

**BONE HISTOLOGY OF A LOWLAND AND MONTANE SPECIES FROM THE  
FAMILY CORDYLIDAE (REPTILIA : SQUAMATA)**

by

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### **Declaration**

I the undersigned hereby declare that the work contained in this thesis is my own original work and has not previously in its entirety or in part been submitted at any university for a degree.

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Date

## ABSTRACT

Ecological adaptations, size and longevity, are known to be reflected as histological variations within vertebrate skeletons. To obtain baseline information on bone histology in the lizard family Cordylidae, two species, which differ considerably in general morphology and biology, were selected as representatives of the family. These were *Cordylus cataphractus*, a lowland lizard found along the western districts of South Africa, and *Pseudocordylus capensis*, a montane lizard occurring in the Cape Fold Mountains of South Africa. Cross-sections of the femora from an ontogenetic range of individuals were examined for each species. In the smallest juveniles of both species, the compacta consisted of typical embryonic bone, while in adults it consisted of parallel-fibred bone. In general, the compacta was poorly vascularized. Endosteally formed lamellar and parallel-fibred bone, lined the medullary cavity in most adults and juveniles of *C. cataphractus*, but was limited in *P. capensis*.

Bone remodeling, which incorporates the removal of old bone as new bone is added, produced distinctive features in both species. Endosteal resorption was intensive but irregular in both species. In *C. cataphractus*, however, resorption was more widespread along the medullary cavity margin, while in *P. capensis*, it was more localized. This observation was supported by the presence of embryonic bone remnants in most juveniles and some adults of *P. capensis*, as well as the shape of the medullary cavity area and number of lines of arrested growth (LAGs) resorped in both species. In *C. cataphractus*, up to four LAGs were estimated to have been resorped, and in *P. capensis*, up to three LAGs were estimated to have been resorped. Endosteal bone was present in 80 % of the *C. cataphractus* sample, but in only 68 % of the *P. capensis* sample. When it occurred in *P. capensis* individuals, it was thinner than the

endosteal deposits of *C. cataphractus* individuals. In general, therefore, the relative bone thickness (RBT) of *P. capensis* was thin (9 - 39 %), compared to the RBT of *C. cataphractus* (18 - 49 %). Genetic and epigenetic processes can explain the differences and variations observed between these two species. *P. capensis* bone morphology, resulting in a lighter skeleton, could possibly be due to the need for speed and agility. *C. cataphractus*, on the other hand, is a heavily armoured, sluggish lizard with a lower basal metabolic rate than any other cordylid species studied to date. A heavier skeleton would, therefore, be an advantage in supporting a heavily armoured body. It is suggested that the intensive, widespread resorption in *C. cataphractus* could be due to food shortages as a result of group-living behaviour and apparent lack of active foraging.

Growth was periodic in both species and growth marks were represented by zones and LAGs. Most *P. capensis* juveniles and even some adults had a clearly defined birth line. A birth line was visible in only one *C. cataphractus* juvenile. LAGs varied in distinctness and spatial arrangement within and between the two species, possibly due to a variety of factors, including altitudinal differences, climatic conditions and individual variation. *C. cataphractus* possessed more double LAGs than *P. capensis*, a feature usually related to cold climates and high altitudes. This phenomenon is suggested to be mainly due to food shortages rather than climatic conditions, as *C. cataphractus* occurs in a warm temperate climate.

Skeletochronology was used to provide information on age, longevity, growth rates and age at sexual maturity for both species. Individual ages were estimated by calculating the number of LAGs resorped and adding them to the number of visible LAGs. The maximum estimated age was 13 years in *C. cataphractus* and 11 years in *P. capensis*. Sexual maturity, assessed through the analysis of femoral microstructure, occurred around six to seven years in *C.*



*cataphractus* and four to six years in *P. capensis*. This estimate corresponded favourably with macroscopic and microscopic assessments of the gonads. Log-transformed variables regressed against log number of LAGs, were used to assess growth rates and sexual dimorphism. In general, there was considerable variation in all variables within age classes among the juvenile cohort, but less variation was noted among adults of both species. In *C. cataphractus*, males and females exhibited different growth rates when snout-vent length (SVL), femoral length (FL) and bone wall area (BWA) were regressed against the number of LAGs (age). In *P. capensis*, sexual dimorphism in growth rate was only observed between BWA and age. Evidence suggests that both species have the potential for long life-spans.

## UITTREKSEL

Dit is bekend dat ekologiese adaptasies, grootte en lewensverwagting as histologiese veranderlikes in die weweldierskelet waarneembaar is. Basiese inligting oor die beenhistologie van die Cordylidae is verkry deur twee spesies, wat grootliks in algemene morfologie en biologie van mekaar verskil, as verteenwoordigend van hierdie familie te kies. *Cordylus cataphractus* is 'n laagland-akkedis wat in die westelike gebiede van Suid-Afrika voorkom en *Pseudocordylus capensis* 'n bergakkedis wat in die Kaapse Plooiberge hou. 'n Ontogenetiese reeks dwarsnitte van die femora is vir elke spesies ondersoek. Die beenkorteks van die kleinste onvolwassenes van beide spesies het tipiese embrioniese been vertoon terwyl die beenweefsel van volwassenes, parallelgerigte vesels vertoon het. Die korteks was oor die algemeen swak gevaskulariseer. Die medulêre holte is by die meeste volwassenes en onvolwassenes van *C. cataphractus* uitgevoer met endosteale gelamineerde been en been met parallelle vesels in terwyl dit in *P. capensis* beperk was.

In beide spesies het die hermodulering van beenweefsel, wat die verwydering van ou been en die toevoeging van nuwe been insluit, kenmerkende eienskappe vertoon. In beide spesies was endosteale resorpsie intensief maar oneweredig. By *C. cataphractus* was die resorpsie meer wydverspreid langs die rand van die medulêre holte terwyl dit by in *P. capensis* meer gelokaliseer was. Hierdie waarneming word ondersteun deur die teenwoordigheid van embrioniese beenoorblyfsels by die meeste onvolwassenes en sommige volwassenes van *P. capensis*, tesame met die vorm van die area van die medulêre holte en die aantal minimale groeilyne (MRGe) wat in beide spesies geresorbeer is. Daar is geskat dat tot vier MGRs by *C. cataphractus* en drie MGRs in *P. capensis* geresorbeer is. Endosteale been was by 80% van

die *C. cataphractus* monster teenwoordig maar slegs by 68% van die *P. capensis* monster.

Waar dit voorgekom het in *P. capensis* individue, was dit dunner as die endosteale neerlae van *C. cataphractus* individue. Die relatiewe beendikte van *P. capensis* was dus oor die algemeen dun (9 - 39 %) in vergelyking met *C. cataphractus* (18 - 49 %). Genetiese en epigenetiese prosesse kan die histologiese verskille en variasies tussen hierdie spesies verklaar. Die beenmorfologie van *P. capensis*, wat 'n ligte skelet veroorsaak kan moontlik as gevolg van die behoefte van speed en ratsheid wees. In teenstelling hiermee is *C. cataphractus* 'n swaar gepantserde, lomp akkedis, wat 'n laer basale metaboliese tempo het as enige ander *Cordylus* spesie wat tot op datum bestudeer is. Dit is voorgestel dat die intensiewe wydverspreide resorpsie in *C. cataphractus* moontlik die gevolg is van voedseltekorte wat gepaard gaan met groeplewende gedrag en die waarskynlike afwesigheid van die aktiewe voeding.

Beide spesies het 'n periodieke groei getoon en groeimerke is verteenwoordig deur sones en MGR. Die meeste onvolwasse eksemplare van *P. capensis* asook sommige volwassenes, het 'n duidelik afgebakende geboortelyn getoon. 'n Geboortelyn was slegs in een onvolwasse *C. cataphractus* sigbaar. MGR het in duidelikheid en spasiering binne en tussen die twee spesies gewissel, waarskynlik as gevolg van 'n verskeidenheid faktore wat verskille in hoogte bo seespieël, klimaatsomstandighede en individuele variasie insluit. *C. cataphractus* vertoon meer dubbele MGR as *P. capensis*, 'n kenmerk wat gewoonlik verband hou met 'n koue klimaat en groot hoogtes bo seespieël. Die stelling word gemaak dat hierdie verskynsel hoofsaaklik te wyte is aan voedseltekorte eerder as klimaatstoestand aangesien *C. cataphractus* in 'n warm, gematigde klimaatstreek voorkom. Skeletokronologie is aangewend om inligting t.o.v. ouderdom, lewensverwagting, groeitempo en ouderdom met geslagsrypheid in beide spesies te bepaal. Individuele ouderdomme is geskat deur die aantal geresorpeerde

MGR<sub>e</sub> te bereken en dit by die aantal waarneembare MGR<sub>e</sub> te tel. Die maksimum geskatte ouderdom vir *C. cataphractus* was 13 jaar en vir *P. capensis* 11 jaar. Geslagsrypheid is bepaal deur die analise van femorale mikrostruktuur en vind plaas vanaf 6 tot 7 jaar by in *C. cataphractus* en 4-6 jaar by *P. capensis*. Dit vergelyk gunstig met beide makroskopiese en mikroskopiese data van die gonades. Die regressielyn van logaritmes-getransformeerde veranderlikes teenoor die logaritme van die getal MGR<sub>e</sub> is bepaal om groeitempo en geslagsdimorfisme te evalueer. Oor die algemeen was daar groot variasie binne ouderdomsklasse tussen onvolwassenes, maar minder variasie is waargeneem tussen volwassenes van beide spesies. In *C. cataphractus*, het mannetjies en wyfies verskillende groeitempos getoon, wanneer snoet-kloak lengte (SKL), femorale lengte (FL) en beenwand area (BWA) teen die getal MGR<sub>e</sub> (ouderdom) geregreseer was. By *P. capensis* is geslagsdimorfisme en groeitempo net tussen BWA en ouderdom waargeneem. Dit suggereer dat beide spesies die potensiaal besit om 'n hoë ouderdom beriek



I would like to dedicate this thesis to the Lord God, to Him be all the glory, and to my mother Louise, who opened my eyes to the world of nature.....

".....to the eyes of the man of imagination,

Nature is imagination itself.

As a man is, so he sees"

*William Blake*



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FL, femoral length; PW, proximal width; DW, distal width; MW, mid-shaft width.

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FIGURE 37. T.s. through the femoral shaft of an adult male *Pseudocordylus capensis*, showing intensive remodeling of the compacta indicated by decreased periosteal primary bone due to resorption and increased secondary endosteal deposition, in the form of endosteal parallel-fibred bone and/or lamellar bone. PB, periosteal bone; ELB, endosteal lamellar bone; EPB, endosteal parallel-fibred bone; RL, resorption line. Scale bar equals 40  $\mu\text{m}$ .

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## CHAPTER 1

### INTRODUCTION AND LITERATURE SURVEY

#### 1.1. Bone structure of Reptiles

The earliest known bone, found in prehistoric fishes, was composed of a hard, fibrous matrix, containing both cells and blood vessels (Enlow, 1969). This basic plan has been retained by most vertebrates of all classes, but a number of divergent specializations in structure have appeared related to different growth rates between taxa, or within an individual's life-span (Enlow, 1969). Reptiles, both living and extinct, have cellular bone which could be avascular, poorly vascularized or highly vascular in appearance (Enlow, 1969; de Ricqlès, 1976). Cellular bone refers to the presence of osteocytes in cortical bone (Enlow, 1969). This is in contrast to the bone of most teleost fish, which apparently exhibits a total lack of bone cells (Enlow, 1969).

It is significant that, although there are distinct similarities among all reptiles, there is no single "reptilian pattern" of bone tissue structure (Enlow, 1969; de Ricqlès, 1976). The shafts of the long bones in lizards are devoid of extensive cancellous tissues, which is a distinguishing characteristic of the Sauria, as opposed to Chelonia and Crocodylia where extensive spongy bone occurs throughout the skeleton (Enlow, 1969; de Ricqlès, 1976; Castanet and Cheylan, 1979; Hua and de Buffrenil, 1996). Spongy bone is often only well developed in epiphyseal and metaphyseal regions of long bones in lizards (de Ricqlès, 1976). Most areas of the saurian skeleton are composed of primary periosteal bone comprising dense, lamellar or parallel-fibred bone, which can be avascular or poorly vascularized (de Ricqlès, 1976). Endosteal bone is deposited at the periphery of medullary cavities and



seldom shows secondary osteons or, specifically in the long bone shafts of saurians, cancellous trabeculae (de Ricqlès, 1976). Primary bone is bone formed without replacement of pre-existing bone, irrespective of whether deposition is periosteal or endosteal (Reid, 1984).

This general pattern of bone microstructure has been demonstrated in many lizards, for example, *Eumeces obsoletus* (Enlow, 1969), *Iguana* (Enlow, 1969), *Gerrhonotus multicarinatus* (Enlow, 1969), *Callopiastes maculatus* (Castanet, Meunier and de Ricqlès, 1977), *Lacerta vivipara* (Pilorge and Castanet, 1981), *Cophosaurus texanus* (Nouira, Maury, Castanet and Barbault, 1982), *Leposoma guianense* (Castanet and Gasc, 1986), *Iguana iguana* (Zug and Rand, 1987), *Gallotia* (Castanet and Baez, 1988; 1991) and *Angolosaurus skoogi* (Chinsamy, Hanrahan, Neto and Seely, 1995). Before hatching or metamorphosis, the composition of bone in lizards and amphibians is that of embryonic bone, for example as found in *Triturus marmoratus* (Caetano, Castanet and Francillon, 1985), *Leposoma guianense* (Castanet and Gasc, 1986), *Rana pipiens* (Leclair and Castanet, 1987), *Gallotia* (Castanet and Baez, 1988, 1991), *Angolosaurus skoogi* (Chinsamy *et al.*, 1995) and *Batrachoseps attenuatus* (Wake and Castanet, 1995). This embryonic cortex is made of a rather woven fibred bone tissue (Castanet and Baez, 1988) which is associated with relatively fast growth rates (Reid, 1984; Castanet *et al.*, 1977; Castanet, Francillon-Vieillot, Meunier and de Ricqlès, 1993). Among ectothermic tetrapods, cyclical deposition of bone is well documented (Peabody, 1961; Enlow, 1969; de Ricqlès, 1976, Castanet *et al.*, 1977; Castanet *et al.*, 1993). Cyclical growth results in the formation of growth marks (GM), such as rest lines, in bones and some other hard tissues and the general conclusion is that the number of rest lines or annuli in bones corresponds to the individual's age (Castanet and



Smirina, 1990). For poikilothermic species living in temperate climates, seasonality is the main factor that directly provides the yearly cyclical growth rhythm of the organism as recorded in its skeleton (Castanet *et al.*, 1993). However, GM are also present in homeothermic animals and in poikilotherms living in more or less constant climatic conditions throughout the year, although in these cases GM are less distinct than in the bone of those animals subjected to contrasting seasonality (Castanet *et al.*, 1993). The current opinion, therefore, is that bone growth variations and associated GM are ultimately caused by an internal (genetically based) rhythm which under natural conditions becomes synchronized with, and reinforced by, the seasonal cycles, even if these cycles have a low amplitude (Meunier, Pascal and Loubens, 1979; Castanet, 1985; Castanet and de Ricqlès, 1986-1987; Castanet *et al.*, 1993).

This annual cyclical growth pattern provides information which has been used in many studies to accurately age amphibians and reptiles (e.g., Castanet and Cheylan, 1979; Leclair and Castanet, 1987; Francillon-Veillot, Arntzen, and Géraudie, 1990; Castanet and Baez, 1991; Castanet *et al.*, 1993; Chinsamy *et al.*, 1995; Wake and Castanet, 1995; Wayne and Gregory, 1998). In cyclical growth, broad zones are associated with periods of rapid growth, while narrower zones or annuli are associated with periods of decreased growth rates (Castanet *et al.*, 1993; Wake and Castanet, 1995; Esteban, Garcia-Paris and Castanet, 1996). These latter periods can also manifest as lines of arrested growth (LAGs), which are functionally defined as rest lines (Castanet, 1981; Castanet *et al.*, 1993). LAGs depict temporary arrests of local osteogenesis, usually of a few weeks, and always have a very narrow thickness (Castanet, 1981; Castanet *et al.*, 1993). Together, a zone plus an annulus and or line of arrested growth correspond to one growth cycle (Castanet and Smirina, 1990;

Castanet *et al.*, 1993).

The bone macrostructure of vertebrates is summarized by Tortora and Grabowski (1993). Structurally, the skeletal system consists of cartilage, bone (osseous) tissue, bone marrow and a periosteum and endosteum (the membranes surrounding bones). A typical long bone (in other words a bone that has a greater length than width) consists of the following parts: 1) Diaphysis, which is the shaft or long main portion of the bone. 2) Epiphyses, or the extremities or ends of the bone. 3) Metaphysis, which is the region in a mature bone where the diaphysis joins the epiphysis. In a growing bone, it is the region that includes the epiphyseal plate where cartilage is replaced by bone. 4) Articular cartilage, which is the thin layer of cartilage that covers the epiphysis where the bone forms a joint with another bone. 5) Periosteum, which is the membrane around the surface of the bone not covered with articular cartilage. The periosteum is essential for bone growth in diameter, repair and nutrition, and also serves as a point of attachment for ligaments and tendons. 6) Medullary cavity, which represents the space within the diaphysis containing the fatty marrow in adults. 7) Endosteum, which is the membrane lining the medullary cavity, containing osteo-progenitor cells (unspecialized cells derived from the mesenchyme which undergo mitosis and develop into osteoblasts). These cells are also found in the inner portion of the periosteum and in vascular canals.

## 1.2. Bone remodeling

Remodeling is fundamental to the general process of skeletal growth and is concerned with the continual reshaping of a bone during growth, as it increases in size (Enlow, 1963).

Remodeling is an orderly, progressive mechanism of adjustment, which maintains the

constant shape, proportions and relationships of a bone during its growth (Enlow, 1963). Because bone is hard, it cannot grow and increase in mass by the internal expansion of existing tissues, as do most soft tissues, it can only grow by an appositional process of surface deposition together with resorption from a contralateral surface (Enlow, 1963). Resorptive removal is essential since proportionate growth would not be possible without a system for removal of old bone as new bone is added (Enlow, 1963). Since a bone cannot grow by interstitial expansion, surfaces become the critical parts of bone which are directly involved in active remodeling changes (Enlow, 1963). A variety of "surfaces" exist in bone, namely periosteal, endosteal, cancellous, the inner surface of a canal, the surface within resorption spaces, and the inner surfaces of lacunae and canaliculi (Enlow, 1963). A surface produced by periosteal or endosteal deposition can be distinguished from active surfaces of resorption, in that the surface which has received additions of new bone, is identified in microscopic sections by its relatively smooth, even contours, while the resorptive surface appears scalloped, eroded and notched (Enlow, 1963).

The diameter of the metaphysis decreases during longitudinal bone growth until it reaches the dimensions of the diaphysis, which is itself increasing in diameter at the same time (Enlow, 1963). When this point is reached, metaphyseal reduction is complete, but the diaphysis continues to increase in diameter as the general dimensions of the entire bone increase (Enlow, 1963). Inward or endosteal growth produces endosteal lamellar bone into parts of the metaphysis or diaphysis which have relatively few or no cancellous trabeculae, and is associated with metaphyseal reduction in diameter or with other remodeling processes related to osseous drift (Enlow, 1963). If variable amounts of cancellous trabeculae occur in the medullary cavity, however, this results in the formation of spongy bone (Enlow, 1969).



Spongy bone can undergo a process of compaction, due to remodeling changes, by the deposition of bone within cancellous spaces (Enlow, 1969). As cancellous bone is incorporated into the growing, inward-moving cortex in the medullary cavity, it is thereby converted, during these remodeling processes, into compacted coarse cancellous bone (Enlow, 1969). The structure of this common and widespread type of bone is distinctive in appearance and easily recognized in all reptiles. It has a characteristically convoluted, irregular structure, which results from the deposition of new bone on the older, irregularly orientated surfaces of the original cancellous trabeculae (Enlow, 1969).

Internal remodeling of the bone cortex, linked to bone vascularization, can completely destroy growth marks (Castanet and Smirina, 1990). Among living reptiles, this mainly occurs in chelonians and crocodilians, and in amphibians, it occurs in some anurans (Castanet and Smirina, 1990). At the medullary cavity margin, endosteal resorption can also remove initial growth marks, in other words the earliest (first few) growth marks deposited, thereby causing individual ages to be underestimated (Castanet and Smirina, 1990). The rate of periosteal bone resorption can be evaluated by using either known age individuals or by a back calculation method (Castanet and Smirina, 1990). Moreover some studies, for example Hemelaar (1985), Castanet and Baez, (1988) and Castanet and Baez (1991), suggest that differences in the intensity of bone resorption can represent distinctive characteristics among different taxa and can perhaps offer a new taxonomical character (Castanet and Baez, 1988; Castanet and Smirina, 1990).

In her study of *Bufo bufo*, for example, Hemelaar (1985) found that the number of growth marks resorped in adults did not increase with increasing age. She concluded that resorption



of periosteal bone decreased sharply once sexual maturity was reached and therefore, that differences among individuals and populations with respect to resorption, must have arisen before they attained sexual maturity (Hemelaar, 1981; Hemelaar, 1985). Sexual dimorphism relating to the age at which sexual maturity is reached, was also reported for *Bufo bufo* and the results showed a delayed maturation for female toads, which went hand in hand with a greater resorption (Hemelaar, 1985). Despite this fact, she still found considerable individual variation. In male toads from Norway, for example, the first rest line in all individuals had been completely removed, whereas occasionally in some, even the third LAG had been partially resorped (Hemelaar, 1985). Yet, in many toads, the line of metamorphosis was present (Hemelaar, 1985). In view of the fact that bone is described as being remodeled continuously, this large individual variation in the degree of resorption was a curious phenomenon, especially the observation that, in a number of toads, bone was not remodeled at all (Hemelaar, 1985). Variation in remodeling has also been observed in lizards, for example, in a study conducted on *Gallotia* lizards from the Canary Islands, Castanet and Baez (1991) found that the species (*G. stehlini*) displaying the most extensive endosteal resorption and greatest LAG removal, also had the most widespread deposition. The other four species had irregular resorption indicated by embryonic bone remnants and individuals had all LAGs present to some degree (Castanet and Baez, 1988; 1991).

### 1.3. Age estimation

Aging individuals, in other words, determining the time elapsed between birth and a specific moment in time, for example, the time between birth and the attainment of sexual maturity or between birth and death (longevity), is a necessity in many fields of research involving biological systems and organisms (Castanet *et al.*, 1993). To date, several methods have been

proposed to assess the ages of wild animals, namely, eye lens weight, tooth grinding, testis lobation, isotopic ratio, size-frequency data, mark-recapture and skeletochronology, (Halliday and Verrell, 1988; Castanet and Smirina, 1990; Castanet *et al.*, 1993). Halliday and Verrell (1988) critically evaluated four of these methods and discussed the extent to which these hypotheses, that assumed a size-age correlation, were tenable. The methods reviewed were mark-release-recapture, skeletochronology, extrapolation from size-frequency data, and testis lobation.

*Mark-recapture* - The method, which potentially provides the most reliable data on age-size relationships, is mark-release-recapture (Halliday and Verrell, 1988; Castanet *et al.*, 1993). This method ideally involves marking a group of newly metamorphosed amphibians or hatchling reptiles, which subsequently would be recaptured at regular intervals over a long period. The virtue of this method, in comparison to others, is that it yields precise data on growth over a specific time interval and, provided the age of individuals are known when they are first marked, accurate growth curves can be obtained (Halliday and Verrell, 1988). The principal disadvantages of this method are that it is very labour-intensive, requires a large juvenile sample size (due to mortality rates) and may take a long time to yield results (Halliday and Verrell, 1988; Castanet *et al.*, 1993).

*Size frequencies* - Extrapolation from size-frequency data requires that body sizes of a large number of individuals be measured, to produce a size-frequency histogram (Halliday and Verrell, 1988). It must be assumed, that all size classes have an equal probability of being captured and that measurements are taken at an instantaneous moment in time (in other words in as short a time as possible), putative age classes are then assigned to the size-frequency

distribution by examining it for discontinuities (Halliday and Verrell, 1988). According to Halliday and Verrell (1988), there are two major problems associated with this method, firstly, the method assumes what it sets out to demonstrate, that age and size are statistically related and secondly, it is often not clear what age should be assigned to the smallest size class. This method would also be difficult, if not impossible to use if individuals in a given species breed on reaching a particular size rather than at a particular age (Halliday and Verrell, 1988). This method requires a precise knowledge of both adult growth rates and age specific variance in body size (Gibbons, 1976) and in cases where body size within particular age classes was highly variable, extrapolation from size-frequency data would be unreliable (Halliday and Verrell, 1988).

*Testis lobation* - The use of testis lobation to estimate age, was originated by Humphrey (1922). He found that in *Desmognathus* salamanders, it took three years for males to develop two lobes and that thereafter, one new lobe was added biennially. However, Tilley (1977), examined testis lobation in a population of *Desmognathus ochrophaeus* for which he had obtained data on individual age by mark-recapture methods. His results showed considerable variation in male age within any one lobe category as did a study done on two *Triturus* species (Dolmen, 1982), where age had been determined by skeletochronology. The conclusion from both studies was that, apart from providing a means for distinguishing younger from older males and possibly assigning a minimum age to individual males, testis lobation and age do not appear to be correlated in any simple way (Halliday and Verrell, 1988). Some other methods, also using change during life of various morphometric data, such as lens weight, tooth grinding, isotopic ration and the progressive changes of bone and other hard tissue structures, are worthy of mention but will not be discussed in any detail in



this study.

All the above mentioned methods (except mark-release-recapture) are more or less accurate and generally allow, at best, the establishment of broad and overlapping age groups or even only the separation of juveniles from adults (Castanet *et al.*, 1993). Moreover, such methods require the use of specimens of known ages to establish a reference sample to be compared with the wild animals to be aged (Castanet *et al.*, 1993). Morphometric criteria imply, in order to be accurate, a great deal of non-variability or standardization of the biological objects (whether organisms or populations) dealt with (Castanet *et al.*, 1993).

*Skeletochronology* - Skeletochronology, assesses individual ages by evaluating the number of rest lines or growth marks occurring in bone (Castanet and Smirina, 1990; Castanet *et al.*, 1993). It is based on the observation that skeletal growth is often a periodic phenomenon in ectothermic animals and that its temporal pattern is recorded in certain bones and epidermal scutes (Halliday and Verrell, 1988, Castanet *et al.*, 1993). Growth periods appear as broad bands of tissue (zones) separated by narrower zones (annuli) and/or rest lines (LAGs). LAGs mark periods when growth is arrested and annuli depict decreased growth rates but not a complete arrest of growth (Castanet, 1981; Halliday and Verrell, 1988; Castanet *et al.*, 1993). The skeletochronological technique assumes that growth marks are durable over time and that they are laid down at reliable intervals (Halliday and Verrell, 1988). Halliday and Verrell (1988) state that in the studies they reviewed, authors assumed that a growth line is laid down each year and, therefore, that chronological age is accurately reflected by the number of growth lines. This hypothesis should be tested for each ecological study, especially when the local biology of the species is unknown (Castanet *et al.*, 1993). The use of skeletochronology



in tropical species or species living in aseasonal environments was anecdotal up until recently but has been shown to be valid in both amphibians and reptiles, through studies such as those of Zug and Rand (1987), Castanet and Baez (1988, 1991), and Chinsamy *et al.* (1995).

The conclusion is that all aging methods present problems, but that mark-release-recapture and skeletochronology are deemed to be the most reliable in principle (Halliday and Verrell, 1988; Castanet and Smirina, 1990; Castanet *et al.*, 1993). However, with the exception of mark-release-recapture, none of the methods are wholly accurate, so it is preferable to conduct studies where more than one method is used in parallel so that each can be validated against the other (Halliday and Verrell, 1988; Wayne and Gregory, 1998). For example, in a study done by Pilorge and Castanet (1981) on the lizard *Lacerta vivipera* where data were attained by size-frequency, mark-release-recapture, and skeletochronology, it was found that size frequency revealed little about age structure, while mark-release-recapture and skeletochronology were found to compliment each other in providing data on size-age relationships, population age structure, longevity, age at sexual maturity and growth rates. The best experimental approaches for validating skeletochronology, which can also be associated with one another, involve the use of animals of known age, mark-release-recapture and fluorescent labeling (Castanet *et al.*, 1993). Fluorescent labeling involves several injections of fluorochromes at different times of the year, whereby the positions of fluorescent lines and of growth marks, especially haematoxylinophylic lines and annuli, can be compared, to find out the time of growth mark formation within the year (Castanet *et al.*, 1993). Studies that have validated age assumptions using one or more of the above methods, include those of Castanet (1978), Castanet and Cheylan (1979), Francillon (1979), Gibbons and McCarthy (1983), Castanet (1985) and Francillon and Castanet (1985).

Where data from other methods are unavailable, however, skeletochronology has been shown to be the best method for providing data on age and growth in individuals (Caetano *et al.*, 1985; Castanet and Baez, 1991; Francillon-Veillot *et al.*; 1990, Marnell, 1997).

Skeletochronology is not, however, without difficulties. The destruction of growth marks by biological processes is a major problem (Castanet *et al.*, 1993). Bone is a living tissue that undergoes many transformations owing to bone remodeling (bone resorption and construction), which is generally considered a consequence of mechanical constraints and physiological demands throughout life (Amprino, 1948; Castanet *et al.*, 1993). When bone remodeling is locally restricted growth marks can be spared and aging remains possible (Castanet *et al.*, 1993). Conversely, if remodeling is intense, one or several of the earliest growth marks deposited may be completely removed, thus age can only be estimated (Castanet *et al.*, 1993). This particular aspect of bone remodeling involves endosteal resorption at the medullary cavity margin. Furthermore, when counting the number of visible rest lines in bone, problems that may effect accuracy include the presence of birth lines, false lines and double lines (Castanet and Smirina, 1990). These phenomena can cause independent observers to reach different estimates for the number of lines in a bone section (Halliday and Verrell, 1988).

