Verification of von Ebner incremental lines in extant and fossil archosaur dentine and tooth replacement rate assessment using counts of von Ebner lines

by Gregory Mark Erickson

A thesis submitted in partial fulfillment of the requirements for the degree of Master of Science in Biological Sciences
Montana State University

Abstract:
Upon finding dentinal incremental lines in fossil archosaur thin sections in the Winter of 1990, a study was undertaken to see if their periodicity of deposition could be determined. It was felt that a method of determining tooth replacement rates could be developed should this periodicity be ascertained.

Using thin sections it was found that teeth from similar sized extant and extinct crocodilians exhibited similar sized incremental lines (average 14 and 16 microns respectively).

Through the use of interperitoneal injections of tetracycline and calcein (flourochrome markers) it was determined that modern archosaurs (Alligator mississippiensis) were depositing incremental lines on a daily basis (von Ebner incremental lines). It was presumed that the fossil crocodilian increments were similarly deposited on a daily basis.

By counting the number of von Ebner incremental lines in a functional alligator tooth and subtracting the number found in the respective replacement tooth it was suspected that the replacement rate could be determined. Theoretical replacement rates were determined in this manner for a growth series of alligators (0.60-3.2 meter lengths). To test the methodology 2(0.85 meter) captive alligators were monitored for tooth replacement rates for 10 months. The average rate of replacement was found to be approximately 120 days. Similar sized alligators from the growth series that were incrementally assessed for replacement rates showed an average of 109 days.

The von Ebner increments in alligators were found to increase in width with tooth size (range 4-16 microns) and the rate of tooth replacement was determined to slow with increased animal size (range 83-260 days).

Thin sections from 11 species of fossil archosaurs revealed that they had incremental lines with similar average widths (11-20 microns) as those confirmed to be of daily deposition in the extant alligators.

By using the tooth replacement methodology using incremental line counts developed for alligators in this study the average tooth replacement rates were determined for the fossil archosaurians (range 46-777 days).

Like the alligator data the fossil archosaurs also exhibited an increase in incremental line widths and a slower replacement rate with increased tooth size.

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This thesis has been read by each member of the thesis committee and has been found to be satisactory regarding content, English usage, format, citations, bibliographic style, and consistency, and is ready for submission to the College of Graduate Studies.

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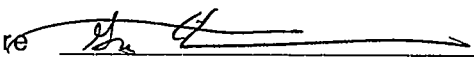
Signature 

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ABSTRACT

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INTRODUCTION

Histologic research of tooth dentine in the past has been focused primarily on the dentition of mammals. Human dentine in particular has received the majority of this attention. One of the more notable features found in tooth dentine as a result of these studies is the presence of incremental lines striating throughout thin sectioned teeth. One of the earliest, if not the first report of dentinal increments came from Owen (1840). Owen found incremental structures (sensu contour lines of Owen) with thicknesses approximating 0.025 centimeters. Contour lines of Owen are believed to be the result of mineral deficiencies experienced by the individual as recorded in the dentine (Ten Cate 1985). Von Ebner (1922) found increments (sensu von Ebner) as small as 18-20 microns apart recorded in the dentine of mammal teeth. Schour and Hoffman (1939a) reported that incremental lines (presumably von Ebner increments) averaged 16 microns in all taxa. Later that same year these researchers (Schour and Hoffman,1939b), through periodic injections of sodium flouride and alizarin Red S (chemical dentinal markers), determined that cats, dogs, and many rodents deposit approximately 16 microns of dentine daily. Their data suggests that the dentinal increments (sensu von Ebner) are the result of daily deposition in tooth development.

Relatively few studies of dentine in reptiles have been undertaken. In particular data on archosaurian dentine (the focus of this study) are even sparser. Some of the more notable works are by Owen (1840), Kvam (1958), Kaye (1966). Interestingly both extant and fossil archosaurs exhibit incremental lines bearing similarity to the daily increments reported by Schour and Hoffman(1939a,b). Schour and Hoffman (1939a), Kaye (1966), and Kariyama et. al. (1969) all reported increments in extant alligators, but none confirmed a rate of deposition. Johnston (1979) also presumably found archosaurian dentinal increments. He believed that he had found contour lines of Owen in fossil archosaurs, but made no mention of von Ebner increments.

