CHAPTER 20

Techniques for Ageing Wild Crocodilians

Jonathan M. Hutton[1]

"How old is that crocodile"? "How long do crocodiles live"? Surely two of the most commonly asked questions at any crocodile farm that caters for tourism. Yet perhaps, by accident, such visitors pose two of the most fundamental questions in crocodile research and management.

To measure and model the dynamics of a population it is essential to derive its age structure and measure age-specific fecundity and mortality. It is therefore important to be able to assign each individual an age, or at least to be able to place individuals into relevant age classes. The importance of ageing was highlighted in Zimbabwe when some apparently thriving populations were demonstrated to be marginal or declining (Hutton 1984). Crocodiles in these populations grew slowly and were susceptible to high juvenile mortality for up to six years. Individuals did not attain sexual maturity until they were thirty-five years old! These findings, from a field study in which individuals were aged by skeletochronology, were sufficient to alter parts of a carefully planned management policy.

Ageing techniques are based on morphological phenomena which change either continuously or discontinuously with age (Caughley 1977; Gibbons 1976). For many small, rapid-growing and short-lived reptiles, the construction of growth curves has produced age estimates with low standard errors. Indeed, comparison of precise lizard growth curves has produced information on environmental control of growth (Dunham 1978) and genetic variation in growth (Ferguson and Brockman 1980). Amongst crocodilians, however, growth rates are highly variable and it is difficult to assign individuals to cohorts after only three or four years (Cott 1961; Dowling and Brazaitis 1966; Hutton 1984; Webb et al. 1983a). That crocodile growth responds dramatically to temperature, population density and food (in terms of both quantity and quality) is, of course, exploited commercially within crocodile farms.

Generally, where the age-size relationship is inaccurate or unknown, the measurement of an individual's age requires the examination of morphological indicators. In reptiles the most useful of such indicators are annual growth rings, which may be manifested externally, as in the epidermal scales of some chelonians, or, more usually, internally as rings in bone (Gibbons 1976).

This chapter briefly assesses the use of growth curves and morphological indicators to determine the age of wild crocodilians.

AGEING FROM GROWTH CURVES

In all capture-recapture studies of wild crocodilians, the size of individuals is routinely recorded, either with a simple measure of length (Gorzula 1978) or with a series of measurements of body dimensions (Webb and Messel 1978). The construction of growth curves usually follows (but is not necessarily of principal interest). Correlations between linear growth, allometric growth, feeding, thermoregulation, movement and home range, mortality and environmental variables such as season, have been reported in crocodilians (Webb and Messel 1978; Webb et al. 1978, 1982, 1983a,b; Hutton 1984). Crocodilian growth is highly variable and, as a consequence, size-age curves constructed directly from the measured growth of wild individuals give large errors if they are used to predict the age of individuals.

In a study of the estuarine crocodile *Crocodylus porosus*, Webb et al. (1978) introduced a new approach to the problem of analysing growth data, and this was later modified and improved in a study of the Australian freshwater crocodile *Crocodylus johnstoni* (Webb et al. 1983a). They examined the relationship between growth rate and mean size between captures.

Head length (HL) is now accepted as something of a standard measure of size in such analyses (Webb et al. 1983a; Hutton 1984), because it can be measured more precisely than total length or snout-vent length. Growth rate (mm HL day^{-1}) is plotted against mean HL between captures. Multiple regression analysis is then applied to this relationship, which allows testing of the extent to which the variation in growth rate can be attributed to variables such as sex, proportion of winter-summer in the capture-recapture period, salinity at capture and recapture sites etc.

Because a good number of such variables do influence growth rate (Webb *et al.* 1978; Hutton 1984), the procedure for ageing individuals is best carried out with capture-recapture data spanning as close to 1.0 or 2.0 years as possible [rather than with data from random intervals (e.g. 1.2, 1.6 years)], or with data that at least span a complete "growing" season if there is strong seasonality in growth (Webb *et al.* 1983a).