A final problem involves the sacrificing of large numbers of animals, especially when the long bones are used for the skeletochronological assessment (Halliday and Verrell, 1988). This is due to the fact that limb bones, and specifically the middle part of the diaphysis where the periosteal cortex is the thickest and the medullary cavity the narrowest, are known to be the most suitable parts of the skeleton for skeletochronological assessment (Castanet and

Smirina, 1990, Castanet *et al.*, 1993). An alternative, involving the examination of the phalanges, has been proposed. This can be coupled to a mark-release-recapture study in which marking is accomplished by toe clipping (Halliday and Verrell, 1988), and has proved reliable in some studies, for example those of Gibbons and McCarthy (1983), Francillon *et al.* (1990) and Marnell (1997). In practice, however, especially when dealing with individuals of unknown age, one will select the bone with the highest and clearest number of growth marks, where the chances of growth mark removal by remodeling will be as small as possible (Castanet *et al.*, 1993). Age estimation based solely on phalangeal growth mark assessments can, therefore, be inaccurate, as phalanges generally stop growing earlier than long bones (e.g. Castanet *et al.*, 1988). It is best to validate results with growth mark assessments from long bones (e.g. Castanet *et al.*, 1988). In snakes, the only bones that can be examined without extermination, are the caudal vertebrae via a tail clip. This is not, however, a feasible method for snakes that depend on the tail tip for defense or have a blunt tip which would leave a big wound if the end were removed (Waye and Gregory, 1998). According to Waye and Gregory (1998), the problem with caudal vertebrae is that, although the centrum and neural arch show annuli, the bands vary in appearance making counting difficult. This problem does not make the technique less valid for snakes than for amphibians and other reptiles, it just makes precise results difficult to obtain (Waye and Gregory, 1998).

In principle, skeletochronology is fundamentally different from techniques involving morphometric data. Since growth marks are attuned to an external rhythm, their deposition may be largely independent of specific populational, or individual variations (Castanet *et al.*, 1993). In that sense, this method does not need a reference system for each study as do morphometric methods and, since growth marks have been shown to be periodical structures



among many ectothermic vertebrates, they can directly provide, at least in theory, individual ages (Castanet *et al.*, 1993). Skeletochronology has also been shown to be a reliable and powerful tool in assessing biological time (Castanet and Smirina, 1990; Castanet and Baez, 1991; Castanet *et al.*, 1993). According to bone morphogenesis, the histological structure and spatial aspect of growth marks, change from place to place in the same bone and between different bones of the same individual (Castanet *et al.*, 1993). Similarly, according to the general evolution of growth rate throughout life, the structure of growth marks and their sequence changes from birth to death (Castanet *et al.*, 1993). Until sexual maturity is reached, body growth rate is high and rest lines (annuli or LAGs) are usually separated by wide zones of relatively fast growing tissues (Castanet *et al.*, 1993). These zones become closer together after sexual maturity is attained and very close together near death, as the growth rate decreases (Castanet *et al.*, 1993). Moreover, it would seem that external pressures are able to induce selection according to an animals longevity, in other words, to favor adaptive strategies in which longevity is a target for selection as well as any other morphological or physiological adaptive character (Castanet and Baez, 1991).

Skeletochronology is also a unique tool for obtaining such data from fossils and subfossils, as bone tissues and teeth are, in most cases, the only structures remaining from which to obtain such information (e.g., Castanet and Baez, 1991; Chinsamy, 1993). However, in spite of its heuristical and practical values, it is not devoid of difficulties and cannot be seen as a “routine” method, as it requires a basic knowledge and understanding of bone histomorphogenesis, which varies within different taxa and different environmental constraints (Castanet and Smirina, 1990).



Bone histology, involving skeletochronology, has provided insight into many areas of study such as population biology, evolutionary mechanics, taxonomy, climatology and seasonality (Castanet *et al.*, 1993). For example, several distinct populations from one species can be studied, promoting interpopulational comparisons (e.g. Hemelaar, 1985; 1988; Castanet and Baez, 1991). Different sympatric species may be simultaneously studied (e.g. Francillon-Veillot *et al.*, 1990). Information on the relationship existing between ecological conditions and patterns of rest lines can be obtained (e.g. Caetano *et al.*, 1985; Castanet and Baez, 1991; Collins and Rodda, 1994). Comparisons between closely related extinct and extant species can be explored, providing valuable data on species-specific growth strategies, palaeoclimatology and the microevolutionary process (e.g. Castanet and Baez, 1991). Comparisons between conspecific populations and closely related species, can provide new insights into interpreting chronological data in terms of internal or external causation and microevolution, thereby leading to a better and more accurate use of the skeletochronological method (Castanet and Baez, 1990).

#### 1.4. Growth rates and growth curves

Compared to similar aging methods such as scalimetry or otolithometry, investigations of bone tissues have some specific advantages (Castanet *et al.*, 1993). For instance, the functional interpretation of the various histological patterns of bone tissues allows construction of detailed hypotheses about the growth dynamics of individual bones, whole skeletons, and extrapolation to the complete organism (Castanet *et al.*, 1993). Moreover, valuable data on bone growth rates can be deduced from the actual type of bone tissue and/or from its vascularization (Castanet *et al.*, 1993). By using bone growth marks as well as other progressive transformations of bone structures closely related to the time flow, skeletal

tissues now appear to be an essential chronological tool in many fields of research (Castanet *et al.*, 1993).

When conducting studies on the relationship between phylogeny and ontogeny, the relationship between size and age is of special importance (Alberch, Gould, Oster and Wake, 1979). In amphibians and reptiles, there is a general correlation between size and age, so when age is not known, size is sometimes substituted (Halliday and Verrell, 1988; Wake and Castanet, 1995). This relationship is usually quite weak and within a given age class there is considerable variance in body size. The largest individuals are not necessarily the oldest, and as a rule the individuals growing slowly and gradually are generally those who live longer (Castanet and Smirina, 1990). Therefore, it is not feasible to use data on body size alone to reliably and accurately predict the age of an individual (Halliday and Verrell, 1988).

Accurate estimation of age in individuals is an important component of the study of populations, because demographic processes (e.g., fecundity and survivorship) are often age-specific (Waye and Gregory, 1998).

For many amphibians and reptiles, body size and fecundity are often positively correlated, particularly in females, so it is important to know if body size is a function of age or of early growth (Halliday and Verrell, 1988; Waye and Gregory, 1998). This hypothesis has been addressed in many studies with interesting results. In their investigation of the smooth newt, *Triturus vulgaris*, Halliday and Verrell (1988) for example, found that there was rapid growth up to the time of first breeding and that thereafter, growth was very slow. They presumed the reason for this to be due to a shift in resource allocation from somatic growth to reproduction. They also found considerable variance in the body size of animals that had reached first-

breeding age and, because of slow subsequent growth, the result was that individuals that were small for their age when they first bred, continued to be small throughout their lives (Halliday and Verrel, 1988). Wake and Castanet (1995) studied the salamander, *Batrachoseps attenuates*, and obtained similar results, the largest animals fell into all age classes and relatively small animals were several years older than much larger animals. Castanet and Baez (1991) found, in their examination of various *Gallotia* species, that until sexual maturity was reached, relative bone growth was highest for the smallest species *G. atlantica*, lowest for the largest species *G. stehlini* and intermediate for the *G. g. galloti* group of medium size. The relative growth rates were significantly different between the various *Gallotia* species and were inversely correlated to the specific adult size of the taxa (Castanet and Baez, 1991). Therefore, the smallest and most short-lived species, *G. atlantica*, grew faster than the largest and more long-lived species, *G. goliath* (Castanet and Baez, 1991). Wayne and Gregory (1998) also found considerable variation in growth rate between individuals and between years in their study of garter snakes (*Thamnophis*). It would therefore appear, at least from the above studies, that body size and fecundity are not positively correlated and that the attainment of sexual maturity generally seems to be more age specific. It would also seem that size is mainly due to early growth, presumably before the onset of sexual maturity.

### 1.5. The family Cordylidae

The Cordyliformes is a clade of scincomorph lizards partitioned into two families, the Gerrhosauridae and Cordylidae (Lang, 1991). The Gerrhosauridae occur on Africa and Madagascar, while the Cordylidae is restricted to Africa (Brygoo, 1985; Lang, 1991; Mouton and Van Wyk, 1997). The family Cordylidae is divided into four genera: *Platysaurus*,



*Chamaesaura*, *Pseudocordylus* and *Cordylus* (FitzSimons, 1943; Loveridge, 1944; Lang, 1991). In his cladistic analysis based on morphological characters, Lang (1991) demonstrated that the genus *Chamaesaura*, a group of snakelike lizards, is the earliest diverging taxon, while *Cordylus* is the sister taxon of a *Pseudocordylus*–*Platysaurus* clade. Subsequent studies by Herselman, Mouton and Van Wyk (1992) and Mouton and Van Wyk (1997), however, showed that *Cordylus* and *Pseudocordylus* are paraphyletic. Based on morphological and molecular data, Mouton and Van Wyk (1997) considered *Platysaurus* the most basal genus in the family in their analysis of adaptive radiation in cordyliform lizards.

While members of Gerrhosauridae are active foragers with the ability to discriminate prey chemicals by tongue flicking, members of Cordylidae are sit-and-wait feeders without the ability to discriminate prey chemicals (Cooper, 1995; Cooper, Whiting and Van Wyk, 1997). Furthermore, while gerrhosaurids are all oviparous and mainly terrestrial, cordylids are, with the exception of the genus *Platysaurus*, all viviparous and mainly rupicolous (Mouton and Van Wyk, 1997). Most gerrhosaurids presently have extensive lowland distributions in subtropical areas while the majority of cordylids have restricted ranges in temperate highland areas (Branch, 1998). Mouton and Van Wyk (op. cit.) are of the opinion that, within Cordyliformes, sit-and-wait foraging, lack of prey chemical discrimination, and a rupicolous lifestyle co-evolved in the immediate ancestor of the Cordylidae, while viviparity evolved in the immediate ancestor of the *Cordylus*–*Chamaesaura*–*Pseudocordylus* clade. The highland distribution and viviparous reproductive mode of the latter clade suggest that the ancestor became adapted to cool regimes, in other words that the clade had a cold origin. The transitions to a sit-and-wait foraging mode, lack of prey chemical discrimination ability and a rupicolous lifestyle in the ancestor of the Cordylidae could have served as preadaptations



which facilitated the invasion of highland areas by the *Cordylus-Chamaesaura-Pseudocordylus* clade.

Members of the genus *Pseudocordylus* (six species) are all montane forms and are restricted to the Cape Fold and Drakensberg ranges. The majority of the 33 *Cordylus* species recognized today, occur along the escarpment mountains of southern Africa, but a few such as *C. polyzonus*, *C. macropholis*, *C. cataphractus*, and *C. tropidosternum*, are lowland forms (Branch, 1998). All three species of *Chamaesaura* occur in escarpment and montane grasslands. Similar to gerrhosaurids, the genus *Platysaurus* mainly has a subtropical distribution (Broadley, 1978).

The transformations that accompanied the Gerrhosauridae- Cordylidae divergence were apparently all energy-related (Mouton and Van Wyk, 1997). The question arose as to whether this hypothesized cold origin of the Cordylidae would also be reflected in the bone structure of this family, as ecological adaptations can be reflected in bone microstructure (de Ricqlès, 1976). Only limited information on bone microstructure is available for the Gerrhosauridae. Chinsamy, Hanrahan, Neto and Seely (1995) conducted a skeletochronological assessment of age in *Angolosaurus skoogi*, a gerrhosaurid living on the coastal dunes of the northern Namib Desert. The environment where this species is found is largely aseasonal and there is a constant food supply provided by the evergreen Nara plant and wind-borne detritus (Mitchell, Seely, Roberts, Pietruszka, McClain, Griffen, and Yeaton, 1987). The results of the study done by Chinsamy *et al.* (1995), showed that *Angolosaurus skoogi* has a similar bone microstructure to that of other lizards, namely a cortex composed of embryonic bone in the youngest individuals, and parallel-fibred bone in older juveniles and

adults. Sublamellar to lamellar bone occurred in the outer cortex of older individuals. The histological data, therefore, showed that the bone depositional rate was initially rapid and subsequently decreased with increasing age (Chinsamy *et al.*, 1995). Growth marks were represented by zones and LAGs (Chinsamy *et al.*, 1995). Longevity and age at sexual maturity were assessed using skeletochronology and, although there was no direct evidence that LAGs are annual in *A. skoogi*, because they are known to be annual in other lizards, they were assumed to be so in this species (Chinsamy *et al.*, 1995). Furthermore, males and females were found to exhibit different growth rates when snout-vent length (SVL) and mass were regressed against the number of LAGs (age) (Chinsamy *et al.*, 1995).

Information on bone histology, longevity and age at sexual maturity is non-existent for Cordylidae. To obtain baseline information on bone morphology in this family, I selected two species, *Cordylus cataphractus* and *Pseudocordylus capensis*, which differ considerably in general morphology and biology, for a detailed histological analysis.

### *Cordylus cataphractus*

The armadillo lizard, *C. cataphractus*, occurs in the western districts of South Africa, from the Orange River in the north to the Piketberg Mountains in the south, reaching as far inland as Matjiesfontein in the western Karoo (Mouton, Oelofsen and Mostert, 1987; Branch, 1998; Mouton, 1988). It is a heavily armoured, sluggish lizard that exhibits the peculiar habit of curling up by gripping its tail firmly in its jaws and rolling into a tight ball when threatened (Peers, 1930; FitzSimons, 1943; Rose, 1950; Mouton, Flemming and Kanga, 1999). This species is also gregarious and up to 40 individuals may be found sharing a crevice for long periods (Peers, 1930; FitzSimons, 1943; Mouton *et al.*, 1999). Males reach larger

asymptotic body sizes than females and also have larger heads and longer tails (Mouton *et al.*, 1999). The maximum SVL recorded for the species is 124 mm and in their analysis of sexual dimorphism in this species, Mouton *et al.* (1999) found that both sexes reach maturity at approximately 95 mm snout-vent length.

Like in other cordylid species, female *C. cataphractus* display autumn or winter vitellogeneic activity, with ovulation during late spring and parturition in April the following year (Flemming and Mouton, unpublished data). In contrast to other *Cordylus* species where three to five young per annum is the norm, *C. cataphractus* produces only one young per annum (Flemming and Mouton, op. cit.). Male *C. cataphractus* exhibit a prenuptial testicular cycle (see Van Wyk and Mouton, 1998) and maximal spermatogenesis is synchronous with ovulation in females (Flemming and Mouton, op. cit.).

By analyzing stomach contents of 126 *C. cataphractus* individuals, Mouton, Geertsema and Visagie (2000) established that termites are the main food item of this species, although a wide range of other invertebrates is also eaten. Mouton *et al.* (2000) found a high percentage of individuals with empty stomachs at the end of the dry season in April. They also found that the incidence of individuals with empty stomachs was much higher among individuals living in groups than among lone individuals, showing that competition for food among group members must be considerable. Mouton *et al.* (op. cit.) confirmed that this species behaves like a typical sit-and-wait feeder, despite living in groups. Fourie (1997) found that its basal metabolic rate is much lower than in other cordylid species for which data are available and concluded that this is an adaptation to facilitate group-living.



*Pseudocordylus capensis*

The graceful crag lizard, *P. capensis*, occurs in isolated populations from Niewoudtville in the north through the Cape Fold Mountains to the Kammanassieberg in the east (Herselman, Mouton and Van Wyk, 1992; Branch, 1998). It is a medium-sized crag lizard reaching a snout-vent length of 108 mm (Van Wyk and Mouton, 1998). Sexual maturity is reached between 80-90 mm snout-vent length in both sexes (Van Wyk and Mouton, 1998). Unlike *C. cataphractus*, it is extremely agile and runs at speed over the smooth rocks and vertical cliff faces it prefers (Branch, 1998). Its long toes and exceptionally long tail is proof of its agility (Garland and Losos, 1994).

*P. capensis* is one of several melanistic cordylid species occurring in the southwestern corner of southern Africa (Mouton and Oelofsen, 1988). Badenhorst (1990) established that along the coast, these melanistic populations are associated with zones of upwelling of cold water, while in the mountains the localities where they occur are characterized by a high incidence of cloud cover or mist. Although mainly occurring at high altitudes in the Cape Fold Mountains, *P. capensis*, at many places, also occur at low altitudes, but then usually in very special types of surroundings such as waterfalls, along ravines, etc (Mouton, pers. comm.). Vitellogenesis in females starts in autumn with ovulation during spring (Van Wyk and Mouton, 1998). Females are gravid during summer (December to January), and males display a prenuptial cycle like *C. cataphractus* (Van Wyk and Mouton, 1998). Information on the diet of this species is anecdotal. Branch (1998) reports that it is mainly insectivorous, particularly feeding on bees and wasps. Cooper *et al.* (1997) report that, although it can be classified as a sit-and-wait feeder, it is much more active than any *Cordylus* species examined.



### 1.6. Key questions

1.) *Does the bone histology of C. cataphractus and P. capensis conform to that of other lizards and to what extent does their bone microstructure reflect their ecology?*

*C. cataphractus* and *P. capensis* are not closely related (Mouton and Van Wyk, 1997) and occur at different altitudes, in different habitats, and exhibit different behavioural adaptations to these environmental conditions (Badenhorst Mouton and Van Wyk, 1992; Herselman *et al.*, 1992; Mouton and Van Wyk, 1995; Mouton and Van Wyk, 1997; Van Wyk and Mouton, 1998; Mouton *et al.*, 1999; Mouton *et al.*, 2000). It has been demonstrated in many previous studies that ecological adaptations, as well as the animal's specific size and longevity, are reflected as histological variations within its skeleton (e.g. de Ricqlès, 1976; Caetano *et al.*, 1985; Castanet and Baez, 1991; Chinsamy *et al.*, 1995; Zug and Rand, 1987; Wayne and Gregory, 1998).

2.) *To what extent do altitudinal differences affect bone histology?*

This question specifically pertains to *P. capensis*, as the material studied contained individuals from low and high altitudes. Samples of *Triturus marmoratus* taken from various elevations (550 m, 650 m, 700 m, 800 m, 1150 m, 1500 m) in the Peneda Geres National Park (North Portugal), showed that older individuals lived in higher stations and that individuals from the highest altitudes showed two periods of arrested growth in one growth cycle, demonstrating the highest number of double lines of arrested growth in the bone cortex (Caetano *et al.*, 1985). Altitude also affects the distinctness of LAGs, as Castanet and Baez (1991) demonstrated, when looking at populations of *Gallotia stehlini* from different altitudes in Gran Canaria. Those specimens from the highest altitude (1800 m) showed the most distinct LAGs, probably due to the greater seasonality at higher altitudes (Castanet and Baez,

1991).

### 3.) *What is the life span or longevity of each species?*

Skeletochronology was used to estimate individual ages and to give an indication as to the possible longevity of each species, which differs markedly in reptiles and amphibians. For example, Caetano *et al.* (1995) found the maximum age in adult *Triturus marmoratus* males to be 14 years, while in females it was 15 or 16 years. In *Gallotia* lizards, longevity differed between species, with *G atlantica* having a maximum age of five years, *G. gallotia*, eight to nine years, *G. stehlini*, 10 to 11 years and *G. goliath* (the extinct species), 20 + years (Castanet and Baez, 1991). In *Angolosaurus skoogi*, the maximum age was 10 years (Chinsamy, *et al.*, 1995). The maximum age in *Rana pipiens* was four or five years (Leclair and Castanet, 1987) while in *Batrachoseps attenuates*, the maximum age was eight years (Wake and Castanet, 1995). Knowledge of longevity within the Cordyliformes can help to explain certain phenomena like times of breeding and body-sizes, as well as to give an indication of how long these animals live in various environments.

### 4.) *At what age does sexual maturity occur?*

This question can also be addressed by skeletochronology as can be seen from the above mentioned studies; in *Triturus marmoratus*, the age at sexual maturity was between four and five years (Caetano *et al.*, 1985), while in *Gallotia* lizards, the age at sexual maturity was between three and four years in *G. gallotia*, four to five years in *G. stehlini*, probably around two years in *G atlantica* and possibly between the 15<sup>th</sup> or 16<sup>th</sup> LAG in *G. goliath*, although this is not a reliable result (Castanet and Baez, 1991). Sexual maturity was possibly reached at around two years in *Rana pipiens* (Leclair and Castanet, 1987), while in *Batrachoseps*

*attenuates*, sexual maturity seemed to be reached around four years of age (Wake and Castanet, 1995). In studies done on *Cordylus cataphractus* and *Pseudocordylus capensis*, the minimum snout-vent length at sexual maturity was determined by macroscopic as well as microscopic observation of the gonads (Van Wyk and Mouton, 1998; Mouton *et al.*, 2000). It was, therefore, of interest to see whether the skeletochronological method would concur with these macroscopic observations, as well as give an approximate age as to when sexual maturity occurred.

#### 5.) *What type of growth rate do C. cataphractus and P. capensis exhibit?*

The type of bone microstructure, along with general body sizes, individual ages and age at sexual maturity allows for growth rates, as well as sexual dimorphism in growth rates, to be assessed for populations and by extension, species (by regression analyses).



## CHAPTER 2

### MATERIALS AND METHODS

#### 2.1. Materials

All material used in this study was provided by the Ellerman Museum of the University of Stellenbosch (see Appendix 1 for a complete list of specimens used and locality data). The *Cordylus cataphractus* specimens used were collected by Mouton *et al.* (2000) in a relatively small geographical area of approximately 100 km<sup>2</sup> in the Graafwater district. The vegetation in the area is classified as Namaqualand Coastal Belt, a subdivision of succulent karoo (Veld Type no. 31a; Acocks, 1988). This is vegetation characteristic of the low altitude, hot, arid areas with rainfall ranging from 50-200 mm per annum, mainly falling in winter (Acocks, 1988). Aridity is, however, reduced by sea mists.

The *Pseudocordylus capensis* specimens were obtained from a range of localities varying in altitude from 360 – 1640 m above sea level along the Cape Fold Mountains (Appendix 1). A sample of 19 lizards from Landdroeskop in the Hottentots Holland Mountains near Somerset West, was used to determine growth curves for this species. At an altitude of 1180 m above sea level, this locality regularly experiences cloud caps of orogenic fog or cloud. Sirgel (1985) reports that misty overcast conditions prevail for at least 200 days per year at this locality.

#### 2.2. Methods

The *C. cataphractus* sample consisted of left femora from 10 males, 12 females and 23 juveniles. The *P. capensis* sample consisted of the left femora from 10 males, 9 females and six juveniles from various localities along the Cape Fold Mountains (Appendix 1) and seven



males, seven females and five juveniles from Landdroskop. For both species, the size at sexual maturity for females was determined as the snout-vent length of the smallest female containing advanced vitellogenic follicles or oviductal eggs, and for males, the smallest male displaying spermatogenesis (Mouton *et al.*, 1999; Van Wyk and Mouton, 1998). For *Cordylus cataphractus*, the size at which sexual maturity is reached was taken as approximately 95 mm for both sexes (Mouton *et al.*, 1999), and for *P. capensis*, between 80-90 mm for both sexes (Van Wyk and Mouton, 1998).

The femora were carefully dissected out and the length of each femur, width of the proximal and distal ends (taken as the greatest diameter at each end of the bone) and width of the mid-shaft region (taken as the smallest diameter along the middle region of the bone) were measured (Figure 1). The snout-vent-length (SVL) of all lizards was also recorded. All measurements were taken with digital callipers to 0.01 mm (Appendix 2).

### 2.2.1 Decalcification

The femora were fixed in 10 % formalin overnight and then washed in running water for approximately 10 hours to remove the formalin, since it was found that the fixative influenced embedding and staining at a later stage. Each femur was cut in half at the mid-shaft region, before decalcification. Previous studies stated that decalcification with 3 % or 5 % nitric acid for five to 12 hours, depending on the size of the bone, was sufficient time to remove all the calcium from the bone (Caetano *et al.*, 1985; Castanet and Gasc, 1986; Leclair and Castanet, 1987; Castanet and Baez, 1991). However, in this study, good wax infiltration was only obtained once the decalcification time was increased to at least 20 hours in 3 % nitric acid at room temperature. In some former studies (e.g., Caetano *et al.*, 1985;

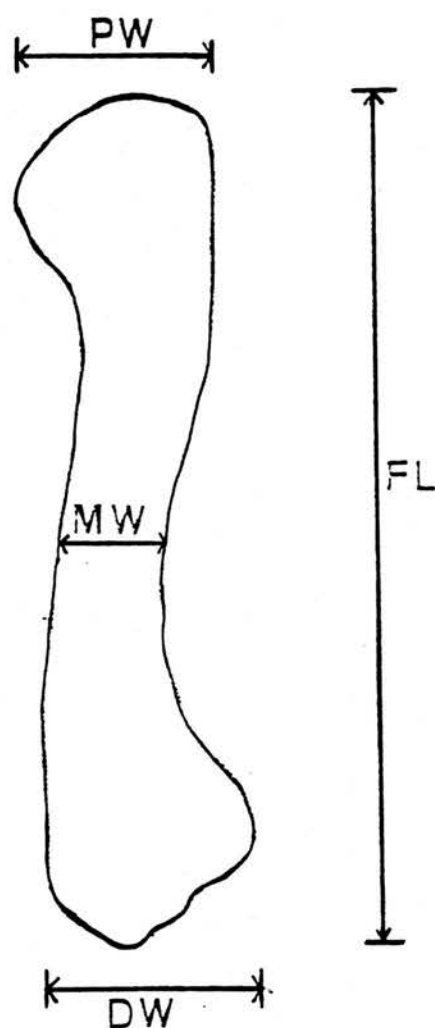


FIGURE 1. Diagrammatic representation indicating macroscopic measurements of a femur.

FL, femoral length; PW, proximal width; DW, distal width; MW, mid-shaft width.

Castanet and Gasc, 1986; Leclair and Castanet, 1987; Castanet and Baez, 1991), bones were cut using a freeze microtome. For both methods (wax embedding and freezing) it is necessary to remove all the calcium, to insure that the bones are very soft and that smooth sections can be obtained. This also insures that wax infiltration is not hindered by the presence of residual calcium.

### 2.2.2. Dehydration

Dehydration requires the gradual increase in the percentage of ethyl alcohol to distilled water to prevent tissue distortion (Humason, 1962). The femora were dehydrated starting with 50 % ethyl alcohol, followed by 70 %, 96 %, 100 % and a final solution of 100 % ethyl alcohol. The duration for each dehydration step was one to one and a half hours, with intermittent periods of agitation. The volume of the alcohol was at least 30 times that of the bone (Humason, 1962). After dehydration, the femora were de-alcoholized and cleared with xylene for one hour to aid wax infiltration.

### 2.2.3. Embedding

After clearing, the femora were placed in a solution of xylene and wax (1 : 1) for one hour. Paraplast Plus (melting point: 56 - 58°C) was used for embedding as it provides the best support for hard material like bone and skin, instead of softer waxes with lower melting points (Humason, 1962; Wallington, 1972). The bones were transferred to three consecutive wax baths for one hour, 12 hours and one hour, respectively. Humason (1962) states that a third wax bath can be added and that the second infiltration time can be increased to overnight if problems are encountered with wax infiltration. After infiltration, the bones were placed in separate embedding containers (each half to its own container) and transferred

to a vacuum oven for two to three hours to remove air, thereby eliminating holes in the final wax block (Humason, 1962). The femora were orientated in the wax with the proximal or distal part facing upward (top of the container) and the mid-shaft region facing the base of the container, so that when the wax block was mounted onto the microtome, cross sections could be cut from the mid-shaft region of each bone. The wax was left to solidify at a cool temperature (approximately 12 °C) overnight.

#### 2.2.4. Sectioning

Approximately 20 transverse sections were cut from the mid-shaft region of each femur and at a thickness of 14 µm, using a rotating microtome. Sections were mounted on microscope slides with a glycerine-egg albumin solution and left to dry overnight. On each slide it was noted in which direction the sections had been cut and the number of sections cut. In other words, from the mid-shaft region section of each femur, a number of sections were cut in a direction extending towards either the proximal or distal end of the bone.

#### 2.2.5 Staining

Sections were stained with Ehrlich's haematoxylin for 20 minutes and eosin for 30 seconds, according to the method set out by Wallington (1972). Permanent mounting onto slides was accomplished using xylene-based E-Z Mount and the slides were left to dry for one to two days on a flat surface.

#### 2.2.6. Microscopic analysis

Slides were labelled with the specimen number and the sections were studied under a transmitting light microscope. The histological composition was recorded and the number of



visible lines of arrested growth (LAGs) were counted using the “blind analysis” method (Castanet *et al.*, 1993). This method requires that the number of visible growth rings is counted three or four times over a few weeks, without prior knowledge of the animal's size or sex. Random slides are then examined by a second person to check the results. A section from each individual was also photographed to validate the direct counting results. In cases where the earliest LAGs were absent due to bone resorption, the estimated number of LAGs resorped was calculated using the back calculation method (Castanet and Cheylan, 1979, Castanet and Smirina, 1990). This method involves comparing the size of the medullary cavities and first rest lines in the youngest juveniles, with the size of medullary cavities and visible rest lines in older individuals (Castanet and Smirina, 1990). To estimate the number of LAGs removed by endosteal resorption, LAG diameters were calculated in juveniles and adults, similar to the method that Hemelaar (1985) used to calculate phalangeal rest line diameters in toads. The longest and shortest perpendicular diameters were recorded for the area enclosed by each visible LAG and the average value was calculated. This value (in microscopic units) was then multiplied with the scale to obtain a value in millimetres. Individual ages were based on the number of LAGs (visible LAGs plus estimated number of LAGs resorped) occurring in the mid-diaphysis of each femur. There is no direct evidence that LAGs are annual in *C. cataphractus* and *P. capensis*, but because they are known to be annual in other lizards, they are assumed to be so in these two species.

Area values were calculated by projecting each slide onto a flat surface at 10 X magnification, using a Nikon microprojector, and tracing the outline of the whole bone section and medullary cavity. A scale was obtained by projecting a micrometer slide in the same manner and calculating how many ruler millimetres corresponded to one projected

millimetre. A square was drawn using this mm measurement, representing one millimetre squared. This square represented the area scale. A portable area meter (Li – 3000 model) was used to determine the area of the scale, medullary cavity areas (MCAs) and total bone areas (TBAs). Areas were obtained by running the diagrammatic representations of the medullary cavities, whole bone sections and scale at least twice through the machine, to obtain a value in  $\text{mm}^2$ . Each value was divided by the scale to obtain a value in  $\text{mm}^2$  (recorded in Appendix 3). To test the accuracy of this method, the area of a perfect circle was calculated manually using the standard area formula ( $\pi r^2$ ). The same circle was put through the portable area meter and the percentage difference between the two values was calculated. The result was a 0.77 % difference between the two values. The same method was used to test the area of a perfect square and the result was a 0.33 % difference between the two values. In both instances, therefore, there was less than one percent difference between the two values. Area meter calculations can thus be considered as 99 % accurate.

The intensity of bone resorption, was estimated with reference to MCAs and number of LAGs resorped. The age for each individual was estimated by adding the estimated number of LAGs removed by bone resorption, to the number of visible LAGs in each section. In undamaged sections, the relative bone thickness (RBT) was calculated by dividing the mean thickness of the compacta by the mean diameter of the whole bone section and then multiplying by 100 (Appendix 3). Bone wall area (BWA) was calculated by subtracting the MCA from the TBA (Appendix 3). MCAs were calculated as percentages of TBAs and the values were recorded in Appendix 4. The endosteal bone area (EBA) surrounding the MCA was calculated in the same way as MCAs and TBAs. All microscopic measurements were recorded in millimetres (mm) and areas were recorded in  $\text{mm}^2$ .