Tooth development and replacement rate studies, not unlike reptile histology studies, are scarce. Edmund (1962,1969) reported on the development time and shedding rates of infant alligators (*Alligator mississippiensis*), iguanas (*Iguana iguana*), gila monsters (*Heloderma suspectum*), and varanids (*Varanus bengalesis*). Kaye (1966) gave replacement rates for infant caimans (*Caiman sclerops*). Westergaard and Ferguson (1990) reported on the rates of tooth replacement in newborn alligators (*Alligator mississippiensis*).

Knowledge of tooth replacement rates could have important implications for interpreting fossil faunal evidence. Incorporating data such as ontogenetic variance and interspecific differences in tooth shedding with counts of shed teeth from geologic formations could perhaps serve as unbiased indicators of biodiversity, and population structure, as well as predator/prey ratios. Interspecific differences in shedding rates of teeth may also help to explain differences in dentitional morphology as it relates to diet (i.e. quick replacement of teeth might suggest extreme wear and an abrasive foodstuff). A better understanding of incremental lines may also help to assess physiological disruptions and environmental influences experienced by the tooth bearer.

In the winter of 1990, while looking at thin sections in the histology comparative collection housed at the Museum of the Rockies (Montana State University), incremental structures were observed in the dentine of a carnosaur tooth. These increments were found to exhibit widths averaging in the tens of microns when measured with a slide-mounted micrometer. Examination of other prepared thin sections of fossil archosaurs (a nodosaur, hadrosaur, and a crocodile) revealed incremental lines of similar magnitude. Thus, an examination was begun in hopes that the periodicity of dentinal increment deposition might be determined in extinct archosaurs. It was suspected that these incremental lines represented daily deposition, but previous research had confirmed this only in mammal teeth (Shour and Hoffman, 1939b).

It was reasoned that a method of tooth replacement rate assessment might be developed using incremental line counts should their rate of deposition be ascertained. By knowing the rate at which increments are formed, it is theorized that total time of a tooth's formation can be determined simply by counting the number of deposited increments. In a similar fashion, replacement rates may also be assessed by differences in the incremental counts between functional and replacement teeth, the latter being less developed (i.e. less increments). As a model for archosaurian dentitions, growth series of extant alligators (*Alligator mississippiensis* and *Caiman sclerops*) were analyzed. To establish expected replacement rates, several captive alligators were periodically assessed for tooth shedding over a 10-month span.

METHODS

Establishing the Model: Testing for Similarity of Dentinal Increment Patterns Between Modern and Fossil Archosaurs.

Since the only extant toothed archosaurs are the crocodilians, it was conjectured that they might serve as suitable models for explaining the deposition of incremental lines in the dentine of fossil archosaurs. Accordingly a comparison of fossil and modern crocodilian dentine was undertaken. It was hypothesized that if similar incremental structures were found in both types of teeth that this would provide evidence that the fossil increments were indeed structural features of the tooth and not products of postmortem diagenesis. Furthermore, if the "model" extant crocodilian teeth have similar incremental structures in both size and form to those found in the fossil crocodilian dentine, it could be assumed that the fossil increments were probably deposited in a similar time frame (presumably daily incremental deposition). Substantiating the rate of increment deposition in extant crocodilians would provide the "model" rate.

The fossil crocodile teeth selected for this phase of the study were from the paleontology collection of the Museum of the Rockies (Montana State University). Two teeth were chosen; both had been shed (most of the root having been resorbed in vivo) and were approximately 2.5 centimeters in height. The teeth were from the Cloverly and Hell Creek Formations respectively, Cretaceous Period. The species types were not determinable. Both were found by the museum staff in Montana, U.S.A.

Each individual tooth was embedded in a clear plastic. A 0.5-1 millimeter thin section was made sagittally through the apex of the tooth using a diamond-bladed lapidary saw. The thin section was mounted on a microscope slide using an epoxy glue. The thin section now mounted on the slide then was sanded down to a 40-100 micron thickness using descending grits of sandpaper (60-600 grit), using water as a lubricant. Subsequent to sanding, the thin section was polished on a felt cloth using wet aluminum oxide powder. The thin section was viewed under a light microscope with a polarizing filter at 40-400 objectives. Dentinal increments were readily visible by this methodology.

