

Changes in Femoral Robusticity and Porosity During the Reproductive Cycle of the Female Alligator (*Alligator mississippiensis*)

CAROLE S. WINK, RUTH M. ELSEY, AND ELIZABETH M. HILL
Departments of Anatomy (C.S.W.) and Biometry (E.M.H.), Louisiana State University Medical Center, New Orleans, Louisiana 70112 (C.S.W.); Louisiana Department of Wildlife and Fisheries, Rockefeller Wildlife Refuge, Grand Chenier, Louisiana 70643 (R.M.E.)

ABSTRACT To help understand the nature of skeletal changes during the reproductive cycle of the female alligator, we compared femoral robusticity (density) and porosity of cross-sections from the midshafts of femora from the following groups of female alligators: 1) immature; 2) pre-ovulatory; 3) post-ovulatory with soft-shelled oviducal eggs; 4) post-ovulatory with hard-shelled oviducal eggs; 5) post-ovulatory with eggs in the nest; 6) post-ovulatory with hatched eggs; and 7) mature, quiescent. Femora from alligators with eggs in the nest were significantly less robust (dense) than those of the other groups except those with hard-shelled oviducal eggs. Cross sections from the midshaft of femora from alligators with eggs in the nest were significantly more porous than those from all the other groups. The results indicated that calcium was mobilized from the femoral shaft shortly before eggs were laid and that femoral density returned to normal levels for mature alligators 1-2 months after egg-laying.

The reproductive cycle of *Alligator mississippiensis* in Louisiana has been described in detail (Joanen, '69; Joanen and McNease, '70, '72, '79, '80; McNease and Joanen, '74; Lance et al., '83; Lance, '84). The reproductive cycle of the female begins in early spring. By early April ovarian follicles are enlarged, and during May follicles reach their pre-ovulatory maximum size. During this phase of follicular growth (April-May), concentrations of estradiol in the blood plasma increase. The plasma becomes "milky" with high circulating levels of vitellogenin, a yolk precursor, and there are increases in plasma levels of total protein, total lipid, cholesterol, vitamin E, magnesium, iron, zinc, and calcium. Ovulation and mating occur in late May or early June. The time interval between ovulation and egg-laying is about 2-3 weeks. During this time the shells of oviducal eggs become calcified, and the female lays her eggs (usually about 40) in a single night. Post-ovulatory follicles form corpora lutea, and no further ovarian growth is evident until the following spring. Plasma chemistry returns to normal by July or August.

In birds, pre-ovulatory estrogen-dependent hypercalcemia and the proliferation of medullary bone are followed by resorption of this endosteal bone. The resorption can be correlated with the formation of the calcareous eggshell (Simkiss, '67; Taylor et al., '71). This mechanism provides calcium for the formation of eggshells without imposing a burden on the female's skeletal calcium reserves. The alligator, which is closely related to birds in evolution, also exhibits a pre-ovulatory hypercalcemia (Lance et al., '83), but recent studies in this laboratory have indicated that the alligator has not evolved the skeleton-sparing mechanism of pre-ovulatory bone formation to provide calcium for the formation of eggshells (Elsey and Wink, '86). Rather, like the turtle (Edgren, '60; Simkiss, '61; Suzuki, '63; Magliola, '84), the alligator resorbs structural bone for the formation of eggshells (Wink and Elsey, '86). It is not known, however, when during the female's

Address reprint requests to Dr. C.S. Wink, Department of Anatomy, Louisiana State Univ. Medical Center, New Orleans, LA 70112.

reproductive cycle bone is resorbed or when (and if) bones return to pre-ovulatory density. The purpose of this study was to investigate changes in the density of femoral bone during the reproductive cycle of the female alligator.

MATERIALS AND METHODS

The methods used in this study follow those described in previous studies (Elsey and Wink, '86; Wink and Elsey; '86). Femora were collected in the field in southwest Louisiana from 7 groups of female alligators of different reproductive status: 1) immature; 2) pre-ovulatory (follicular growth); 3) post-ovulatory with soft-shelled oviducal eggs; 4) post-ovulatory with hard-shelled oviducal eggs; 5) post-ovulatory with eggs in the nest (femora were collected 2 weeks after eggs had been laid); 6) post-ovulatory with hatched eggs; and 7) mature, quiescent (nonproductive for the present season). Femora were chosen because Ferguson ('82) showed that the femur undergoes less remodeling during aging than other bones. Baited hooks were set at nest sites to catch egg-producing females, and immature and quiescent females were caught with nooses at night as described by Chabreck ('63). Some femora were obtained from hunter-killed alligators taken during Louisiana's month-long alligator harvest each September and from alligators taken for other research purposes at Rockefeller Wildlife Refuge and Marsh Island Wildlife Refuge in southwest Louisiana. Necropsies were performed on all females to verify reproductive status. The bones were collected from May through October (Fig. 1) over a 3-year period. Ambient temperatures affect the timing of nesting and egg-laying so the dates of these activities may vary slightly from year to year.