#### 2.2.7. Statistical methods

The data were analysed using the Statistica (Version 5) and Jandel Scientific Sigma Stat (Version 2.0) statistical packages. All values were log-transformed to remove the possible effects of allometric growth. Least square regression analysis of log-transformed variables, namely snout-vent length (SVL), femoral length (FL), relative bone thickness (RBT), medullary cavity area (MCA), total bone area (TBA) and bone wall area (BWA), versus log number of LAGs, was used to assess any correlation between these variables and increasing age. MCA was regressed against SVL, FL, RBT and TBA, to assess any correlation between MCA and these variables. Regression equations for juveniles, adult males and adult females for the various morphometric variables were compared using Analysis of Covariance (ANCOVA) to determine any significant differences between the sexes. Growth rates were assessed from growth curves, which were plotted using the Jandel Scientific Sigma Plot (Version 2.0) package. Probability values (P) less than 0.05 were considered as significant in all the tests performed.



## CHAPTER 3

### Histological analysis of bone in *Cordylus cataphractus*

#### 3.1. Bone histology and growth mark features

##### *Juveniles*

Transverse sections of the femora of 23 juveniles and subadults (SVL < 95 mm), revealed a layer of compact bone tissue surrounding a medullary cavity which lacked cancellous bone (spongy bone) and which was filled with adipose tissue, blood vessels, blood cells and connective tissue (Figure 2). The compact bone of young individuals was thin, with a relative bone thickness (RBT) ranging from 18 % (SVL = 69.78 mm) to 33 % (SVL = 79.27 mm), and essentially consisted of parallel-fibred bone (Figure 3). The area of the compacta was small in comparison to adults, ranging from 0.10 mm<sup>2</sup> (SVL = 58.27 mm) to 0.32 mm<sup>2</sup> (SVL = 79.04 mm), with an average of 0.23 mm<sup>2</sup> ± 0.06 SD. In the smallest juvenile examined (SVL = 58.27 mm), the compacta consisted of embryonic bone, which is characterized by the presence of numerous, enlarged, globular osteocytic lacunae (Figure 4). In 15 juveniles, the medullary cavity was lined, in places, with a narrow region of endosteal lamellar tissue, which was clearly separated from periosteal bone by an uneven resorption line (Figure 5). In 18 juveniles, the medullary cavity was uneven due to bone resorption (Figure 6). The compacta, in all but six individuals, was avascular. Five of the latter six juveniles had one large simple vascular canal (Figure 7A). The remaining juvenile had a transverse vascular canal, generally classified as a Volkmann's canal, because it originated from the periosteum and was surrounded by endosteal lamellar bone. These vascular canals are typically associated with haversian systems, however, although this was not the case here, the transverse canal still retained features characteristic of Volkmann's canals.

FIGURE 2. Transverse section (T.s.) through the mid-diaphysis of the femur of a juvenile female *Cordylus cataphractus*. MC, medullary cavity; C, Compacta. Scale bar equals 100  $\mu\text{m}$ .

FIGURE 3. T.s. through the mid-diaphysis of a femur of a juvenile female *Cordylus cataphractus*, indicating a compacta composed of parallel-fibred bone. MC, medullary cavity; PB, parallel-fibred bone. Scale bar equals 40  $\mu\text{m}$ .

FIGURE 4. T.s. through the mid-diaphysis of the femur of a juvenile male *Cordylus cataphractus*, showing the compacta composed of embryonic bone. EB, embryonic bone; arrow indicates osteocyte within globular osteocytic lacunae. Scale bar equals 40  $\mu$ m.

FIGURE 5. T.s. through the mid-diaphysis of the femur of a juvenile female *Cordylus cataphractus*, showing the medullary cavity lined in various regions by endosteal lamellar bone. ELB, endosteal lamellar bone; RL, resorption line. Scale bar equals 40  $\mu$ m.



FIGURE 6. T.s. through the mid-diaphysis of the femur of a juvenile male *Cordylus cataphractus*, showing an uneven medullary cavity due to intense endosteal resorption. Arrows indicate resorption. Scale bar equals 100  $\mu\text{m}$ .

**FIGURE 7. A.** T.s. through the mid-diaphysis of the femur of a juvenile female *Cordylus cataphractus*, indicating a longitudinal vascular canal. **B.** T.s. through the mid-diaphysis of a juvenile female *Cordylus cataphractus*, indicating a Volkmann's canal. VC, vascular canal; ELB, endosteal lamellar bone; RL, resorption line. Scale bar serves A and B and equals 40  $\mu\text{m}$ .

FIGURE 8. T.s. through the mid-diaphysis of the femur of an adult female *Cordylus cataphractus*, showing the periosteal cortex composed of parallel-fibred bone. PB, parallel-fibred bone; ELB, endosteal lamellar bone; arrows indicate lines of arrested growth (LAGs). Scale bar equals 100  $\mu\text{m}$ .

FIGURE 9. T.s. through the mid-diaphysis of the femur of an adult female *Cordylus cataphractus*, showing endosteal lamellar bone lining the medullary cavity. ELB, endosteal lamellar bone; EPB, endosteal parallel-fibred bone; RL, resorption line; ?, indistinct "line". Arrows indicate LAGs. Scale bar equals 40  $\mu\text{m}$ .



FIGURE 10. T.s. through the mid-diaphysis of the femur of an adult female *Cordylus cataphractus*, indicating the decrease in size and abundance of osteocytic lacunae near the periosteum. OL, osteocytic lacunae; series of arrows indicate LAGs. Scale bar equals 40  $\mu\text{m}$ .

FIGURE 11. T.s. through the femoral shaft of an adult male *Cordylus cataphractus*, showing intensive remodeling of the compacta indicated by decreased periosteal primary bone due to resorption and increased secondary endosteal deposition, in the form of endosteal parallel-fibred bone and/or lamellar bone. PB, periosteal bone; ELB, endosteal lamellar bone; EPB, endosteal parallel-fibred bone; RL, resorption line. Scale bar equals 40  $\mu\text{m}$ .

**FIGURE 12. A.** T.s. through the mid-diaphysis of the femur of an adult male *Cordylus cataphractus*, indicating a longitudinal vascular canal. **B.** T.s. through the mid-diaphysis of an adult female *Cordylus cataphractus*, indicating a Volkmann's canal. VC, vascular canal; ELB, endosteal lamellar bone; RL, resorption line. Scale bar serves A and B and equals 40  $\mu\text{m}$ .

appeared to have not yet reached their first period of arrested growth. A birth line was present in only one of the smallest juveniles and not in adults (Figure 13). LAGs were distinct in all individuals, although some were more distinct than others in the same bone section (Figure 14). In 30 individuals, one or more double LAGs were present (Figure 15). In three individuals, a double LAG appeared to split into three thin indistinct lines in the broadest parts of the compacta. A double LAG represents two periods of arrested growth in one growth cycle, but it is still counted as a single growth cycle, in other words, one year (Castanet and Baez, 1988). In one juvenile male, two out of three LAGs were double (67 %), and in two adult males, two thirds of the visible number of LAGs were double (71 % and 75 %). In seven individuals, half the number of visible LAGs (50 %) were double. The mean percentage of double LAGs for the sampled population as a whole, was  $28 \% \pm 22$  SD. The mean for juveniles was  $22 \% \pm 25$  SD and for adults,  $34 \% \pm 16$  SD (adult males had a mean of  $39 \% \pm 21$  SD, and adult females  $29 \% \pm 10$  SD). Indistinct "lines", which could be false LAGs, indistinct LAGs or staining anomalies, were present in the largest adults making accurate LAG counting difficult (Figure 9). False LAGs resemble genuine LAGs in appearance and indicate a temporary reduction in growth rate (Castanet and Smirina, 1990; Sagor, Oullet, Barten and Green, 1998). These false lines, however, are thought to result from injury or reduced food supply (Hemelaar, 1988) which compel the animal to focus available resources on sustenance rather than growth (Sagor *et al.*, 1998). False LAGs are generally fainter than LAGs and do not form a complete ring around the bone section (Sagor *et al.*, 1998). In cases where indecision still persisted regarding the origin of a line, following numerous re-examinations of all bone sections, it was excluded from the skeletochronological assessment.

FIGURE 13. T.s. through the mid-diaphysis of the left femur of a juvenile male *Cordylus cataphractus*, displaying a possible birth line. MC, medullary cavity; EB, embryonic bone, BL, birth line. Scale bar equals 25  $\mu\text{m}$ .

FIGURE 14. T.s. through the mid-diaphysis of the left femur of an adult female *Cordylus cataphractus*, indicating lines of arrested growth (LAGs). Circles indicate distinct LAGs; arrow indicates indistinct LAG. Scale equals 40  $\mu\text{m}$ .



**FIGURE 15. A:** T.s. through the mid-diaphysis of the femur of an adult female *Cordylus cataphractus*, showing a double LAG. **B:** T.s. through the mid-diaphysis of the femur of an adult female *Cordylus cataphractus*, showing numerous double LAGs. Arrows indicate LAGs; squares indicate double LAGs. Scale bar serves A and B and equals 40  $\mu\text{m}$ .

### 3.2. Bone remodeling

#### *Endosteal resorption*

In both juveniles and adults, the medullary cavity was uneven due to endosteal resorption, which took place around the entire circumference (Figures 6, 8 and 16). In older juveniles and all adults the medullary cavity was slightly off-center as a result of osseous drift (Figures 6 and 16). In adults, MCA was calculated as a percentage of TBA (Appendix 4). The mean MCA percentage of TBA was  $52 \% \pm 8 \text{ SD}$ .

There was a statistically significant correlation between MCA and snout-vent length (SVL), as well as between MCA and total bone area (TBA) for the *C. cataphractus* population as a whole (Table 1, Figure 17). In juveniles, there was a significant correlation between MCA and SVL as well as between MCA and TBA (Table 2, Figures 18A and 18B). No significant correlation between MCA and any of the variables was recorded for adult males but in adult females, there was a significant correlation between MCA and TBA (Table 2, Figure 18B). There was no statistically significant difference between adult and juvenile males and females (Table 3), and the data could thus be pooled to test for sexual dimorphism. The only statistically significant case of sexual dimorphism was MCA over TBA (Table 4, Figure 19).

In this study, the intensity of resorption was estimated by calculating the number of LAGs resorped (Appendix 4). Of the 44 individuals examined, six were not included in this assessment. In three of these six specimens, the bone cortex was damaged and LAG diameters were not obtained. In two others, remodeling was so intense in the bone cortex, that LAG diameters could not be obtained, while in the last individual, no LAGs had yet been deposited.

FIGURE 16. T.s. through the mid-diaphysis of the femur of an adult female *Cordylus cataphractus*, showing an uneven medullary cavity as a result of endosteal resorption. MC, medullary cavity; arrows indicate resorption. Scale bar equals 100  $\mu\text{m}$ .

Table 1. Regression statistics of log-transformed medullary cavity area versus snout-vent length and total bone area for a population of *Cordylus cataphractus* from the Graafwater district.

Dimension	N	R <sup>2</sup>	F	df	P	Slope	Intercept
Snout-vent length	40	0.379	23.1	1,38	< 0.0001	1.09	-2.53
Total bone area	40	0.573	51.1	1,38	< 0.0001	0.611	-0.295



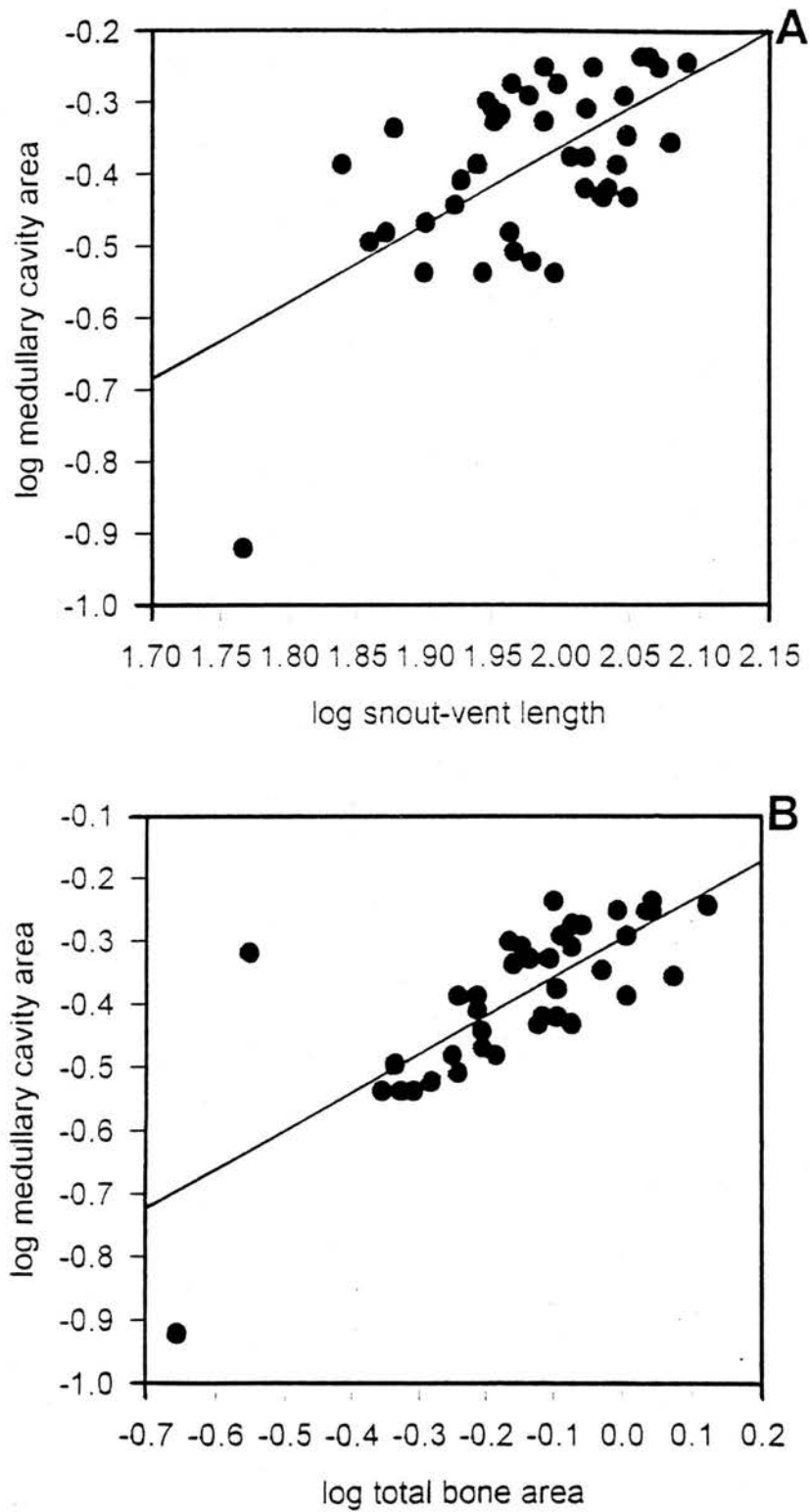


FIGURE 17. Correlations of log-transformed medullary cavity area versus: **A**, log snout-vent length and **B**, log total bone area, for the *Cordylus cataphractus* population sample from the Graafwater district.

Table 2. Regression statistics of log-transformed medullary cavity area versus snout-vent length, femoral length, relative bone thickness and total bone area of adult and juvenile *Cordylus cataphractus* males and females.

Dimension	Sex	N	R <sup>2</sup>	F	df	P	Slope	Intercept
Snout-vent length	Males	8	0.006	0.0388	1,6	> 0.05	0.154	-0.625
	Females	12	0.111	1.249	1,10	> 0.05	1.232	-2.850
	Juveniles	20	0.386	11.302	1,18	<b>&lt; 0.005</b>	1.652	-3.607
Femoral length	Males	8	0.002	0.0138	1,6	> 0.05	0.115	-0.166
	Females	12	0.242	3.190	1,10	> 0.05	1.748	-2.501
	Juveniles	20	0.131	2.707	1,18	> 0.05	1.100	-1.690
Relative bone thickness	Males	8	0.062	0.401	1,6	> 0.05	0.206	-0.397
	Females	11	0.209	2.383	1,9	> 0.05	0.650	-0.0538
	Juveniles	18	0.205	4.123	1,16	> 0.05	0.656	-0.799
Total bone area	Males	8	0.287	2.414	1,6	> 0.05	0.503	-0.314
	Females	12	0.570	13.281	1,10	= 0.005	0.765	-0.219
	Juveniles	20	0.518	19.308	1,18	<b>&lt; 0.001</b>	0.730	-0.252

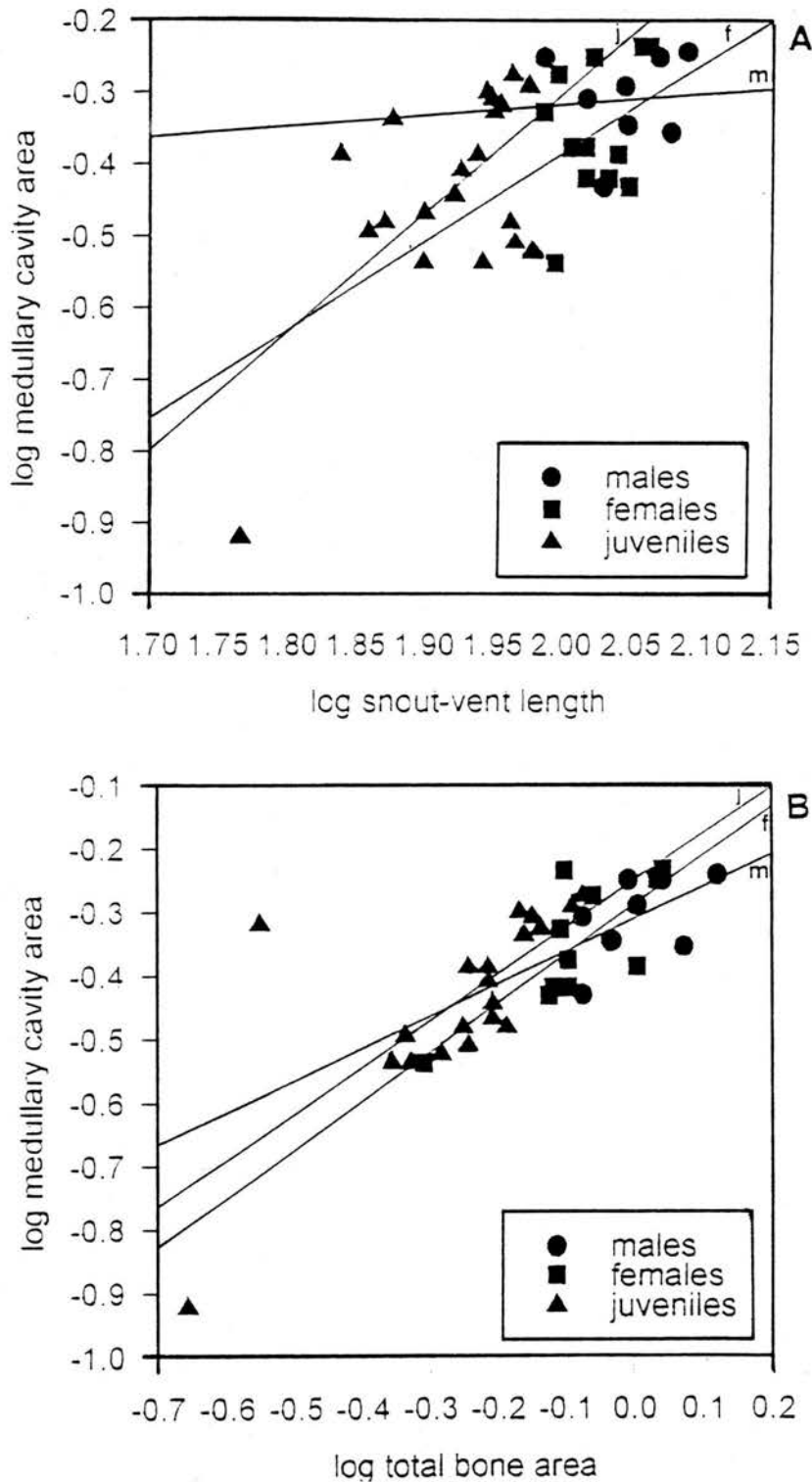


FIGURE 18. Correlations of log-transformed medullary cavity area versus: **A**, log snout-vent length and **B**, log total bone area, for juvenile and adult *Cordylus cataphractus* individuals, from the Graafwater district.

Table 3. Comparison of regression slopes of log-transformed medullary cavity area versus snout-vent length, femoral length, relative bone thickness and total bone area among males, females and juveniles of *Cordylus cataphractus*.

Comparison	Dimension	F	df	P
Adult males versus juvenile males	Snout-vent length	1.8028	1,14	> 0.05
	Femoral length	0.7909	1,14	> 0.05
	Relative bone thickness	0.5829	1,12	> 0.05
	Total bone area	4.2074	1,14	> 0.05
Adult females versus juvenile females	Snout-vent length	0.9558	1,17	> 0.05
	Femoral length	0.0622	1,17	> 0.05
	Relative bone thickness	3.5684	1,15	> 0.05
	Total bone area	0.4207	1,17	> 0.05



Table 4. Comparison of regression slopes of medullary cavity area versus snout-vent length, femoral length, relative bone thickness and total bone area among males and females of *Cordylus cataphractus*.

Comparison	Dimension	F	Df	P
Males versus females	Snout-vent length	2.7188	1,35	> 0.05
	Femoral length	1.6240	1,35	> 0.05
	Relative bone thickness	0.6089	1,31	> 0.05
	Total bone area	7.6001	1,35	< <b>0.01</b>

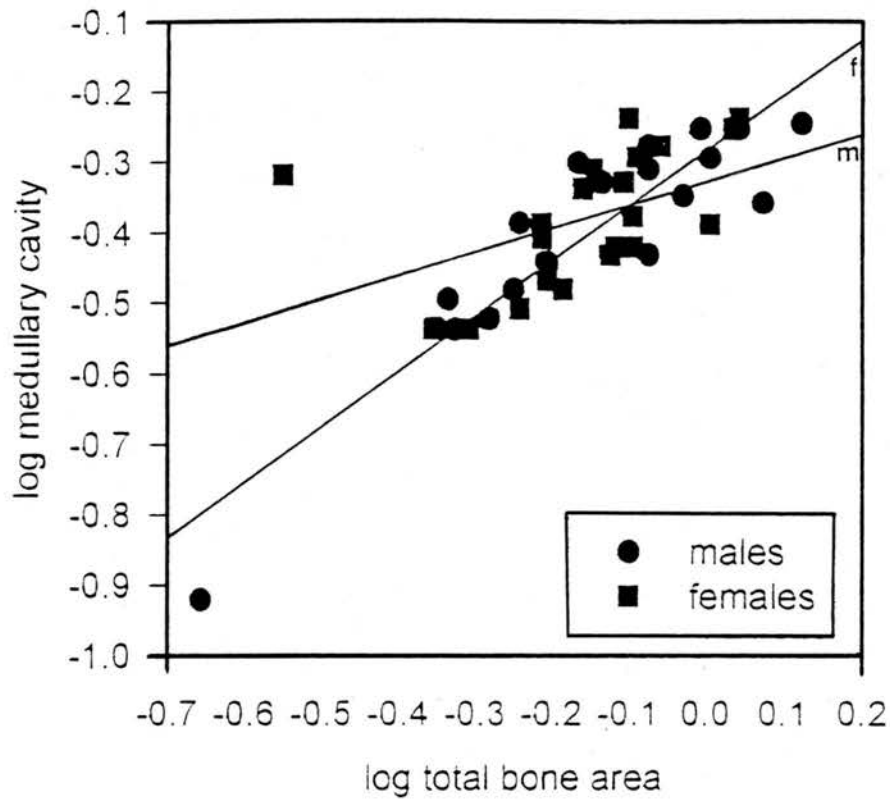


FIGURE 19. Correlations of log-transformed medullary cavity area versus log total bone area for *Cordylus cataphractus* males and females, from the Graafwater district

In all individuals ( $N = 38$ ), except for the two smallest juvenile males, the birth line had been completely resorped. There was considerable variation in LAG resorption and MCAs in all three groups (Appendix 4). For example, up to four LAGs were estimated to have been resorped in two adult males (SVL = 122.90 and 112.71 mm, respectively), two pregnant females (SVL = 114.01 and 101.38 mm), and the second largest juvenile male (SVL = 91.83 mm). On the other hand, in the largest adult female (SVL = 115.48 mm), only one LAG was estimated to have been resorped and in the largest juvenile male (SVL = 94.98 mm), only the birth line (Appendix 4). The only difference observed between males and females, was that in adult females ( $N = 12$ ), two individuals seemed to have retained all their year rings with only the birth line missing. However, in adult males ( $N = 7$ ), from the second to the fourth LAG were estimated to have been resorped (Appendix 4). There was no noticeable difference between pregnant and non-pregnant females in LAG resorption or MCAs (Appendix 4). The only noteworthy correlation observed between LAG resorption and medullary cavity size, was that in those individuals where only the birth line was estimated to have been resorped, the MCA was small, ranging from 0.29 to 0.38 mm<sup>2</sup> (Appendix 4). In these individuals, therefore, little bone had been resorped. As for the rest, individuals with the same number of LAGs resorped had MCAs of varying size.

Of the 19 juveniles examined, one individual exhibited partial resorption of the first two visible LAGs and 12 others the first visible LAG. The remaining six juveniles showed no LAG resorption and the LAGs, therefore, occurred around the entire circumference of the cortex. In the 19 adults examined, two individuals exhibited partial resorption of the first three visible LAGs, four individuals of the first two visible LAGs and 11 individuals, of the first visible LAG. This partial resorption of LAGs in one area in each individual and not

around the entire circumference of the bone cortex, indicated a slightly more intensive resorption of bone in that area.

### *Endosteal deposition*

Of the 44 individuals examined, four were not suitable for this assessment due to intensive remodeling and damage. In all individuals where endosteal bone was deposited, resorption of periosteal bone had first taken place. This was evident from the scalloped or notched resorption line separating endosteal from periosteal bone (Figures 9, 10, 11 and 20). In four adults, an additional thin section of endosteal lamellar bone had been deposited on top of the secondary endosteal bone (Figure 21). The area with the most endosteal deposition (thickest part of endosteal bone) always corresponded to the area of least periosteal deposition (Figures 6, 7B and 22).

In 18 adults (90 %) and 11 juveniles (55 %), in other words 72.5 % of the sample (N = 40), endosteal bone surrounded almost the entire medullary cavity (Figures 22). In one adult (5 %) and two juveniles (10 %), in other words 7.5 % of the sample, only a part of the medullary cavity was bordered by endosteal bone (Figure 6 and 8). In one adult (5 %) and seven juveniles (35 %), in other words 20 % of the sample, no endosteal bone was present at all (Figure 2). Adults had thicker endosteal bone deposits than juveniles. The maximum thickness ranged from 0.03 to 0.09 mm in adults, with a mean of  $0.05 \text{ mm} \pm 0.02 \text{ SD}$ , while the juvenile range was 0.005 to 0.05 mm, with a mean of  $0.02 \text{ mm} \pm 0.01 \text{ mm}$ . Endosteal bone area in adults ranged from 0.03 to  $0.08 \text{ mm}^2$ , with a mean of  $0.05 \text{ mm}^2 \pm 0.02 \text{ SD}$ .



FIGURE 20. T.s. through the mid-diaphysis of the femur of an adult female *Cordylus cataphractus*, indicating LAG resorption. LAG, line of arrested growth; ELB, endosteal lamellar bone; RL, resorption line; R, resorption. Scale bar represents 40  $\mu\text{m}$ .

FIGURE 21. T.s. through the mid-diaphysis of the femur of an adult male *Cordylus cataphractus*, indicating additional deposition of endosteal lamellar bone. ELB, endosteal lamellar bone, RL, resorption line. Scale bar equals 40  $\mu\text{m}$ .

**FIGURE 22.** T.s. through the mid-diaphysis of the femora of **A:** a juvenile *Cordylus cataphractus*, and **B:** an adult *Cordylus cataphractus*, indicating endosteal bone surrounding the medullary cavity. MC, medullary cavity; EB, endosteal bone. Scale bar equals 100  $\mu\text{m}$ .

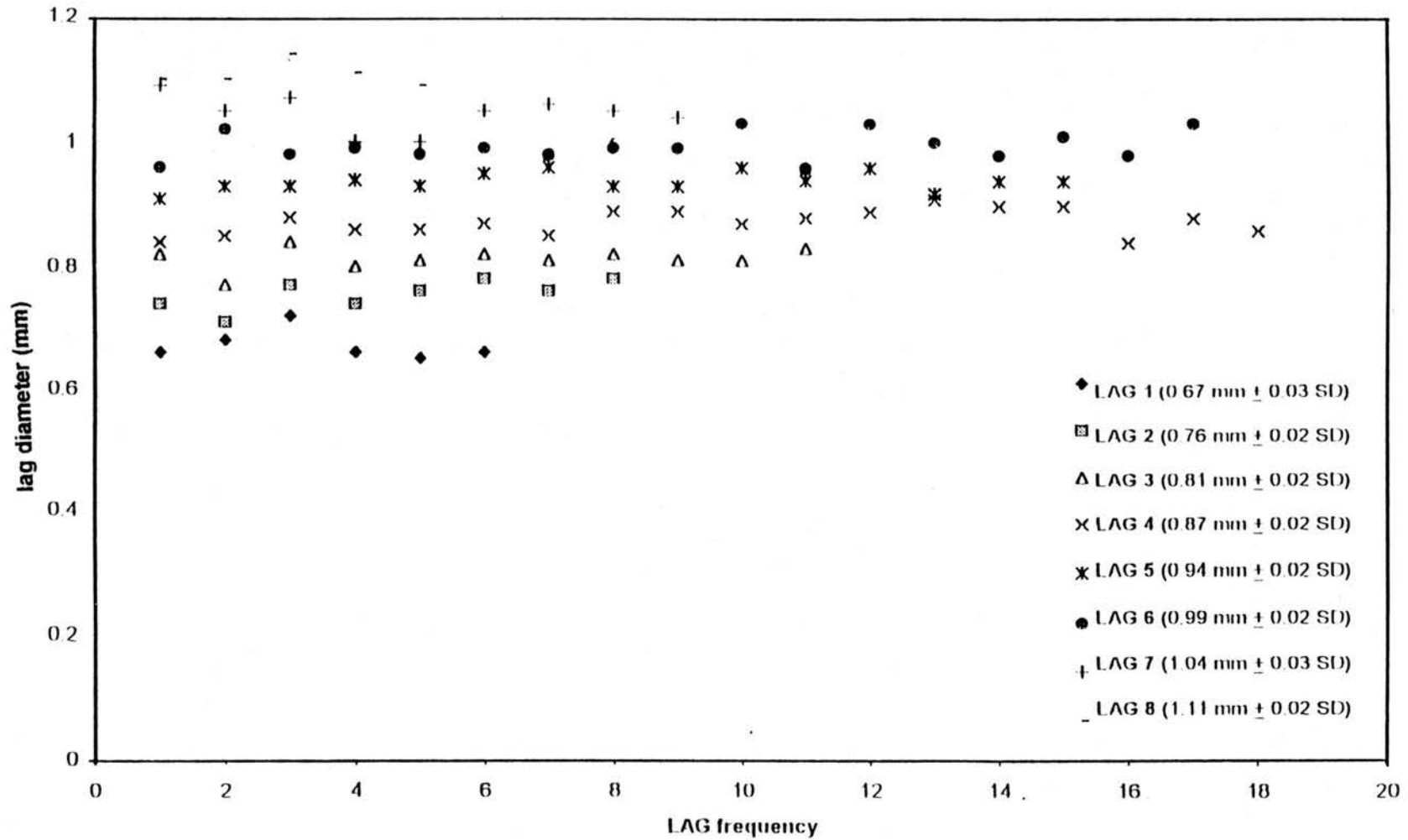
### *Periosteal deposition*

Periosteal deposition refers to the amount of bone deposited along the periosteum per growth season. In both juveniles and adults, the amount of bone deposited between periods of arrested growth was not the same around the entire circumference of the bone cortex. A higher rate of osteogenesis took place in one area of the compacta, represented by thicker deposits of zonal bone between LAGs in this area (Figures 16 and 22). The area of greatest osteogenesis always had the highest rate of endosteal resorption (and thus least endosteal deposition) (Figure 6, 8, 16 and 22).