Next, several similar sized teeth (selected to avoid possible ontogenetic differences), were extracted from a 3.2 meter length alligator specimen (*Alligator mississippiensis*) belonging to the Museum of the Rockies (Montana State University)

comparative collection. These teeth were similarily prepared for microscopic viewing. The only change in methodology was the use of 70% ethanol in place of water at all applicable steps. Water often causes the shrinkage of non-fossilized dentinal tissues.

A comparison of the dentine of the fossil and extant dentine was performed both visually and by comparison of incremental widths. The visual comparison stressed similarity in increment trajectories as well as increment contrast. The average widths of the incremental lines in each tooth were found by using the width measurements attained by an image analysis computer program (IM300A version 3.0, Analytical Imaging Concepts, Irvine, California) with a microscope objective setting of 40, 100, or 400x. Groups of increments, usually between 5 and 15 (depending on increment size and visibility) at a time, were measured perpendicular to their deposition plane (Figure 1). The number of increments in the field of view in the objective were divided by their respective distance spanned, thereby giving the mean for that particular section of dentine (Figure 1). The means of the increments throughout the tooth were averaged to give an idea of the whole tooth's mean increment size. Averaging of the increments was necessary due to individual variation in increment widths. Sections of some teeth did not show good incremental structure, probably due to non-uniform sanding of the thin section. If a section is too thick light doesn't penetrate it sufficiently for proper increment differentiation. In these cases, the total distance across the unknown span of dentine was divided by the mean increment width of the dentine in which the lines were visible. Rarely did unknown spans exceed 20% of the total dentine cross section for any of the thin sections made in this study. It should be noted that on occasion incremental lines were found that far exceeded the width (usually double) found in the adjacent dentine. In these instances, the increment was followed along the entire expanse of the tooth to see if the accentuation was simply just two or more incremental lines whose boundaries were not well delineated. In almost all instances this was found to be the case. If the seemingly extra wide increments were not proven to be two or more increments, then the extra wide line was simply included into the mean calculation as a single increment.

