recaptured turtle and the humerus bones were processed by histological techniques followed by Zug *et al* (1986). Their results confirmed growth layers being annually deposited by the fluorescent OTC marking found in the growth layers.

5.1 Humerus bone section

Zug *et al* (1986) conducted several test on different bones to determine which ones had growth layers present and the least amount of resorption. It was concluded that humerus bones have the least amount of resorption in the middle of the shaft, just distal to the deltopectoral crest. Thus, the humerus bone was selected for this study. In this study, one femur bone and a pair of phalanges was used to compare the number of growth rings found in the individual bones. Comparing the number of observable LAGs in the humerus to the femur for the same individual showed no difference, the same number of growth rings where found in both bones; while the phalanges showed a difference of 2 LAGs less than that observed in the humerus. It was confirmed that both the femur and phalanges can also be used to determine age; however, using the phalanges is a time taking process. Although the phalangial bones are smaller and they decalcify faster than the humerus bones, to have a more accurate account of the number of growth rings observed, the second and third intermediate and proximal digits have to be analyzed and several cross sections has to be taken in a series which is then compared to each other. In majority of the dead turtles encountered, the back flippers were not present; therefore obtaining the femur bone was not possible for all samples.

5.2 Morphological characteristics of the dead olive ridley turtles

In sea turtle species, males and females differ in size, usually females are larger. According to the Mann-Whitney U Test, the difference in the straight carapace lengths between males and females were not statistically significant. However, males were not recorded in size classes beyond 68 cm (SCL).

5.3 Age estimation: Ranking and Correction Factor Protocol

The ranking protocol (RP) and correction factor protocol (CF) yielded age estimates that were not remarkably different from each other. For females age was estimated at 24 - 49 (RP) and 19.9 - 51.8 (CF) while males were 27 - 41 (RP) and 21- 46 (CF). There was no correlation between either protocols to SCL. Zug *et al* (2006) found a high correlation between the ranking age estimates with the SCL for olive ridleys, and the age estimates from correction factor were found to be biologically plausible. Since no correlation was found in my study and the two protocols yielded age estimates that were not significantly different from each other, both the age estimates are accepted as biological plausible age for the Indian Ocean olive ridley turtles. The results from this study, mirrors the results of investigations on the American toad *Bufo americnus,* northern crested newt *Triturus cristatus* and marbled newt *Triturus marmoratus* where they found body size is a poor predictor of age and larger individuals are not necessarily older (Acker *et al.*, 1986; Francillon - Vieillot *et al.*,1990). Discrepancies found for age estimates for different size class could be attributed to the low sample collection. Age differences observed in cases where larger sized individuals were found younger than smaller sized individuals can be attributed to injury, disease or being the 'runt of the litter" (Zug *et al.*, 2006). At a certain point these turtles also stop growing, however, this does not mean they do not grow older, therefore, in cases where small sized individuals are aged older than larger size individuals, it suggest that these individuals may have reached the maximum growth length. For example, individuals in the size class 63 cm were found to be as old as 41 (RP) and 45.5 (CF) years old, while individuals in the size class 64 cm was found to be 27 (RP) and 24.4 (CF) years old. Although it is a matter of 1

cm difference between these two individuals there is a difference of more than 20 years using the correction factor protocol and 14 years by the ranking protocol.

5.4 Size and age relationship

Previous studies by Zug *et al* (1986) study on loggerheads, Zug *et al* (1997); kemp's ridley, Zug & Glor (1998); green turtles, Zug *et al* (2006); olive ridley turtles found a positive correlation between age and size class. Contrary to these findings, this study did not establish such correlation between age and size class. In all the previous studies, the size class represented smaller sized individuals; loggerheads were from size class of 80 - 90 cm SCL and age was estimated at 13 - 15 years for attaining sexual maturity, Kemp's ridley (Zug *et al.*, 1997) were 18.8 - 72 cm; age estimates 2 -15 years, green turtles (Zug & Glor 1998) 28 - 74 cm; age estimated 3 - 14 years, with the sample lacking adults. Olive ridleys (Zug *et al.*, 2006) 20.5 - 64.4 cm, aged 5 - 38 years by ranking protocol and 7.3 - 24.1 years by correction factor protocol. In this study the size class was 56 - 74 cm and they were aged as 24 - 49 years (RP) and 19.9 - 51.8 years (CF). Reasons for lack of correlation may be because the samples are all from an adult breeding population as compared to the other studies where the sample collections were from a mixed population.