### 3.3. Age classes and longevity

Of the 44 individuals sampled, 38 were suitable for skeletochronological assessment. The remaining six individuals were excluded due to the various reasons listed in section 3.2. In 13 juveniles and 17 adults, the earliest LAGs were removed due to endosteal resorption across the entire border of the medullary cavity (Figures 6, 16 and 20). In the remaining 8 individuals, only the birth line was estimated to have been removed. The number of LAGs removed, were estimated by calculating LAG diameters in juveniles and comparing them to adult values. Those individuals with the smallest medullary cavity sizes were assumed to have had the least amount of bone resorped (Appendix 4). LAG diameters with similar values were grouped together and a mean plus standard deviation were calculated (Figure 23). In cases where a LAG diameter could fall into two age classes, the lesser age was assumed. Age could therefore be underestimated by one year for at least 8 individuals (21 %). However, as individual variations in growth can influence size and thus LAG diameters, it must be stressed that individual ages are only estimates and have not been validated with known age individuals or a mark-recapture study. Individual ages ranged up to an estimated

FIGURE 23. LAG diameters representing age classes for the *Cordylus cataphractus* population sample from the Graafwater district.





13 years (number of visible LAGs plus estimated number of LAGs removed, see Appendix 4).

LAGs near the outer perimeter of the cortex were frequently very close together in some of the oldest individuals, making LAG counting, and the associated age estimation difficult (Figures 10, 14 and 15B). This could, in rare cases, have resulted in a double LAG being mistaken for two separate LAGs and, therefore, in overestimation of age. Another problem was that a LAG might have been overlooked, especially if it occurred at or very near the periosteum, or was indistinct. Age estimation may further have been encumbered by false LAGs being mistaken for true LAGs, or by the appearance of localized, numerous, closely packed rest lines in regions where periosteal deposition was the most intense (Figure 24).

### 3.4. Sexual maturity

In adults, the first three or four visible LAGs were separated by a large increment of bone, suggesting fast bone growth. After the third or fourth LAG, the bone thickness between successive LAGs decreased (Figure 25). This phenomenon is related to the decrease in growth rate of adults and is considered to depict the general age at which sexual maturity is reached (Castanet and Smirina, 1990; Castanet and Baez, 1991). Four out of nine adult males (the largest males) exhibited a decrease in bone thickness from the fifth visible LAG (Figure 25C), the remaining five had less than five visible LAGs so this phenomenon was not apparent. Eight out of 12 females showed the same phenomenon from the fourth visible LAG (Figure 25B), two from the second (Figure 10) and one from the third (Figure 25A). Once the estimated number of LAGs resorped were added to the visible number of LAGs, the general age at which sexual maturity was reached, was estimated as being approximately six

FIGURE 24. T.s. through the mid-diaphysis of the femur of an adult female *Cordylus cataphractus*, indicating localized, numerous, closely packed LAGs near the periosteum. LAG, line of arrested growth. Scale bar equals 40  $\mu\text{m}$ .

**FIGURE 25.** T.s. through the mid-diaphysis of the femur of an adult *Cordylus cataphractus*, indicating the decrease in spacing between successive LAGs, brought on by the decrease in osteogenesis after the attainment of sexual maturity. **A**, after the third LAG; **B**, after the fourth LAG; **C**, after the fifth LAG. Arrows indicate LAGs. Scale bar serves A,B and C and equals 40  $\mu\text{m}$ .

years ( $\pm$  one year) for females and seven years ( $\pm$  one year) for males. Again it must be stressed that this is just an estimated age, further studies on known age individuals are needed to validate these results. The estimated age at sexual maturity did, however, correspond favourably to the general body size estimate of approximately 95 mm SVL proposed by Mouton *et al.* (1999a) for the attainment of sexual maturity in both sexes (Figure 26).

### 3.5. Growth rates, scaling and sexual dimorphism

The log-transformed variables that were used to assess growth rates and differences between the sexes, were snout-vent length (SVL), femoral length (FL), relative bone thickness (RBT), medullary cavity area (MCA), bone wall area (BWA) and total bone area (TBA) versus the log number of LAGs (age in years). FL and TBA were also regressed against SVL to obtain a clearer indication of differences in growth rates and sexual dimorphism. The correlation between SVL and number of LAGs (age) is depicted Table 5. There was a statistically significant correlation between SVL and number of LAGs in adult males and juveniles (Table 5). SVL varied considerably within age classes among the juvenile cohort, but less variation was noted among adults (Figure 26). Males were generally larger than females of a similar age (Figure 26). The correlation between FL and number of LAGs was only statistically significant for adult males (Table 5). FL varied considerably within age classes among juveniles but showed less variation among adults of similar ages. There was also a highly significant correlation between FL and SVL for all groups (Table 6, Figure 28A). RBT, MCA and BWA also showed considerable variation within age classes. There was a statistically significant correlation between RBT and number of LAGs in adult males and females (Table 5). The only statistically significant correlation between MCA and number of LAGs was found in juveniles and adult females. The correlation between BWA and TBA



Table 5. Regression statistics of log-transformed snout-vent length and femoral macroscopic and microscopic measurements versus log number of lines of arrested growth (LAGs) of adult and juvenile *Cordylus cataphractus* males and females.

Dimension	Sex	N	R <sup>2</sup>	F	df	P	Slope	Intercept
Snout-vent length	Males	7	0.988	401.7	1,5	< 0.001	0.291	1.77
	Females	12	0.208	2.63	1,10	> 0.05	0.041	1.99
	Juveniles	20	0.220	5.07	1,18	< 0.05	0.118	1.86
Femoral length	Males	7	0.863	31.5	1,5	< 0.005	0.227	1.04
	Females	12	0.159	1.89	1,10	> 0.05	0.0375	1.19
	Juveniles	20	0.156	3.32	1,18	< 0.05	0.109	1.08
Relative bone thickness	Males	6	0.867	26.1	1,4	< 0.01	0.634	-1.04
	Females	11	0.366	5.19	1,9	< 0.05	0.141	-0.591
	Juveniles	19	0.0930	1.74	1,17	> 0.05	0.124	-0.522
Medullary cavity area	Males	6	0.0031	0.0126	1,4	> 0.05	0.0213	-0.273
	Females	12	0.426	7.43	1,10	< 0.05	0.218	-0.544
	Juveniles	19	0.580	23.5	1,17	< 0.005	0.448	-0.656
Bone wall area	Males	6	0.814	17.5	1,4	< 0.05	0.916	-1.17
	Females	12	0.646	18.2	1,10	< 0.005	0.384	0.73
	Juveniles	19	0.264	6.10	1,17	< 0.05	0.545	-0.924
Total bone area	Males	6	0.682	8.57	1,4	< 0.05	0.442	-0.409
	Females	12	0.711	24.6	1,10	< 0.001	0.278	-0.326
	Juveniles	19	0.306	7.49	1,17	< 0.05	0.387	-0.444

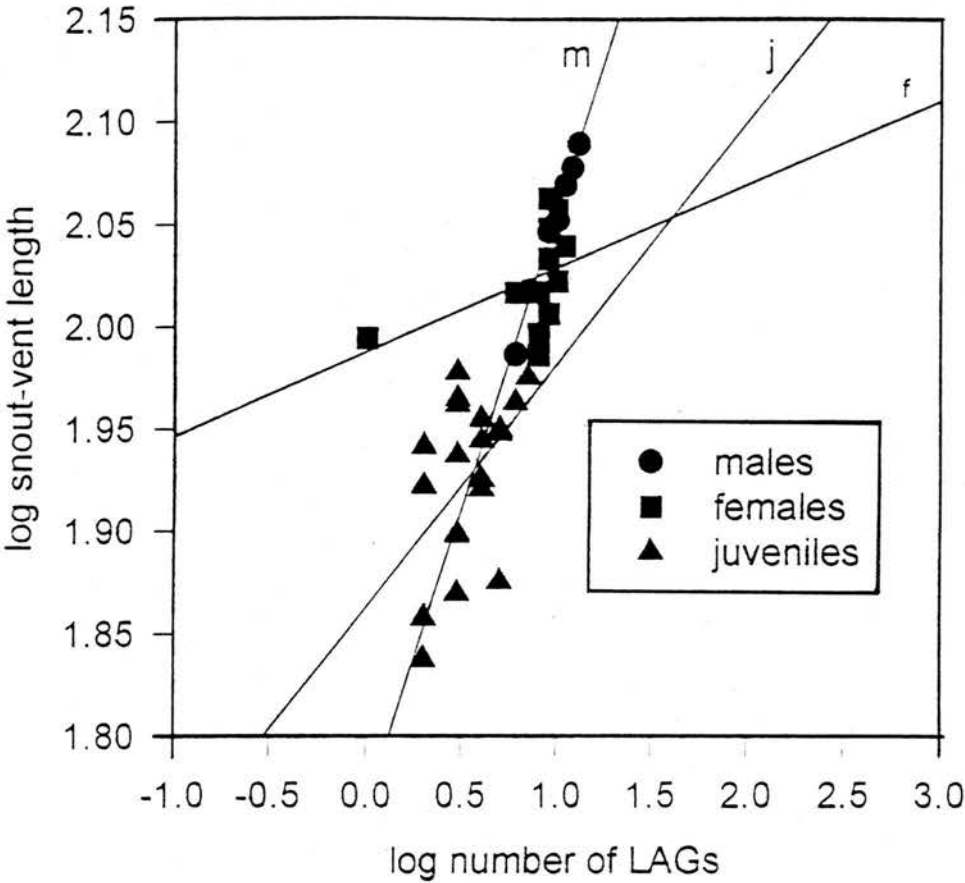


FIGURE 26. Correlation of snout-vent length versus log number of LAGs, for juvenile and adult *Cordylus cataphractus* individuals, from the Graafwater district.

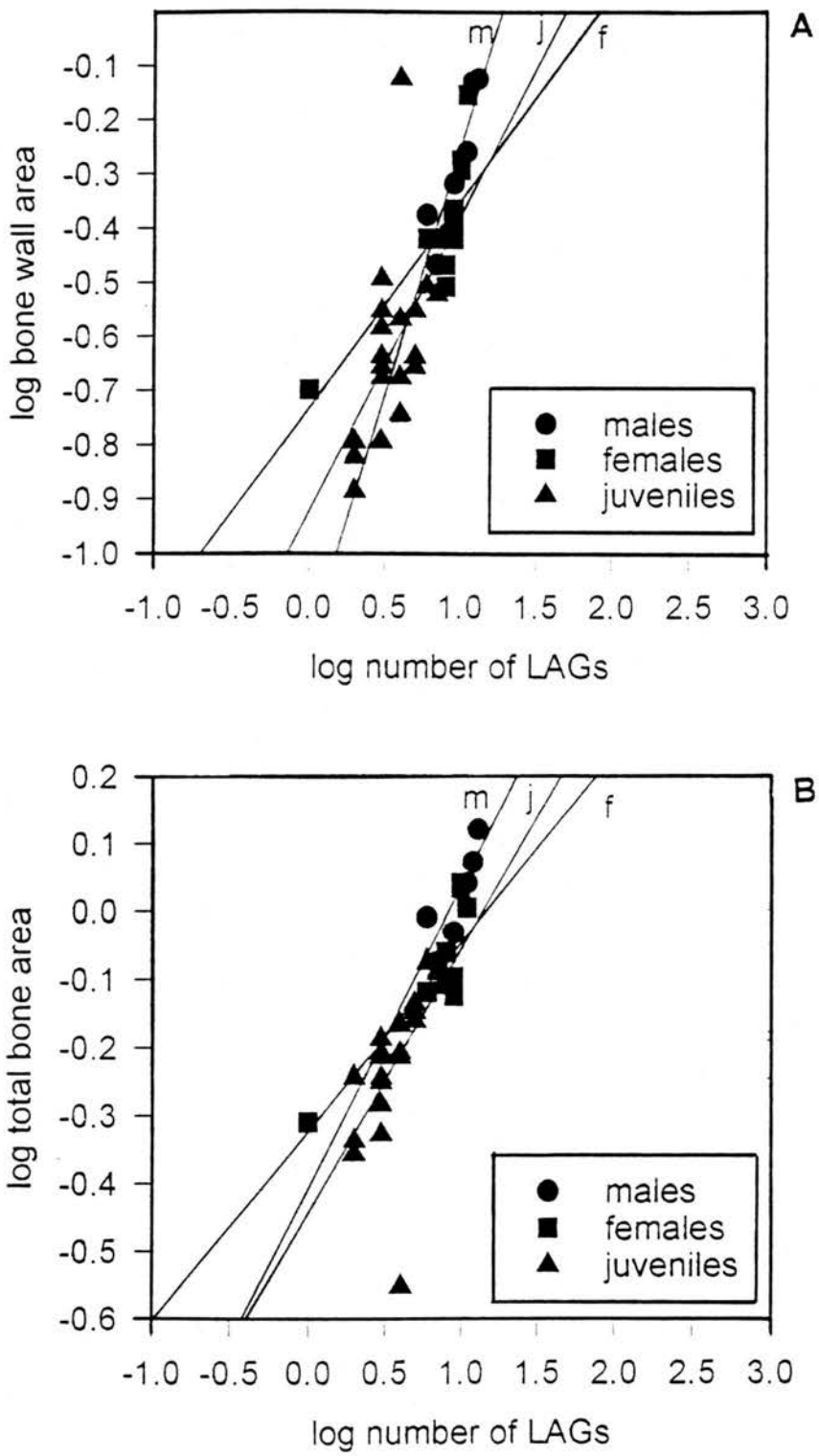


FIGURE 27. Correlations of **A**: log bone wall area and **B**: log total bone area versus log number of LAGs, for juvenile and adult *Cordylus cataphractus* individuals, from the Graafwater district.

Table 6. Regression statistics of log-transformed femoral length and total bone area versus snout-vent length, of adult and juvenile *Cordylus cataphractus* males and females.

Dimension	Sex	N	R <sup>2</sup>	F	df	P	Slope	Intercept
Femoral length	Males	9	0.863	44.038	1,7	< 0.001	1.113	0.639
	Females	12	0.607	15.438	1,10	< 0.005	0.810	-0.413
	Juveniles	23	0.799	83.696	1,21	< 0.001	0.813	-0.423
Total bone area	Males	8	0.571	8.001	1,6	< 0.05	1.541	-3.147
	Females	12	0.188	2.308	1,10	> 0.05	1.582	-3.286
	Juveniles	20	0.240	5.673	1,18	< 0.05	1.284	-2.717



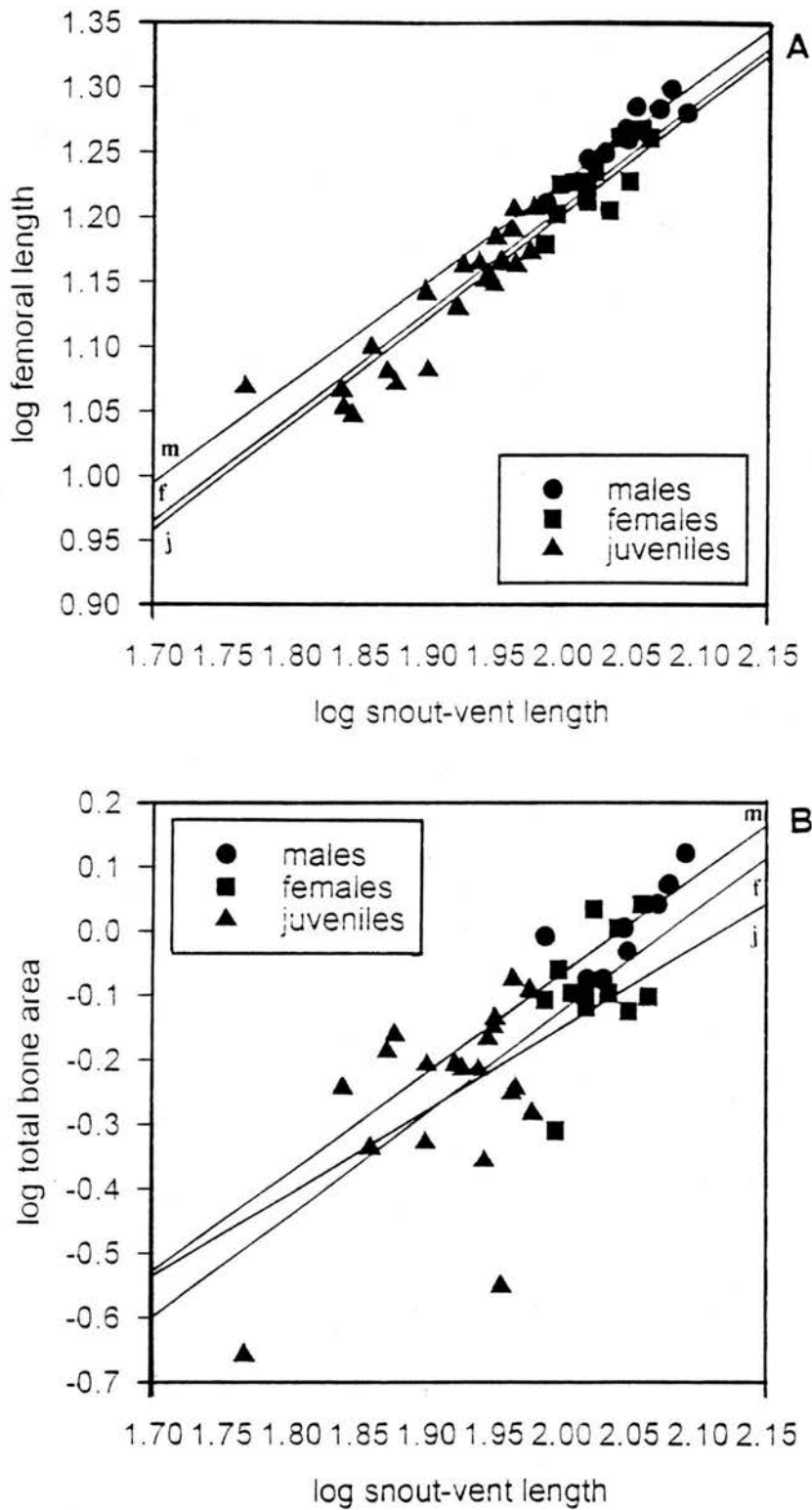


FIGURE 28. Correlations of **A**: log femoral length and **B**: log total bone area versus log snout-vent length, for juvenile and adult *Cordylus cataphractus* individuals, from the Graafwater district.

versus number of LAGs was statistically significant in all groups (Table 5, Figure 27). Both BWA and TBA showed less variation within age classes for all groups (Figure 27). Total bone area was highly correlated with SVL (Figure 28B). However, only adult males and juveniles showed a statistically significant correlation between these two variables (Table 6). No significant difference was found between juvenile and adult males and juvenile and adult females for the above mentioned variables versus number of LAGs (Table 7). Similarly, there was no significant difference found between adults and juveniles for FL and TBA versus SVL (Table 8). Therefore, the data for adults and juveniles was pooled to test for sexual dimorphism. There was a statistically significant difference between males and females for SVL, FL and BWA versus number of LAGs (Table 9, Figure 29). There was, however, no significant difference observed between males and females for FL and TBA versus SVL (Table 10).

Table 7. Comparison of regression slopes of log-transformed body and femoral dimensions versus number of lines of arrested growth (LAGs) among males females and juveniles of *Cordylus cataphractus*.

Comparison	Dimension	F	Df	P
Adult males versus juvenile males	Snout-vent length	0.37500	1,12	> 0.05
	Femoral length	0.02843	1,12	> 0.05
	Relative bone thickness	3.54933	1,11	> 0.05
	Medullary cavity area	1.91337	1,11	> 0.05
	Bone wall area	0.55608	1,11	> 0.05
	Total bone area	0.00882	1,11	> 0.05
Adult females versus juvenile females	Snout-vent length	0.13639	1,19	> 0.05
	Femoral length	0.00323	1,19	> 0.05
	Relative bone thickness	3.38096	1,18	> 0.05
	Medullary cavity area	4.01209	1,18	> 0.05
	Bone wall area	0.00132	1,18	> 0.05
	Total bone area	0.17978	1,18	> 0.05

Table 8. Comparison of regression slopes of log-transformed femoral length and total body area versus snout-vent length, among males, females and juveniles of *Cordylus cataphractus*.

Comparison	Dimension	F	Df	P
Adult males versus juvenile males	Femoral length	0.08699	1,17	> 0.05
	Total bone area	0.00455	1,14	> 0.05
Adult females versus juvenile females	Femoral length	0.76104	1,19	> 0.05
	Total body area	0.98957	1,18	> 0.05



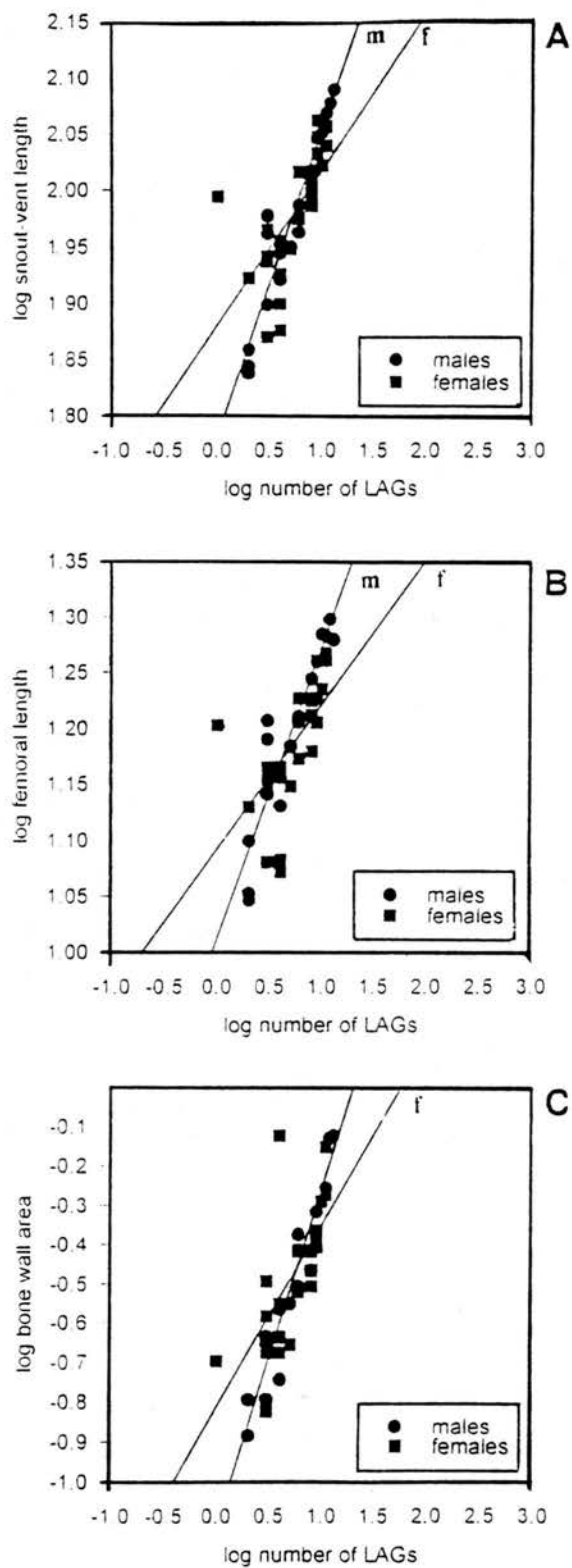


FIGURE 29. Correlations of **A:** log snout-vent length, **B:** log femoral length and **C:** log bone wall area versus log number of LAGs, for *Cordylus cataphractus* males and females from the Graafwater district.

Table 10. Comparison of regression slopes of femoral length and total bone area versus snout-vent length, among males and females of *Cordylus cataphractus*.

Comparison	Dimension	F	df	P
Males versus females	Femoral length	1.21196	1,40	> 0.05
	Total bone area	0.13224	1,36	> 0.05

## CHAPTER 4

### Histological analysis of bone in *Pseudocordylus capensis*

#### 4.1. Bone histology and growth mark features

##### *Juveniles*

Transverse sections of the femora of 11 juveniles (SVL < 90 mm) showed a medullary cavity, filled with blood vessels, blood cells, connective tissue and adipose tissue, and surrounded by a layer of compact bone (Figure 30). As in *C. cataphractus*, no cancellous bone was evident in the mid-shaft region. The relative bone thickness (RBT) of the five juveniles from Landdroskop ranged from 12 % (SVL = 46.79 mm) to 19 % (SVL = 73.29 mm). The bone wall area (BWA) was small in these juveniles in comparison to that of adults, ranging from 0.05 mm<sup>2</sup> (SVL = 46.79 mm) to 0.21 mm<sup>2</sup> (SVL = 86.32 mm), with a mean of 0.10 mm<sup>2</sup> ± 0.07 SD. The RBT was thin in the six juveniles from the remaining localities, ranging from 9 % (SVL = 43.78 mm) to 13 % (SVL = 66.99 mm). The BWA was also small, ranging from 0.06 mm<sup>2</sup> (SVL = 43.78 mm) to 0.21 mm<sup>2</sup> (SVL = 66.99 mm), with a mean of 0.12 mm<sup>2</sup> ± 0.08 SD.

In two of 11 juveniles (43.78 and 42.74 mm SVL, respectively), the compacta was composed exclusively of embryonic bone, which was characterized by enlarged globular osteocytic lacunae (Figure 31). In seven juveniles, the compacta consisted of both parallel-fibred and embryonic bone, separated by a birth line (Figure 32). The matrix of this embryonic bone appeared to have stained lighter than the corresponding parallel-fibred bone (Figure 32). In the remaining two juveniles, the compacta was composed of parallel-fibred bone only (Figure 33). A single longitudinal vascular canal was present in two juveniles, all other individuals

FIGURE 30. Transverse section (T.s.) through the mid-diaphysis of the femur of a juvenile female *Pseudocordylus capensis*, showing a bone cortex (compacta) surrounding a medullary cavity. C, compacta; MC, medullary cavity. Scale bar equals 100  $\mu\text{m}$ .

FIGURE 31. T.s. through the mid-diaphysis of the femur of a juvenile male *Pseudocordylus capensis*, showing a compacta composed exclusively of embryonic bone. EB, embryonic bone; arrows indicate osteocyte within globular osteocytic lacunae. Scale bar equals 25  $\mu\text{m}$ .



FIGURE 32. T.s. through the mid-diaphysis of the femur of a juvenile male *Pseudocordylus capensis*, showing embryonic bone and parallel-fibred bone separated by a distinct birth line. EB, embryonic bone; PB, parallel-fibred bone; BL, birth line; ELB, endosteal lamellar bone; RL, resorption line. Scale bar equals 25  $\mu\text{m}$ .

FIGURE 33. T.s. through the mid-diaphysis of the femur of a juvenile female *Pseudocordylus capensis*, indicating a compacta composed entirely of parallel-fibred bone. PB, parallel-fibred bone. Scale bar equals 40  $\mu\text{m}$ .

examined had avascular bone (Figure 34). Endosteal bone was absent in all juveniles, except for two individuals, which each have a thin, isolated patch of endosteal lamellar bone along the medullary cavity margin (Figure 32).

### *Adults*

In adult lizards (N = 32, SVL > 90 mm), the compacta was thicker than in juveniles. In the Landdroskop sample, adult RBT ranged from 18 % (SVL = 95.66 mm) to 39 % (SVL = 91.96 mm). The adults from the remaining localities had RBTs ranging from 26 % (SVL = 101.75 mm) to 39 % (SVL = 95.59 mm and 85.51 mm). The BWA was larger in adults than in juveniles, with a mean of  $0.39 \text{ mm}^2 \pm 0.10 \text{ SD}$ . In all individuals, the periosteal cortex was composed of parallel-fibred bone (Figure 35), and in eight adults, remnants of embryonic bone were visible, separated from the parallel-fibred bone by a birth line (Figure 36). Most adults displayed a thin layer of endosteal lamellar bone lining the medullary cavity in varying degrees (Figures 35 and 36A; this is discussed in detail in section 4.2.). Endosteal bone was separated from the periosteal cortex by an undulating resorption line (Figures 35 and 36A). No lamellar or pseudolamellar bone was found inside the periosteum of the outer cortex in any individual, although the osteocytic lacunae become smaller and less numerous with increasing age (Figure 35). As in juveniles, cancellous bone was lacking in all adults. In three adults, remodeling appeared more intensive than in the other individuals, most likely due to these sections being cut more towards the proximal or distal ends of the femur, rather than from the mid-shaft region (Figure 37). Nine adults had one large longitudinal vascular canal (Figure 38A), one adult had two, and one adult had a Volkmann's canal (Figure 38B). These vascular canals were lined, in some instances, by endosteal lamellar bone and the Volkmann's canal, although not associated with haversian systems, originated from the

FIGURE 34. T.s. through the mid-diaphysis of the femur of a juvenile male *Pseudocordylus capensis*, indicating an enlarged longitudinal vascular canal in the poorly vascularized compacta. VC, vascular canal. Scale bar equals 40  $\mu\text{m}$ .

