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Accepted 27 June 1985.

Copeia, 1986(2), pp. 332–341

Age Determination of Living Nile Crocodiles from the Cortical Stratification of Bone

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Vital staining experiments with captive, known-age Nile crocodiles, *Crocodylus niloticus*, in Zimbabwe, show that all skeletal elements have laminae formed by a broad zone deposited in the hot season of rapid growth and a narrow annulus deposited in the cool non-growing season. Each lamina indicates the passage of one year. To age living crocodiles, a method was developed to estimate age from laminae in osteoderms. Internally, the osteoderms of all animals, except breeding females, have well represented laminae with only the most deep-seated having been eroded. The osteoderms of breeding females show considerable internal remodeling. They probably act as a store for calcium used during oogenesis. Age based on number of laminae in osteoderms was tested in adult animals of known ages up to 46 yr. Errors in the most satisfactory method of estimation varied between 9 and 15%. This technique was applied to a population of wild crocodiles at Ngezi, Zimbabwe.

IN a short-term study of a long-lived reptile, the only method of obtaining age estimates where the age/size relationship is unknown or inaccurate is by the use of morphological indices which vary discontinuously with age. The most useful of such ageing criteria are annual growth layers, which may be manifested externally, as in the epidermal scales of some testudines, or internally as layers in bone.

The cortex of reptilian bone generally is composed of laminae (stratified zones and annuli) which, unlike those in mammals, are not associated with remodeling processes, but are rather related to the intermittent nature of skeletal growth (Enlow, 1969). The estimation of age from these zones, termed skeletochronology (Castanet et al., 1977), has a history of controversy, but for some reptiles the seasonal nature of bone deposition has been documented (Ricqlès, 1976; Castanet et al., 1977).

The cortex of crocodilian bone has distinct laminae that are related to incremental growth (Enlow, 1969; Peabody, 1961), but the use of laminae for age determination has been complicated by: 1) the unquantified relationship between laminae and environmental influences; 2) the internal resorption of laminar bone during growth; 3) the remodeling of bone during growth; and 4) the allometric growth and development of bones.

Buffrénil (1980a, b) concluded that crocodilian bone is deposited as in other reptiles and that in Siamese crocodiles, *Crocodylus siamensis*, a close relationship exists between number of growth zones in long bones and age. Using vital staining techniques and a series of known-age

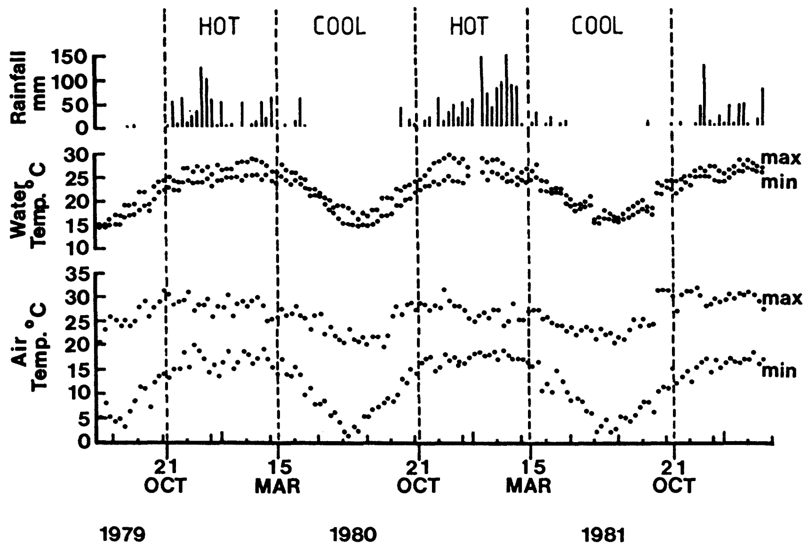

Fig. 1. Seasonal cycles of rainfall, air and water temperatures at Ngezi during the study period.

animals, M. W. J. Ferguson (pers. com.) demonstrated a similar relationship in the American alligator, *Alligator mississippiensis* and established an accurate post-mortem ageing method based on number of growth zones in the cortex of the femur.

This paper reports an evaluation of skeletochronology for determining age in living Nile crocodiles, *Crocodylus niloticus.* Vital-staining was used over a period of 4 yr to examine the nature of bone deposition in a number of skeletal elements, including long bones and osteoderms, of young, known-age captive animals and the relationship between number of laminae and age was determined for large, known-age animals. After establishing that osteoderm sections could be removed from live animals, a technique derived from this relationship was used to determine ages of wild crocodiles in Zimbabwe.