5.5 Aging arribada population

Arribada is a unique phenomenon amongst the ridley turtles only. Females synchronously arrive at nesting beaches *en masse* to nest. To date no skeletochronological study has been conducted to age an arribada population. Therefore, the information provided by this study is a baseline data set for future monitoring. Since this breeding population has no positive correlation between age and size, age ranges has been given for size classes. Studies conducted by Pandav & Choudhury (2000) has documented a wide range of size classes (50 - 84 cm), however in the past ten years, there has been reports of decline in size class. In this study, size classes recorded were from 58 - 74 cm. Data provided from the skeletochronological method accounts for individuals within this

size class, however, the number of individuals from larger size (70 - 74 cm) and smaller size class (56 - 60 cm) was less. Therefore, age estimations for these size classes are limited by sample size. Age range for the larger size class (70 - 74 cm) is 35 - 43 and 28.9 - 34.8 by the ranking and correction factor protocols respectively. These estimates of age were derived from two (2) individuals from the dead population. Because there is no correlation between size and age for the breeding population, the arribada population does not have an external morphological feature which can be used as a predictor of age. The variation of age in this investigation gives an understanding of how healthy the breeding population is and shows this adult breeding population to be healthy since age ranges between 24 - 49 years (RP) and 20 - 52 (CF). These findings, although limited by sample size have provided much needed information into the demography of olive ridleys in the Indian Ocean.

6 Conclusion

My investigations into age estimation of a breeding population of olive ridleys along the coast of Odisha documented ages from 19.9 -51.8 and 24 - 49 years by the correction factor and ranking protocol respectively. It is clear there is no correlation between size class and age, which disagrees with studies by Zug *et al* (1986, 1997, 2002, and 2006). In those studies they have established a positive correlation between age and size class. The sample collection from those studies is representative of several populations (hatchling, juveniles, sub adults and adults) and thus positive correlations exist between size class and age; whereas in the adult breeding population this relationship is non-existent. In smaller sized individuals the resorption core is smaller than in larger individuals and the number of LAGs resorbed is also lesser in smaller size class. In adult breeding population, these individuals at some point would stop growing, but resorption and deposition of growth layers would not cease thus a positive correlation would not be established between the size and age of these turtles. Arribada populations do not have an external morphometric feature which can be used as a predictor of age. The variation in the age across size class overlaps; however, this study shows the breeding population of olive ridleys has a huge variation in age even though they are subjected to high mortality on a yearly basis. A long term study needs to be done to have a comprehensive understanding of the age variation within the breeding population.

7 References

Acker PM, Kruse KC and Krehbiel EB 1986 Aging *Bufo americanus* by skeletochronology. *Journal of Herpetology* **20** 570-574.

Avens L, Taylor JC, Goshe LR, Jones TT and Hastings M 2009 Use of skeletochronological analysis to estimate the age of leatherback sea turtles *Dermochelys coriacea* in the western North Atlantic. *Endangered Species Research* **8** 165-177.

Baker TT and Timmons LS 1991 Precision of ages estimated from five bony structures of Arctic char (*Salvelinus alpinus*) from the Wood River System, Alaska. *Canadian Journal of Fisheries and Aquatic Sciences* **48** 1007-1014.

Bastien H and Leclair R 1992 Aging wood frogs (*Rana sylvatica*) by skeletochronology. *Journal of Herpetology* **26** 222-225.

Bjorndal KA, Bolten AB, Bennett RA, Jacobson ER, Wronski TJ, Valeski JJ and Eliazar PJ 1998 Age and growth in sea turtles: limitations of skeletochronology for demographic studies. *Copeia* **1998** 23-30.