FIGURE 35. T.s. through the mid-diaphysis of the femur of an adult female *Pseudocordylus capensis*, showing adult bone tissue. MC, medullary cavity; PB, parallel-fibred bone; ELB, endosteal lamellar bone; RL, resorption line; R, resorption. Arrows indicate lines of arrested growth (LAGs); ? indicates indistinct "line". Scale bar equals 40  $\mu\text{m}$ .

FIGURE 36. T.s. through the mid-diaphysis of the femora of **A**, an adult male

*Pseudocordylus capensis*, showing a remnant of embryonic bone in the periosteal cortex with a relatively thick layer of endosteal lamellar bone deposited below, and **B**, an adult female *Pseudocordylus capensis*, showing a remnant of embryonic bone in the periosteal cortex, with no endosteal bone deposited below. MC, medullary cavity; ELB, endosteal lamellar bone; EB, embryonic bone; RL, resorption line; BL, birth line. Scale bar serves A and B and equals 40  $\mu\text{m}$ .



FIGURE 37. T.s. through the femoral shaft of an adult male *Pseudocordylus capensis*, showing intensive remodeling of the compacta indicated by decreased periosteal primary bone due to resorption and increased secondary endosteal deposition, in the form of endosteal parallel-fibred bone and/or lamellar bone. PB, periosteal bone; ELB, endosteal lamellar bone; EPB, endosteal parallel-fibred bone; RL, resorption line. Scale bar equals 40  $\mu\text{m}$ .

FIGURE 38. T.s. through the mid-diaphysis of the femora of **A**, an adult male *Pseudocordylus capensis*, indicating a large longitudinal vascular canal filled with three blood vessels, and **B**, an adult female *Pseudocordylus capensis*, indicating a Volkmann's canal. VC, vascular canal; ELB, endosteal lamellar bone; RL, resorption line. Scale bar serves A and B and equals 40  $\mu\text{m}$ .

periosteum (Figure 38). The remaining adults had an avascular cortex.

### *Growth marks*

Of the 43 individuals examined, periosteal lines of arrested growth (LAGs) were present in 42 (Figure 35 and 39), the remaining two being the smallest juveniles not having yet reached their first period of arrested growth (Figure 31). These two juveniles also lacked a birth line (Figure 31), which was present in seven juveniles (Figure 32) and eight adults (Figure 36). Of these seven juveniles (four from Landdroskop and three from other localities), a double birth line occurred in three individuals (Figure 32), two from Landdroskop and one from Jonaskop at 1133 m and 1640 m above sea level, respectively. The remaining four juveniles and all eight adults possessed a single birth line. LAGs were distinct in 38 individuals (Figures 35 and 39), and indistinct in four individuals (Figure 40).

In 23 individuals examined, one or more double LAGs were present (Figure 41), representing two periods of arrested growth in one growth cycle (one year) (Castanet and Baez, 1988). Of the 11 juveniles studied, only two possessed double LAGs, one with 33 % (one out of three) and one with 100 % (one out of one). In adults, the highest number of double LAGs were found in an adult male (50 %), followed by an adult female with 40 %. In five individuals, 25 % of the visible LAGs were double and in another five adults, 20 % were double. The mean percentage of double LAGs for the adults sampled from Landdroskop was  $20 \% \pm 22$  SD, with adult males having a mean of  $24 \% \pm 17$  SD, and adult females a mean of  $18 \% \pm 9$  SD. The mean for those adults from the remaining localities, was  $13 \% \pm 12$  SD, with adult males having a mean of  $14 \% \pm 10$  SD, and adult females a mean of  $11 \% \pm 15$  SD.

Indistinct "lines", which could represent false LAGs, indistinct LAGs or staining anomalies,

FIGURE 39. T.s. through the mid-diaphysis of the femur of an adult male *Pseudocordylus capensis*, showing distinct lines of arrested growth. MC, medullary cavity; series of arrows indicate LAGs. Scale bar equals 40  $\mu\text{m}$ .



FIGURE 40. T.s. through the mid-diaphysis of the femora of: **A**, an adult female *Pseudocordylus capensis*, indicating weakly expressed lines of arrested growth, and **B**, an adult female *Pseudocordylus capensis*, indicating highly indistinct lines of arrested growth. MC, medullary cavity; R = resorption. Arrows indicate LAGs. Scale bar serves A and B and equals 40  $\mu\text{m}$ .

were also present in the largest adults and made the counting of true LAGs difficult (Figure 35).

## 4.2. Bone remodeling

### *Endosteal resorption*

Of the 44 individuals studied (11 juveniles and 33 adults), the medullary cavity was even in 17 individuals (38,5 %; Figure 42). Of these, 13 had a thin layer of endosteal lamellar bone lining small regions of the medullary cavity (Figure 42). The remaining four had no endosteal bone (Figures 43). In 21 individuals (48 %), the medullary cavity was slightly uneven, either in one region only (Figure 44) or around the whole medullary cavity (Figures 30 and 45). Of these, 11 had a thin layer of endosteal lamellar bone bordering small sections of the medullary cavity (Figure 44), the rest had no endosteal bone lining the medullary cavity (Figure 30). In the remaining six individuals (13,5 %), the bone cortex was badly damaged for four and extensively remodeled for two.

Endosteal resorption seemed to be localized in all individuals, in other words it did not take place with equal intensity across the entire border of the medullary cavity, but was focused in one area (Figure 45). This observation is supported by the fact that in eight juveniles (73 %) and eight adults (24 %), a remnant of embryonic bone and birth line remained present in one part of the bone cortex (Figure 36). Osseous drift resulted in the off-center appearance of the medullary cavity (Figure 45).

Only the Landdroskop sample was used to determine possible correlations between medullary cavity area (MCA) versus snout-vent length (SVL), femoral length (FL), relative

**FIGURE 41.** T.s. through the mid-diaphysis of the femora of: **A**, an adult female and **B**, an adult male *Pseudocordylus capensis*, indicating double lines of arrested growth. Arrows indicate LAGs; squares indicate double LAGs. Scale bar serves A and B and equals 40  $\mu\text{m}$ .

FIGURE 42. T.s. through the mid-diaphysis of the femur of an adult male *Pseudocordylus capensis*, indicating an even medullary cavity surrounded by a thin layer of endosteal lamellar bone in some regions. MC, medullary cavity; ELB, endosteal lamellar bone; RL, resorption line. Scale bar equals 100  $\mu\text{m}$ .

FIGURE 43. T.s. through the mid-diaphysis of the femur of an adult male *Pseudocordylus capensis*, indicating an even medullary cavity with no endosteal bone present. MC, medullary cavity. Scale bar equals 100  $\mu\text{m}$ .



FIGURE 44. T.s. through the mid-diaphysis of the femur of an adult male *Pseudocordylus capensis*, indicating a slightly uneven medullary cavity surrounded by a thin layer of endosteal lamellar bone in some regions. MC, medullary cavity; R, resorption; ELB, endosteal lamellar bone; RL, resorption line. Scale bar equals 100  $\mu\text{m}$ .

FIGURE 45. T.s. through the mid-diaphysis of the femur of an adult male *Pseudocordylus capensis*, showing more intense resorption in a localized area of the compacta. R, resorption; PD, periosteal deposition. Scale bar equals 100  $\mu\text{m}$ .

bone thickness (RBT) and total bone area (TBA). There was a highly significant correlation between MCA and SVL, as well as between MCA and TBA for the *P. capensis* population sample as a whole (Table 11, Figure 46). In juveniles, there was a statistically significant correlation between MCA and TBA (Table 12, Figure 47). In adult males, there was a statistically significant correlation between MCA and TBA (Table 12, Figure 47), as well as between MCA and RBT (Table 12, Figure 47). In adult females, there was no statistically significant correlation between MCA and any of the variables (Table 12).

Due to the small number of juveniles, only adult males and females were used to assess possible sexual differences using the above mentioned correlations. There was no statistically significant case of sexual dimorphism between MCA and any of the variables (Table 13). MCA was calculated as a percentage of TBA in adults from Landdroskop (Appendix 4). The average percentage MCA of TBA was  $56 \% \pm 13 \text{ SD}$ .

The intensity of resorption was estimated for the Landdroskop population, by calculating the number of LAGs resorped (Appendix 4). It was not possible to estimate LAG resorption in four of the 19 individuals, due to damaged sections. Males had a greater number of LAGs resorped than females, having up to an estimated three LAGs resorped in three of the six individuals (Appendix 4). The maximum number of LAGs resorped in females was an estimated two in the largest female (Appendix 4). Two adults had partial or complete visibility of all LAGs since birth present (Figure 48). Five individuals (three juveniles and two adults) had all or part of the birth line still present. The only link between LAG resorption and MCA, was that in those individuals with the smallest MCAs, no LAGs were estimated to have been resorped (Appendix 4).

Table 11. Regression statistics of log-transformed medullary cavity area versus snout-vent length and total bone area for a population of *Pseudocordylus capensis* from Landdrooskop.

Dimension	N	R <sup>2</sup>	F	df	P	Slope	Intercept
Snout-vent length	19	0.940	266.517	1,17	< 0.001	0.902	-0.305
Total bone area	19	0.687	37.290	1,17	< 0.001	2.605	-4.427

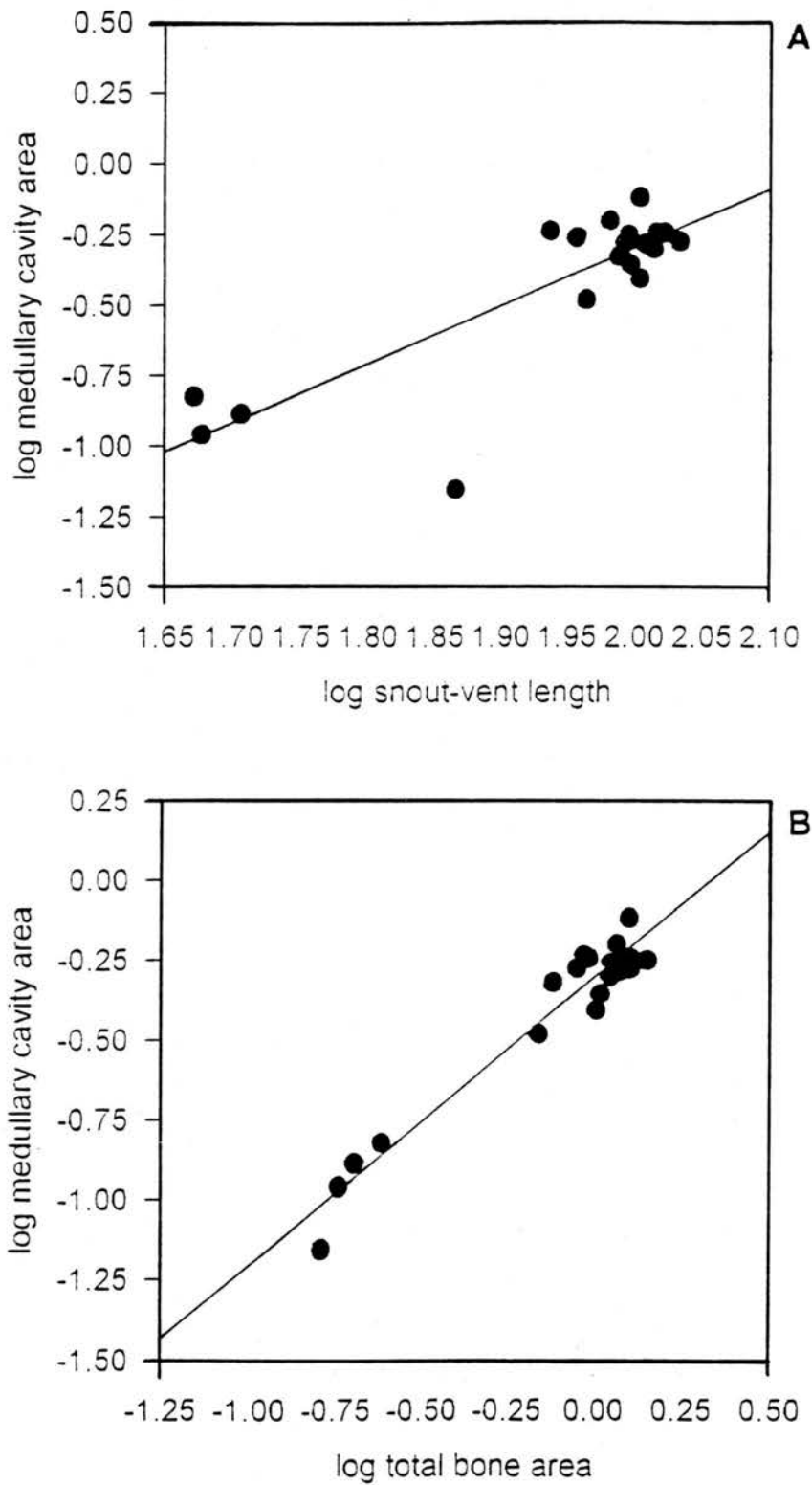


FIGURE 46. Correlations of log-transformed medullary cavity area versus: **A**, log snout-vent length and **B**, log total bone area, for the *Pseudocordylus capensis* population sample from Landdrooskop.



Table 12. Regression statistics of log-transformed medullary cavity area versus snout-vent length, femoral length, relative bone thickness and total bone area of adult and juvenile *Pseudocordylus capensis* males and females from Landdrooskop.

Dimension	Sex	N	R <sup>2</sup>	F	df	P	slope	Intercept
Snout-vent length	Males	6	0.062	0.263	1,4	> 0.05	1.443	-3.162
	Females	8	0.026	0.162	1,6	> 0.05	0.998	-2.282
	Juveniles	5	0.241	0.955	1,3	> 0.05	1.387	-3.268
Femoral length	Males	6	0.057	0.243	1,4	> 0.05	0.968	0.963
	Females	8	0.073	0.473	1,6	> 0.05	1.349	-2.056
	Juveniles	5	0.264	1.074	1,3	> 0.05	1.404	-2.336
Total bone area	Males	6	0.767	13.189	1,4	<b>&lt; 0.05</b>	0.815	-0.335
	Females	8	0.222	1.710	1,6	> 0.05	0.493	-0.283
	Juveniles	5	0.964	79.926	1,3	<b>&lt; 0.01</b>	1.084	-0.185
Relative bone thickness	Males	6	0.848	22.251	1,4	<b>&lt; 0.01</b>	0.801	-0.798
	Females	8	0.133	0.919	1,6	> 0.05	0.195	-0.384
	Juveniles	5	0.239	0.944	1,3	> 0.05	2.321	-2.750

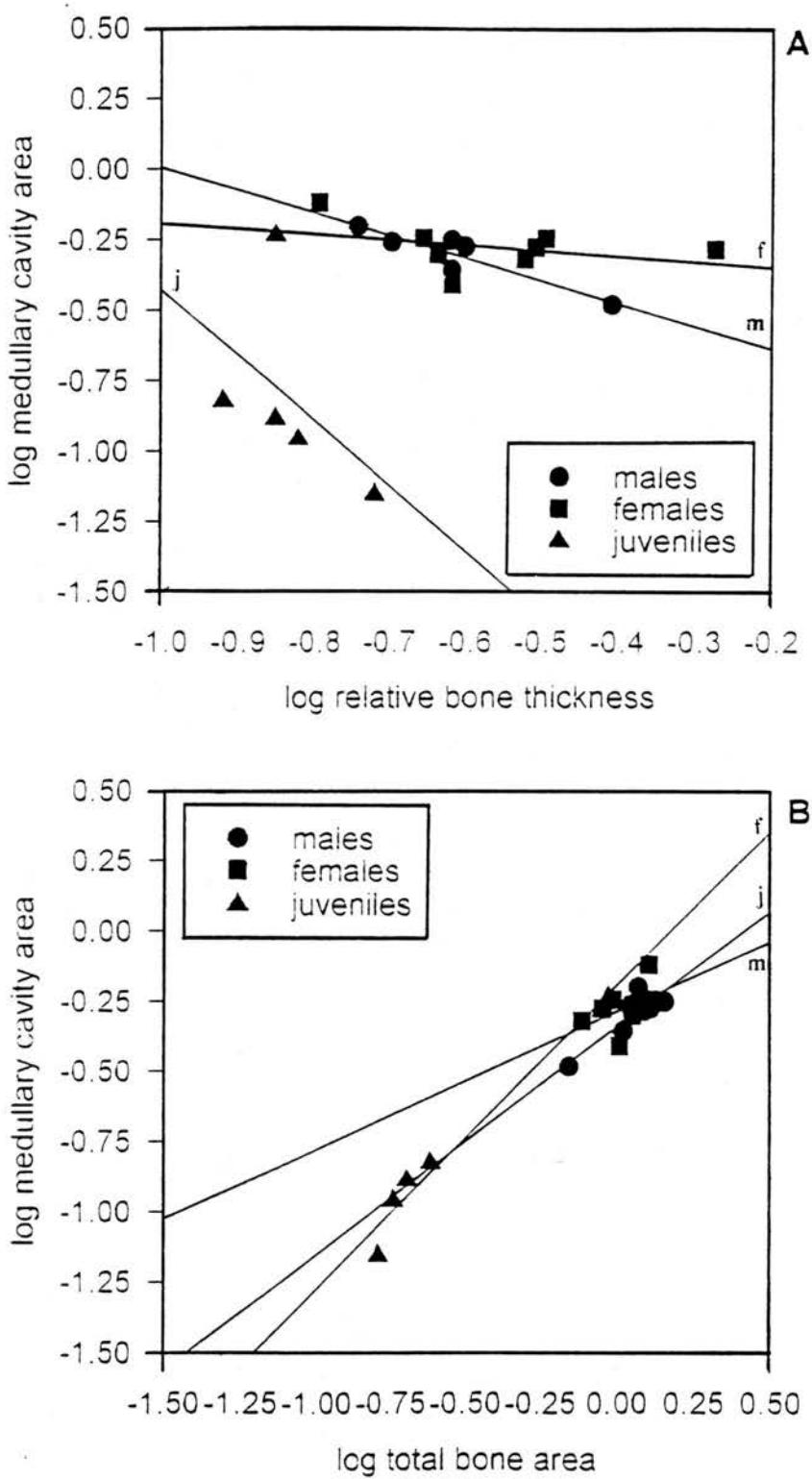


FIGURE 47. Correlations of log-transformed medullary cavity area versus: **A**, log relative bone thickness and **B**, log total bone area, for juvenile and adult *Pseudocordylus capensis* individuals, from Landdrooskop.

Table 13. Comparison of regression slopes of log-transformed medullary cavity area versus log transformed snout-vent length, femoral length, relative bone thickness and total bone area among males and females from a population of *Pseudocordylus capensis* from Landdrooskop.

Comparison	Dimension	F	df	P
Adult males versus adult females	Snout-vent length	0.1181	1,33	> 0.05
	Femoral length	1.1962	1,33	> 0.05
	Total bone area	2.7458	1,33	> 0.05
	Relative bone thickness	3.7343	1,33	> 0.05

FIGURE 48. T.s. through the mid-diaphysis of the femur of an adult female *Pseudocordylus capensis*, indicating subsequent LAGs. Series of arrows indicate LAGs. Scale Bar equals 40  $\mu\text{m}$ .



The partial resorption of visible LAGs varied between individuals. Of the 11 juveniles studied (five from Landdrooskop and six from other localities), the first visible LAG was partially resorped in four individuals. Of the remaining seven, the birth line was partially resorped in two individuals. The rest showed no LAG resorption. In the 32 adults investigated (13 from Landdrooskop and 19 from other localities) a maximum of two visible LAGs were partially resorped in four individuals (three females, one male). Of those remaining, 20 had the first visible LAG partially resorped and six the birth line. There was no notable difference between the visible LAG resorption of the Landdrooskop population and individuals from other localities. This partial resorption of LAGs in one area and not around the entire circumference of the bone cortex, indicates a more intense resorption of bone in one area of the cortex for each individual.

### *Endosteal deposition*

Of the 43 individuals examined, two were excluded from this assessment due to damaged sections. In all individuals where endosteal bone was deposited, resorption of periosteal bone had first taken place. This was evident due to the uneven resorption line separating endosteal from periosteal bone (Figures 35, 36A and 38A). The area with the most endosteal lamellar bone deposited did not necessarily correspond to the area of least periosteal deposition (Figure 49). In those adults with a remnant of embryonic bone ( $N = 7$ ), two had a relatively thick layer of endosteal lamellar bone deposited below the embryonic bone (Figure 36A), the rest had none (Figure 36B).

In three out of 30 adults (10 %) and 10 out of 11 juveniles (91 %), in other words 32 % of the total sample size, there was no endosteal bone lining the medullary cavity (Figures 30 and

FIGURE 49. T.s. through the mid-diaphysis of the femur of an adult male *Pseudocordylus capensis*, showing endosteal bone. ELB, endosteal lamellar bone; RL, resorption line; ED, endosteal deposition; PD, periosteal deposition. Scale Bar equals 100  $\mu\text{m}$ .

FIGURE 50. T.s. through the mid-diaphysis of the femur of an adult male *Pseudocordylus capensis*, showing endosteal lamellar bone almost completely surrounding the medullary cavity. ELB, endosteal lamellar bone. Scale Bar equals 100  $\mu\text{m}$ .

43). In 18 (60 %) adults and one (9 %) juvenile, in other words 46 % of the total sample size, the medullary cavity was partially lined with endosteal lamellar bone (Figures 42 and 44). This ranged from a minute patch (Figure 35), to almost totally surrounding the MCA (Figures 49 and 50). In nine adults (30 %), i.e. 22 % of the total sample size, the MCA was totally surrounded by a thin layer of endosteal lamellar bone. As there is only one juvenile with endosteal bone, adults have the most endosteal bone deposited. The maximum thickness of endosteal lamellar bone (widest part), ranged from 0.005 mm to 0.04 mm, with a mean of  $0.02 \text{ mm} \pm 0.01 \text{ SD}$ . Endosteal bone area in adults ranged from  $0.004 \text{ mm}^2$  to  $0.06 \text{ mm}^2$ , with a mean of  $0.02 \text{ mm}^2 \pm 0.015 \text{ SD}$ .

### *Periosteal deposition*

Periosteal deposition refers to bone that is deposited along the periosteum per growth season. The amount of bone deposited between successive LAGs (zonal bone) was not the same around the circumference of the bone cortex. Zonal bone differed in thickness, indicating that a higher rate of osteogenesis (bone deposition) had taken place in one area. This area did not necessarily correspond to the thickest (widest) part of the compacta (Figure 45). It did, however, correlate with the area of most intense resorption (Figure 45).

### 4.3. Age classes and longevity

Of the 19 individuals collected from Landdroskop, 15 were suitable for skeletochronological assessment. The remaining four individuals were excluded, as LAG diameters could not be calculated due to damaged thin sections. In two adults ( $N = 11$ ) and three juveniles ( $N = 4$ ), all LAGs were partially or completely present and individual ages were accurately determined without the necessity of back calculation. In seven adults, the earliest LAGs were

resorped, while in the remaining two adults and one juvenile, only the birth line was estimated to have been resorped (Appendix 4). The number of LAGs removed, were estimated by calculating LAG diameters in juveniles and in those adults with all LAGs still present, and then comparing them with the values obtained for the remaining individuals. Those individuals with the smallest medullary cavity sizes had all LAGs either partially or completely present (Appendix 4).

Similar LAG diameter values were grouped together and a mean plus standard deviation were obtained (Figures 51 and 52). It was assumed that each LAG corresponded to an age class. All LAGs were thus placed within a specific age class, if the diameter value fell within the range of that age class. Males and females deviated in terms of LAG diameters in that females had larger LAG diameters for the same age class (LAG group) than males (Figures 51 and 52). In cases where LAG values could fall within two ranges, in other words two age groups, the lesser age was assumed. It must be stressed, however, that, as individual variations in growth can influence size and thus LAG diameters, individual ages (where a back calculation was necessary) are only estimates and have not been validated with known age individuals or a mark-recapture study. Ages ranged up to an estimated 11 years (number of visible LAGs plus estimated number of LAGs removed, see Appendix 4). The two adults (SVL = 91.96 and 97.43 mm, respectively) with a birth line still present and thus all corresponding LAGs until death, were both five years old (Appendix 4).

LAGs near the outer perimeter of the cortex are frequently very close together in some of the oldest individuals, making LAG counting and the associated age estimation difficult (Figures 35 and 41A). This can be due to, as was previously mentioned for *C. cataphractus*, a double



FIGURE 51. LAG diameters representing age classes for individual *Pseudocordylus capensis* adult males and three smallest juveniles from Landdrooskop.

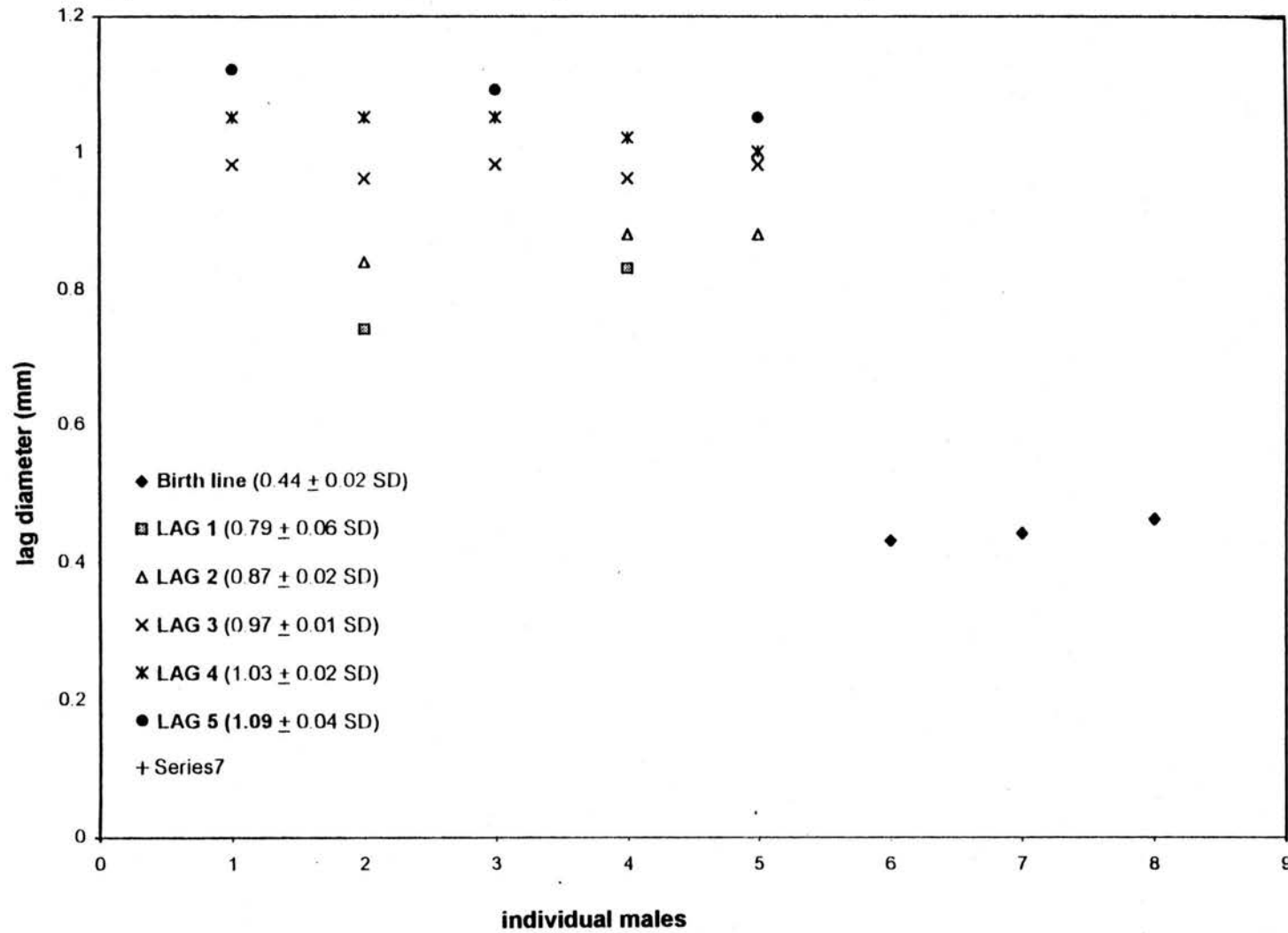
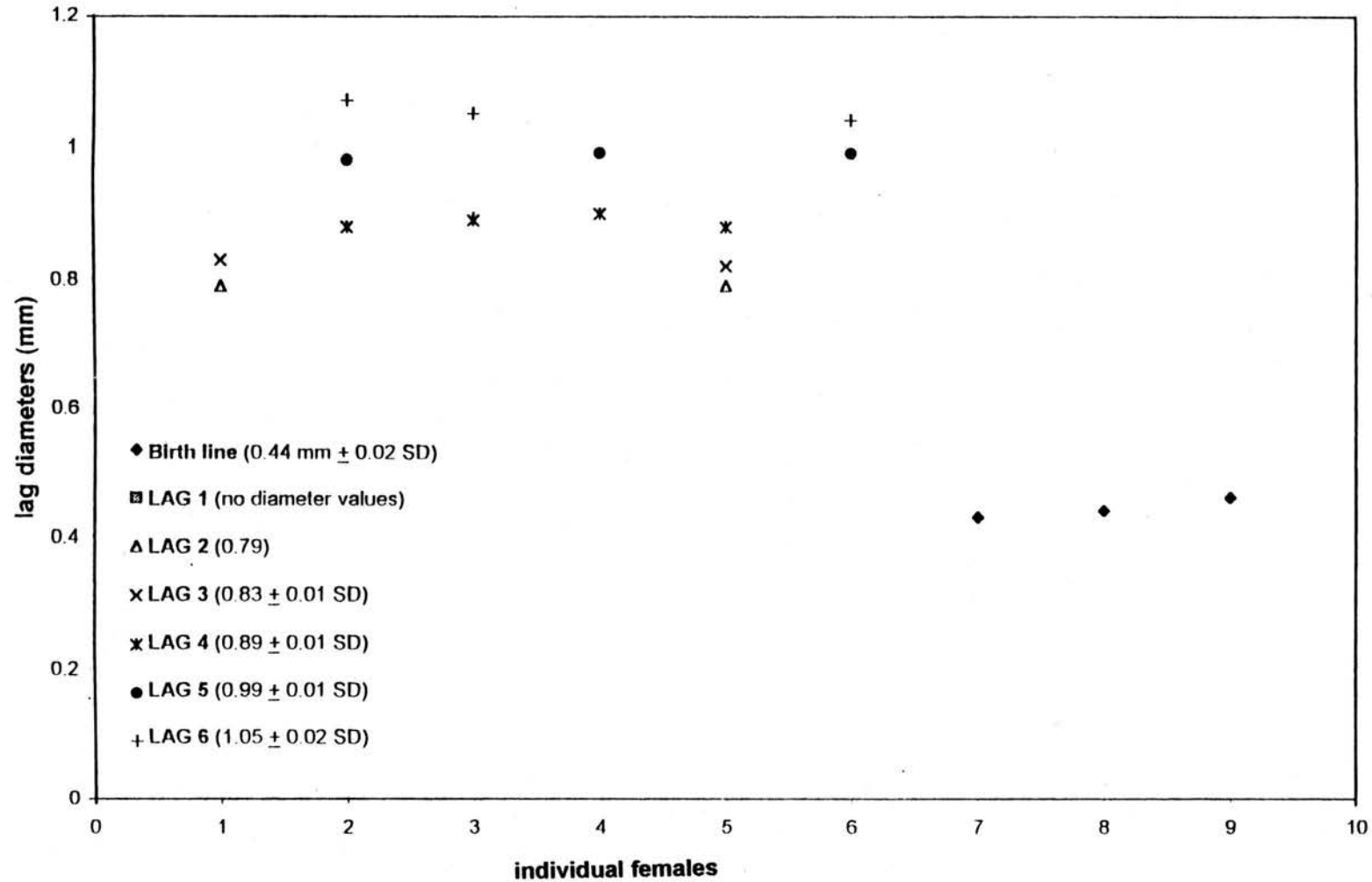


FIGURE 52. LAG diameters representing age classes for individual *Pseudocordylus capensis* adult females and three smallest juveniles from Landdroeskop.