Study Areas

Lake Ngezi is an artificial impoundment, 1220 m above sea level, in central Zimbabwe, between 30°20′/30°29′S and 18°39′/18°44′E. The area has a hot/rainy season between Nov. and March, followed by a cool/dry season until Sept. and a hot/dry season until the rains (Fig. 1). Growth of crocodiles is strictly confined to the hot season when water temperatures reach a maximum, approximately the period 21 Oct. until 15 March. A decline of physical condition occurs during the cool season (Hutton, 1984).

Spencer's Creek Crocodile Ranch (Victoria Falls) lies adjacent to the Zambezi River at 980 m a.s.l., in extreme northwestern Zimbabwe. Growth of captive crocodiles is seasonal, with a pattern similar to that of Ngezi animals (Hutton, 1984).

Hwange National Park also lies in northwestern Zimbabwe. The area experiences seasons similar to those of Ngezi, but with higher mean temperatures.

Materials and Methods

Twenty-two juveniles, aged 1.5 yr, were isolated at Spencer's Creek and given intramuscular injections with commercially obtainable tetracycline at a rate of 50 mg/kg. Tetracycline labels growing bone with fluorescence detectable in thin undecalcified sections under ultraviolet (UV) excitation (Milch et al., 1957).

All of the 1.5 yr-old animals were first labelled in June 1979 (cool/dry season), but several were lost as a result of inadequate tagging and only 15 were re-labelled in June 1980 (Table 1). One larger juvenile of 3.5 yr was added to the sample at this time. Eight individuals were labelled again in Jan. 1981 (hot/wet season) and four of these were killed in March 1981, along with five which had last been labelled in June 1980. Of the remaining seven animals the three which had not been labelled in Jan. 1981 were labelled in March 1981 and all were collected in June 1982. The experiment was designed to

TABLE 1. DOSING SCHEDULE AND HISTORY OF 23 CAPTIVE-REARED JUVENILES IN A VITAL-STAINING EXPERIMENT.

	Date				
	27 May 1979 Non-growing period	10 June 1980 Non-growing period	10 Jan. 1981 Growing period	7 Mar. 1981 Growing period	7 June 1982 Non-growing period
Animal	Treatment				
F1, F5	100 mg/kg	50 mg/kg	50 mg/kg	50 mg/kg	Collected
F2, F11	100 mg/kg	50 mg/kg	50 mg/kg	Collected	
F3	100 mg/kg	50 mg/kg	—	50 mg/kg	Collected
F4, F12, F15	100 mg/kg	50 mg/kg	—	Collected	
F6, F9	50 mg/kg	50 mg/kg	50 mg/kg	Collected	
F7	50 mg/kg	50 mg/kg	50 mg/kg	50 mg/kg	Collected
F8, F10	50 mg/kg	50 mg/kg	—	Collected	
F21, F22	50 mg/kg	50 mg/kg	—	50 mg/kg	Collected
F23	—	50 mg/kg	—	Collected	

accommodate ranch management activity and all animals were killed as part of the cropping program.

Several skeletal elements were taken from experimental animals, including long bones, osteoderms, ribs and the lower jaw. As the stability of the labelling was unknown, specimens were stored in the dark at −12 C. Serial sections were cut from frozen bones at 5 mm intervals using a fine-toothed hacksaw. Those from long bones were taken along the diaphysis, while those from osteoderms were taken longitudinally. Sections were ground by hand on silicon carbide paper to approximately 200 μm for stained mounts and 100 μm for unstained mounts. Fluorescence was visible in unstained sections under UV excitation, but staining with 0.3% basic fuchsin in 40% alcohol, at 37C, for 8 h (Frost, 1959a) suppressed auto-fluorescence and produced superior mounts. After staining, sections were again ground to 100 μm and were examined, without mounting medium, under transmitted UV light with appropriate filters for yellow fluorescence (Frost, 1959b; Ibsen and Urist, 1964). The same sections were compared under normal transmitted and reflected light and photomicrographs were taken to aid description. All bone sections and growth rings were measured and, wherever possible, the number of growth rings in sections of different skeletal elements from the same individual was compared.

Preliminary experimentation revealed that dermal osteoderms contain dense and largely intact laminae and vital staining showed that the laminae are deposited in consecutive seasonal rings. To obtain osteoderm bone from living animals, the animals were immobilised with Flaxedil (Loveridge and Blake, 1972) and a local anaesthetic was infused into the immediate area of the bone. The osteoderm was washed with 95% alcohol and a section approximately 5 mm thick was cut with an amputation saw. The bone sliver was preserved in 75% alcohol and was ground and mounted as above. Care was taken not to damage subcutaneous tissue during the operation and the wound was packed with antibiotic powder. Wounds healed rapidly and infection was never recorded. Osteoderm sections were taken from 39 Ngezi animals, including four juveniles which had been labelled with tetracycline on an earlier occasion. These were useful for comparison with faster-growing captive-reared juveniles.