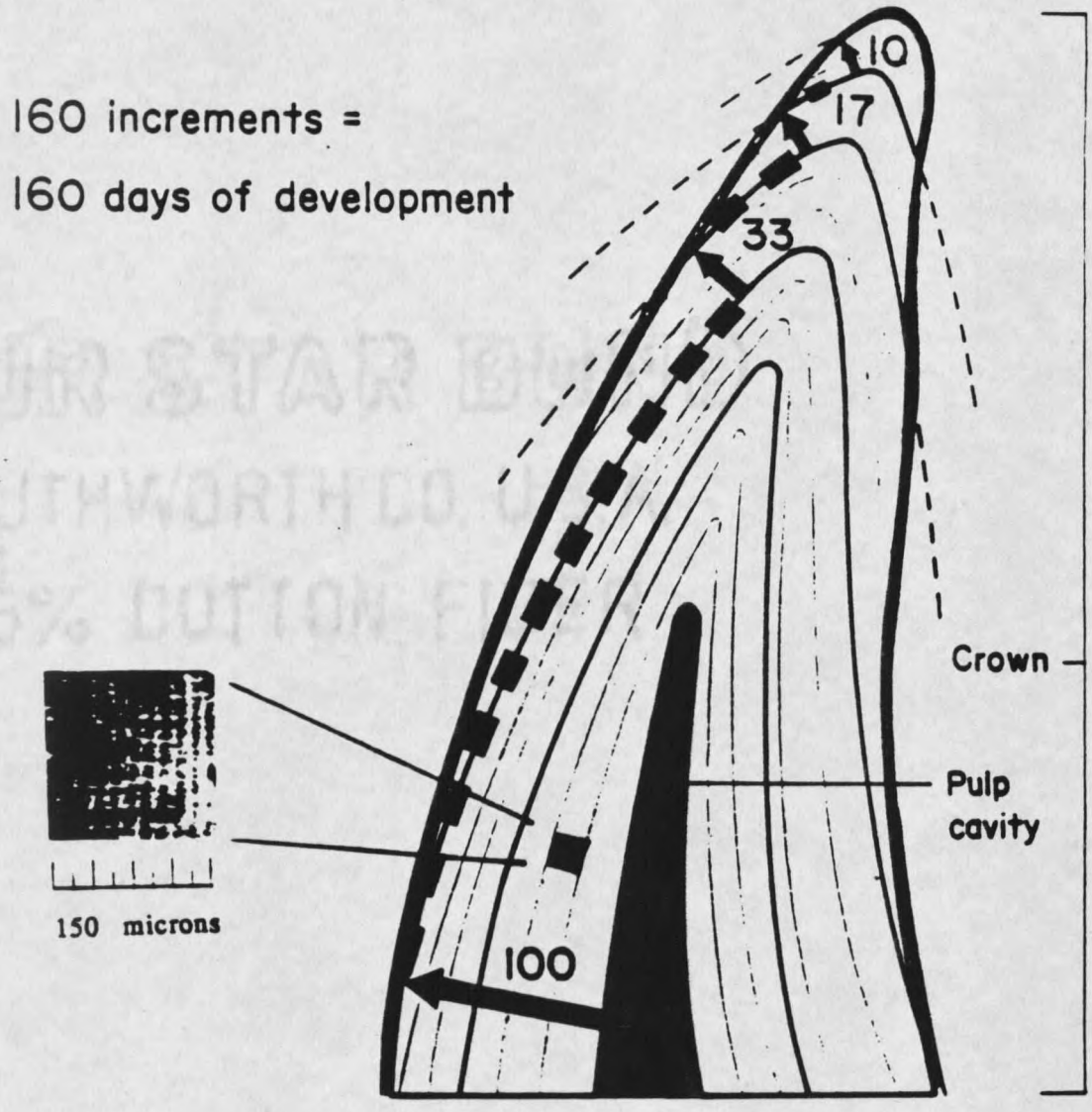

Figure 1- **Sagittal Section of a Typical Archosaur Tooth (faint lines indicate incremental line trajectories; counts are made from the pulp cavity to the tooth exterior where an incremental line is followed toward the coronal end of the tooth [rectangular boxes], the process is repeated until all increments are recorded).**

Calibrating the Model: Determining the Rate of Dentinal Increment Deposition in Modern Archosaurs.

In April of 1991, 6 alligators (*Alligator mississippiensis*) were selected for an increment depositional rate study. This was done to test the hypothesis that there is a daily deposition of the archosaurian incremental lines. The animals were provided by the Rockefeller Wildlife Refuge (Louisiana Department of Wildlife and Fisheries; Louisiana, U.S.A.) and a commercial alligator farm in Louisiana. The sizes of alligators selected were pairs of approximately 0.60, 0.85, and 1.40 meter long animals (total length- tip of snout to end of tail). The animals were given interperitoneal injections of oxytetracycline (30 mg./Kg.of body weight) and/or calcein (20 mg./Kg. of body weight). These chemicals are deposited with dentine at the time of injection, marking the incremental line being incorporated into the tooth at the time of injection. Kariyama et al. (1969) used these two chemicals in labelling caiman teeth and Kaye (1966) used just tetracycline, also in work on caimans. After 7 days had elapsed, one of the 1.4 meter long alligators that had received an oxytetracycline injection, was given a calcein injection. Kariyama et al. (1969) used a similar multi-labelling method on individual caimans. The following day (8 days from the first injection) the alligators were sacrificed with the exception of the single labelled 1.4 meter alligator. This alligator was sacrificed 127 days after the first injection. The jaws were secured and fixed in an 80% ethanol solution.

Following fixation the teeth were prepared for microscopic viewing with the individual tooth families intact. A "tooth family" is the functional tooth and its replacements, which are found in the jaw slightly lingual and ventral to the functional tooth. This was done by sawing between the individual tooth positions (and families) through what would generally be called the transverse plane (Figure 2). The jaws with tooth families intact were prepared for microscopy as described for extant alligator teeth in the Methods section. The plane of thin sectioning was slightly oblique to the transverse plane of each functional tooth so as to cut through the apex of both the functional and replacement teeth (Figure 3). The marked dentine was readily visible with natural lighting under 40-100x magnification and appeared as thin olive-brown colored incremental lines. When viewed under u.v. lighting (~410 nm.) at 100x magnification the colored increments fluoresce a grayish green (oxytetracycline) or a