The relationship between growth rate and mean size is described by one or two linear regression lines. In the species examined to date the mean growth rate to size relationship changes around 4-5 years of age, so that there is one regression for younger animals and one for older ones. These regression lines are then used to predict the real (or hypothetical in the case of younger animals) maximum HL size (S_m), which is when growth rate equals zero. The inverse of the slope of each regression line is used to predict the two time constants (τ). The initial size (S_o) for the regression for younger animals is the real mean hatchling HL (mm) and for the older animals is predicted by extrapolating the regression back to the hypothetical HL at zero time. With these three coefficients estimated for each regression line, mean size at time t (in days)[$S_{(t)}$] can be predicted from the formula:

$$S_{(t)} = (S_m - S_o)[1 - \exp(-t/\tau)] + S_o$$

Where two regression lines fit the growth rate to size relationship, two intersecting curves will model the mean size-age relationship. If there is a significant effect of sex in the growth rate to mean size relationship, then separate regressions can be made for males and females, which will generate separate mean size-age curves. If there are significant differences between two areas, again separate regressions can be calculated accordingly.

Errors for these ageing curves are large because growth shows considerable variation (Webb *et al.* 1978). The curves are adequate for comparing size-age relationships between populations or sexes, but give large errors around the estimated size of individuals, which limits their utility for this purpose; i.e. for ageing individuals from size alone.

However, if recapture data are available for an individual, the prediction errors can be greatly reduced. The growth rate of that individual relative to the mean relationship in the population can be quantified, and a separate size-age curve, which is consistent with a particular animal's measured growth rate, can be generated. This "individual" size-age curve can in turn be used to estimate the animal's age (Webb *et al.* 1983a).

The principle behind the method is biologically simple. If there are *no* data on an individuals growth rate, and it is being aged from size alone, then the full variation in the population must be incorporated

into the prediction error. If growth data are available, one can say this individual is growing faster or slower than the mean trend, and a gross correction can be made on that basis. The more growth data available, the more confidence in the correction. However, measured growth over short intervals is unsatisfactory, because the variation in measurement error is high relative to the real growth, and this leads to spurious results.

Ageing crocodilians from size and growth rates is a reasonably complex undertaking, and it is based on a number of assumptions that need further attention (Webb *et al.* 1983a). Ideally, captures and recaptures need to be carried out as close to 1.0 or 2.0 years as possible, to reduce errors due to seasonality. Data collected over varying time periods are not handled very well at all by the technique. Similarly, large samples of adults are needed to define the mean size-age relationship in larger animals. To construct a population age structure from such data requires recapture data to be a random sample of the population, or to be corrected for capture bias. It is perhaps better suited to species such as *C. johnstoni* (Webb *et al.* 1983a) and *Caiman crocodilus* (Gorzula 1978) which congregate in large numbers during dry seasons, and which lend themselves to capture-recapture studies in which large numbers and high proportions of the total population can be obtained.

AGEING FROM SKELETOCHRONOLOGY

The cortex of reptilian bone is generally composed of laminae (stratified zones and annuli) which, unlike those in mammals, are not associated with remodelling processes but are rather related to the intermittant nature of skeletal growth (Enlow 1969). The estimation of age from these zones, termed skeletochronology by Castanet *et al.* (1977), has a history of controversy. However, for many reptiles the seasonal nature of bone deposition is now well documented (e.g. Ricqles 1976; Castanet *et al.* 1977).

The cortex of crocodilian bone has distinct laminae and it has long been accepted that these are related to incremental growth (Enlow 1969; Peabody 1961). Use of this banding for determining age has been complicated by several factors, particularly:

1. The unquantified relationship between laminae and environmental influence;
2. The internal resorption of laminar bone during growth;
3. The remodelling of bone during growth; and,
4. The allometric growth and development of bones.

