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RATE OF SCUTE ANNULI DEPOSITION OF EASTERN BOX TURTLES (*TERRAPENE CAROLINA CAROLINA)* HELD IN CAPTIVITY AND IN THEIR NATURAL HABITAT

Eastern box turtles (terappene Carolina carolina) are one of many species of turtles that lays down an annual growth ring in each scute, called a scute annuli. This study investigates whether Eastern box turtles lay down new growth rings due to environmental factors, such as temperature drops that induce brumation, or if they are caused by physiological factors, such as the size of the turtle. The appearance of scute annuli in turtles in the wild that underwent annual brumation was compared to the appearance in turtles that were kept in captivity and prevented from bromating. The turtles kept in captivity who grew at a faster rate since they did not brumate formed more than one new annuli within a year.

INTRODUCTION

The scutes on a turtle are the carapace and plastron of a turtle are the equivalent of scales on other reptiles. The epidermis that covers the body shell of turtles forms the scutes. When new layers of dermis are deposited over the bone, old epidermal cells are pushed towards the outside. As these epidermal cells die, they are keratinized and form the scutes. (See *Fig. 1).*In *Terrapene* turtles, old layers of scute are retained, as opposed to other species of turtles that shed old scutes. After growth is halted for a certain period of time, an entirely new layer of dermis begins to grow, pushing the deratinized scutes towards the margins. A depression forms between old, keratinzed layers and new layers of epidermis. These depressions are called scute annuli (Wilson, 2003). Counting the number of scute annuli on the carapace or plastron of a turtle is a common way of determining the age of turtles (Nazdrowicz, 2003). Eastern box turtles (*Terrapene carolina)* is one species of turtles in which scute annuli number is utilized to approximate their age. It has been reported that in box turtles usually one annulus is formed each year (Ewing, 1939), presumably after the halt of growth that occurs when turtles in seasonal regions brumate when the temperature drops.

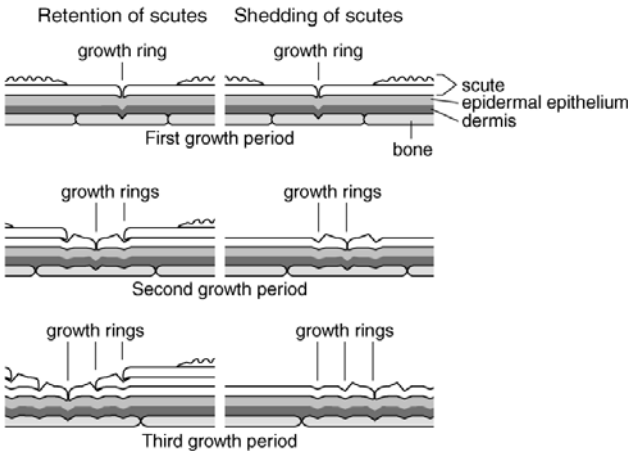

Figure 1. Diagram showing the process of scute annuli formation in turtles that retain old scutes (left) and turtles that shed old scutes (right), (Wilson, 2003).

However, some species of turtles that live in tropical regions and do not brumate lay down scute annuli as well. Some turtles lay down more than 1 annulus per year, even though they do not undergo an annual cessation of growth (Germano & Bury, 1998). Based on studies like this one, an alternative hypothesis states that growth rings are deposited as a function of body size, not age ((Litzgus and Brooks, 1998; Tracy and Tracy, 1995) OR (Wilson, 2003).

To determine whether environmental or physiological factors cause the deposition of new growth rings we monitored their appearance on Eastern box turtles found in their natural habitat that underwent annual brumation, and monitored the appearance of new rings on turtles of the same species that were kept in captivity and prevented from brumating.

QUESTION

Are scute annuli seen on the carapaces and plastrons of eastern box turtles (*Terrapene Carolina carolina*) deposited due to environmental factors or to physiological factors? The environmental factor investigated will be seasonal temperature changes which induce brumation when the temperature decreases in the fall and winter months. The physiological factor investigated will be size and whether new rings appear when the animals reach a certain size.

MATERIALS AND METHODS

Experiments were conducted from July, 2003 through June, 2006. Eight, three-year old, male turtles that had been monitored since hatching, to ensure they all were the same age, and were determined to have three scute annuli on the 1^{st}, 2^{nd}, and 3^{rd} dorsal scutes, to ensure they all had undergone similar growth rates and conditions, were chosen to conduct the study. All turtles were weighed and measured (length and width of plastron and of carapace) before the experiment began.

Three of the turtles, numbered 1-3, were kept in captivity where they were fed year round and ambient temperature was maintained constant at 24˚ Celsius to prevent brumation. Radio transmitters were placed on the other five turtles, labeled A-E, and

were released to their natural habitat around the Barnstable High School area in April, 2003. Before they went into brumation, radiotransmitters were replaced by new ones in October, before the turtles went into brumation. At this time measurements of weight, length and number of annuli were taken.