Known-age adults are rare, but one breeding pen at Spencer's Creek contains 22 such females and osteoderm sections were removed from four of these (FA1, FA2, FA3 and FA4), in March 1981, when they were 13 yr and 3 months old. The only free-living adult Nile crocodile of known-age is "Beadle," a female which hatched in Dec. 1935. For most of its life this animal has lived in a water-hole in Hwange National Park, Zimbabwe and its early growth is reported by Cott (1961). Beadle was recaptured in Oct. 1981, at the age of 45 yr 10 months and an osteoderm section was removed for examination.

As crocodiles grow, the upper surface of their osteoderms is sculptured into pits and grooves by simultaneous processes of resorption and accretion (Buffrénil, 1982). As a consequence, dorsal lamine are broken-up and eroded. Although laminae of the ventral surface are free from this sculpturing, secondary or Haversian remodeling is more common than in long bones and this, together with widening of primary vascular canals, erodes deep-seated ventral laminae. To estimate age it was necessary to estimate the number of laminae which had disappeared since the animal's birth.

As laminae generally narrow as the crocodile grows, I attempted to construct curves describing the progressive widening of the annuli from the periosteal surface to the point at which they disappeared. Extrapolation of these curves to the centre of the osteoderm allows estimation of the number of eroded laminae and, by inference, total age of the individual. A second approach was to average the separation of the innermost two, three or five laminae, using the mean derived to estimate the number of eroded laminae. Both methods were applied to the five known-age adults; thus, four separate age estimates were made for each animal.

Total depth of each osteoderm, total depth of laminae and depths of individual laminae were measured by two observers, independently, with a graticule eyepiece, under 25 power magnification, in three of the deepest parts of the section. These measurements were used to estimate age in the above four ways and for each, the three individual estimates were averaged to give a mean.

The age of 35 Ngezi animals of known sex and reproductive status, ranging in size from 1.44–4.04 m total length (TL), was estimated using the technique derived from known-age adults. As many serial sections as possible were cut from the osteoderm sample of each animal. The total depth (TD) and depth of the ventral laminae (DL) were measured in three places in the section which had the greatest number of annuli. The number of missing laminae was calculated by division of the depth of ventral bone without laminae (TD/2-DL) by a mean yearly increment calculated from the depth of the innermost five laminae.

Results

Structure and seasonality of laminae.—All skeletal elements of both wild and captive Nile crocodiles contain laminae of the type described from the Siamese crocodile by Buffrénil (1980b). Laminae are composed of a regular alternation of two types of osseous deposit: a) wide zones of well vascularised and poorly organised non-lamellar bone, characteristic of rapid growth; and b) narrow annuli of non-vascular lamellar bone, characteristic of slow growth. The number and clarity of laminae was highly variable

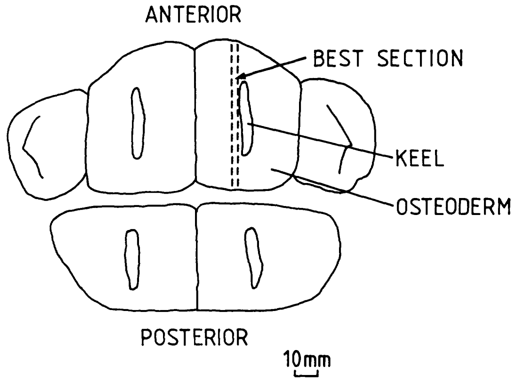

Fig. 2. The dorsal neck osteoderms of a crocodile showing the most useful section for ageing a live animal.

between different parts of the skeleton. Although mandibular bone has been used in preliminary attempts at ageing (Graham, unpubl.; Peabody, 1961), many bones, notably the mandibles and ribs, had considerable secondary bone indistinguishable from Haversian remodeling in mammals, making them unsuitable for skeletochronology. Long bones and osteoderms generally contained the most laminae, the most distinct laminae and the least remodeling of all bones examined. Osteoderm sections with the best represented laminae were those from inside the keel of the largest anterior osteoderm of the neck region (Fig. 2).

All animals of 1.5 yr were given the vital-stain in two consecutive cool, non-growing seasons and in each case two inner annuli were marked with fluorescence. The bone of those animals which were killed at the end of the 1980/81 growing period, without having been given further vital-stain, showed similar fluorescence, with a subsequent unmarked zone. The bone of animals which were killed after being labelled in the growing season of 1980/81 had fluorescence in the two annuli as above, but also a broad fluorescent band in the outermost zone (Fig. 3). The bone of all crocodiles which were labelled in the 1980/81 growing period, but which were not collected for a further year, had the zone with fluorescence and a further, more distal, unmarked annulus and zone. The bone of the individual which was labelled in one non-growing season, at the age of 3.5 yr and then killed at the end of the subsequent growing season, had one fluorescent annulus and an outer, unmarked zone. These results are summarized in Fig. 4.