Buffrenil (1980a) concluded that crocodilian bone is deposited in a manner similar to that observed in other reptiles. He also demonstrated a close relationship between the number of growth

zones in long bones and age for several young, known-age Siamese crocodiles, *Crocodylus siamensis* (Buffrenil 1980b). Using vital staining experiments and a series of known-aged animals up to 46 years, Hutton (1984) assessed the above factors in the Nile crocodile *Crocodylus niloticus*, and found a definite relationship between laminae and age, which led to an ageing technique for living animals. Ferguson (1984) has established a post-mortem ageng technique for the American alligator *Alligator mississippiensis*, based on the number of growth rings in the cortex of the femur.

All skeletal elements of both wild and captive Nile crocodiles contain laminae of the type described in the Siamese crocodile (Hutton 1984). Laminae are composed of a regular alternation of two types of osseous deposits:
1. Wide zones of well vascularised and poorly organized non-lamellar bone, characteristic of rapid growth, of the type described by Enlow (1969); and,
2. Narrow annuli of non-vascular lamellar bone, charcteristic of slow growth.

The number and clarity of laminae is highly variable between different parts of the skeleton and, although mandibular bone has been used in preliminary attempts at ageing (Graham 1968; Peabody 1961), many bones (notably the mandibles and ribs) have considerable secondary bone, indistinguishable from Haversian remodelling in mammals; this makes them unsuitable for skeletochronology. Long bones and osteoderms generally contain the most laminae, the most distinct laminae and the least amount of remodelling of all bones. Buffrenil (1980b) found that growth laminae appeared in all long bones of *C. siamensis* but preferentially used the fibula for counting the rings. In the fibula, with a small diameter diaphysis, it is easy to examine a whole cross-section and follow the same annuli around the circumference. The contrast between zones and annuli is good, vascularization is poor, and the annuli are particularly well preserved.

Laminae occur, and are well preserved, in the long bones of all species of crocodilian so far examined, and they are also clear in the osteoderms of most species (Buffrenil, pers. comm.). Seidel (1979) described laminae in the osteoderms of *A. mississippiensis*, and made useful observations on the function of osteoderms. However, Ferguson (1984) found that laminae in the neck osteoderms of *A. mississippiensis* were poorly preserved relative to those in the femur.

Whenever skeletochronology is to be applied to crocodilians, the relationship between zones, annuli and the environment ideally should be established. However, from the available information, it would appear safe to assume that laminae are seasonal when animals suffer reduced growth during one season of the year. As a post-mortem technique, as many long bones as possible should be sampled, with age being determined on the basis of several sections taken mid-diaphysis.

To remove sections of osteoderms from live *C. niloticus*, we immobilize the crocodiles with Flaxedil (Loveridge and Blake 1972) and infuse a local anaesthetic into the immediate area of the target bone. The osteoderm is then washed with 95% alcohol and a section approximately 5 mm thick is cut with an amputation saw. The resultant bone sliver is preserved in 75% alcohol and is eventually ground and mounted (see below). Care is taken not to damage subcutaneous tissue during the operation and the wound is packed with antibiotic powder. Wounds heal rapidly, and no infection has yet been recorded from captive animals nor from wild animals that have been subsequently recaptured. With *C. niloticus* in Zimbabwe, osteoderm sections with the best represented laminae are those from the inside of the keel of the largest anterior osteoderm on the neck (Fig. 1).
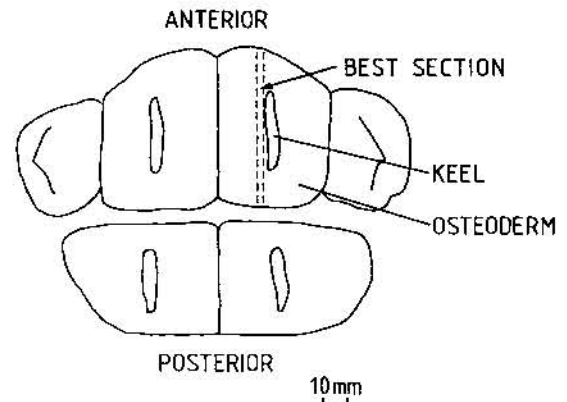

Fig. 1. The dorsal neck osteoderms of *Crocodylus niloticus* showing the most useful section for ageing live animals.