Braun-McNeill J, Epperly SP, Avens L, Snover ML and Taylor JC 2008 Growth rates of loggerhead sea turtles (*Caretta caretta*) from the western North Atlantic. *Herpetological Conservation and Biology* **3** 273-281.

Brown CA and Gruber SH 1988 Age assessment of the lemon shark, *Negaprion brevirostris*, using tetracycline validated vertebral centra. *Copeia* **1988** 747-753.

Campana S 2001 Accuracy, precision and quality control in age determination, including a review of the use and abuse of age validation methods. *Journal of Fish Biology* **59** 197-242.

Campana SE 1983 Feeding periodicity and the production of daily growth increments in otoliths of steelhead trout (Salmo gairdneri) and starry flounder (*Platichthys stellatus*). *Canadian Journal of Zoology* **61** 1591-1597.

Casale P, Conte N, Freggi N, Cioni C, and Argano R 2011 Age and growth determination by skeletochronology in loggerhead sea turtles (*Caretta caretta*) from the Mediterranean Sea. *Scientia Marina* **75**: 197-203.

Cass AJ and Beamish RJ 1983 First evidence of validity of the fin-ray method of age determination for marine fishes. *North American Journal of Fisheries Management* **3** 182-188.

Casselman JM 1974 Analysis of hard tissue of pike *Esox lucius* with special reference to age and growth. In *The Ageing of Fish* (Bagenal, T. B., ed.), pp. 13–27. Surrey: Unwin Brothers.

Castanet J 1982 Recherches sur la Croissance du Tissu Osseux des Reptiles. Application: La Methode Squelettochronologique. PhD thesis, University of Paris, Paris.

Castanet J and Cheylan M 1979 Les marques de croissance des os et des ecailles comme indicateur de l'age chez *Testudo hermanni* et *Testudo graeca* (Reptilia, Chelonia, Testudinidae). *Canadian Journal of Zoology* **57** 1649- 1665.

Castanet J and Naulleau G 1974 Donnees experimentales sur la valeur des marques squelettiques comme indicateur de l'age chez Vipera aspis (L.) (Ophidia, Viperidae). *Zoologica Scripta* **3** 201-208.

Castanet J, Francillon-Viellot H, Meunier FJ and De Ricqles A 1993 Bone and individual aging; in *Bone* (ed) BK Hall (Boca Raton: CRC Press) pp. 245 - 283.

Castanet J, Jean-Pierre G, Meunier FJ, and De Ricqles A 1970 Calcium et nature des zones de croissance cyclique dans I'os des vertebres poikilothermes. *Compte Rendu Academie de Science Paris*, series D, **270**: 2853-2856.

Castanet J, Meumier FJ and De Ricqles A 1977 L'enregistrement de la croissance cyclique par le tissu osseux chez les vertebres poilkilothermes: donnees comparatives et essai de synthese. *Bulletin Biologique de la France et dela Beligique* **111** 183 - 202.

Castanet, J., G. Jean-Pierre, F. J Meunier, and A. De Ricqles, 1970. Calcium et nature des zones de croissance cyclique dans I'os des vertebres poikilothermes. Compte Rendu Academie de Science Paris, series D, 270:2853-2856.

Chapskii K 1941 Marine animals of the Soviet Arctic. (Leningrad: Arctic Library Press of the Northern Sea Route).

Chinsamy A and Valenzuela N 2008 Skeletochronology of the endangered side-neck turtle, *Podocnemis expansa*. *South African Journal of Science* **104** 311-314.

Dawe EG, O'Dor RK, O'Dense PH and Hurley GV 1985 Validation and application of an ageing technique for short-finned squid (*Illex illecebrosus*). *Journal of the Northwest Atlantic Fisheries Science* **6** 107–116.

de Buffrenil V 1980 Mise en evidence de l'incidence des conditions de milieu sur la croissance de *Crocodylus siamensis* (Schneider, 1801) et valeur des marques de croissance squelettiques pour revaluation de l'age individuel. *Archives de Zoologie experimentale et generate* **121** 63-76.

Dobie JL 1971 Reproduction and Growth in Alligator Snapping Turtle, *Macroclemys temminckii* (Troost). *Copeia* **1971** 645 - 658.