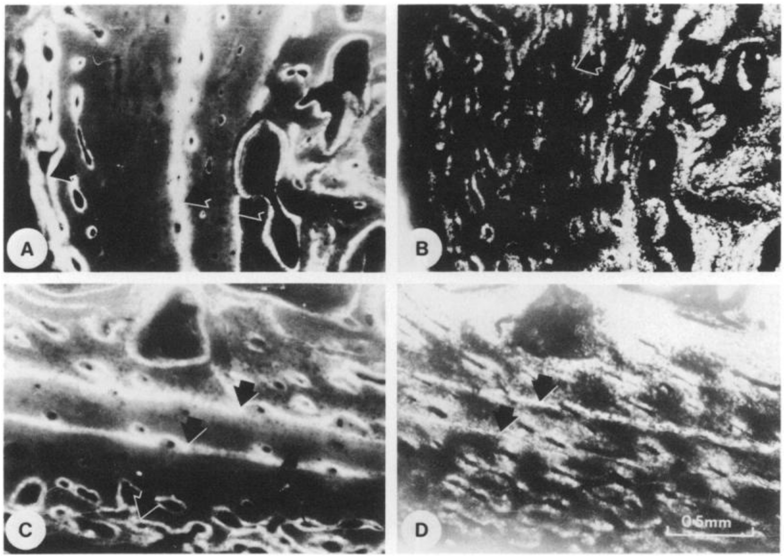

Fig. 3. Photomicrographs in transmitted (TR) and ultraviolet (UV) light of thin sections of a femur and osteoderm of crocodile F2. A = Femur UV, B = Femur TR, C = Osteoderm UV, D = Osteoderm TR. In A and C note two narrow fluorescent bands corresponding with two annuli arrowed in B and D and a broad fluorescent band in the outer zone.

Examination of bone deposited before the crocodiles were dosed with tetracycline provides information on the resorption of laminae during growth. If laminae were of seasonal origin and none had been lost, the number of zones and annuli which should have been present in the bone of experimental animals is shown in Fig. 4. All individuals which were labelled at 1.5 yr should have had an unmarked annulus within the innermost marked one. In the first animals killed, this annulus could be seen, at least in part, in all long bone sections and occasionally in osteoderms. By the time the last of the experimental animals were killed, almost 4 yr after being labelled, the innermost ring was only visible in localised parts of some long bone sections and never in osteoderms. The bone of the animal which was first labelled at 3.5 yr was expected to have three unmarked annuli on the inner side of the marked one. There were three in osteoderms but only two in long bones.

Griffiths (1962) cited the fact that early laminae in long bones disappear as they are engulfed by the marrow cavity during growth as an objection to the use of laminae for ageing. However, Haversian bone, characteristic of remodeling, is rare in crocodiles. In addition, resorption from the endosteal surface of long bones during enlargement of the marrow cavity does not involve remodeling as it does in mammals. Instead, periosteal cortical bone, complete with laminae, is converted directly into cancellous bone by the enlargement of primary canals (Enlow, 1969). Since this bone is largely non-reworked, inner laminae may be seen right up to the time that they are engulfed by the enlarging marrow cavity. Further, because long bones are shaped and curved during growth (the cortex of the diaphysis changing by a combination of deposition and resorption to shift laterally in line with the curvature of the growing shaft) it is possible, within sections, to find regions where remodelling and growth have had only a small effect on laminae. In a series of long bones I found that the marrow cavity drifted towards the dorsal side where growth was most rapid. In mid-diaphysis sections, laminae on the ventral side were closely packed and generally intact while those on the dorsal side were broad and being eroded. The area of the section where the number of laminae showed the best agreement with age was actually that between these extremes (Fig. 5).