LAG being mistaken for two separate LAGs, resulting in overestimation of age or a LAG being overlooked, especially if it occurs at or very near the periosteum or is indistinct. Age estimation may further be encumbered by false LAGs being mistaken for true LAGs.

#### 4.4. Sexual maturity

Of the 19 individuals examined from Landdroskop, seven individuals were excluded from this assessment. Three of these seven were juveniles with only a birth line present. Thin sections were damaged for the remaining four (one juvenile and three adults). Two adults from the 12 individuals remaining, exhibited relatively closely spaced visible LAGs and a decrease in LAG width was thus not clearly discernable (Figure 39). Age at sexual maturity was thus difficult to estimate for these two adults.

In the largest juvenile (SVL = 86.32 mm), there was a large increment of bone separating the three visible LAGs from each other. In the remaining nine adults examined (excluding the two mentioned above), four individuals exhibited a decrease in zonal bone width from the third visible LAG (Figure 53A), and five from the fourth visible LAG (Figure 53B). This phenomenon is related to the decrease in growth rate of adults and is considered to depict the general age at which sexual maturity is reached (Castanet and Smirina, 1990; Castanet and Baez, 1991). Of these nine adults, the two individuals still possessing a birth line, showed a decrease in zonal bone width from the fourth LAG. In the two adults where only the birth line was estimated to have been resorped (Appendix 4), a decrease in zonal bone width also took place from the fourth visible LAG (Figure 48). In these four individuals, sexual maturity was estimated to have occurred around four years of age. In the three adult males where the most number of LAGs were estimated to have been resorped (Appendix 4), the

**FIGURE 53.** T.s. through the mid-diaphysis of the femora of *Pseudocordylus capensis* adults, indicating the decrease in spacing between successive LAGs, brought on by the decrease in osteogenesis after the attainment of sexual maturity. **A**, after the third LAG: **B**, after the fourth LAG. Arrows indicate LAGs. Scale bar serves A and B and equals 40  $\mu\text{m}$ .



decrease in zonal bone occurred from the third visible LAG. The age at sexual maturity was thus estimated at around six years for these three adult males. There was no clear difference in zonal bone width reduction between males and females. The relative age at sexual maturity for this population of *P. capensis* was therefore estimated as being between four to six years. Again it must be stressed that this is just an estimated age, further studies on known age individuals are needed to validate these results.

#### 4.5. Growth rates, scaling and sexual dimorphism

The log-transformed variables that were used to assess growth rates and differences between the sexes, were snout-vent length (SVL), femoral length (FL), relative bone thickness (RBT), medullary cavity area (MCA), total bone area (TBA) and bone wall area (BWA) versus the log number of LAGs (age in years). FL and TBA were also regressed against SVL to obtain a clearer indication of differences in growth rates and sexual dimorphism. The correlation between the above mentioned variables and number of LAGs (age) is depicted in Table 14. There was no statistically significant correlation between SVL and number of LAGs nor between FL and number of LAGs, for any of the groups (Table 14). SVL and FL varied considerably within age classes among juveniles, but showed less variation among adults. FL was highly correlated with SVL (Figure 55A). However, juveniles showed the only statistically significant correlation between FL and SVL (Table 15), displaying more variation than adults between these two variables (Figure 55A).

There was no statistically significant correlation between RBT and number of LAGs for any of the groups (Table 14). MCA, TBA and BWA displayed similar results, with adult males and juveniles showing a statistically significant correlation between these three variables and

Table 14. Regression statistics of log-transformed snout-vent length and femoral macroscopic and microscopic measurements versus log number of lines of arrested growth (LAGs) of adult and juvenile *Pseudocordylus capensis* males and females from Landdrooskop.

Dimension	Sex	N	R <sup>2</sup>	F	Df	P	Slope	Intercept
Snout-vent length	Males	6	0.216	1.104	1,4	> 0.05	0.0839	1.907
	Females	5	0.543	3.560	1,3	> 0.05	0.0706	1.946
	Juveniles	5	0.575	4.058	1,3	> 0.05	0.434	1.729
Femoral length	Males	6	0.0131	0.0531	1,4	> 0.05	0.0296	1.335
	Females	5	0.638	5.282	1,3	> 0.05	0.0963	1.239
	Juveniles	5	0.575	4.054	1,3	> 0.05	0.448	1.043
Relative bone thickness	Males	6	0.562	5.132	1,4	> 0.05	0.902	0.175
	Females	5	0.098	0.328	1,3	> 0.05	0.464	-0.148
	Juveniles	5	0.0216	0.0663	1,3	> 0.05	0.0501	-0.830
Medullary cavity area	Males	6	0.810	17.016	1,4	<b>&lt; 0.05</b>	0.942	-1.130
	Females	5	0.270	1.111	1,3	> 0.05	0.298	-0.500
	Juveniles	5	0.870	20.057	1,3	<b>&lt; 0.05</b>	1.058	-0.956
Total bone area	Males	6	0.970	129.741	1,4	<b>&lt; 0.001</b>	1.109	-0.934
	Females	5	0.131	0.451	1,3	> 0.05	0.263	-0.200
	Juveniles	5	0.957	66.914	1,3	> 0.005	1.432	-0.715
Bone wall area	Males	6	0.820	18.188	1,4	<b>&lt; 0.05</b>	1.230	-1.308
	Females	5	0.009	0.0271	1,3	> 0.05	0.0871	-0.216
	Juveniles	5	0.965	83.660	1,3	<b>&lt; 0.005</b>	1.351	-1.100

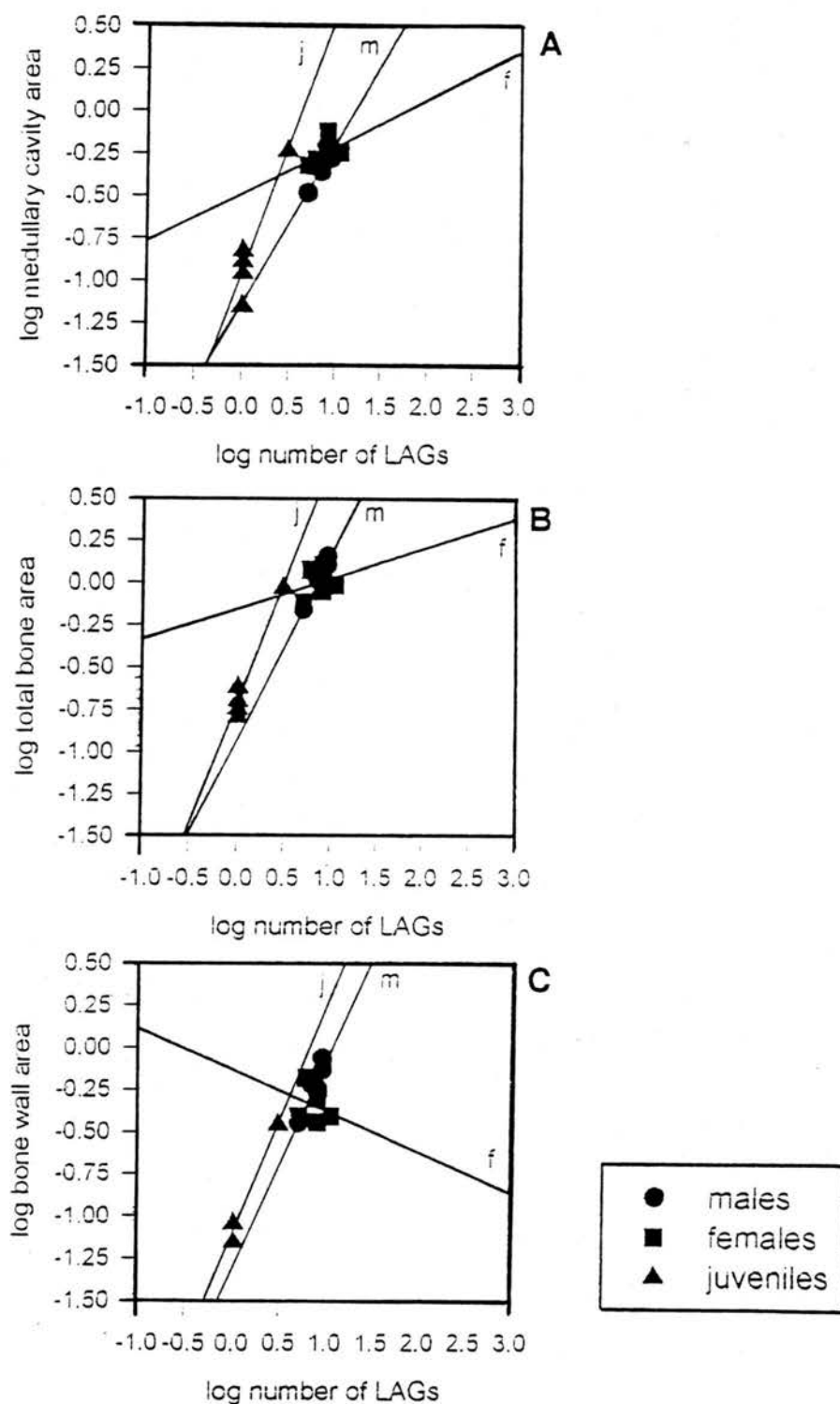


FIGURE 54. Correlations of **A**: log medullary cavity area **B**: log total bone area and **C**: log bone wall area versus log number of LAGs, for juvenile and adult *Pseudocordylus capensis* individuals, from Landdrooskop.

Table 15. Regression statistics of log-transformed femoral length and total bone area versus snout-vent length, of adult and juvenile *Pseudocordylus capensis* males and females from Landdrooskop.

Dimension	Sex	N	R <sup>2</sup>	F	df	P	slope	Intercept
Femoral length	Males	6	0.462	3.428	1,4	> 0.05	0.975	-0.621
	Females	7	0.353	3.279	1,6	> 0.05	0.733	-0.154
	Juveniles	5	0.959	69.588	1,3	< <b>0.005</b>	1.011	-0.704
Total bone area	Males	6	0.305	1.758	1,4	> 0.05	3.450	-6.796
	Females	7	0.051	0.321	1,6	> 0.05	2.046	-4.108
	Juveniles	5	0.411	2.090	1,3	> 0.05	1.639	-3.479



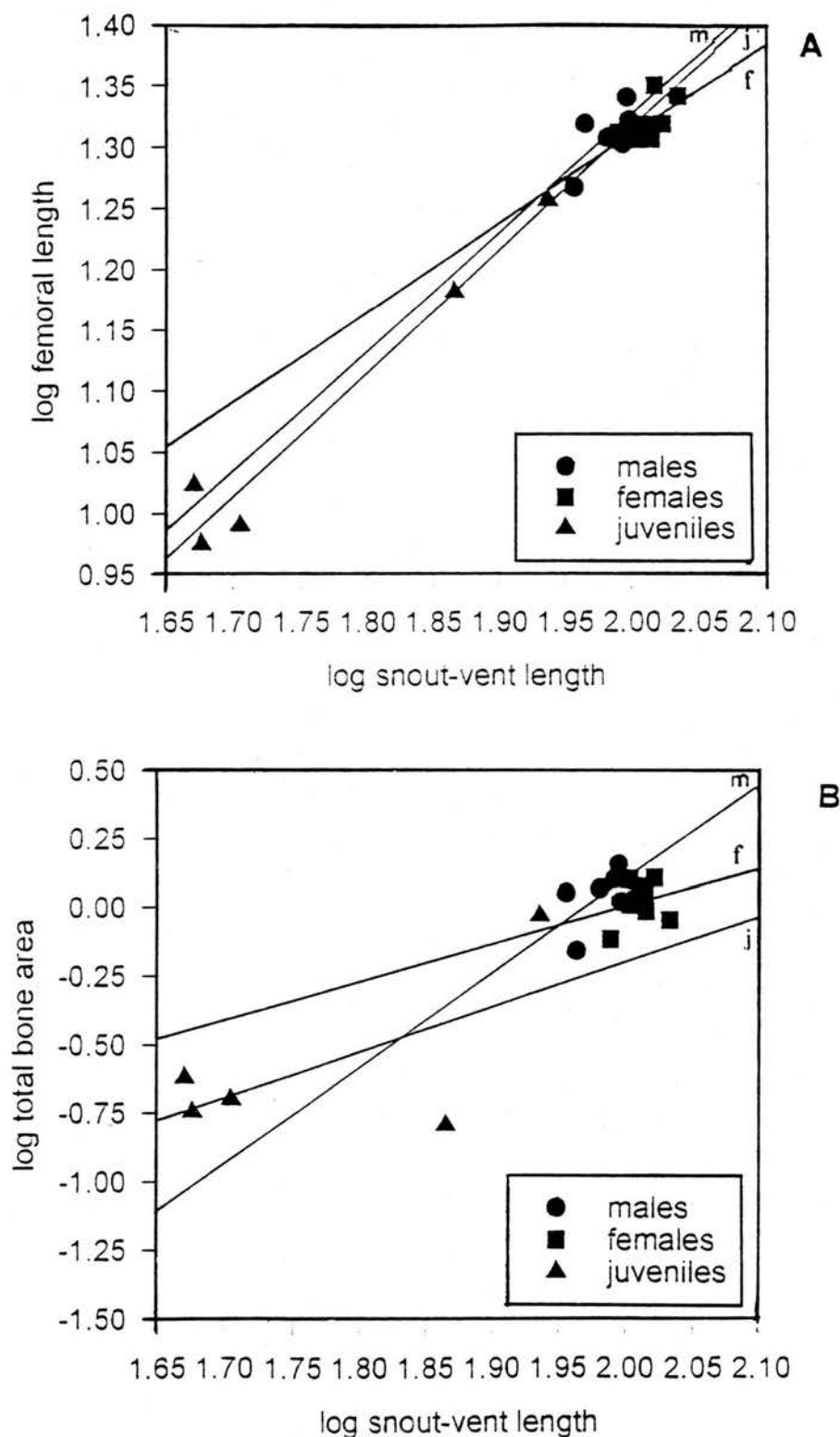


FIGURE 55. Correlations of **A**: log femoral length and **B**: log total bone area versus log snout-vent length, for juvenile and adult *Pseudocordylus capensis* individuals, from Landdroskop.

number of LAGs (Table 14). For all three variables, adults showed less variation within age classes than juveniles (Figure 54). There was no statistically significant correlation between TBA and SVL for all groups (Table 15), although adults displayed less variation within size classes than juveniles (Figure 55B).

To test for sexual dimorphism, only adult males and females were used due to the poor subadult and older juvenile representation in the population sample (Table 16). The only statistically significant correlation was between BWA and number of LAGs (Table 16, Figure 56). There was no statistically significant correlation between FL and SVL or between TBA and SVL for males and females (Table 17).

Table 16. Comparison of regression slopes of body and femoral dimensions versus number of lines of arrested growth among adult males and females of *Pseudocordylus capensis* from Landdroskop.

Comparison	Dimension	F	df	P
Adult males versus adult females	Snout-vent length	0.005497	1,7	> 0.05
	Femoral length	1.072022	1,7	> 0.05
	Relative bone thickness	0.336045	1,7	> 0.05
	Medullary cavity area	3.03892	1,7	> 0.05
	Bone wall area	7.272762	1,7	< 0.05
	Total bone area	5.263500	1,7	> 0.05

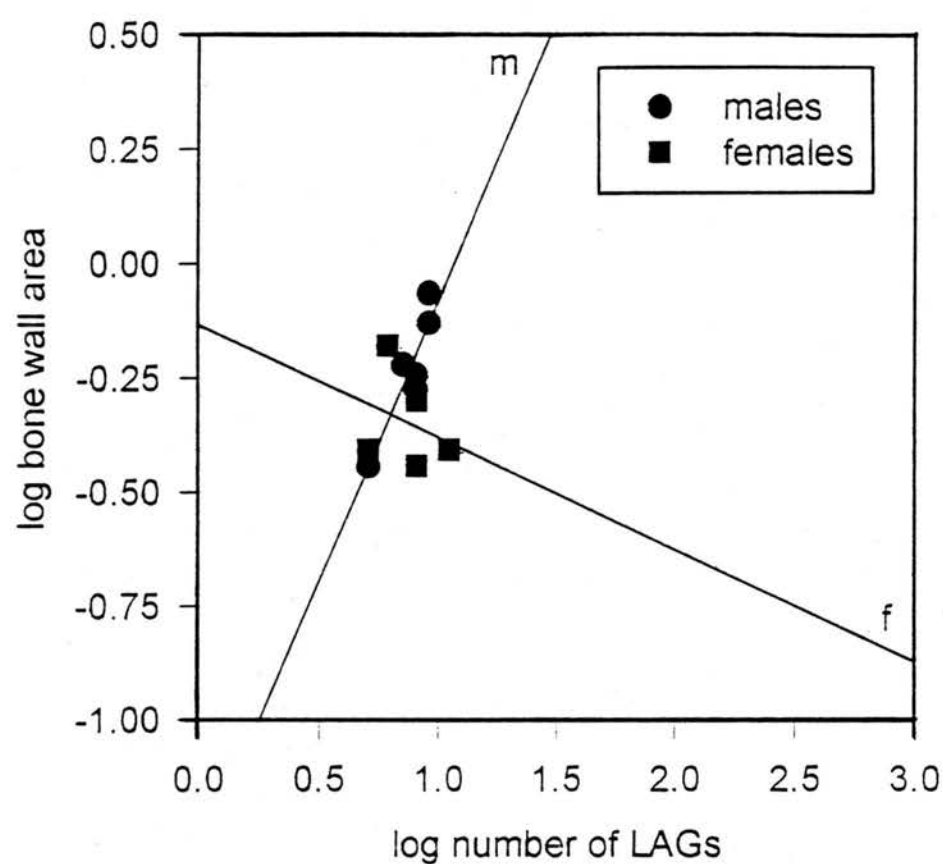


FIGURE 56. Correlation of log bone wall area versus log number of LAGs, for *Pseudocordylus capensis* males and females from Landdrooskop.

Table 17. Comparison of regression slopes of femoral length and total bone area versus snout-vent length, among males and females of *Pseudocordylus capensis*.

Comparison	Dimension	F	Df	P
Adult males versus adult females	Femoral length	0.13796	1,10	> 0.05
	Total bone area	0.358265	1,10	> 0.05



## CHAPTER 5

### DISCUSSION AND CONCLUSIONS

#### 5.1. Bone Histology

The bone histology of *Cordylus cataphractus* and *Pseudocordylus capensis* is similar to that of most other lizards. For example, the compacta of both species is poorly vascularized or avascular and is primarily composed of parallel-fibred bone. The only other periosteal bone tissue encountered in the two species, was embryonic bone. This general bone histology has been reported in other lizards and snakes (e.g. de Ricqlès, 1976; Castanet and Cheylan, 1979; Caetano *et al.*, 1985; Zug and Rand, 1987; Castanet and Baez, 1991; Chinsamy *et al.*, 1995; Wayne and Gregory, 1998). Since bone is a living tissue, the bone microstructure reflects overall growth rates and growth strategies (Chinsamy, 1994). According to Castanet and Baez (1988), embryonic bone is made from a type of woven fibred bone tissue. A woven bone matrix indicates a high rate of osteogenesis and thus fast growth (Castanet *et al.*, 1993). A parallel-fibred bone matrix, on the other hand, indicates an intermediate rate of bone deposition, while a lamellar bone matrix (like that occurring in endosteal lamellar bone), indicates a low rate of osteogenesis and thus slow growth (Castanet *et al.*, 1993). Each type of bone matrix can also gradually merge into the other (Castanet *et al.*, 1993). Embryonic bone and periosteal parallel-fibred bone are primary bone tissues, and the most valuable information on growth is obtained from primary bone (Chinsamy, 1994). The smallest juveniles of both species, therefore, displayed fast growth until birth, after which the growth rate decreased and growth was more intermediate. There was no transition from parallel-fibred bone to lamellar or pseudolamellar bone inside the periosteum of the oldest individuals from both species, which would have indicated that the growth rate had

decreased considerably. Therefore, intermediate growth was maintained in adults, even in the oldest individuals from both species.

## 5.2. Bone Remodeling

Bone remodeling is essential since proportionate growth would not be possible without a system for removal of old bone as new bone is added (Enlow, 1963). Because bone is hard, it can only grow and increase in mass by an appositional process of surface deposition (periosteal deposition), together with resorption from a contralateral surface (endosteal resorption) (Enlow, 1963). During bone growth, widespread rebuilding also occurs in the form of endosteal growth (endosteal deposition) (Enlow, 1963). The patterns of femoral remodeling in *C. cataphractus* and *P. capensis* will first be discussed, before commenting on causes or explanations for the remodeling differences between the two species.

### *Endosteal resorption*

*Resorption intensity and localization* - In both species, endosteal resorption varied in intensity along the medullary cavity margin and was more intense in some areas than in others. This localized resorption was more prominent in *P. capensis*. Partial LAG resorption in both species, together with the presence of a birth line and embryonic bone remnants in the cortex of most juveniles and even some adult of *P. capensis*, and the lack thereof in *C. cataphractus*, is evidence of this. Variation in endosteal resorption intensity around the medullary cavity margin has been demonstrated in previous studies. Castanet and Baez (1988, 1991) found that, in four out of the five *Gallotia* species studied, endosteal resorption was irregular, indicated by a remnant of embryonic bone which remained intact in some region of the bone, in 90 to 100 % of all individuals.

*Resorption intensity and total LAG resorption* - Both species demonstrated variation in the number of LAGs resorped. In *P. capensis*, individuals of similar sizes ranged from having all LAGs present from birth (at least to some degree), to having up to an estimated three LAGs resorped (Appendix 4). *C. cataphractus*, demonstrated similar results, although resorption was slightly more intensive in terms of the number of LAGs resorped. Some individuals were estimated as having had up to four LAGs resorped (Appendix 4). LAG resorption as a result of intensive localized resorption has been observed in previous studies. Castanet and Baez (1988; 1991), in their study of five extant *Gallotia* species from the Canary Islands, observed that one species (*Gallotia stehlini*) displayed a more extensive endosteal resorption than the other four. They also noted that older specimens of *G. stehlini* showed complete removal of up to the second LAG, while the other four species had all LAGs present to some degree (Castanet and Baez, 1991). Similarly, in a study conducted on *Lacerta lepida*, it was found the first and sometimes second LAG were removed and that endosteal resorption had occurred across the entire border of the medullary cavity (Cheylan, 1984).

*Resorption intensity and medullary cavity areas* - The shape and size of the medullary cavity provides further evidence for intensive but irregular resorption in both species. In *C. cataphractus* resorption was widespread along the whole medullary cavity border, indicated by the overall uneven shape of the medullary cavity in most individuals. *P. capensis* also displayed irregular endosteal resorption, although resorption intensity differed in various regions along the medullary cavity margin. However, because resorption was not as widespread along the medullary cavity margin in *P. capensis* as it was in *C. cataphractus*, it must not be assumed that endosteal resorption was more intensive in the latter species. When looking at total bone areas in adults and the percentages of total bone area (TBA) that



consisted of medullary cavity areas (MCAs), it was generally found that MCAs constituted a larger portion of TBAs than did bone wall areas (BWAs). Relative MCA percentages were, however, higher in *P. capensis* adults than in *C. cataphractus* adults. Furthermore, similar results were found when medullary cavity area (MCA) was regressed against total bone area (TBA). *P. capensis* ( $R^2 = 0.69$ ) displayed larger overall MCAs than did *C. cataphractus* ( $R^2 = 0.57$ ). Therefore, although endosteal resorption was more irregular across a wider spectrum of the medullary cavity margin in *C. cataphractus* than in *P. capensis*, the latter species seems to demonstrate greater overall (albeit localized) endosteal resorption, when one compares the sizes of MCAs relative to TBAs.

*Resorption intensity and sexual dimorphism* - In this study, sexual dimorphism in endosteal resorption was observed in *C. cataphractus*, but not in *P. capensis*. The relative medullary cavity area was significantly larger in females than in males, possibly indicating more intensive resorption. However, adult males seemed to have had a more intensive resorption of early LAGs, than adult females (Appendix 4). Further studies using known age individuals need to be done to ascertain the exact number of early LAGs resorped and to establish whether sexual differences in relative medullary cavity area and LAG resorption are only valid for the Graafwater population or for the species as a whole. In *P. capensis*, the lack of sexual dimorphism (Table 13) could be due to the small sample size. Sexual dimorphism in LAG resorption has been demonstrated in previous studies. For example, both Smirina (1983) and Hemelaar (1983; 1985), found a sexual difference in *Bufo bufo*, which was expressed as a difference in the number of rest lines removed by endosteal resorption among males and females in a given population. The results showed a delayed maturation of female toads, which accompanied a greater resorption in female toads than in

male toads (Smirina, 1983; Hemelaar, 1985). Furthermore, the trend of greater resorption in populations in which the youngest adults reached sexual maturity at an older age, also indicated that resorption increased with increasing age of maturation (Hemelaar, 1985). The localization and intensity of endosteal remodeling (incorporating LAG removal) can, therefore, represent distinguishing characteristics between species, sexes and possibly even populations.

### *Endosteal deposition*

A noteworthy difference between the two species was that in almost all *C. cataphractus* individuals examined, endosteal bone surrounded most of the medullary cavity, while in *P. capensis*, it was more limited. Where it did occur in the latter species, it was thinner than in the former species. The data, therefore, indicated that endosteal bone deposition was less in *P. capensis* than in *C. cataphractus*, and seems to point to a more intensive endosteal resorption in the former than in the latter species (although more localized). This would explain the limited and thin endosteal bone deposits, as well as the generally thinner bone walls observed in *P. capensis*, as opposed to the thicker, more abundant endosteal bone deposits and relatively larger bone wall thicknesses in *C. cataphractus*. It would also explain the larger medullary cavity areas and relatively smaller bone wall areas in *P. capensis* (Appendix 3).

Most of the *P. capensis* specimens examined, displayed thinner bone walls, larger medullary cavity areas and limited endosteal deposition. There were, however, not enough individuals from the different populations to allow statistical assessments of possible altitudinal variation in these traits. In previous studies, the presence and distribution of endosteal deposits has



also proved to be distinctive. For example, Castanet and Baez (1991) observed that endosteal lamellar bone was centripetally deposited around the medullary cavity in *G. stehlini*, which had also displayed the most intensive endosteal resorption of the five *Gallotia* species studied. *C. cataphractus* displayed a similar distribution of endosteal deposition to *G. stehlini*. Cheylan (1984), however, found that, despite a relatively extensive and intense removal of bone in *L. lepida*, endosteal bone deposition was restricted or lacking altogether (Cheylan, 1984). The relationship between endosteal resorption and deposition is, therefore, a distinctive yet complex feature of species.

In previous studies, authors deemed the presence of endosteal bone deposits below embryonic bone a noteworthy feature. For example, in *G. galloti*, some endosteal lamellar bone occurred below embryonic bone in adults, indicating that endosteal resorption was not intense in those areas (Castanet and Baez, 1988). Endosteal deposits, below embryonic bone, thus indicate limited resorption. In this study, endosteal lamellar bone was not always present below embryonic bone in *P. capensis*. This was not surprising because the occurrence of endosteal bone in *P. capensis* was generally limited. However, in those adults where it did occur below embryonic bone, it was the thickest deposition of this bone tissue. This observation further supports the occurrence of irregular, off-center resorption in *P. capensis*.

In light of this, if remodeling involves two opposing processes to maintain the shape and proportions of the bone as it grows in length and diameter (Enlow, 1963), then one would expect endosteal deposition to take place opposite areas of resorption. However, despite the fact that endosteal resorption was intensive in *P. capensis*, endosteal deposition was limited

or lacking altogether. This created disproportionately large medullary cavity areas in most *P. capensis* individuals, which was initially difficult to interpret. Variation in the remodeling process was also found by Hemelaar (1985) in her study of different toad populations.

Within one population, she found large individual variation in the degree of resorption, even within age classes, which to her was an enigma. What she found most remarkable, however, was that bone remodeling appeared to be lacking in a number of these toads and she stated that the “factors responsible for this phenomenon should be investigated” (Hemelaar, 1985). From this and all previously mentioned studies, one can conclude that the extent of bone remodeling is the result of complex interaction between resorption, deposition and growth. However, the reasons behind species or even populational differences are most often difficult to interpret. Possible explanations for remodeling differences observed between the two cordylid species, are discussed below.

*Causes and explanations* - Bone remodeling can be seen as the result of two kinds of processes, namely genetic and epigenetic processes. The presence or absence of all the various types and patterns of bone, is largely determined by the nature of growth and complex remodeling processes that occur during the development of each bone, regardless of species (Enlow, 1969). The combinations of the basic kinds of bone tissue in any species, vertebrate group, age, individual bone, or part of any bone are dependent on several factors (Enlow, 1969). These include the shape of the entire bone, the rate of localized growth in any area of the bone, the rate of growth of the whole body, the sequence of remodeling changes that an individual undergoes during its growth, the life-span of the animal, the nature of muscle attachments, the extent of cortical vascularization, and the size of the animal and its skeleton (Enlow, 1969). "These circumstances can be considered as phylogenetic in the

sense that they become genetically established within the framework of growth in any given species" (Enlow, 1969).