Dodge RE and Thomson J 1974 The natural radiochemical and growth records in contemporary hermatypic corals from the Atlantic and Caribbean. *Earth and Planetary Science Letters* **23** 313-322.

Eden CJ, Whiteman HH, Duobinis-Gray L and Wissinger SA 2007 Accuracy assessment of skeletochronology in the Arizona tiger salamander (*Ambystoma tigrinum nebulosum*). *Copiea* **2007** 471-477.

Francillon-Vieillot H, Arntzen J and Géraudie J 1990 Age, growth and longevity of sympatric Triturus cristatus, T. marmoratus and their hybrids (Amphibia, Urodela): a skeletochronological comparison. *Journal of Herpetology* **24** 13-22.

Francillon-Vieillot H, Buffrénil V. de, Castanet J, Géraudie J, Meunier FJ, Sire JY, Zylberberg L, Ricqlès A de 1990. Microstructure and mineralization of vertebrate skeletal tissues. In: **Carter Jr. (ed). Skeletal biomineralization: Patterns, processes and evolutionary trends. New York**: *Van Nostrand Reinhold*, 471-530.

Goshe LR 2009 Age at maturation and growth rates of Green sea turtles (*Chelonia mydas*) along the Southeastern U.S Atlantic Coast estimated using skeletochronology' M.Sc thesis, University of North Carolina, Wilmington.

Goshe LR, Avens L, Bybee J and Hohn AA 2009 An evaluation of histological techniques used in skeletochronological age estimation of sea turtles. *Chelonian Conservation and Biology* **8** 217-222.

Guarino FM, Andreone F and Angelini F 1998 Growth and longevity by skeletochronological analysis in *Mantidactylus microtympanum*, a rain-forest anuran from southern Madagascar. *Copeia* **1998** 194-198.

Guarino FM, Di Maio A and Caputo V 2004 Age estimation by phalangeal skeletochronology of *Caretta caretta* from the Mediterranean Sea. *Italian Journal of Zoology* **71** 175-179.

Hagstrom T 1977 Growth studies and ageing methods for adult *Triturus vulgaris* L. and *T.*

cristatus Laurenti (Urodela, Salamandridae). *Zoologica Scripta* **6** 61-68.

Halliday TR and Verrell P 1988 Body size and age in amphibians and reptiles. *Journal of Herpetology* **22** 253-265.

Hammer DA 1969 Parameters of a marsh snapping turtle population Lacreek Refuge, South Dakota. *The Journal of Wildlife Management* **33** 995-1005.

Hemelaar A 198- An improved method to estimate the number of year rings resorbed in phalanges of *Bufo bufo* (L.) and its apphcation to populations from different latitudes and altitudes. *Amphibia-Reptilia* **6**: 323- 341.

Hemelaar AS 1980 Age determination of male *Bufo bufo* (Amphibia, Anura) from the Netherlands, based on year rings in phalanges. *Amphibia-Reptilia* **1** 3-4.

Hemelaar ASM and Van Gelder J 1980 Annual Growth Rings in Phalanges of *Bufo bufo* (Anura, Amphibia) from the Netherlands and Their Use for Age Determination. *Netherlands Journal of Zoology,* **30 (l)**: 129-135.

Jones C 1986 Determining age of larval fish with the otolith increment technique. *Fishery Bulletin* **84** 91-104.

Kar CS 1982 Discovery of a second mass nesting ground of the Pacific olive ridley turtles (*Lepidocheyls olivacea*) in Orissa, India; *Tiger Paper* **9** 6-7.

Kellner A, Green DM 1995 Age structure and Age at Maturity in Fowler's Toad, *Bufo woodhousii fowleri*, at Their Northern Range Limit. *Journal of Herpetology* **29** (3).

Klevezal□ G and Kleinenberg SE 1969 Age determination of mammals from annual layers in teeth and bones: Israel Program for Scientific Translations (available from US Dept. of Commerce, Clearinghouse for Federal Scientific and Technical Information, Springfield, Va.).

Klevezal□ GA 1996 Recording structures of mammals: determination of age and reconstruction of life history (Rotterdam: CRC Press Llc).