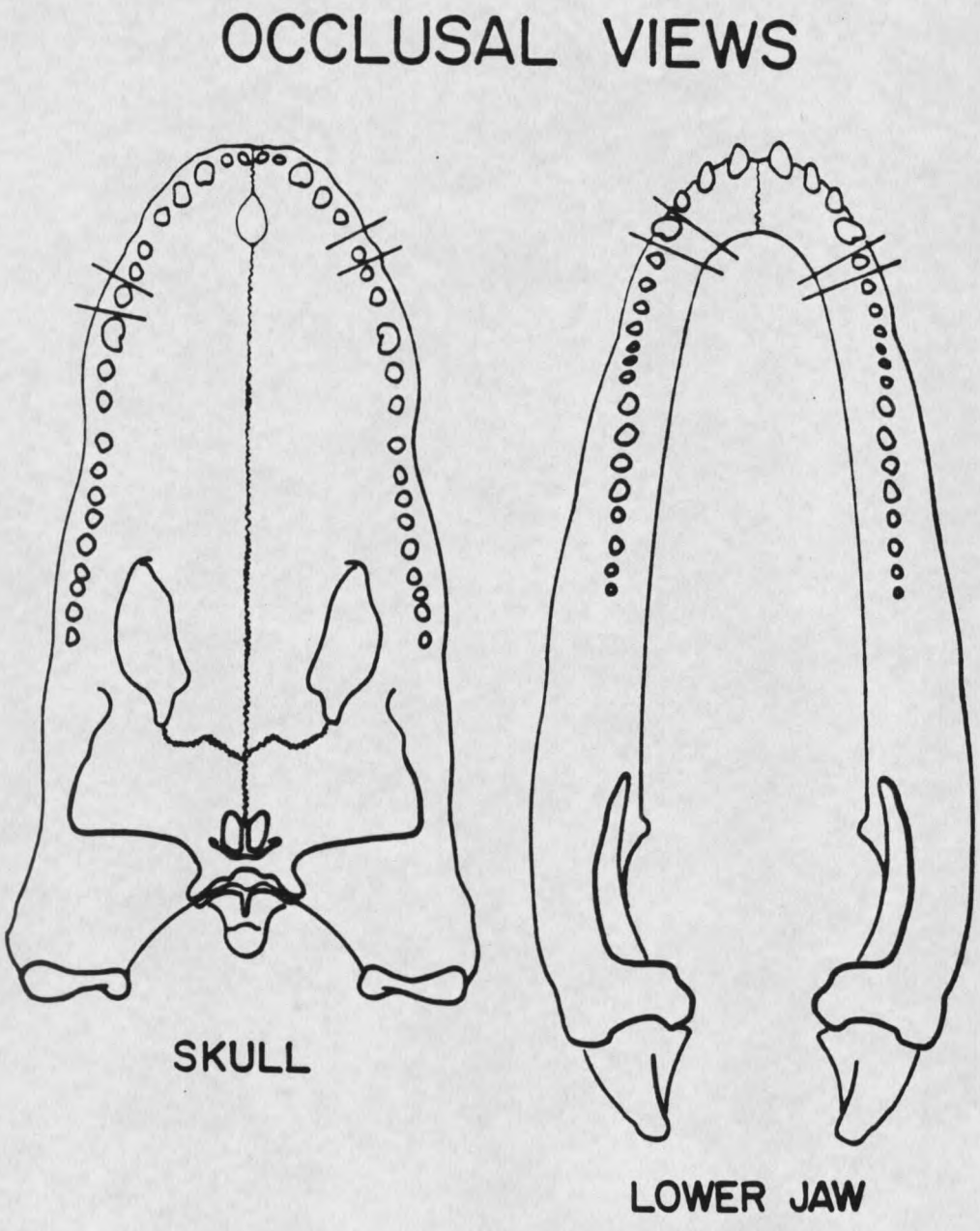

Figure 2- Planes of Sawing to Secure Alligator Tooth Families (example of planes of thin sectioning are denoted by pairs of lines).