The turtles were located using radio telemetry and measurements were taken in the spring of 2004. At this point, one of the turtles kept in captivity was released after placing a radio transmitter on it. Measurements were again taken in the fall and in the spring the following year in 2005. The turtles kept in captivity were measure and their annuli were checked at the same times as the released turtles were checked. The radio transmitters were replaced every six months when the during the months the turtles were not bromating.

RESULTS

It was noted that the turtles that were left in their natural habitat, except for one, only showed one new growth ring each year. It was noted that most of the growth was done between April and October. The growth done from October through April was negligible. The average growth rate was 1.23 $\pm$ 0.12cm annually.

	April, 2003		October, 2003		April, 2004		October, 2004	
Turtle	length plastron (cm)	Annuli	length plastron (cm)	Annuli	length plastron (cm)	Annuli	length plastron (cm)	Annuli
A	3.25	3	4.55	4	4.55	4	5.70	5
B	3.35	3	4.60	4	4.65	4	5.85	5
C	3.35	3	4.70	4	4.70	4	5.90	5
D	3.20	3	4.55	4	4.50	4	5.70	5
E	3.15	3	4.65	3	4.70	3	5.85	4
1	3.20	3	4.75	4	6.20	5	7.50	6
2	3.55	3	5.15	5	6.35	6	7.75	7
3	3.30	3	4.80	4	6.30	5	7.60	6
	April, 2005		**October, 2005**		**April, 2006**		**October, 2006**	
Turtle	length plastron (cm)	Annuli	length plastron(cm)	Annuli	length plastron (cm)	Annuli	length plastron(cm)	Annuli
A	5.75	5	6.95	6	6.95	6	8.10	7
B	5.80	5	7.15	6	7.15	6	8.35	7
C	5.85	5	7.05	6	7.05	6	8.30	7
D	5.75	5	6.95	6	6.95	6	8.15	7
E	5.85	4	6.90	5	6.90	5	8.05	6
1	7.50	6	8.80	7	8.80	7	9.95	8
2	9.20	8	10.35	9	10.35	9	11.55	10
3	8.85	7	10.05	8	11.15	10	12.20	11

Table 1: Data collected from each turtle in 2005 including plastron length and annuli. Note that turtle 1 was released in April 2004, and turtle 2 was released in April 2005.

The turtles kept in captivity exhibited a faster growth rate. Once released into the wild, they grew 1.19 $\pm$ 0.11 cm each year after being released. The growth of only one new annulus was seen each subsequent year. While in captivity, the turtles grew an average of 2.75 $\pm$ 0.45 cm annually. Most showed the appearance of two new annuli each year, with the exception of one turtle one year who grew three new annuli within that year.

DISCUSSION

There was a difference in scute annuli appearance per year between the turtles kept in captivity and the turtles left in their natural habitat. It seems that the new layer of epidermis that causes the annuli to appear begins to grow after the turtle reaches a certain size and not caused by the halt in growth the turtle undergo during brumation, as was originally thought.

One of the turtles kept in captivity grew more than the other two within a single year. In that same year, the turtle exhibited the appearance of three new annuli within that same year. All subsequent years that turtle exhibited the same growth rate as the other two captive turtles, and all exhibited the addition of 2 annuli each year they remained captive.

Once the turtles that had been held captive were released into their natural habitat, their annual growth rates decreased from the other captive turtles and were closer to the growth rate exhibited by the turtles left in their natural habitat. They underwent brumation and ceased to grow during this time, like the other turtles had done. Once the turtles were released one annuli was added per year, so being in captivity did not permanently accelerate their metabolism or cause them to lay more than one growth ring annually for an indefinite period.

Evidently, the reason new epithelial layers grow below the surface is a factor of size and not due to environmental factors. After the turtle reaches a certain size, a new

layer of skin begins to grow. Perhaps as the skin grows further away from the center, the skin begins to die and so it is necessary for a new layer to grow.

The number of growth rings does not necessarily tell how old a turtle is. If the turtle is placed in conditions where it can grow more than the usual annually, more than one ring will be seen within that period of time. Many turtles studies use growth rings to determine the age of turtles. Since the turtles studied have been in their natural habitat their entire lives, using growth rings is in fact an accurate way of telling their age. However, it is important to note if the turtle was head started or kept in captivity during winter months before reaching adulthood. If either of these two cases is true, then the turtle may be younger than what the annuli indicate. It would also be important to note the size and refer to the average age of turtles that size. If the turtle is bigger than turtles of the age the growth rings indicate it could be an indication that the turtle was in deed head started or held in captivity for several years.

Eastern box turtles tend to lay down around 11 growth rings when they reach adulthood, at which point old rings tend to fade away. In the future, it would be interesting to see if captive turtles only lay down a maximum number of annuli before the average age that the turtles reach adult hood.

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