The epigenetic processes which influence bone remodeling and thus bone structure, involve external factors (mainly related to function) and include biomechanical and metabolic constraints (Tortora and Grabowski, 1993; Chinsamy *et al.*, 1995). If bone remodeling is mainly related to biomechanical processes, then differences in the intensity and localization of endosteal resorption and rebuilding, due to changes in the load or pressure placed on the bone, can be explained (refer to Enlow, 1963, and Alexander, 1971, for a more in-depth discussion). Within limits, bone has the ability to alter its strength in response to mechanical stress, the bone cortex becomes thicker, and thus stronger, through increased deposition of mineral salts and collagen fiber production (Tortora and Grabowski, 1993). The main mechanical stresses placed on bone are those that result from the pull of skeletal muscles and gravity (Tortora and Grabowski, 1993). On the other hand, if endosteal remodeling is mainly metabolic in origin, this can explain differences observed between males and females, endothermic and ectothermic organisms, and between various periods in life, for example, sexual maturity or immaturity (Castanet, pers.comm.). Bone tissue stores several minerals, especially calcium and phosphorus, which are important in muscle contraction and nerve activity (among other functions), moreover, bone marrow stores lipids, an important chemical energy reserve (Tortora and Grabowski, 1993). Remodeling allows bone to serve as the body's reservoir for calcium because normal bone growth in the young, and bone replacement in the adult, depends on the presence of sufficient amounts of calcium and phosphorus (Tortora and Grabowski, 1993). These elements are components of hydroxyapatite, the primary component that makes bone matrix hard (Tortora and Grabowski, 1993).



Therefore, both genetic and epigenetic processes are responsible for skeletal macro- and microstructure. An animal's evolutionary history, would have established the basic foundation of bone remodeling and bone morphology, and would directly be related to the animal's longevity, a hypothesis discussed later in this chapter. Naturally, its environment, and the stresses placed on it by that environment, would play a major role in this regard. It is possible that the larger MCAs and thinner bone walls in *P. capensis* (producing a lighter skeleton), could be the result of a need for speed and agility in these animals. When MCA was regressed against snout-vent length in both species (Table 1 and Table 11), the results indicated that, in general, MCA tended to increase as body size increased. In *P. capensis*, however, ( $R^2 = 0.94$ ) this phenomenon was more pronounced than in *C. cataphractus* ( $R^2 = 0.38$ ). The results, therefore, tend to support the proposed need for a lighter skeleton in *P. capensis*, and the processes involved would thus be genetically established. *P. capensis* has been referred to as an agile and fast-running species (Branch, 1998) and, although classified as sit-and-wait feeders, are known to be faster than any *Cordylus* species studied to date (Cooper *et al.*, 1997). Many studies have found a positive correlation between limb length and sprint speed (refer to Garland and Losos (1994) for a more detailed discussion). A lighter skeleton with longer limb bones would, thus, be an advantage.

*C. cataphractus*, on the other hand, is a heavily armoured, sluggish lizard (Mouton *et al.*, 2000), with a sit-and wait foraging mode and a lower basal metabolic rate than any other cordylid species studied to date (Fourie, 1997; Mouton *et al.*, 2000). Moreover, although *C. cataphractus* reaches larger asymptotic body sizes than does *P. capensis*, individuals have shorter and thicker femora than *P. capensis* individuals (Appendix 2). Therefore, a heavier skeleton would be an advantage in supporting a heavily armoured body and would be

genetically established.

The intense endosteal resorption in *C. cataphractus*, however, can largely be explained on epigenetic grounds. As Chinsamy *et al.* (1995) states, biological (mainly metabolic) and biomechanical constraints directly induce remodeling changes on the overall size of the medullary cavity area. I propose that the intensity of endosteal resorption, could be as a result of food shortages known to be experienced by *C. cataphractus* as a result of group-living conditions (Mouton *et al.*, 1999; Mouton *et al.*, 2000). This could, consequently, promote the removal of vital minerals from bone (via resorption) for the maintenance of normal biological functions. The dimorphism in relative cavity size between *C. cataphractus* males and females can also largely be due to epigenetic constraints. The results showed that when medullary cavity area (MCA) was regressed against total bone area (TBA), adult females were the only group to show a statistically significant correlation between these two variables. Furthermore, when testing for sexual dimorphism in medullary cavity size, the only statistically significant correlation was between MCA and TBA. This indicated that in females, as TBA increased, MCA increased significantly as well. A possible explanation for this observation is that sexually mature females would require more biologically vital minerals than males, especially if food was lacking, to support pregnancy, this would then promote the resorption of bone, causing MCAs to increase in size as bone wall areas decreased.

Both species demonstrated a broad variation in the number of early LAGs resorped within the same size and estimated age classes (Appendix 4), similar to those discovered by Hemelaar (1985) in toads. It is suggested, that the answer lies within the genetic and epigenetic



processes controlling remodeling in each individual. Nonetheless, it is obvious that further investigation into these processes needs to be done.

### 5.3. Growth mark features

*Growth mark identification* - Growth was periodic in both the cordylid species studied, represented by zones and LAGs in the compacta. According to Castanet *et al.* (1993), there are three categories of growth marks recognized. Zones, which are fast growing layers, correspond to the periods of general active osteogenesis and are thus the widest marks. Annuli, correspond to periods of slow osteogenesis and always appear narrower than adjacent zones. Annuli are usually composed of lamellar bone. Lastly, lines of arrested growth (LAGs), which are functionally defined as rest lines, depict temporary arrests of local osteogenesis (Castanet, 1979; Castanet *et al.*, 1993). LAGs are weaker than zonal bone due to their ultrastructure, which depicts a thin irregular network of loose and poorly organized collagen fibers when compared to neighbouring bone, which underlie the boundary between old and new bone (Castanet, 1981). This is probably why, where bone sections tore in histological preparation, it either happened along the LAGs (Figure 45) or along resorption lines (Figure 44), which have a similar ultrastructure to LAGs (Castanet, 1981).

*LAG expression* - In *C. cataphractus*, LAGs are well defined, which would be expected as this species lives in a temperate climate. In *P. capensis*, however, LAGs were generally more inconspicuous in specimens from the lowest altitudes than in those from higher altitudes. One possible explanation could be that, even though all localities where specimens were collected fall under a temperate climate, the microclimate under which those lowland specimens lived was more constant than those specimens coming from higher altitudes. In

general, LAGs appear strongly expressed under contrasting seasonality (Castanet and Smirina, 1990). However, according to Castanet and Smirina (1990), optical sharpness can decrease, with LAGs becoming more inconspicuous in bones of specimens living under more constant climatic conditions. Therefore, rest line distinctness has been positively correlated with the length and severity of winter periods (Hemelaar, 1985). In her study involving different populations of toads from different climates, Hemelaar (1985), however, found that toads from southern France had very distinct LAGs, although they came from the mildest climate, while half the toads with indistinct LAGs came from Norway, a country with long, harsh winters. Hemelaar (1985) concluded that distinctness of LAGs in *Bufo bufo*, provided no evidence for the correlation of LAG distinctness with the length and severity of winters.

LAG distinctness has, however, been linked to altitude. For example, in their study of *Gallotia* species from the Canary Islands, Castanet and Baez (1991), found that *G. stehlini* showed the clearest LAGs and, of the four populations studied from different altitudes, the one from the highest altitude (1800 m), showed the most distinct LAGs. An inter-population study on LAG distinctness in a species like *P. capensis*, where populations can be separated by altitudinal differences of over 1000 meters, should yield interesting results, especially as the present study seems to indicate that altitude does effect LAG expression. Those *P. capensis* individuals from the lowest altitudes displayed less distinct LAGs, than those individuals from the highest altitudes. It must, however, be taken into consideration that most of the *P. capensis* specimens used, came from a museum collection and had already been preserved in alcohol for many years (Appendix 1). This probably influenced the staining procedure, and consequently LAG expression, as many of the sections came out lighter than expected even after being stained for longer than required periods. The

population sample from Landdrooskop was fresh material and all individuals showed highly distinct LAGs. The *C. cataphractus* sample was in alcohol for approximately one year before removal of the femora and showed distinct LAGs, although not as distinct as the Landdrooskop population. Therefore, although there appears to be an altitudinal difference, fresh material should be examined to make comparisons more meaningful.

The variability of LAG expression between different taxa or populations, still remains difficult to understand (Castanet and Baez, 1991). For example, in the *Gallotia* lizards, four of the five species studied showed clear LAGs (although variation was found in these species as well), but one species, without any clear explanation, showed much weaker LAG expression (Castanet and Baez, 1991). The general assessment, however, is that LAGs originate from an endogenous (genetic) biological rhythm, but are reinforced and synchronized by external factors, the main factor being seasonality (Castanet and Baez, 1991; Castanet *et al.*, 1993).

*LAG spacing* - The great variability in spatial arrangement of LAGs, is another noteworthy feature. In some *C. cataphractus* and *P. capensis* individuals, all the LAGs were either relatively close together or far apart. According to Castanet and Baez (1988), it is difficult to interpret variation in LAG spacing and to propose some causal relationship with any particular parameter, such as sex or environmental conditions, because in their study on *Gallotia* lizards, this phenomenon occurred in males and females, as well as in lizards from different altitudes and localities. They speculate that several factors could explain these features, for example, that the variation of growth rate is directly related to the genetic program of each individual, or that it may be connected with individual competition for food



(Castanet and Baez, 1988). The latter explanation would certainly be a factor for *C. cataphractus*, as was previously mentioned, due to their group-living behaviour and apparent lack of active foraging (Mouton *et al.*, 2000). As far as *P. capensis* is concerned, most of the individuals used in this study were not from a single population, but were sampled from various localities and altitudes, so variations in LAG spacing could be due to individual growth patterns. The population that was sampled from Landdrooskop also showed this variation in LAG spacing, however, and the reasoning behind this is most likely similar to that for the *C. cataphractus* population. In other words, lack of food and individual growth patterns, as well as possible climatic influences due to the high altitude where they occur.

#### 5.4. Double LAGs

On the whole, *C. cataphractus* displayed a greater number of double LAGs than *P. capensis*. *C. cataphractus* adults had a higher overall percentage of double LAGs than juveniles, and adult males had a slightly larger number of double LAGs than adult females. In *P. capensis*, the average number of double LAGs was higher in the population from Landdrooskop than in those individuals from other localities. There was no noticeable difference between males and females, although adult males had a marginally higher percentage of double LAGs than adult females from the Landdrooskop population. When most of the rest lines appear double in a majority of individuals, the likelihood of a double annual growth cycle, including an estivating and/or hibernating period (Castanet and Smirina, 1990), is supported. For example, Caetano *et al.* (1985), found a double annual growth cycle among populations of *Triturus marmoratus* living in high altitudes. This directly demonstrated the adaptive plasticity of a species to its environment, since lowland populations of the same species, showed only a single growth ring per year in bones (Caetano *et al.*, 1985). A double annual

growth cycle did not appear in either of the two species studied here. Although *C. cataphractus* individuals displayed a high percentage of double LAGs, the majority of visible LAGs were, however, not double. The cause of these randomly distributed double LAGs remains uncertain, but they may be related to individual variations, pathologic injury or atypical periods of climatic conditions (Castanet and Baez, 1988). Altitude and by extension climatic conditions, has been shown to play a major role in double LAG formation (for example Caetano *et al.*, 1985). In *P. capensis*, the randomly distributed double LAGs were most likely caused by the cool climates and high altitudes in which these lizards live (Mouton and Van Wyk, 1997; Van Wyk and Mouton, 1998) reflecting atypical periods of arrested growth. More individuals from different localities, however, need to be studied to gain accurate insight into the effect of altitude on double LAG formation in *P. capensis*. A lack of food may also induce periods of atypical arrested growth (Castanet and Baez, 1991). For example, Collins and Rodda (1994) found that periods of low feeding levels or ecdysis, were linked to rest line formation in *Boiga irregularis*. In *C. cataphractus*, where populations live in groups, with one population occupying a single rocky outcrop, competition for food would be high (Mouton *et al.*, 2000). Observations of daily activity patterns in these lizards, have shown them to be sit-and-wait feeders (Mouton and Van Wyk, 1997; Mouton *et al.*, 2000), which would have serious consequences in terms of food availability. Lack of food could, thus, be the main cause of double LAG formation in *C. cataphractus*.

### 5.5. Age estimation

*LAG expression* - Even with good histological preparation, it is rarely possible to determine individual ages simply by counting LAGs present in bone sections (Castanet and Smirina, 1990). Bone sections have many peculiarities that one has to become acquainted with, such



as the appearance of false LAGs and double LAGs. False LAGs are always difficult to detect and interpret in terms of their determinism (Castanet and Smirina, 1990; Castanet and Baez, 1991), especially when trying to decipher between false LAGs and indistinct LAGs, either due to insufficient staining or variation in LAG expression. Double LAGs differed in thickness and distinctness between individuals and sometimes even within a single bone section, this lead to some difficulty in interpreting whether the LAG was a double or just two LAGs occurring close together.

*Birth lines and subsequent LAGs* - The position of the first LAG and its relation to the birth or hatching line can also complicate age determination. The thickness of the bony crown between these two LAGs equals the duration and intensity of growth during this period (Castanet and Gasc, 1986; Castanet and Baez, 1988, 1991). The birth period for *C. cataphractus* and *P. capensis* is in autumn around March to May (Flemming and Mouton, unpublished data; Van Wyk and Mouton, 1998). In some individuals, specifically pertaining to *P. capensis* as only one juvenile in *C. cataphractus* displayed a birth line, there was a noticeable variation in the amount of bone deposited between the birth line and first LAG (Figures 51 and 52). Growth generally stops at the same time of year (usually winter) in lizards living in a seasonal climate (Castanet and Baez, 1988), so one would expect juveniles to show more or less the same increment of bone between the birth or hatching line and first LAG. However, Castanet and Baez (1988, 1991), found that the hatching period in *Gallotia* lizards, spread between early spring and autumn, thereby influencing the amount of bone deposited before the first period of arrested growth. Despite this, in some lizards the distance between the hatching line and first LAG was so large that it could only correspond to a full year of growth. In these conditions, the first LAG represented a pause after the second

season of growth (Castanet and Baez, 1988). Two hypotheses were proposed to explain this phenomenon: (a) that the lizard hatched late in autumn and that no osteogenesis occurred until after the first winter, so the hatching line and first winter LAG were merged, or (b), that the LAG of the first winter was generally less distinct than later ones or sometimes completely lacking. This was as a result of continued growth during the first winter, owing to the strong intrinsic growth of juveniles and or reduced influence of adverse environmental conditions (Castanet and Baez, 1988). Castanet and Baez (1988), proposed that the first hypothesis was more probable than the second, in explaining this phenomenon in *Gallotia* lizards.

In *P. capensis*, however, the variation in spatial arrangement between the birth line and first LAG was most likely due to the time of birth, which would have influenced the amount of bone deposited before the first period of arrested growth. It was also noteworthy that in some *P. capensis* juveniles from the highest altitudes (one from 1640 m and two from 1133 m above sea level), a double birth line was observed (Figure 32). The second period of arrested growth after birth probably corresponded to the first winter growth arrest, as these lizards are born in autumn and would have experienced their first winter shortly after birth. This double birth line is unlikely to be due to food shortages, as *P. capensis* is known to have adequate resource availability in late summer through autumn months (Van Wyk and Mouton, 1998) and in winter as well (Mouton, pers.comm.).

*Age and body size* - In general, lizards tend to increase in body size until they reach sexual maturity, after which a decrease in growth is observed (Castanet and Baez, 1991; Castanet *et al.*, 1993; Chinsamy *et al.*, 1995). Size correlated relatively well with age in *C. cataphractus*

and *P. capensis* (Figures 26A and 54A), but some individuals of both sexes, although larger in size, were younger in age than other smaller individuals. Body size, therefore, does not account for the individual variation observed. In *C. cataphractus*, sexual dimorphism was evident when snout-vent length (SVL) and femoral length (FL) were regressed against number of LAGs (age). The results indicated that males tended to have larger body sizes and longer femora at younger ages than females (Figure 29). The results also showed that the correlation between SVL and age was statistically significant in adult males but not in adult females. Therefore in adult males, body size increased significantly with age (Figure 26). There was no statistically significant correlation between SVL and FL versus age in *P. capensis* and the only sexual dimorphism observed in this species, was between bone wall area (BWA) and age. Adult males tended to show an increase in BWA with increasing age, while in adult females, these two variables seemed to be negatively correlated (Figure 55). More individuals need to be examined, however, to gain accurate insight into the interaction between age and body size variables, because in such a small population sample, individual variation can have a large impact on results.

The spacing between successive growth marks is not constant throughout life, but reveals variation in bone and body growth, and is more a record of an individual's growth curve through life than is body size (Castanet and Baez, 1990). The general trend is that regular spacing of growth rings decreases as the animal matures, which can lead to difficulties counting closely packed marks in long living old individuals (Castanet and Smirina, 1990). Slow body growth in old individuals, however, still contributes to the final body size (Castanet and Baez, 1991) and further indicates that size is not a reliable indicator of age, especially after the attainment of sexual maturity, as individual growth rates are not constant



throughout life (Castanet and Baez, 1988).

### 5.6. Sexual maturity

A decrease in LAG spacing occurred in both *C. cataphractus* and *P. capensis* and allowed the general age at the onset of sexual maturity, to be estimated. Sexual maturity can be estimated in this way because a distinct difference in the spacing patterns of LAGs appears, when the slopes of growth curves decrease after the attainment of sexual maturity (Castanet and Smirina, 1990; Francillon-Veillot *et al.*, 1990; Castanet and Baez, 1991). When looking at the spacing of LAGs in adult *C. cataphractus* specimens, there seemed to be a general decrease in the zonal bone thickness around the fourth visible LAG in females and fifth visible LAG in males. After back calculation, this corresponded to the sixth or seventh year of life. In *P. capensis* this corresponded to the fourth to sixth year of life.

Variation in snout-vent length within age classes was observed for both species. Although age at sexual maturity generally corresponded to the approximated snout-vent length at the onset of sexual maturity (Van Wyk and Mouton, 1998; Mouton *et al.*, 1999), the variation within age classes seemed to point to it being more a function of age rather than purely the attainment of a specific body size. This variation in body size at first breeding age was also discovered in previous studies, for example, Halliday and Verrel (1988), Castanet and Baez (1991), Wake and Castanet (1995) and Waye and Gregory (1998).

### 5.7. Longevity

In both species, LAGs near the outer margin of the compacta were difficult to count. This is usually the case in long-lived animals in which the growth rate at old age is so low that



annual layers are formed very close together and are extremely thin (Castanet and Smirina, 1990). In some studies, principally among reptiles, it was shown that local bone growth stops well before the end of life (Castanet and Baez, 1988), and moreover not necessarily at the same moment for different bones of the same individual (Castanet and Smirina, 1990). It is doubtful that bone growth had stopped in any of the oldest individuals investigated in this study, as one would expect to find lamellar or pseudolamellar bone inside the periosteum of the cortex, prior to the cessation of bone growth. Lamellar bone is associated with very slow growth but the oldest individuals still displayed intermediate growth rates, reflected by their bone tissue. I propose that both species have the potential for long life-spans, especially *C. cataphractus* which appears to reach sexual maturity at an older age, but more studies on age at sexual maturity and longevity in different populations need to be done to test this theory.

In terms of longevity, body size is accorded importance as an adaptive character (e.g. Barbault, 1988 and Castanet and Baez, 1991). Three factors are involved in the attainment of body size, firstly, the initial size at hatching, secondly, the post-embryonic growth rate and lastly, the duration of growth (Castanet and Baez, 1991). According to Castanet and Baez (1991), interspecific body sizes can be accounted for, irrespective of differences in body sizes at hatching or birth, by differences in longevity and time of sexual maturity, not differences in growth rates. In line with intra and interspecific phenotypic (ecological) plasticity of reptiles, are differences concerning factors such as local population density, competition, predation, food supply and climates, which could easily account for small individual variations of longevity (Castanet and Baez, 1991). External limiting pressures, for example intensity of seasonality and food availability, can disturb the usual relationships between biological parameters, as they indirectly affect bone growth (Castanet and Baez, 1991). This

was demonstrated in two allopatric populations of *Lacerta viridis* (Saint Girons *et al.*, 1989). Castanet and Baez (1991), hypothesize that external pressures are able to induce selection of lizards according to their longevity, for example, to favour adaptive strategies in which longevity is the target for selection as well as any other morphological or physiological adaptive character. This appeared to be the case in their study of extant and extinct *Gallotia* lizards from the Canary Islands.

The girdled lizards (*Cordylus*) are the most characteristic lizards of southern Africa, and are known to be long-lived (up to 25 years is known in captivity; Branch, 1998). They also possess very large species, like the armadillo lizard, *Cordylus cataphractus*, and the giant girdled lizard or sungazer, *Cordylus giganteus*, which reaches sizes of over 200 mm snout-vent length (Branch, 1998). The crag lizards (*Pseudocordylus*) are also known to have some of the largest cordylid species (Branch, 1998), surpassed in size only by the sungazer.

According to Branch, these rock-living crag lizards have tremendously strong jaws and an unusual, hinged skull structure that allows the shape of the head to thicken if the jaws are clamped shut. This is effectively used as a defense mechanism, in that these lizards wedge their heads into the narrow parts of a crack, and as long as its jaws are clenched shut, the top of the head (protected with thickened scales and osteoderms) and narrow jaws will be tightly jammed against the rock walls (Branch, 1998). Branch (1998) states, that their bull-dog like tenacity makes it almost impossible to pull them from their retreats and although large and aggressive, they are very wary and retreat quickly into their cracks at the first sign of danger. The girdled lizards, on the other hand, possess stocky bodies, with heads that are covered in shields (fused to the skull) and body scales that have thick, keeled or spiny osteoderms (body armour) for protection against predators (Branch, 1998). To evade predators, many species

jam themselves into rock crags by inflating their bodies and shortening and thickening their skull, which (like *Pseudocordylus*) has an unusual, hinged structure (Branch, 1998).

Both *Cordylus* and *Pseudocordylus*, therefore, have very effective anti-predator or defense mechanisms, which could play an important role in influencing selection for longer life spans. Both genera would, therefore, constitute interesting case studies to further investigate the hypothesis that external pressures are able to induce selection of lizards according to their longevity, specifically, by favouring adaptive strategies in which longevity is the target for selection. This type of study would obviously require fossil or ancestral material for any genus investigated, but could yield some interesting results on the range of longevity within genera and warrants further investigation within the Cordylidae.

### 5.8. Conclusions

To conclude, I shall briefly summarize the results to the key questions posed in chapter 1:

1.) *Does the bone histology of C. cataphractus and P. capensis conform to that of other lizards and to what extent does their bone microstructure reflect their ecology?*

The bone tissue structure of both species does conform, in general, to that of other lizards.

The process of remodeling is, however, distinctly different between the two species. This is related to genetic and epigenetic (external) factors and could, with further investigation, offer a new taxonomic character. Bone tissue structure did not seem to correlate with any ecological aspect. Growth mark expression, however, could be related to climatic conditions.

The occurrence of randomly distributed double LAGs in both species, could be related to various factors including atypical climatic conditions, injury or food shortages.



2.) *To what extent do altitudinal differences affect bone histology?*

The histological feature investigated in this regard, was growth marks. *C. cataphractus*, despite occurring in a warm, relatively low altitude environment, had distinct LAGs. This was equated to the strong seasonality of climates in the Western Cape and possible also food shortages during summer. *P. capensis* seemed to show altitudinal differences in LAG expression, despite all populations occurring in a cool, temperate climate. Individuals from lower altitudes generally displayed less distinct LAGs than higher altitude individuals, but more individuals from the different populations need to be studied before a definite conclusion can be reached.

3.) *What is the life span or longevity of each species?*

The oldest individuals from both species were found to be over 10 years of age and still maintained an intermediate growth rate at time of capture. If both species were short-lived, then the growth rate would have decreased in the oldest individuals and growth would have been slow. *Pseudocordylus*, and especially *Cordylus* are considered to be long-lived genera (ages up to 25+ years is known in captivity for *Cordylus*) and the evolution of very effective anti-predator features attests to this. No actual studies on longevity in wild populations has been done before now.

4.) *At what age does sexual maturity occur?*

The age at sexual maturity was estimated as being between four to six years in *P. capensis* and around six years in female and seven years in male *C. cataphractus*. The variation in SVL at the onset of sexual maturity recorded in both species, however, suggests that age is the main criteria for initiating sexual maturity rather than strictly body size. The relatively



late onset of sexual maturity in both species, lends support to the hypothesis that these two species could have long life-spans.

5.) *What type of growth rate do C. cataphractus and P. capensis exhibit?*

Evidence from bone histology and skeletochronology suggests that both species have the potential for long life-spans. When various macroscopic and microscopic measurements were regressed against age (estimated number of LAGs), there was generally considerable variation in all variables among juveniles, but less among adults from both species. In *C. cataphractus*, males displayed significantly higher growth rates than females when snout-vent length (SVL), femoral length (FL) and bone wall area (BWA) were regressed against age. In *P. capensis*, sexual dimorphism in growth rate was only observed between BWA and age. Larger samples need to be examined to reduce the effect of individual variation and thereby gain more accurate information on species growth rates and sexual dimorphism in growth rates.

## SUMMARY

1. Ecological adaptations, as well as the animal's specific size and longevity, are known to be reflected as histological variations within its skeleton. Limited information on bone histology, longevity and age at sexual maturity is available for the Gerrhosauridae, but is non-existent for its sister family the Cordylidae. To obtain baseline information on bone morphology in the Cordylidae, two species which differ considerably in general morphology and biology were selected for a detailed histological analysis.
2. A population of *Cordylus cataphractus*, a lowland lizard, was sampled from the Graafwater district, and a population of *Pseudocordylus capensis*, a montane lizard, from Landdroeskop in the Hottentots Holland Mountains. Both localities are in the Western Cape Province, South Africa. A few *P. capensis* individuals from other localities were also examined. The left femora from an ontogenetic range of individuals from both species were used. The size at sexual maturity in *P. capensis* and *C. cataphractus*, had been previously determined by macroscopic and microscopic investigations of the gonads. The femora were decalcified and processed according to standard histological procedure. Cross sections from the mid-shaft region of each bone were stained with haematoxylin and eosin. Skeletochronology was used to age individuals by assessing the number of LAGs occurring in each femur. A back calculation method was used to estimate the number of LAGs that had been resorped by bone remodeling. Various area values and microscopic measurements from each bone section were calculated. Least square regression analysis of log-transformed variables were used to assess any correlation between these variables and increasing age. ANCOVA was used to determine any significant differences between the sexes.

3. The bone histology of *C. cataphractus* and *P. capensis* was similar to the bone microstructure of other lizards. The compacta of both species was poorly vascularized or avascular and was primarily composed of parallel-fibred bone. The only other primary periosteal bone encountered was embryonic bone. The smallest individuals of both species displayed fast growth until birth, represented by embryonic bone, after which growth became intermediate, represented by parallel-fibred bone. There was no primary lamellar bone inside the periosteum, so growth had not slowed even in the oldest individuals of both species.
4. The femoral mid-shaft region in most individuals from both species had undergone some remodeling. Endosteal resorption varied in intensity along the medullary cavity periphery in both species, and was generally more intense in some areas than in others. This localized resorption was more prominent in *P. capensis*, while *C. cataphractus* displayed a more widespread resorption along the medullary cavity border. The shape and size of the medullary cavity, partial LAG resorption, and the presence of a birth line and remnants of embryonic bone in the cortex of most juveniles and some adults of *P. capensis*, is evidence of this. Both species demonstrated variation in the number of LAGs resorped. *P. capensis* seemed to demonstrate greater overall (albeit localized) endosteal resorption than *C. cataphractus*, when the sizes of the medullary cavity areas (MCAs) relative to the total bone areas (TBAs), were compared. Sexual dimorphism in endosteal resorption was observed in *C. cataphractus* but not in *P. capensis*.
5. In almost all *C. cataphractus* individuals examined, endosteal lamellar bone surrounded most of the medullary cavity, while in *P. capensis*, it was more limited. Where it did occur in the latter species, the data indicated that endosteal deposition was less than in the former species. This together with the more intensive resorption in *P. capensis*, would explain the generally



thinner bone walls and larger MCAs as opposed to the generally thicker bone walls and smaller MCAs, relative to TBAs, in *C. cataphractus*.

6. The causes or explanations for the differences between these two cordylid species, lies in genetic and epigenetic processes. The presence or absence of various types and patterns of bone, is largely determined by the nature of growth and complex remodeling processes that occur during the development of each bone, regardless of species. The combinations of the basic kinds of bone tissue in any species, vertebrate group, age, bone or part of a bone depend on several factors which are genetically established within the framework of growth in any given species.
7. The epigenetic processes, which influence bone remodeling and thus bone structure, include biomechanical and metabolic constraints. Biomechanical processes, involving changes in the load or pressure placed on the bone, explain differences in the intensity and localization of endosteal resorption and rebuilding. Metabolic constraints explain differences observed between males and females, endothermic and ectothermic organisms and between various periods in life like sexual maturity or immaturity.
8. It is possible that the longer limbs, larger MCAs and thinner bone walls in *P. capensis*, could be the result of a need for speed and agility in this lizard. Limb length is known to be positively correlated with speed and *P. capensis* is known to be faster than any *Cordylus* species studied to date, although it, like *C. cataphractus*, is classified as a sit-and-wait feeder. These features, producing a lighter skeleton, would thus be genetically established. *C. cataphractus*, on the other hand, is a heavily armoured sluggish lizard, with a lower metabolic rate than any other cordylid species studied to date. A heavier skeleton would, thus, be an advantage in supporting a heavily armoured body and would be also be genetically



established.

9. The intense endosteal resorption in *C. cataphractus*, however, can largely be explained on epigenetic grounds. Food shortages due to group-living conditions, could have resulted in intensive resorption in this species, as this would have promoted the removal of vital minerals, like calcium and phosphorus, from bone for the maintenance of normal biological functions.
10. LAGs were well defined in *C. cataphractus*, while in *P. capensis*, LAGs were generally more inconspicuous in those individuals from the lowest altitudes than in those from higher latitudes. This could have been due to a more constant microclimate at lower latitudes, as LAGs generally appear strongly expressed under contrasting seasonality and or high altitudes. There was also great variation in the spatial arrangement of LAGs in both species, which could have been due to growth rate variation, directly related to the genetic program of each individual, or to individual competition for food. The latter explanation would have been a factor for *C. cataphractus* individuals due to their group-living behavior.
11. On the whole, *C. cataphractus* displayed a greater number of double LAGs than *P. capensis*. Double LAGs represent two periods of arrested growth in one growth cycle. This phenomenon is usually related to individual variation, pathologic injury or atypical periods of climatic conditions. Altitude has also been shown to play a major role in double LAG formation. The randomly distributed double LAGs in *P. capensis*, were most likely caused by the cool climates and high altitudes at which these lizards live. A lack of food may also induce periods of atypical arrested growth, and was most likely the cause of double LAG formation in *C. cataphractus*.
12. It is rarely possible to determine individual ages by simply counting LAGs present in bone

sections. There are many difficulties which effect age estimation such as the appearance of false LAGs, double LAGs, insufficient staining or indistinct LAGs, the position of the birth line and first LAG, and closely packed LAGs near the periosteum in the oldest individuals. In very old individuals, age may also be underestimated due the cessation of local osteogenesis some time before death.