Techniques for the preparation of sections are well established (e.g. Castanet 1978). No expensive facilities are required and sections can easily be prepared in the field. In Zimbabwe, bones are first cleaned in boiling water with sodium perborate (15-20 g l⁻¹) and are then allowed to dry. Sections are then cut with a fine-toothed hackway and ground to 100 to 140μ (approximately the thickness of 80 g bond paper) and polished on silicon carbide paper (Frost 1958). Sections are generally mounted dry between two glass slides held together with adhesive tape. Dry sections can be examined in transmitted, reflected and polarised light, and even photographing dry sections is straight-forward. They can be inserted into an enlarger and used as "negatives", producing high quality and accurate prints. Decalcified preparations, stained with Ehrlich's Hematoxylin, are particularly useful for highlighting growth-arrest lines, and these are also prepared

easily in the field (Enlow 1961). Few will have facilities comparable to those of the research team "Formations squelettiques" of Paris, where micro-radiographs of undecalcified sections are used to demonstrate differences in mineralisation between zones and annuli.

In the long bones of large animals, some of the early laminae will have been lost by medullary resorption, and Griffiths (1962) cited this as an objection to the use of laminae for ageing. However, Haversian bone characteristic of remodelling is rare in crocodiles. In addition, resorption from the endosteal surface of long bones during enlargement of the marrow cavity does not involve rebuilding, 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 more, 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 curva-ture of the growing shaft) it is possible within sec-tions to find regions where remodelling and growth have had only a small effect on laminae. In a series of long bones of the Nile crocodile it was found that marrow cavity drifted towards the dorsal side where growth was most rapid (Hutton 1984). In sections taken mid-diaphysis, laminae on the ventral side were closely packed and generally intact while those on the dorsal side were broad and were 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. 2).

As crocodiles grow, the upper surface of their osteoderms becomes sculptured into pits and grooves by simultaneous processes of resorption and accretion (Buffrenil 1982). As a consequence, dorsal laminae are broken-up and eroded. Laminae on the ventral surface are free from this sculpturing, but secondary remodelling is more common in osteoderms than in long bones, and this, together with widening of primary vascular canals, erodes early deep-seated ventral laminae.

Laminar preservation in the osteoderms of breed-ing females is poorer than that in males and immature females. The large amount of resorption, expansion of vascular canals and reworking in the osteoderms of large females may be due to the mobilization of minerals from non-structural skeletal elements for the formation of egg shells (Hutton 1984). A clutch of 60 eggs of the Nile croco-dile will require approximately 700 g of calcium car-bonate.

In both long bones and osteoderms it is clear that simple addition of annuli, even in sections where
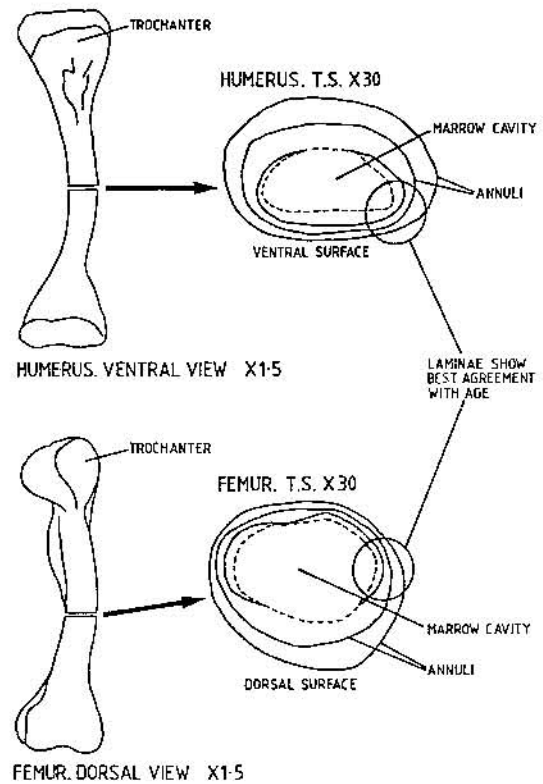