Klinger REC, Musick JA 1992 Annular growth layers in juvenile loggerhead turtles (*Caretta caretta*) (Caldwell , 1962□; Uchida , 1967) to Florida .

Kuniholm PI, Kromer B, Manning SW, Newton M, Latini CE and Bruce MJ 1996 Anatolian tree rings and the absolute chronology of the eastern Mediterranean, 2220-718 BC. *Nature* **381** 780-783.

Kusano T, Fukuyama K and Miyashita N 1995 Age determination of the stream frog, *Rana*

sakuraii, by skeletochronology. *Journal of Herpetology* **29** 625-628.

Kuzmin SL and Ischenko VG 1997 Skeletochronology of *Bufo raddei* from the Gobi Desert. *Journal of Herpetology* **31** 306-309.

Landon DB, Waite CA, Peterson RO and Mech LD 1998 Evaluation of age determination techniques for gray wolves. *The Journal of Wildlife Management* **62** 674-682.

Laws R 1952 A new method of age determination for mammals.

Lutz RA and Rhoads DC 1980 Growth patterns within the molluscan shell. An overview; in *Skeletal growth of aquatic organisms: biological records of environmental change* (ed) DC Rhoads (New York: Plenum Press) pp. 203-254.

Marquez R, and Van Dissel H 1982 A method for evaluation the number massed nesting olive ridley sea turtles, *Lepidocheyls olivacea*, during an arribazon, with comments on arribada behavior. *Neth. J. of Zool.* **32**(3): 419 - 425.

Márquez R, Peñaflores C and Vasconcelos J 1996 Olive ridley turtles (*Lepidochelys olivacea*) show signs of recovery at La Escobilla, Oaxaca. *Marine Turtle Newsletter* **73** 5-7.

Matthews KR and Miaud C 2007 A skeletochronological study of the age structure, growth, and longevity of the mountain yellow-legged frog, *Rana muscosa*, in the Sierra Nevada, California. *Copeia* **2007** 986 - 993.

Mattox NT 1936 Annular rings in the long bones of turtles and their correlation with size. *Transactions of the Illinois State Academy of Science* **28** 225-226.

Meylan A 1981 Estimation of population size in sea turtles. In: **Biology and Conservation of sea turtles. Proceedings of the World Conference on Sea Turtle Conservation held at the U.S. State Department Building, Washington,** D.C., 26 - 30 November, 1979, edited by K. Bjorndal. Washington D.C., *Smithsonian Institution Press*: 135 - 138.

Minakami K 1979 An estimation of age and life-span of the genus *Trimeresurus* (Reptilia, Serpentes, Viperidae) on Amami Oshima Island, Japan. *Journal of Herpetology* **13** 147-152.

Myrick Jr AC, Shallenberger EW, Kang I and MacKay DB 1984 Calibration of dental layers in seven captive Hawaiian spinner dolphins, *Stenella longirostris*, based on tetracycline labeling. *Fishery Bulletin* **82** 207-225.

Pandav B, Choudhury B and Kar C 1994 Discovery of a new sea turtle rookery in Orissa, India. *Marine Turtle Newsletter* **67** 15-16.

Pandav B, Choudhury B and Shanker K 1998 The Olive Ridley sea turtle (*Lepidochelys olivacea*) in Orissa: an urgent call for an intensive and integrated conservation programme. *Current Science* **75** 1323-1328.

Pandav B, Choudhury BC, Kar CS 1997 Mortality of olive ridley turtles *Lepidochelys olivacea* due to incidental capture in fishing nets along the Orissa coast, **India.** *Orxy* Vol **31** No 1.

Parham JF and Zug GR 1997 Age and growth of loggerhead sea turtles (*Caretta caretta*) of coastal Georgia: an assessment of skeletochronological age-estimates. *Bulletin of Marine Science* **61** 287-304.

Peabody FE 1961 Annual growth zones in living and fossil vertebrates. *Journal of Morphology* **108** 11-62.