Bone robusticity

Femora were cleaned and placed in an 85°C water bath for 1-2 weeks to divest them of soft tissue. Then they were defatted and dehydrated through a graded series of alcohols and ethers and finally dried in a vacuum oven. Bones were weighed and lengths measured with vernier calipers. Femoral length was measured from the articular surface of the head to the articular surface of the lateral condyle (maximum length). Bone robustness was determined using the formula:

$$\text{robusticity index} = \frac{\text{bone length}}{\sqrt[3]{\text{bone weight}}}$$

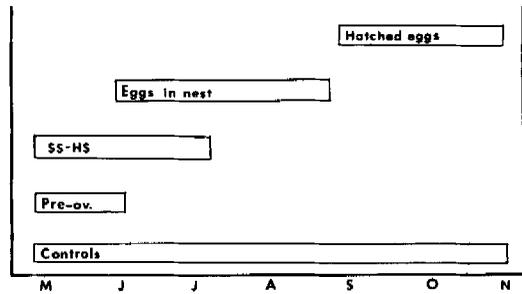


Fig. 1. Months in which femora were collected from female alligators. Bones from control (immature and mature, quiescent) animals were collected from May (M) to November (N); those from pre-ovulatory alligators in May; those from alligators with soft-shelled (SS) or hard-shelled (HS) oviducal eggs in May, June (J), July (J), and August (A), those from animals with hatched eggs in September (S) and October (O), and those from alligators with eggs in the nest in June, July, and August.

This robusticity or ponderal index was first proposed by Riesenfeld ('72) and has been used to indicate the density or robustness of a bone as a whole (Riesenfeld, '75, '81; Simon, '84). The lower the index, the denser or more robust the bone; the higher the index, the less dense or more porous the bone.

Porosity of bone sections

After robusticity indices were determined, pieces 1 cm long were cut from the middle of each femoral shaft and embedded in Ward's Bioplastic. Cross-sections 200 m thick were cut from each embedded piece of femoral shaft with a Bronwill Hard Tissue Cutting Machine and diamond wheel. Microradiographs of the sections were made with a Faxitron X-ray machine and Kodak High Resolution Plates 649-0. After routine processing the microradiographs were projected with a microprojector and bone sections were drawn at $\times 20$. Drawings of sections were analyzed for porosity with a Numonics Electronics Graphics Calculator. Porosity was calculated as:

$$\begin{aligned} & \text{percent area unoccupied by bone} \\ &= \frac{\text{total area unoccupied by bone}}{\text{total area of section}} \times 100 \end{aligned}$$

The total area of section was determined by marking around the periphery of the entire cross section (excluding the marrow cavity). Total area unoccupied by bone was determined by marking around the "holes" or radiolucent areas in the bone and adding them together. The higher the percent area unoc-

cupied by bone, the greater the porosity of the section.

Statistical methods

One-way analyses of variance were used to determine overall differences in robusticity and porosity among the seven groups. Differences between specific group means were subsequently tested with Duncan's multiple range tests; the significance level was set at .05 for these tests (Snedecor and Cochran, '80).

RESULTS

Robusticity and porosity values differed significantly among the 7 groups (robusticity: $F(6,45) = 2.99$, $p = .015$; porosity: $F(6,38) = 8.69$, $p = .0001$). Results from subsequent tests are shown in Figure 2. The robusticity index of femora from alligators with eggs in the nest was significantly higher (that is, bones had a lower density) than that for all other groups, except those with hard-shelled eggs.

Animals with eggs in the nest also had more porosity in the midshaft of the femur than the other groups. The midshafts of immature alligators were the least porous of all.

DISCUSSION

Edgren ('60) reported a seasonal change in the density of the tibia, fibula, and femur in female musk turtles, *Sternotherus odoratus*. During early May, at the time of ovulation, densities of bones were equivalent for males and females. In June, decreased densities showed in the bones of turtles with hard-shelled oviducal eggs and eggs in the nest. By the end of August, bone densities were near the early May levels. The present study indicates that the femur of the female *Alligator mississippiensis* undergoes seasonal changes in density similar to those of female musk turtles. Although significant increases in robusticity index and porosity at the midshaft of the femur were not noted until after the alligators had laid their eggs, there appeared to be a trend toward increased porosity and decreased robusticity in the bones from alligators that were nesting and progressing from ovulation to calcification of eggshells (Fig. 2). The robusticity indices of femora from the hard-shelled group were not significantly different from those of any of the other groups. However, they were intermediate between those with eggs in the nest