Age versus number of laminae.—There were problems in the application of an ageing technique which relied on the tendency of laminae to narrow with increasing age. Labelling showed

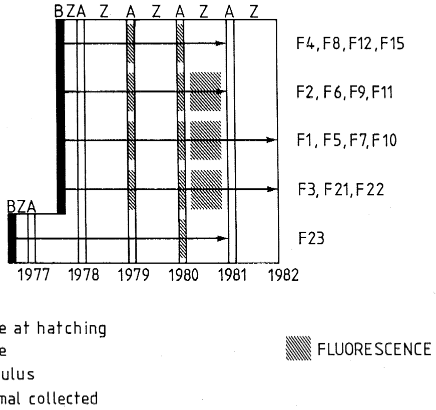

Fig. 4. Schematic representation of fluorescence in zones and annuli of the periosteal bone of experimentally labelled crocodiles (see text).

that osteoderms of the neck develop at an equal rate on all four edges, the dorsal keel of the bone remaining central. In large osteoderms the keel, which could be up to 20 mm wide, would therefore completely overlay the crocodile's original bone, the width of which could be as little as 5 mm. As the section with the clearest laminae was that taken longitudinally from the inside edge of the keel, osteoderm samples from large animals contained no bone from the first three or so years of growth. In addition, although there was in all adult animals a definite tendency for zones to narrow, this was highly variable and curves were largely subjective.

To estimate the number of missing laminae in juvenile animals it was adequate to use only the average of the separation between the innermost two or three annuli. Five-year-old captive juveniles had osteoderms with a mean depth of 4.61 mm and a mean annulus separation of 0.28 mm, while those of Ngezi juveniles sampled at the same age had a mean depth of 2.03 mm and a mean annulus separation of 0.22 mm. This method aged them all as exactly five years and was therefore not affected by the differing rate of growth of juveniles from different environments.

The following details the estimation of age of the five known-age adults by each of the four methods described above:

1) The osteoderm of FA1 (actual age 13 yr and 3 months) had a mean depth of 10.84 mm and nine obvious annuli (Fig. 6). The estimated age from the averaging of the innermost two,

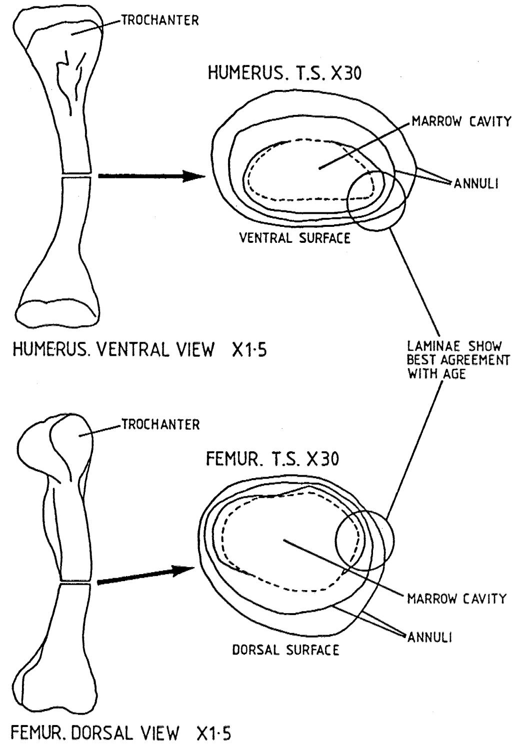

Fig. 5. Cross-sections taken mid-diaphysis from the long bones of a young Nile crocodile. Growth is unequal and the marrow activity is drifting towards the dorsal surface. Lateral laminae show the best agreement with age.

three and five laminae was 14, 14 and 15 yr, respectively, although the highest single estimate was 16 yr and the lowest 13. A curve describing the progressive narrowing of laminae with age was drawn by eye, but in fact there was a great deal of variation and little to indicate the true depth of the innermost missing laminae. The curve was therefore drawn to approach an asymptote at 0.55 mm—the mean depth of 20 inner laminae measured in juveniles raised at the same locality as FA1. From this curve, the total number of laminae was estimated as 13, the true age of the animal.

2) The osteoderm of FA2 (actual age 13 yr and 3 months) had a mean depth of 7.38 mm and eight annuli (Fig. 6). Age, as estimated from the averaging of the innermost laminae, was 12, 12 and 13 yr. The highest single estimate was 14 yr and the lowest was 11. When the size of the innermost missing laminae was assumed to be 0.55 mm, the curve which characterized nar-