Platz JE, Lathrop A 1993 Body Size and Age Assessment of Male Chorus Frog. *Journal of Herpetology* **27 (1).**

Reichart HA 1993 Synopsis of biological data on the olive ridley sea turtle Lepidochelys olivacea (Eschscholtz, 1829) in the western Atlantic. Pages 78: US Department of Commerce, National Oceanic and Atmospheric Administration, National Marine Fisheries Service, Southeast Fisheries Science Center.

Robillard SR and Ellen Marsden J 1996 Comparison of otolith and scale ages for yellow perch from Lake Michigan. *Journal of Great Lakes Research* **22** 429-435.

Scheffer VB 1950 Growth layers on the teeth of Pinnipedia as an indication of age. *Science* **112** 309-311.

Schmid JR and Witzell W 1997 Age and growth of wild Kemp's ridley turtles (*Lepidochelys kempii*): cumulative results of tagging studies in Florida. *Chelonian Conservation and Biology* **2** 532-537.

Simmons D 1992 Circadian aspects of bone biology. *Bone* **6** 91-128.

Snover ML 2002 Growth and ontogeny of sea turtles using skeletochronology: methods, validation and application to conservation, PhD thesis, Duke University, North Carolina.

Snover ML and Hohn AA 2004 Validation and interpretation of annual skeletal marks in loggerhead (*Caretta caretta*) and Kemp's ridley (*Lepidochelys kempii*) sea turtles. *Fisheries Bulletin* **102** 682-692.

Snover ML, Avens L and Hohn AA 2007 Back-calculating length from skeletal growth marks in loggerhead sea turtles *Caretta caretta*. *Endangered Species Research* **3** 95-104.

Stiles FG 1972 Age and sex determination in Rufous and Allen hummingbirds. *The Condor* **74** 25-32.

Suzuki HK 1963 Studies on the osseous system of the slider turtle. *Annals of the New York Academy of Sciences* **109** 351-410.

Tucker JK, Maher RJ and Theiling CH 1995 Year-to-year variation in growth in the red-eared turtle, *Trachemys scripta elegans*. *Herpetologica* **51** 354-358.

Yang W, Hixon R, Turk P, Krejci M, Hulet W and Hanlon R 1986 Growth, behavior, and sexual maturation of the market squid, *Loligo opalescens* cultured through the life cycle. *Fishery Bulletin* **84** 771-798.

Zug G, Rand AS, Wynn AH, and Bock B 1983 Skeletochronology of Panamanian *Iguana iguana:* A Test of Annual Periosteal Growth [abstract]. Program of Twenty-sixth Annual Meeting (SSAR) and Thirty-first Annual Meeting, HL, page 100.

Zug GR 1990 Age determination of long-lived reptiles: some techniques for seaturtles. *Annales des Sciences Naturelles, Zoologie* **11** 219-222.

Zug GR and Glor RE 1998 Estimates of age and growth in a population of green sea turtles (*Chelonia mydas*) from the Indian River lagoon system, Florida: a skeletochronological analysis. *Canadian Journal of Zoology* **76** 1497-1506.

Zug GR, Balazs GH and Wetherall JA 1995 Growth in juvenile loggerhead seaturtles (*Caretta caretta*) in the north Pacific pelagic habitat. *Copeia* **1995** 484-487.

Zug GR, Balazs GH, Wetherall JA, Parker DM, Shawn K and Murakavua K 2002 Age and growth of Hawaiian green seaturtles (*Chelonia mydas*): an analysis based on skeletochronology. *Fishery Bulletin* **100** 117-127.

Zug GR, Chaloupka M and Balazs GH 2006 Age and growth in olive ridley seaturtles (*Lepidochelys olivacea*) from the North-central Pacific: a skeletochronological analysis. *Marine Ecology* **27** 263-270.

Zug GR, Kalb HJ and Luzar SJ 1997 Age and growth in wild Kemp's ridley sea turtles (*Lepidochelys kempii*) from skeletochronological data. *Biological Conservation* **80** 261-268.

Zug GR, Wynn AH and Ruckdeschel C 1986 Age determination of loggerhead sea turtles, *Caretta caretta*, by incremental growth marks in the skeleton. *Smithsonian Contributions to Zoology* **427** 1-34.