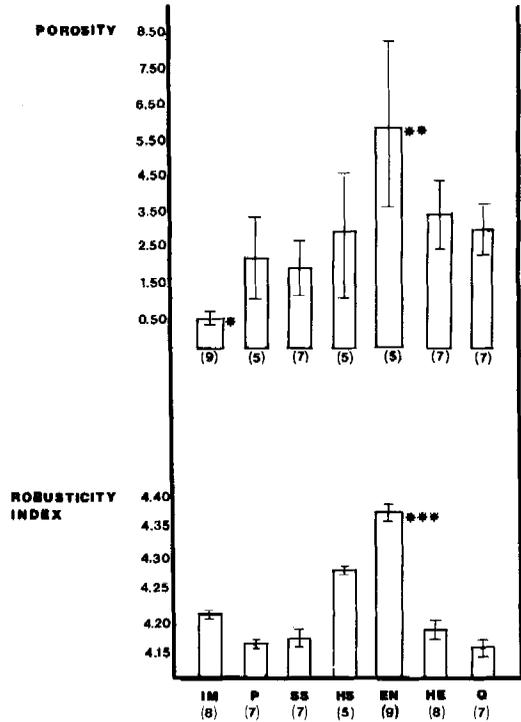


Fig. 2. Femoral robusticity index and porosity of femoral diaphyseal cross sections (mean \pm SD) from the following groups of female alligators: immature (IM); pre-ovulatory (P); post-ovulatory with soft-shelled oviducal eggs (SS); hard-shelled oviducal eggs (HS); eggs in the nest (EN); hatched eggs (HE); and quiescent (Q). *Significantly different from all groups except pre-ovulatory and SS, $P < 0.05$. **Significantly different from all groups, $P < 0.05$. ***Significantly different from all other groups except HS, $P < 0.05$. (X) = No. of animals.

and the other groups, indicating a possible trend toward decreased robusticity. Also, there may have been resorption along the peripheries of the marrow cavities of femora from the hard-shelled group which were not reflected in our measurements of porosity; we did not measure changes in the diameters of the marrow cavities of the bones. And, although the oviducal eggs were classified as hard-shelled at autopsy, it is possible that the shells may not have received their full complement of calcium from the skeleton, and significant differences in robusticity and porosity were not observed until after laying, when the shells were fully calcified.

Lance et al. ('83) reported a rise in plasma calcium in pre-ovulatory female alligators in April and May, followed by a drop to normal levels of plasma calcium after ovulation in June. The present study showed that loss of

femoral robusticity and increased porosity did not occur until June, July, and August, after pre-ovulatory levels of plasma calcium had returned to normal. Apparently, calcium mobilized from the femur during June, July, and August passed immediately into eggshells so that there was no net rise in plasma calcium during these months. These results agree with Urist et al. ('58) who suggested that the hypercalcemia of estrogen-treated birds and the pre-ovulatory hypercalcemia of fishes, amphibians, and birds is associated with the formation of yolk rather than eggshells in these groups.

An unexpected finding of this study was that cross sections of the femoral midshaft were significantly less porous in immature female alligators (0.50 ± 0.33) than in the mature, nonproducing (quiescent) animals (2.63 ± 0.80) (Fig. 2). One possible explanation for this is that the immature alligators had never mobilized calcium from the skeleton to form eggshells because they had never laid eggs. Once mature alligators lay eggs and calcium is mobilized from the skeleton, femoral density may never return to pre-lay levels. Examination of the ovaries at autopsy revealed that the mature, quiescent alligators in this study had laid eggs during previous seasons. Another explanation for the low porosity in the femora from immature animals may be the size of the animal. The immature alligators were 1.81 ± 0.10 m long; mature, quiescent animals were 2.31 ± 0.19 m long. In a previous study (Wink and Elsey, '86) we reported that porosity of the femoral midshaft in male alligators was 2.10 ± 1.23 , which is significantly greater than that of the immature females (0.50 ± 0.33). The male alligators were 2.83 ± 0.38 m long, indicating that as the animals grow larger (male or female) porosity may increase in the midshaft of the femur. This would also explain why porosity of the femoral midshaft in the immature females was not significantly different from that of the pre-ovulatory and soft-shelled oviducal egg groups. Both these latter groups were not much larger than the immature females; the pre-ovulatory group, 2.08 ± 0.22 m long, and the soft-shelled oviducal egg group, 2.13 ± 0.17 m long. Large body size apparently did not contribute to porosity and loss of robusticity in the hard-shelled oviducal egg group and the eggs in nest group; these animals were 2.17 ± 0.12 m long and 2.14 ± 0.16 m long, respectively.

In conclusion, our study indicates that femoral robusticity (density) decreased and porosity of the midshaft of the femur increased prior to and during egg-laying in the alligator. These skeletal changes were most significant in alligators with eggs in the nest. The bones appeared to return to normal pre-lay densities 1–2 months after eggs had been laid.