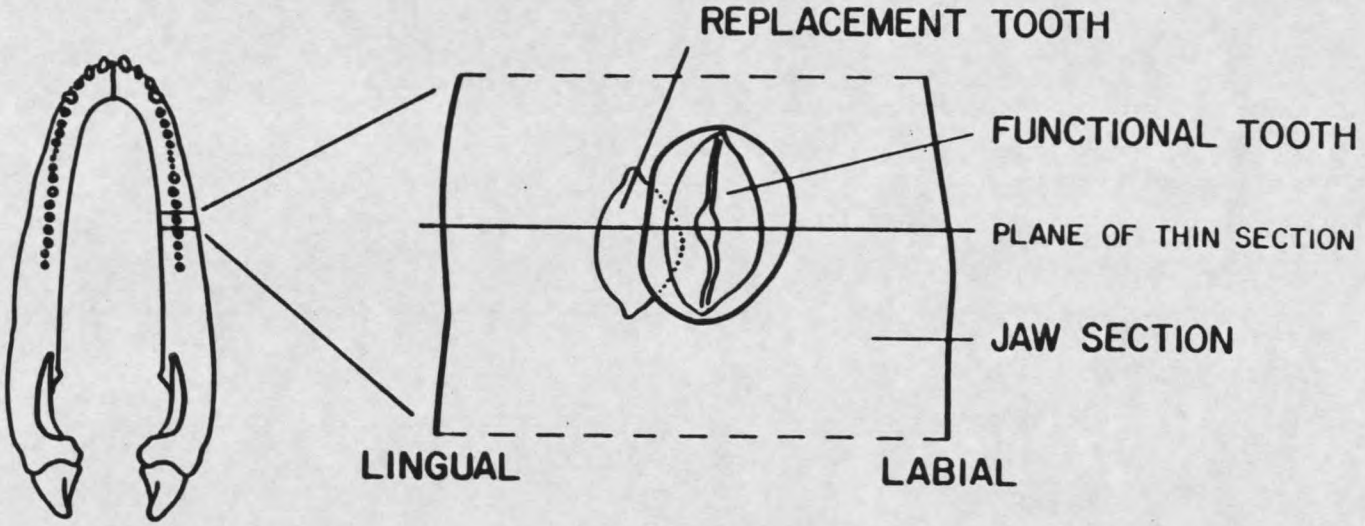

Figure 3- Plane of Thin Sectioning of an Alligator Tooth Family (occlusal view)

bright chartreuse green (calcein), as similarly reported by Kariyama et al. (1969); (Figure 4). The fluorescence confirms that the colored incremental lines are in fact the result of chemical injection of the respective fluorochrome markers. The number of incremental lines deposited between the chemical labels (1.4-meter long alligator) and/or the number of increments recorded between injection day and death were counted and recorded in all the alligators from the growth series.

Experimental Control: Determining Actual Replacement Rates of Teeth of Modern Archosaurs.

Two 0.85 meter long alligators (age seven months at start of study) were analyzed to assess tooth replacement rate. A "replacement" refers to the shedding of a functional tooth and its positional replacement by its successor. The alligators were provided by the Rockefeller Wildlife Refuge (Louisiana Department of Wildlife and Fisheries, Louisiana, USA) and were kept in captivity at the Museum of the Rockies (Montana State University). They were housed in a 150-gallon (39.6 litre) aquarium kept at 82-85c. Each animal was fed approximately 0.5 Kilograms of trout and goldfish two times weekly. From April 1991 through February 1992, the tooth eruptions of these two alligators were monitored. Periodic x-rays, jaw casting (with dental putty and cast with epoxy), photography, and manual measurements were made to document the tooth replacement phenomenon. The average rate of tooth replacement was determined. Also, life information such as body length and mass were recorded throughout the testing period.

Testing the Model: Comparison of Actual Shedding Rates with Incrementally Assessed Shedding Rates in Modern Archosaurs and a Determination of Body Length Influences on Increment Size.

Average tooth replacement rates recorded in previous literature for alligators and the results from the captive animals in this study were compared with rates found by using incremental line counts. Incremental line counting proceeded as follows: Using the thin sectioned jaws from the Methods section above (tooth families of 0.60, 0.85, and 1.4 meter alligators) plus 1.5, 2.5 and 3.2-meter alligators from the comparative collection of the Museum of the Rockies (Montana State University), total incremental line counts were recorded in "representative teeth", both functional and replacement