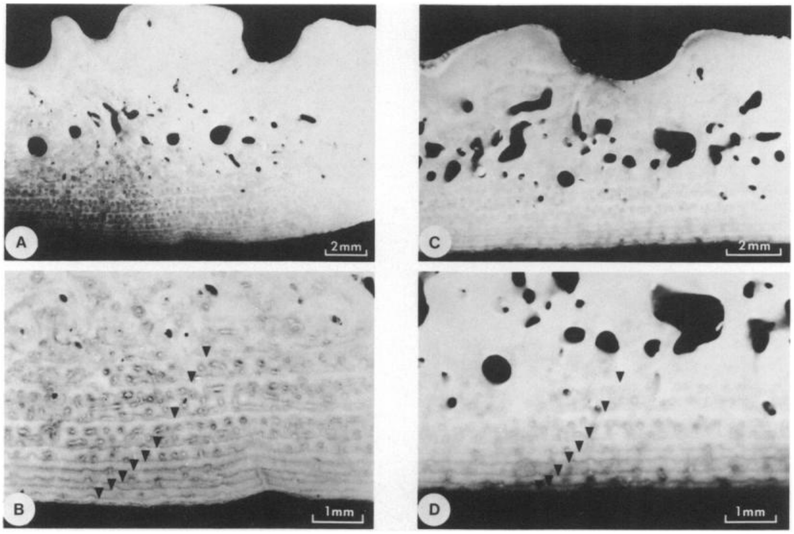

Fig. 6. Thin section of the neck osteoderms of crocodiles FA1 and FA2, both aged 13 years and 3 months. Sculpturing of the dorsal surface, internal reworking and the ventral laminar band of FA1 are shown in A, those of FA2 in C. The nine distinct annuli in the ventral laminar band of FA1 are arrowed in enlargement B, the eight of FA2 in enlargement D.

rowing laminae gave a mean estimated age of 10 yr.

3) The osteoderm of FA3 (actual age 13 yr and 3 months) had a mean depth of 8.30 mm and seven annuli. From the averaging of laminae the age of this animal was estimated as 15, 14 and 14 yr. The highest single estimate was 15 yr and the lowest was 13. Even by assuming the depth of the innermost missing laminae to have been 0.55 mm, it was not possible to find any pattern in the depth of consecutive laminae from which a curve could be constructed.

4) The osteoderm of FA4 (actual age 13 yr and 3 months) was cut too close to the keel. It had a mean depth of 8.84 mm but the deepest part measured 11.11 mm. There were eight annuli in the anterior part of the section but only seven elsewhere. The estimated age from the averaging of laminae was 12 yr in each instance, the highest single estimate being 15 yr and the lowest 10. The depth of laminae varied throughout the section; however, one curve, fitted by eye, satisfied all the data when the asymptote was assumed to be 0.55 mm. The mean age estimated in this way was 11 yr.

5) The osteoderm of Beadle (actual age 45 yr and 10 months) had a mean depth of 10.44 mm and 17 or 18 obvious annuli, depending on the area examined (Fig. 7). The estimated age from the averaging of the innermost two, three and five laminae was 42, 45 and 49 years, respectively, although the highest single estimate was 56 yr and the lowest 38 hr. The curve describing the narrowing of laminae was constructed by assuming an asymptote at a value equivalent to the mean depth of the innermost laminae of wild juveniles from Ngezi. The curve gave a mean estimated age of 40 yr.

Estimation from the innermost five laminae was considered to be consistently the most accurate. The range of errors of individual estimates was approximately 23% in animals of 13 yr and 22% in the 46 yr old animal, while errors of mean estimates were approximately 15% in 13 yr olds and 9% in the 46 yr old (Fig. 8).

Age of Ngezi crocodiles.—Of the 35 Ngezi animals examined, 20 were immature or of non-breeding status for social reasons (Hutton, 1984). Four were mature males and 11 were reproductive females (Table 2). Osteoderm sections from these animals revealed a fundamental difference in the structure of osteoderms between animals of different sex and reproductive status. Those of breeding females showed poor laminar preservation with a great deal of resorption, expansion of vascular canals and reworking (as in Fig. 7), while those of males and non-breeding females showed much less (Fig. 9) and had a significantly greater proportion of laminar

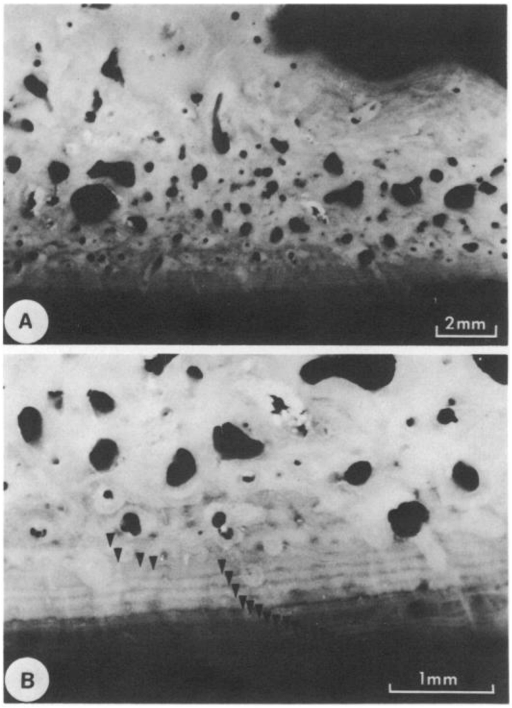

Fig. 7. Thin section of the neck osteoderm of "Beadle" aged 45 years and 10 months. The internal reworking, expansive vascular canals and narrow ventral laminar band, shown in A, are typical of a breeding female. 18 distinct annuli are arrowed in enlargement B.