Fig. 2. Cross-section taken mid-diaphysis from the long bones of a young *Crocodylus niloticus*. Growth is unequal and the marrow cavity is drifting towards the dorsal surface. Lateral laminae show the best agreement with age.

they are best represented, gives only a minimum estimate of age. It is necessary to calculate the number of laminae which have disappeared since the animal's birth. Laminae generally narrow as the crocodile grows, and curves can be constructed that describe this progressive narrowing. These in turn can be extrapolated back to the centre of the bone and used to predict the number of laminae which have been eroded, and by inference, the total age of the individual can be calculated. A simpler approach is to average the separation of the innermost three or five annuli, and use the mean to estimate the number of eroded laminae. While the construction of curves is particularly accurate in long bones, it has been found that the simpler method is the most accurate for osteoderms (Hutton 1984). Errors in estimating the age of living known-age Nile crocodiles from the laminae in osteoderms increases from 9% at 13 years to 15% at 46 years (Hutton 1984).

CONCLUSIONS

Although many demographic parameters of crocodiles are scaled to their size rather than their age (see Nichols Chapter 17), there is no doubt that considerable improvements in management can be achieved if crocodiles can be assigned ages.

Growth rates are notoriously variable, even within single populations, and ages estimated from growth curves alone are generally unsatisfactory for all but gross demographic analyses. However, ageing from growth has the distinct advantage of simplicity during data gathering. Skeletochronology would appear to offer some advantages for detailed work, such as the study of age specific fecundity (Ferguson 1984), and is an excellent post-mortem technique. While the application of skeletochronology to living animals is in its infancy, it certainly works in *C. niloticus* and is likely to find a much wider application in other species.

There is an urgent need for detailed studies of the dynamics of bone growth in all species. It is usually the case that large known-age individuals are scarce and vital staining experiments, in which bone growth at a particular time/s is marked by staining (to define definitively the relationship between annuli and age), often refer only to juvenile growth. There is, of course, much to be said for an integrated approach to the problem of estimating ages. Growth is a fundamental process which is usually measured as a matter of course. Such studies commonly reveal seasonal growth and thus suggest that seasonally deposited laminae can be expected in bone. Measurement of age by skeletochronology can then be used to check the errors associated with growth curves.

ACKNOWLEDGEMENTS

REFERENCES

BUFFRENIL, V. de., 1980a. Donnees preliminaires sur la structure des marques de croissance squelettiques chez les crocodilians actuels et fossiles. *Bull. Soc. zool. Fr.* 105: 355-61.

BUFFRENIL, V. de., 1980b. 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 l'evaluation de l'age individuel. *Archs. Zool. exp. gen.* 121: 63-76.

BUFFRENIL, V. de., 1982. Morphogenesis of bone ornamentation in extant and extinct crocodilians. *Zoomorphology* 99: 155-66.

CASTANET, J., 1978. Les marques de croissance osseuse comme indicators de l'age chez les lezards. *Acta Zool., Stockh.* 59: 35-48.

CASTANET, J., MEUNIER, F. J. AND RICQLÈS, A. de., 1977. L'enregistrement de la croissance cyclique par le tissu osseux chez les vertebres poikilothermes: donnees comparatives et essai de synthese. *Bull. biol. Fr. Belg.* 3: 183-202.

CAUGHLEY, G., 1977. "Analysis of Vertebrate Populations". John Wiley and Sons: London and New York.

COTT, H. B., 1961. Scientific results of an enquiry into the ecology and economic status of the Nile crocodile (*Crocodilus niloticus*) in Uganda and Northern Rhodesia. *Trans. zool. Soc. Lond.* 29: 211-356.

DOWLING, H. G. AND BRAZAITIS, P., 1966. Size and growth in captive crocodilians. *Int. Zoo Yearb.* 6: 265-70

DUNHAM, A. E., 1978. Food availability as a proximate factor influencing individual growth rates in the iguanid lizard *Sceloporus meriami. Ecology* 59: 770-8.