ACKNOWLEDGMENTS

The authors wish to thank Ted Joanen and Larry McNease of the Louisiana Department of Wildlife and Fisheries for assistance in sampling the alligators. Appreciation is also extended to Valentine Lance for his advice and suggestions throughout this study. We also wish to thank Mr. Garbis Kerimian for making the photographs and Mrs. Mary Ann Wilde for typing the manuscript. The project was funded in part by the Louisiana Department of Wildlife and Fisheries.

LITERATURE CITED

- Chabreck, R.H. (1963) Methods of capturing, marking and sexing alligators. Proc. A. Southeast Assoc. Game Fish Comm. 17:47–50.
- Edgren, R.A. (1960) A seasonal change in bone density in female musk turtles, *Sternotherus odoratus* (Latreille). Comp. Biochem. Physiol. 1:213–217.
- Elsey, R.M., and C.S. Wink (1986) The effects of estradiol on plasma calcium and femoral bone structure in alligators (*Alligator mississippiensis*). Comp. Biochem. Physiol. 84A:107–110.
- Ferguson, M.W.J. (1982) *The structure and development of the palate in Alligator mississippiensis*. Doctoral dissertation, Queens University, Belfast.
- Joaanen, T. (1969) Nesting ecology of alligators in Louisiana. Proc. A. Conf. Southeast. Assoc. Game Fish Comm. 23:141–151.
- Joaanen, T., and L. McNease (1970) A telemetric study of nesting female alligators on Rockefeller Refuge, Louisiana. Proc. A. Conf. Southeast. Assoc. Game Fish Comm. 24:175–193.
- Joaanen, T., and L. McNease (1972) A telemetric study of adult male alligators on Rockefeller Refuge, Louisiana. Proc. A. Conf. Southeast. Game Fish Comm. 26:252–275.
- Joaanen, T., and L. McNease (1979) Time of egg deposition for the American alligator. Proc. A. Conf. Southeast. Assoc. Game Fish Comm. 29:407–415.
- Joaanen, T., and L. McNease (1980) Reproductive biology of the American alligator in Southwest Louisiana. In J.B. Murphy and J.T. Collins (eds): Reproductive Biology and Diseases of Captive Reptiles. Society for the Study of Amphibians and Reptiles, Lawrence, Kansas: pp. 153–159.
- Lance, V. (1984) Endocrinology of reproduction in male reptiles. Symp. Zool. Soc. Lond. 52:357–383.
- Lance, V., T. Joanen, and L. McNease (1983) Selenium, Vitamin E, and trace elements in the plasma of wild and farm-reared alligators during the reproductive cycle. Can. J. Zool. 61:1744–1751.
- Magliola, L. (1984) The effects of estrogen on skeletal calcium metabolism and on plasma parameters of vi-

- tellogenesis in the male, three-toed box turtle (*Terropene carolina triumquis*). Gen. Comp. Endocrinol. 54:162-170.
- McNease, L., and T. Joanen (1974) A study of immature alligators on Rockefeller Refuge, Louisiana. Proc. A. Southeast. Assoc. Game Fish Comm. 28:482-500.
- Riesenfeld, A. (1972) Metatarsal robusticity in bipedal rats. Am. J. Phys. Anthropol. 36:529-534.
- Riesenfeld, A. (1975) Endocrine control of skeletal robusticity. Acta Anat. 91:481-499.
- Riesenfeld, A. (1981) Age changes in bone size and mass in two strains of senescent rats. Acta Anat. 109:64-69.
- Simkiss, K. (1961) The influence of large doses of oestrogens upon the structure of the bones of some reptiles. Nature 190:1217-1218.
- Simkiss, K. (1967) Calcium in Reproductive Physiology. New York: Rheinhold.
- Simon, M.R. (1984) The rat as a model for the study of senile idiopathic osteoporosis. Acta Anat. 119:248-250.
- Snedecor, G.W., and W.G. Cochran (1980) Statistical Methods, 7th Ed. Ames, Iowa: Iowa State University Press.
- Suzuki, H.K. (1963) Studies on the osseous system of the slider turtle. N.Y. Acad. Sci. 109:351-410.
- Taylor, T.G., K. Simkiss, and D.A. Stringer (1971) The skeleton: Its structure and metabolism. In D.J. Bill and B.M. Freeman (eds): Physiology and Biochemistry of the Domestic Fowl, Vol. 2. Academic Press, New York; pp. 621-640.
- Urist, M.R., O.A. Schjeide, and F.C. McLean (1958) The partition and binding of calcium in the serum of the laying hen and of the estrogenized rooster. Endocrinology 63:570-585.
- Wink, C.S., and R.M. Elsey (1986) Changes in femoral morphology during egg-laying in *Alligator mississippiensis*. J. Morphol. 189:183-188.