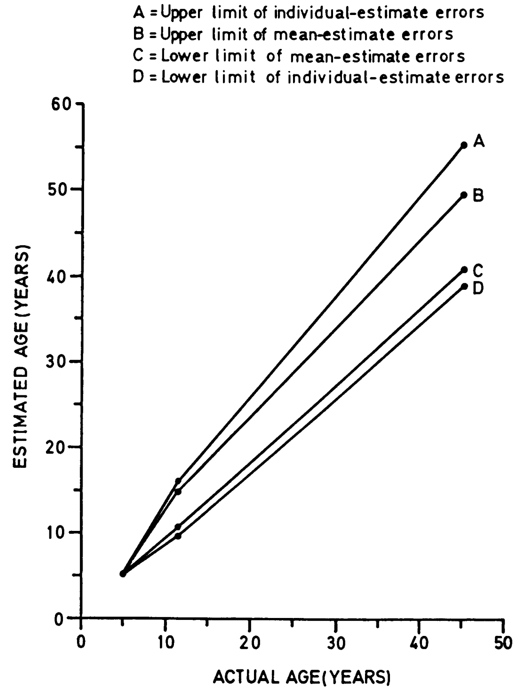

Fig. 8. Errors in the estimation of age of known-age animals by skeletochronology.

bone (Arc sin transformation, F = 4.258; df = 2, 18; $P < 0.05$). For example:

1) The osteoderm of breeding female N78 (3.1 m TL) had a mean depth of 11.04 mm, a ventral laminar band with a mean depth of 2.00 mm and a ratio between the two of 0.182. The mean depth of the innermost five laminae was 0.12 mm. There were an estimated 30 missing laminae and 18 intact laminae, giving a total age of 48 yr (Table 2).

2) The osteoderm of non-breeding female N79 (2.77 m TL) had a mean depth of 8.64 mm, a ventral laminar band with a mean depth of 2.42 mm and a ratio between the two of 0.280. The mean depth of the innermost five laminae was 0.17 mm. There were an estimated 11 missing laminae and 16 intact annuli (Fig. 9), giving a total age of 27 yr (Table 2).

3) The osteoderm of male N95 (3.65 m TL) had a mean depth of 13.26 mm, a ventral laminar band with a mean depth of 3.39 mm and a ratio between the two of 0.256. The mean depth of the five innermost laminae was 0.15 mm. There were an estimated 22 missing laminae and 23 intact laminae (Fig. 9), giving a total age of 45 yr (Table 2).

Discussion

Tetracycline given to captive juvenile crocodiles during the hot season labelled zones within growing bone, while that given in the cool season labelled annuli. It was thus concluded that the environment and cortical stratification within the bones of the Nile crocodile are correlated, with a zone being deposited in the season of rapid growth and an annulus in the non-growing season, together indicating the passage of one year. This agrees with the inferential findings of Buffrénil (1980a) for bone deposition within the Siamese crocodile.

Although there were no animals older than 6.5 yr in the labelling experiment, growth in adult animals follows the same seasonal pattern as that of juveniles (Hutton, 1984) and the laminar structure of the bone of animals of all sizes is microscopically identical. There is no indication that bone growth ceases to be seasonal at any stage in the crocodile's life and with care-

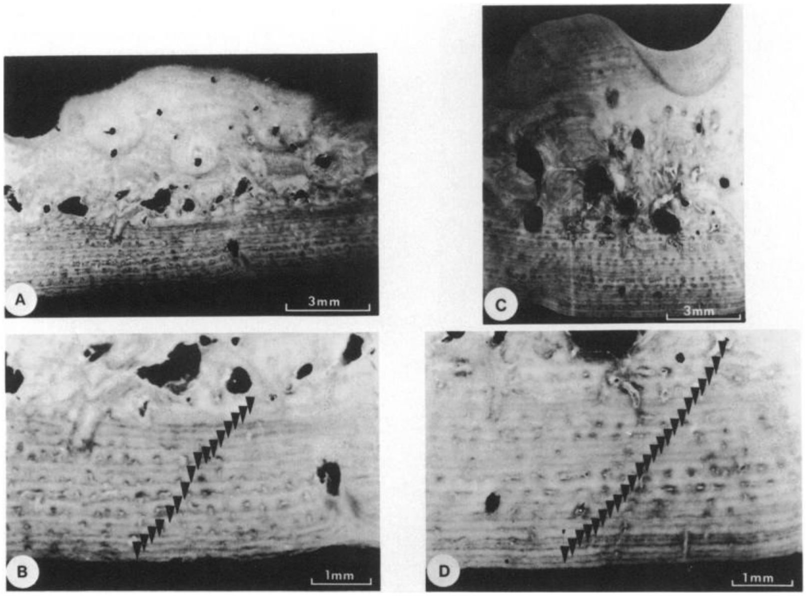

Fig. 9. Thin sections of neck osteoderms of an immature female (N79, A and B) and a male (N95, C and D) from Ngezi. A total of 16 annuli are arrowed in the enlargement of N79 (B) and 23 in that of N95 (D). The large proportion of ventral laminar bone is typical of males and immature females.