ENLOW, D. H., 1961. Decalcification and staining of ground thin sections of bone. *Stain Technol.* 36: 250-1.

ENLOW, D. H., 1969. The bone of reptiles. Pp. 45-80 *in* "Biology of the Reptilia" Vol 1 ed by C. Gans, A. d'A. Bellairs and T. S. Parsons. Academic Press: London and New York.

FERGUSON, G. J. AND BROCKMAN, T., 1980. Geographic differences of growth rate of *Sceloporus* lizards (Sauria: Iguanidae). *Copeia* 1980: 259-64.

FERGUSON, M. W. J., 1984. Craniofacial development in *Alligator mississippiensis. Symp. zool. Soc. Lond.* No. 52: 223-73.

FROST, H. M., 1958. Preparation of thin undecalcified bone sections by rapid manual method. *Stain Technol.* 33: 273-6.

GIBBONS, J. W., 1976. Ageing phenomena in reptiles. Pp. 453-75 *in* "Special review of experimental aging research" ed by M. F. Elias, B. E. Eleftherion and P. K. Elias. Ear Inc.: Bar Harbour, Maine.

GORZULA, S. J., 1978. An ecological study of *Caiman crocodilus crocodilus* inhabiting savanna lagoons in the Venezuelan Guayana. *Oecologia (Berl.)* 35: 21-34.

GRAHAM, A., 1968. The Lake Rudolf crocodile (*Crocodylus niloticus* Laurenti) population. Mimeographed report to Kenya Game Department, Nairobi.

GRIFFITHS, I., 1962. Skeletal lamellae as an index of age in heterothermous tetrapods. *Ann. Mag. nat. Hist.* 4: 449-65.

HUTTON, J. M., 1984. The population ecology of the Nile crocodile *Crocodylus niloticus* Laurenti, 1768, at Ngezi, Zimbabwe. Unpublished Ph.D. Thesis, University of Zimbabwe, Zimbabwe.

LOVERIDGE, J. P. AND BLAKE, D. K., 1972. Techniques in the immobilizing and handling of the Nile crocodile, *Crocodylus niloticus. Arnoldia (Rhod.)* 5: 1-14.

PEABODY, F. E., 1961. Annual growth zones in living and fossil vertebrates. *J. Morph.* 108: 11-62.

RICQLÈS, A. J. de., 1976. On bone histology of fossil and living reptiles, with comments on its functional and evolutionary significance. Pp. 123-50 *in* "Morphology and Biology of Reptiles" ed by A. d'A. Bellairs and C. B. Cox. Linnean Society Symposium No. 3.

SEIDAL, M. R., 1979. The osteoderms of the American alligator and their functional significance. *Herpetologica* 35: 375-80.

WEBB, G. J. W., BUCKWORTH, R. AND MANOLIS, S. C., 1983a. *Crocodylus johnstoni* in the McKinlay River area, N.T. III. Growth, movement and the population age structure. *Aust. Wildl. Res.* 10: 383-402.

WEBB, G. J. W., BUCKWORTH, R. AND MANOLIS, S. C., 1983b. *Crocodylus johnstoni* in the McKinlay River area, N.T. IV. A demonstration of homing. *Aust. Wildl. Res.* 10: 403-6.

WEBB, G. J. W., MANOLIS, S. C. AND BUCKWORTH, R., 1982. *Crocodylus johnstoni* in the McKinlay River area, N.T. I. Variation in the diet and a new method of assessing the relative importance of prey. *Aust. J. Zool.* 30: 877-99.

WEBB, G. J. W. AND MESSEL, H., 1978. Morphometric analysis of *Crocodylus porosus* from the north coast of Arnhem Land, Northern Australia. *Aust. J. Zool.* 26: 1-27.

WEBB, G. J. W., MESSEL, H., CRAWFORD, J. AND YERBURY, M. J., 1978. Growth rates of *Crocodylus porosus* (Reptilia: Crocodilia) from Arnhem Land, Northern Australia. *Aust. Wildl. Res.* 5: 389-99.