13. The author doubts that bone growth had stopped in any of the oldest individuals investigated in this study, as this phenomenon is usually associated with lamellar or pseudolamellar bone inside the periosteum. These bone tissues are associated with very slow growth but were absent in the periosteal cortex of the oldest individuals.
14. The author speculates that both species have the potential for long life-spans, as the oldest individuals from both species had age estimates of between 11 and 13 years and still maintained an intermediate growth rate. Moreover, both species were estimated as reaching sexual maturity at relatively old ages, i.e. between four and seven years. Furthermore, both the *Cordylus* and *Pseudocordylus* genera display very effective anti-predator mechanisms, which could play an important role in influencing selection for greater longevity.
15. This study has provided insight into the bone histology, age, longevity and growth rates of *C. cataphractus* and *P. capensis*. This, however, needs to be expanded upon by looking at different populations and related species, which would provide valuable information regarding microevolution and scope of biological time within the Cordylidae as a whole.

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**APPENDIX 1. Locality data, collectors and collection dates for *Cordylus cataphractus* and *Pseudocordylus capensis*.**

*Cordylus cataphractus*

<b>Museum number</b>	<b>Locality</b>	<b>Locality co-ordinates</b>	<b>Altitude</b>	<b>Collectors and date of collection</b>
Usec-H3096	2 km from farm house	31°52'57"S 18°29'10"E	300 m	05 Dec. 1996 L. Mouton
Usec-H3080	Koppie on Lamberts Bay	31°50'13"S 18°26'22"E	226 m	24 Oct. 1996 L. Mouton
Usec-H3083	Koppie on Lamberts Bay	31°50'13"S 18°26'22"E	226 m	24 Oct. 1996 L. Mouton
Usec-H3083	2 km from farmhouse	31°52'57"S 18°29'10"E	300 m	05 Dec. 1996 L. Mouton
Usec-H3202	Klipfontein	31°59'57"S 18°29'10"E	270 m	02 April 1997 L. Mouton
Usec-H3160	Klipfontein	31°59'57"S 18°29'10"E	270 m	02 April 1997 L. Mouton
Usec-H3166	Klipfontein	31°59'57"S 18°29'10"E	270 m	02 April 1997 L. Mouton
Usec-H3130	Klipfontein	31°59'57"S 18°29'10"E	270 m	02 April 1997 L. Mouton
Usec-H3075	Koppie on Lamberts Bay	31°50'13"S 18°26'22"E	226 m	24 Oct. 1996 L. Mouton
Usec-H3148	Klipfontein	31°59'57"S 18°29'10"E	270 m	02 April 1997 L. Mouton
Usec-H3110	2 km from farmhouse	31°52'57"S 18°29'10"E	300 m	05 Dec. 1996 L. Mouton
Usec-H3149	Klipfontein	31°59'57"S 18°29'10"E	270 m	02 April 1997 L. Mouton
Usec-H3201	Klipfontein	31°59'57"S 18°29'10"E	270 m	02 April 1997 L. Mouton

Usec-H3101	2 km from farmhouse	31°52'57"S 18°29'10"E	300 m	05 Dec. 1996 L. Mouton
Usec-H3093	Koppie on Lamberts Bay	31°50'13"S 18°26'22"E	226 m	24 Oct. 1996 L. Mouton
Usec-H3135	Klipfontein	31°59'57"S 18°29'10"E	270 m	02 April 1997 L. Mouton
Usec-H3100	2 km from farmhouse	31°52'57"S 18°29'10"E	300 m	05 Dec. 1996 L. Mouton
Usec-H3129	Klipfontein	31°59'57"S 18°29'10"E	270 m	02 April 1997 L. Mouton
Usec-H3118	Klipfontein	31°59'57"S 18°29'10"E	270 m	02 April 1997 L. Mouton
Usec-H3147	Klipfontein	31°59'57"S 18°29'10"E	270 m	02 April 1997 L. Mouton
Usec-H3189	Klipfontein	31°59'57"S 18°29'10"E	270 m	02 April 1997 L. Mouton
Usec-H3143	Klipfontein	31°59'57"S 18°29'10"E	270 m	02 April 1997 L. Mouton
Usec-H3183	Klipfontein	31°59'57"S 18°29'10"E	270 m	02 April 1997 L. Mouton
Usec-H3105	2 km from farmhouse	31°52'57"S 18°29'10"E	300 m	05 Dec. 1996 L. Mouton
Usec-H3113	2 km from farmhouse	31°52'57"S 18°29'10"E	300 m	05 Dec. 1996 L. Mouton
Usec-H3175	Klipfontein	31°59'57"S 18°29'10"E	270 m	02 April 1997 L. Mouton
Usec-H3140	Klipfontein	31°59'57"S 18°29'10"E	270 m	02 April 1997 L. Mouton
Usec-H3122	Klipfontein	31°59'57"S 18°29'10"E	270 m	02 April 1997 L. Mouton
Usec-H3176	Klipfontein	31°59'57"S 18°29'10"E	270 m	02 April 1997 L. Mouton

Usec-H3167	Klipfontein	31°59'57"S 18°29'10"E	270 m	02 April 1997 L. Mouton
Usec-H1090	De Meul, Ionkiespoort	32°49'03"S 19°27'58"E	1067 m	14 April 1985 Mouton and Mostert
Usec-H3186	Klipfontein	31°59'57"S 18°29'10"E	270 m	02 April 1997 L. Mouton
Usec-H3091	Koppie on Lamberts Bay	31°50'13"S 18°26'22"E	226 m	24 Oct. 1996 L. Mouton
Usec-H3165	Klipfontein	31°59'57"S 18°29'10"E	270 m	02 April 1997 L. Mouton
Usec-H3124	Klipfontein	31°59'57"S 18°29'10"E	270 m	02 April 1997 L. Mouton
Usec-H3076	Koppie on Lamberts Bay	31°50'13"S 18°26'22"E	226 m	24 Oct. 1996 L. Mouton
Usec-H3153	Klipfontein	31°59'57"S 18°29'10"E	270 m	02 April 1997 L. Mouton
Usec-H3088	Koppie on Lamberts Bay	31°50'13"S 18°26'22"E	226 m	24 Oct. 1996 L. Mouton
Usec-H3108	2 km from farmhouse	31°52'57"S 18°29'10"E	300 m	05 Dec. 1996 L. Mouton
Usec-H3139	Klipfontein	31°59'57"S 18°29'10"E	270 m	02 April 1997 L. Mouton
Usec-H3196	Klipfontein	31°59'57"S 18°29'10"E	270 m	02 April 1997 L. Mouton
Usec-H3073	Koppie on Lamberts Bay	31°50'13"S 18°26'22"E	226 m	24 Oct. 1996 L. Mouton
Usec-H3084	Koppie on Lamberts Bay	31°50'13"S 18°26'22"E	226 m	24 Oct. 1996 L. Mouton
Usec-H1067	Eendekuil, Piekenierskloof	32°37'21"S 19°27'58"E	980 m	10 March 1985 Mouton and Mostert
Usec-H3089	Koppie on Lamberts Bay	31°50'13"S 18°26'22"E	226 m	24 Oct. 1996 L. Mouton



*Pseudocordylus capensis*

<b>Museum number</b>	<b>Locality</b>	<b>Locality co-ordinates</b>	<b>Altitude</b>	<b>Collectors</b>
Usec-H1738	Dehoop (waterfall)	31°33'16"S 19°07'38"E	660 m	02 Dec. 1988 Mouton and Van Wyk
Usec-H2623	Grootvaderbosch	33°56'16"S 20°51'35"E	1060	30 April 1994 Mouton
Usec-H1475	Seekoeivlei	32°07'45"S 18°44'02"E	360 m	25 March 1986 Mouton and Mostert
Usec-H1476	Seekoeivlei	32°07'45"S 18°44'02"E	360 m	25 March 1986 Mouton and Mostert
Usec-H2070	Kamanasieberge	33°36'21"S 22°52'42"E	1576 m	26 March 1990 Herselman
Usec-H1786	Zuurvlakte	32°46'19"S 19°30'44"E	760 m	07 April 1989 Mouton
Usec-H2225	Visgat, Skurweberge	33°05'08"S 19°14'02"E	800 m	16 Feb. 1991 Mouton and Herselman
Usec-H816	Keurbosberg	32°40'05"S 18°45'04"E	455 m	18 Nov. 1984 Mouton and Mostert
Usec-H1740	Dehoop (Waterval)	31°33'16"S 19°07'38"E	660 m	02 Dec. 1988 Mouton and Van Wyk
Usec-H2067	Kamanasieberge	33°36'21"S 22°52'42"E	1576 m	26 March 1990 Herselman
Usec-H875	Langvlei, Jonaskop	33°58'19"S 19°30'35"E	1640 m	29 Nov. 1984 Mouton and Mostert
Usec-H2224	Visgat, Skurweberge	33°05'08"S 19°14'02"E	800 m	16 Feb. 1991 Mouton and Herselman
Usec-H1478	Seekoeivlei	32°07'45"S 18°44'02"E	360 m	25 March 1986 Mouton and Mostert
Usec-H1474	Seekoeivlei	32°07'45"S 18°44'02"E	360 m	25 March 1986 Mouton and Mostert



Usec-H941	Langvlei, Jonaskop	33°58'19"S 19°30'35"E	1640 m	29 Nov. 1984 Mouton and Mostert
Usec-H1477	Seekoeivlei	32°07'45"S 18°44'02"E	360 m	25 March 1986 Mouton and Mostert
Usec-H817	Keurbosberg	32°40'05"S 18°45'04"E	455 m	18 Nov. 1984 Mouton and Mostert
Usec-H2054	Robinson pass, Jonkersberg	33°52'53"S 22°01'30"E	900 m	25 March 1990 Herselman
Usec-H932	Sneeuberghut, Wuppertal	32°29'13"S 19°10'30"E	1340 m	18 Oct. 1984 Botha
Usec-H2068	Kamanasieberge	33°36'21"S 22°52'42"E	1576 m	26 March 1990 Herselman
Usec-H2081	Kamanasieberge	33°36'21"S 22°52'42"E	1576 m	26 March 1990 Herselman
Usec-H1473	Seekoeivlei	32°07'45"S 18°44'02"E	360 m	25 March 1986 Mouton and Mostert
Usec-H942	Langvlei, Jonaskop	33°58'19"S 19°30'35"E	1640 m	29 Nov. 1984 Mouton and Mostert
Usec-H2069	Kamanasieberge	33°36'21"S 22°52'42"E	1576 m	26 March 1990 Herselman
Usec-H3216	Berghut, Landdroskop	34°03'00"S 19°00'22"E	1133 m	06 Nov. 1998 Mouton and Searby
Usec-H3217	Berghut, Landdroskop	34°03'00"S 19°00'22"E	1133 m	06 Nov. 1998 Mouton and Searby
Usec-H3218	Berghut, Landdroskop	34°03'00"S 19°00'22"E	1133 m	06 Nov. 1998 Mouton and Searby
Usec-H3213	Berghut, Landdroskop	34°03'00"S 19°00'22"E	1133 m	06 Nov. 1998 Mouton and Searby
Usec-H1400	Berghut, Landdroskop	34°03'00"S 19°00'22"E	1133 m	07 Dec. 1986 Cronje

Usec-H3215	Berghut, Landdrooskop	34°03'00"S 19°00'22"E	1133 m	06 Nov. 1998 Mouton and Searby
Usec-H3206	Berghut, Landdrooskop	34°03'00"S 19°00'22"E	1133 m	06 Nov. 1998 Mouton and Searby
Usec-H3209	Berghut, Landdrooskop	34°03'00"S 19°00'22"E	1133 m	06 Nov. 1998 Mouton and Searby
Usec-H609	Berghut, Landdrooskop	34°03'00"S 19°00'22"E	1133 m	08 Jan. 1982 Mouton
Usec-H3205	Berghut, Landdrooskop	34°03'00"S 19°00'22"E	1133 m	06 Nov. 1998 Mouton and Searby
Usec-H3208	Berghut, Landdrooskop	34°03'00"S 19°00'22"E	1133 m	06 Nov. 1998 Mouton and Searby
Usec-H3210	Berghut, Landdrooskop	34°03'00"S 19°00'22"E	1133 m	06 Nov. 1998 Mouton and Searby
Usec-H3207	Berghut, Landdrooskop	34°03'00"S 19°00'22"E	1133 m	06 Nov. 1998 Mouton and Searby
Usec-H2619	Berghut, Landdrooskop	34°03'00"S 19°00'22"E	1133 m	19 April. 1994 Mouton
Usec-H3211	Berghut, Landdrooskop	34°03'00"S 19°00'22"E	1133 m	06 Nov. 1998 Mouton and Searby
Usec-H3212	Berghut, Landdrooskop	34°03'00"S 19°00'22"E	1133 m	06 Nov. 1998 Mouton and Searby
Usec-H3220	Berghut, Landdrooskop	34°03'00"S 19°00'22"E	1133 m	06 Nov. 1998 Mouton and Searby
Usec-H3219	Berghut, Landdrooskop	34°03'00"S 19°00'22"E	1133 m	06 Nov. 1998 Mouton and Searby
Usec-H3214	Berghut, Landdrooskop	34°03'00"S 19°00'22"E	1133 m	06 Nov. 1998 Mouton and Searby

**APPENDIX 2. Standard macroscopic measurements of the femur and body size of juvenile and adult males and females of *Cordylus cataphractus* and *Pseudocordylus capensis*.**

*Cordylus cataphractus*

Specimen number	Sex (m/f)	Ontogenetic status	Snout-vent length (mm)	Femur length (mm)	Proximal width (mm)	Mid-shaft width (mm)	Distal width (mm)
Usec-H3096	M	sexually mature adult	122.90	19.01	4.01	1.41	3.72
Usec-H3080	M	sexually mature adult	119.64	19.83	4.03	1.42	3.81
Usec-H3083	M	sexually mature adult	117.32	19.15	3.66	1.43	3.52
Usec-H3083	M	sexually mature adult	115.38	19.17	4.15	1.42	3.72
Usec-H3202	M	sexually mature adult	112.71	19.23	3.62	1.33	3.45
Usec-H3160	M	sexually mature adult	111.31	18.16	3.44	1.21	3.31
Usec-H3166	M	sexually mature adult	110.78	18.47	3.44	1.23	3.21
Usec-H3130	M	sexually mature adult	107.01	17.73	3.31	1.25	3.26
Usec-H3075	M	sexually mature adult	104.01	17.53	3.33	1.24	3.26
Usec-H3148	M	sexually mature adult	96.94	16.21	3.20	1.28	3.15
Usec-H3110	F	sexually mature adult	115.48	18.20	3.73	1.34	3.64
Usec-H3149	F	sexually mature adult, pregnant	114.01	18.48	3.73	1.32	3.21
Usec-H3201	F	Sexually mature adult, pregnant	111.61	16.85	3.46	1.25	3.32
Usec-H3101	F	sexually mature adult	109.53	18.22	3.52	1.20	3.31
Usec-H3093	F	sexually mature adult	107.91	16.02	3.33	1.22	3.30
Usec-H3135	F	sexually mature adult, pregnant	105.22	17.16	3.38	1.20	3.25
Usec-H3100	F	sexually mature adult	103.85	16.84	3.35	1.21	3.06



Usec-H3129	F	sexually mature adult, pregnant	103.92	16.27	3.33	1.22	3.03
Usec-H3118	F	sexually mature adult, pregnant	101.38	16.83	3.31	1.21	3.10
Usec-H3147	F	sexually mature adult,	99.14	16.76	3.15	1.20	3.04
Usec-H3189	F	sexually mature adult	98.67	15.93	3.13	1.18	3.04
Usec-H3143	F	sexually mature adult	96.85	15.09	3.08	1.15	2.09
Usec-H3183	M	Juvenile	94.98	16.08	3.25	1.27	3.09
Usec-H3105	M	Juvenile	91.83	16.05	3.18	1.30	3.07
Usec-H3113	M	Juvenile	91.60	15.47	3.21	1.19	3.07
Usec-H3175	M	Juvenile	89.10	15.25	3.22	1.21	3.04
Usec-H3140	M	Juvenile	88.03	14.29	2.87	1.21	2.47
Usec-H3122	M	Juvenile	83.33	13.48	3.10	1.15	2.41
Usec-H3176	M	Juvenile	79.10	13.82	3.07	1.08	2.82
Usec-H3167	M	Juvenile	72.10	12.55	2.56	1.05	2.25
Usec-H1090	M	Juvenile	69.78	11.12	2.22	0.91	2.06
Usec-H3186	M	Juvenile	68.77	11.27	2.25	1.04	2.22
Usec-H3091	M	Juvenile	68.58	11.62	2.08	0.84	2.03
Usec-H3165	M	Juvenile	58.27	11.71	2.22	0.88	2.13
Usec-H3124	F	Juvenile	94.50	14.86	3.18	1.27	2.87
Usec-H3076	F	Juvenile	92.24	14.53	2.84	1.19	2.85
Usec-H3153	F	Juvenile	90.14	14.61	3.20	1.18	3.04
Usec-H3088	F	Juvenile	88.76	14.05	2.92	1.18	2.83
Usec-H3108	F	Juvenile	87.44	14.15	2.86	1.41	2.81
Usec-H3139	F	Juvenile	86.58	14.60	3.10	1.27	3.05



Usec-H3196	F	Juvenile	84.24	14.52	3.05	1.25	2.81
Usec-H3073	F	Juvenile	83.58	13.46	3.03	1.16	2.55
Usec-H3084	F	Juvenile	79.27	12.07	2.55	1.13	2.52
Usec-H1067	F	Juvenile	75.08	11.78	2.52	1.08	2.32
Usec-H3089	F	Juvenile	74.09	12.02	2.46	1.08	2.41

*Pseudocordylus capensis*

Specimen number	Sex (m/f)	Ontogenetic status	Snout vent length (mm)	Femur length (mm)	Proximal width (mm)	Mid-shaft width (mm)	Distal width (mm)
Usec-H1738	M	Sexually mature adult	104.16	22.85	3.46	1.83	3.24
Usec-H2623	M	Sexually mature adult	101.75	22.07	3.28	1.19	3.06
Usec-H1475	M	Sexually mature adult	96.62	22.03	3.22	1.21	3.04
Usec-H1476	M	Sexually mature adult	95.59	21.88	3.22	1.20	2.95
Usec-H2070	M	Sexually mature adult	95.40	24.53	3.71	1.40	3.36
Usec-H1786	M	Sexually mature adult	93.60	20.55	2.93	1.19	2.87
Usec-H2225	M	Sexually mature adult	93.26	20.18	3.01	1.31	2.83
Usec-H816	M	Sexually mature adult	90.76	20.75	3.10	1.25	3.07
Usec-H1740	M	Sexually mature adult	85.82	20.45	2.97	1.19	2.88
Usec-H2067	M	Sexually mature adult	84.09	18.87	2.94	1.15	2.85
Usec-H875	F	Sexually mature adult	94.41	21.52	3.25	1.24	2.96
Usec-H2224	F	Sexually mature adult	94.26	20.28	3.25	1.18	3.05
Usec-H1478	F	Sexually mature adult	92.31	21.81	3.22	1.21	2.91
Usec-H1474	F	Sexually mature adult	89.50	20.09	3.17	1.20	2.88

Usec-H941	F	Sexually mature adult	88.46	18.08	3.21	1.30	2.92
Usec-H1477	F	Sexually mature adult	85.81	19.01	3.04	1.20	2.90
Usec-H817	F	Sexually mature adult	85.13	19.82	3.02	1.22	2.92
Usec-H2054	F	Sexually mature adult	83.10	17.94	2.92	1.17	2.78
Usec-H932	M	Juvenile	67.84	15.80	2.25	1.36	2.41
Usec-H2068	M	Juvenile	60.46	12.74	2.17	0.88	2.04
Usec-H2081	M	Juvenile	43.78	9.09	1.23	0.65	1.34
Usec-H1473	F	Juvenile	66.99	14.58	2.18	0.86	1.93
Usec-H942	F	Juvenile	44.02	9.63	1.44	0.67	1.38
Usec-H2069	F	Juvenile	42.74	8.42	1.04	0.50	0.91
Usec-H3216	M	Sexually mature adult	99.22	20.97	3.25	1.16	3.13
Usec-H3217	M	Sexually mature adult	98.83	21.90	3.72	1.24	3.56
Usec-H3218	M	Sexually mature adult	98.18	20.04	3.28	1.09	3.16
Usec-H3213	M	Sexually mature adult	95.66	20.28	3.44	1.19	3.26
Usec-H1400	M	Sexually mature adult	91.96	20.82	3.03	1.28	2.91
Usec-H3215	M	Sexually mature adult	90.32	18.48	3.28	1.15	3.13
Usec-H3206	F	Sexually mature adult	107.83	21.94	3.17	1.22	3.14
Usec-H3209	F	Sexually mature adult	105.11	20.80	3.17	1.15	3.04
Usec-H609	F	Sexually mature adult	103.63	22.38	3.41	1.35	3.17
Usec-H3205	F	Sexually mature adult	103.18	20.23	3.31	1.16	2.90
Usec-H3208	F	Sexually mature adult	101.84	20.76	3.40	1.21	2.93
Usec-H3210	F	Sexually mature adult	100.79	20.58	3.38	1.19	3.01
Usec-H3207	F	Sexually mature adult	100.77	20.21	3.34	1.16	2.94

Usec-H2619	F	Sexually mature adult	97.43	20.47	3.36	1.29	3.09
Usec-H3211	F	Juvenile	86.32	18.05	2.72	1.11	2.70
Usec-H3212	F	Juvenile	73.29	15.20	2.57	0.83	2.41
Usec-H3220	M	Juvenile	50.67	9.78	2.07	0.62	1.76
Usec-H3219	M	Juvenile	47.42	9.44	1.66	0.58	1.61
Usec-H3214	M	Juvenile	46.79	10.56	2.11	0.68	1.96



**APPENDIX 3. Microscopic measurements of transverse sections through the left femurs of juvenile and adult males and females of *Cordylus cataphractus* and *Pseudocordylus capensis*.**

*Cordylus cataphractus*

Specimen	Sex	Relative Bone Thickness (%)	Medullary cavity area (mm <sup>2</sup> )	Total bone area (mm <sup>2</sup> )	Bone wall area (mm <sup>2</sup> )
Usec-H3096	M	45	0.57	1.32	0.75
Usec-H3080	M	49	0.44	1.18	0.74
Usec-H3083	M	38	0.56	1.10	0.55
Usec-H3083	M				
Usec-H3202	M	36	0.45	0.93	0.48
Usec-H3160	M	40	0.51	1.01	0.50
Usec-H3166	M	41			
Usec-H3130	M	29	0.49	0.84	0.34
Usec-H3075	M	30	0.56	0.98	0.42
Usec-H3148	F	41	0.58	0.79	0.43
Usec-H3110	F	37	0.56	1.10	0.53
Usec-H3149	F		0.37	0.75	0.39
Usec-H3201	F	36	0.41		
Usec-H3101	F	38	0.38	0.80	0.42
Usec-H3093	F	37	0.56	1.08	0.51
Usec-H3135	F	39	0.38	0.76	0.38
Usec-H3100	F	35	0.41	0.80	0.38
Usec-H3129	F	33	0.42	0.80	0.37
Usec-H3118	F	27	0.53	0.87	0.34
Usec-H3147	F	28	0.29	0.49	0.20
Usec-H3189	F	26	0.47	0.78	0.31
Usec-H3143	M	30		0.52	
Usec-H3183	M	27	0.53	0.84	0.31
Usec-H3105	M	31		0.46	
Usec-H3113	M	25	0.47	0.73	0.28
Usec-H3175	M	25		0.68	
Usec-H3140	M	26	0.36	0.62	0.27
Usec-H3122	M	22	0.29	0.47	0.16
Usec-H3176	M	26		0.46	
Usec-H3167	M				
Usec-H1090	M	18	0.41	0.57	0.16
Usec-H3186	M				
Usec-H3091	M	31	0.12	0.22	0.10



Usec-H3165	F	24	0.51	0.81	0.30
Usec-H3124	F	32	0.31	0.57	0.26
Usec-H3076	F	25	0.48	0.75	0.28
Usec-H3153	F	21	0.49	0.71	0.22
Usec-H3088	F	25	0.29	0.44	0.15
Usec-H3108	F	22	0.41	0.61	0.21
Usec-H3139	F	22	0.39	0.61	0.21
Usec-H3196	F	25			
Usec-H3073	F	32	0.34	0.62	0.28
Usec-H3084	F	33	0.33	0.65	0.32
Usec-H1067	F	22	0.46	0.69	0.23
Usec-H3089	F				

*Pseudocordylus capensis*

Specimen	Sex	Relative bone thickness (%)	Medullary cavity area (mm <sup>2</sup> )	Total bone area (mm <sup>2</sup> )	Bone wall area (mm <sup>2</sup> )
Usec-H1738	M	28	0.72	1.21	0.49
Usec-H2623	M	26	0.62	1.02	0.40
Usec-H1475	M	33	0.56	1.03	0.47
Usec-H1476	M	39	0.51	1.01	0.50
Usec-H2070	M	30	0.45	0.75	0.30
Usec-H1786	M	34	0.44	0.89	0.35
Usec-H2225	M	31	0.55	0.88	0.33
Usec-H816	M				
Usec-H1740	M	33	0.36	0.85	0.49
Usec-H2067	M	33	0.33	0.66	0.30
Usec-H875	M				
Usec-H2224	F	31	0.33	0.63	0.30
Usec-H1478	F	28	0.49	0.72	0.35
Usec-H1474	F	36	0.45	0.87	0.42
Usec-H941	F	36	0.53	0.81	0.28
Usec-H1477	F	39	0.36	0.71	0.35
Usec-H817	F	36	0.31	0.68	0.37
Usec-H2054	F	30	0.34	0.55	0.21
Usec-H932	F				
Usec-H2068	F	12	0.19	0.38	0.19
Usec-H2081	F	09			
Usec-H1473	M	13	0.26	0.48	0.22
Usec-H942	M				
Usec-H2069	M	11			
Usec-H3216	M	24	0.44	1.04	0.60
Usec-H3217	M	24	0.56	1.42	0.86

Usec-H3218	M	25	0.53	1.27	0.74
Usec-H3213	M	18	0.63	1.16	0.53
Usec-H1400	M	39	0.31	0.63	0.32
Usec-H3215	M	20	0.60	0.89	0.29
Usec-H3206	F	31	0.21	0.57	0.36
Usec-H3209	F	22			
Usec-H609	F	32	0.49	0.86	0.37
Usec-H3205	F	23			
Usec-H3208	F	21	0.55	0.87	0.32
Usec-H3210	F	16	0.76	1.04	0.28
Usec-H3207	F	24			
Usec-H2619	F	30	0.49	0.72	0.37
Usec-H3211	F	14	0.55	0.72	0.21
Usec-H3212	F	19	0.07	0.16	0.09
Usec-H3220	M	14	0.13	0.19	0.06
Usec-H3219	M	15	0.11	0.18	0.07
Usec-H3214	M	12	0.14	0.19	0.05

**APPENDIX 4. The number of LAGs resorped in relation to medullary cavity area (MCA) and snout-vent length (SVL) in *Cordylus cataphractus* and *Pseudocordylus capensis* adults and juveniles**

**Cordylus cataphractus**

Individual	SVL (mm)	MCA (mm <sup>2</sup> )	MCA as a % of TBA	Visible LAGs	LAGs possibly resorped
Adult male	122.90	0.57	43	9	4?
Adult male	119.64	0.44	37	9	3?
Adult male	117.32	0.56	51	7	3?
Adult male	115.38	0.50		7	3
Adult male	112.71	0.45	48	5	4
Adult male	110.78	0.51	51	4	Remodeling
Adult male	107.01			3	Damaged
Adult male	104.01	0.49	58	4	3
Adult male	96.94	0.56	57	4	2
Adult female	115.48	0.36	73	8	1
Adult female (pregnant)	114.01	0.58	51	7	4?
Adult female (pregnant)	111.61	0.37	41	6	3
Adult female	109.53	0.41		8	3
Adult female	107.91	0.38	48	7	2
Adult female (pregnant)	105.22	0.38	52	6	Birth line
Adult female (pregnant)	103.85	0.42	50	6	2
Adult female (pregnant)	103.92	0.42	51	6	2
Adult female (pregnant)	101.38	0.56	53	6	4?
Adult female	99.14	0.53	61	5	3
Adult female	98.67	0.29	59	1	Birth line
Adult female	96.85	0.47	60	5	3
Juvenile male	94.98	0.30		3	Birth line
Juvenile male	91.83	0.53		2	4?
Juvenile male	91.60	0.33		3	Birth line
Juvenile male	89.10	0.47		2	Remodeling
Juvenile male	88.03	0.50		2	2
Juvenile male	83.33	0.36		3	1
Juvenile male	79.10	0.29		2	1
Juvenile male	72.10	0.32		2	Birth line
Juvenile male	69.78			1	Damaged



Juvenile male	68.77	0.41	1	1
Juvenile male	68.58		2	Damaged
Juvenile male	58.27	0.12	0	No LAGs
Juvenile female	94.50	0.51	4	3
Juvenile female	92.24	0.31	3	Birth line
Juvenile female	90.14	0.48	2	2
Juvenile female	88.76	0.49	2	3
Juvenile female	87.44	0.29	2	Birth line
Juvenile female	86.58	0.41	1	2
Juvenile female	84.24	0.39	2	2
Juvenile female	83.58	0.34	2	Birth line/1?
Juvenile female	79.27	0.34	2	1 / 2?
Juvenile female	75.08	0.46	3	2
Juvenile female	74.09	0.33	3	Birth line

*Pseudocordylus capensis* (Landdroskop)

Individual	SVL (mm)	MCA (mm <sup>2</sup> )	MCA as a % of TBA	Visible LAGs	LAGs possibly resorped
Adult male	99.22	0.44	39	7	Birth line
Adult male	98.83	0.56	42	6	3
Adult male	98.18	0.53	58	6	3
Adult male	95.66	0.63	49	6	2
Adult male	91.96	0.33	67	5	None
Adult male	90.32	0.55	37	5	3
Adult female	107.83	0.53	63	8	2
Adult female	105.11			6	Damaged
Adult female	103.63	0.57	61	10	1
Adult female	103.18			7	Damaged
Adult female	101.84	0.52	55	6	Birth line
Adult female	100.79	0.76	73	7	1
Adult female	100.77			4	Damaged
Adult female	97.43	0.48	76	5	None
Juvenile male	50.67	0.13		0	None
Juvenile male	47.42	0.11		0	None
Juvenile male	46.79	0.15		0	None
Juvenile female	86.32	0.58		3	Birth line
Juvenile female	73.29			1	Damaged