TABLE 2. THE PROPORTION OF VENTRAL LAMINAR BONE IN THE OSTEODERMS OF LARGE CROCODILES OF DIFFERENT SEX AND REPRODUCTIVE STATUS AND THEIR AGES AS ESTIMATED BY SKELETOCHRONOLOGY.

Individual	Sex	Reproductive status*	Total length (mm)	Mean depth of osteoderm section (TD) (mm)	Mean depth of ventral laminar band (DL) (mm)	DL/TD	Mean depth of five inner-laminae (mm)	Estimated number of missing laminae	Number of visible laminae	Estimated age
N72	♀	NB	2510	7.05	3.04	0.431	0.25	2	24	26
N73	♀	NB	2755	8.97	3.48	0.388	0.17	6	17	23
N74	♀	B	3339	10.00	3.98	0.398	0.08	13	50	63
N75	♀	B	3065	13.89	1.83	0.132	0.16	35	9	44
N76	♂	B	4040	16.57	5.49	0.331	0.19	15	32	47
N77	♀	NB	2180	5.99	1.83	0.306	0.17	7	9	16
N78	♀	B	3100	11.04	2.00	0.182	0.12	30	18	48
N79	♀	NB	2770	8.64	2.42	0.280	0.17	11	16	27
N80	♂	B	3320	9.10	2.80	0.308	0.13	14	24	38
N81	♀	B	2950	8.94	1.75	0.196	0.10	27	15	42
N85	♀	NB	2480	6.90	1.32	0.191	0.13	17	10	27
N87	♀	NB	2680	6.09	1.22	0.200	0.10	18	12	30
N88	♀	NB	2825	7.11	1.72	0.242	0.10	18	16	34
N89	♂	B	3910	15.54	3.85	0.248	0.11	36	21	57
N90	♀	NB	2750	8.32	2.33	0.280	0.10	18	10	28
N91	♀	B	2650	6.89	1.94	0.282	0.13	12	20	32
N92	♀	B	2680	7.22	2.11	0.292	0.12	13	18	31
N93	♀	B	3320	14.23	1.97	0.138	0.11	45	19	64
N94	♀	B	3330	9.90	1.04	0.105	0.13	30	12	42
N95	♂	B	3650	13.26	3.39	0.256	0.15	22	23	45
N97	♀	B	2870	7.94	1.55	0.145	0.12	20	18	38
N98	♀	B	2940	11.06	1.13	0.102	0.14	31	8	39
N100	♀	B	3250	10.22	1.83	0.179	0.13	26	11	37

* B = Breeding, NB = Non-breeding.

ful interpretation the cortical stratification within osteoderms can be used to estimate age.

Age estimation from the extrapolation of curves describing the progressive narrowing of laminae is more complicated and seems less accurate than estimation from measurement of the innermost two, three or five laminae. In addition, the curves cannot be applied unless the asymptote is assumed to be equivalent to the depth of the earliest laminae in juveniles, a parameter which varies with environment and growth regime. The simpler method of estimation was rapid and readily repeatable by a second observer and was applied to wild animals.

That the cortical structure of osteoderms was related to the crocodile's sex and reproductive status may be due to mobilisation of minerals from non-structural skeletal elements in breeding females, for the formation of egg shells. A clutch of 60 eggs requires approximately 36 g of calcium (Hutton, 1984). Although not tested in this study, errors are likely to be larger in breeding females than in males and non-breeding females. In order to establish better confidence limits for this ageing technique the ontogeny and dynamics of osteoderm growth require further study.

Acknowledgments

I wish to thank the staff of the Department of National Parks and Wildlife Management of Zimbabwe, especially L. Chingwendere, for assistance, the Management of Spencer's Creek Crocodile Ranch ranch for their co-operation, S. Mazena and J. Lindsay for typing and D. H. M. Cumming for commenting on the manuscript. The work was supported by the Yvonne Parfitt Wildlife Fellowship, The University of Zimbabwe, The Department of National Parks and Wildlife Management and The New York Zoological Society.

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