An Examination of the Accuracy of Using Plastral Scute Rings to Age Spotted Turtles

(Clemmys guttata)

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ABSTRACT. – The use of plastral scute rings to estimate age in spotted turtles is a widely practiced technique, but a lack of rigorous field testing of this method has led to critiques of its usefulness and accuracy. We tested the method’s effectiveness for aging spotted turtles (Clemmys guttata) by calculating an Age-Increase Ratio and by recording changes in the number of scutes within a year. We found no correlation between the change in the number of scute rings and the number of years between captures, which likely stems from the observer error associated with the difficulty of accurately counting scutes on older turtles; however, we did record a significant correlation between the number of scute rings and body size.

The use of plastral scute rings to estimate age in turtles is a widespread and well-known practice. The technique has been used for well over 100 yrs (Agassiz 1857) and has been touted as an easy, effective, and nondestructive method of determining the age of individual turtles within a population (Wilson et al. 2003). However, multiple studies have confirmed that growth rings are dependent on many other factors than just age (e.g., drought, body size, bone density, length of hibernation; Moll and Legler 1971; Tracy and Tracy 1995; Litzgus and Brooks 1998b; Wilson et al. 2003). Obviously, counts of plastral scute rings are also affected by observer error. Often it is difficult to distinguish which rings are “true rings” (indicating yearly cessation of growth) and which are “false rings” (indicating smaller intra-annual growth spurts). It is also possible during years with multiple distinct growing seasons that more than 1 ring may be laid down, or that during drought years, 0 rings may be laid down (Zug 1991; Aresco and Guyer 1998; Germano and Bury 1998; Berry 2002).

Because the technique has become so widespread, some researchers have forgone the necessary verification or “ground truthing” to ensure that the 1-ring/yr hypothesis matches up with their study organism and site. Wilson et al. (2003) completed a literature review and found that of 150 case studies, 101 of them did not present any evidence of a pretest of the method. Overall, Wilson et al. (2003) concluded that there is no general trend that can be used to age turtles across species, and that few studies have assessed the accuracy of using plastral growth rings to age turtles. Despite these issues, counts of scute rings have been shown to be accurate in many cases for aging juvenile turtles that are growing rapidly (see Wilson et al. 2003, table 3, for a review). It is also possible to create site-specific calibration factors to estimate the true age of an individual at a site if enough interannual recaptures are made (Germano and Bury 1998; Wilson et al. 2003; Stone and Babb 2005; Rodríguez-Caro et al. 2015).

Because spotted turtle populations continue to decline and the species is under review for federal listing (US Fish and Wildlife Service [US FWS] 2015), it is critical that we understand the accuracy of the age estimates necessary for demographic models. To date, only 1 article (Litzgus and Brooks 1998a) has assessed the efficacy of using plastral scutes to age spotted turtles (Clemmys guttata) and found that this was an inaccurate method for aging spotted turtles in Ontario. Here we assess the accuracy of plastral scute rings during a 3-yr population survey on spotted turtles at the center of their range.

Methods. — We conducted a mark–recapture study of C. guttata in central Maryland (site not specified owing to poaching concerns) between 2015 and 2017. These years were all generally comparable in climactic variables and there were no drastic weather events (e.g., drought, exceptional rainfall) that could have affected growth annulus deposition. The study site was composed of 2 wetland complexes divided by a commercial and residential road (Howell et al. 2016). To the north of the road there are 4 woodland vernal pools, totaling 2.28 ha, that we termed the North Wetland Complex (NWC). To the south are 3 permanent wetlands and 1 ephemeral vernal pool, totaling 1.35 ha, that we termed the South Wetland Complex (SWC). Turtles were collected by visual encounter surveys and by passive trapping during their active season between early February and the end of June (for more information on collection protocol, collection frequency, and study site see Howell et al. 2016). After capture, turtles were sexed using secondary sexual characteristics (Ernst 1976), marked using a unique notch code filed into the marginal scutes, measured with dial calipers (midline straight-line carapace length, accurate to the nearest 0.1 mm), weighed with a 300-g Pesola scale (accurate to the nearest 1 g), and aged by counting growth rings (a magnifying glass was used to provide increased accuracy). To provide a more accurate estimate of the number of plastral scute rings, the 3 scutes with the clearest rings were chosen to be counted and averaged. Scutes were counted by H.J.H. and by 3 different field technicians across the years. Field technicians were trained for at least 1 mo, with 2 or 3 sampling periods per week
(total of approximately 10 sampling periods), before counting scutes on their own.

After a spotted turtle had developed roughly 20 scute annuli, it became impossible to accurately count their number because of wear and shell erosion. Such turtles were removed from all analyses. This process was facilitated by a historical mark–recapture study at this site between 1987 and 1992, as captured individuals that had been marked during that time frame were excluded from analyses. Additionally, individuals considered to be adults (carapace length > 80 mm) that exhibited excessive wear on their plastrons (i.e., either no rings were visible for counting or only a few rings remained) were excluded from analyses.

To determine if plastral scute rings were laid down on a yearly basis, we compared changes in number of annuli as a function of time between captures. This comparison provides a metric developed by Litzgus and Brooks (1998a) called the “Age-Increase Ratio” (AIR):

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\text{AIR} = \frac{\text{Change in number of scute rings}}{\text{Number of years between counts}}
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Hypothetically, the change in number of scute rings divided by the number of years between multiple captures should equal 1 (1 growth ring laid down per year). If growth rings are not laid down on a yearly basis, then the slope between AIR and estimated age should be negative (Litzgus and Brooks 1998a). We calculated the AIR value for each turtle and performed a linear regression against each turtle’s recorded number of annuli.

We also conducted a linear regression analysis between the number of growth rings and the body size of each individual (using straight-line carapace length; Litzgus and Brooks 1998a). Because multiple captures were not required for this analysis, all individuals with countable scute rings were included. We used the most recent age and size estimate for each individual in the analysis. We verified the assumptions of linear relationships for all regression equations shown.

**Results.** — Between 2015 and 2017, we captured 104 individual turtles. During the final year of the study we had a 97% recapture rate in the NWC (82 recaptures vs. 3 new captures) and an 89% recapture rate in the SWC (64 recaptures vs. 8 new captures). The average carapace length for all individuals was 99.6 ± 12.27 mm standard deviation (SD). During the 3 yrs, only 1 juvenile was captured with the necessary multiyear encounter history needed to be included in the analysis. Unfortunately, either our collection protocols were heavily biased towards adults or our population has a small number of juveniles. Consequently, we were unable to analyze the efficacy of the method for estimating the age of juveniles at our site.

Of the 104 individuals, 33 were included within the AIR analyses. The other 71 did not have capture records that spanned multiple years \((n = 14)\), were known to be older than the age of 20 yrs \((n = 48)\) from the mark–recapture study between 1987 and 1992, or had too much plastral wear to count any growth annuli \((n = 9)\). The 33 individuals used in the analyses had a mean carapace length of 100.6 ± 10.33 mm SD (Fig. 1). Within our
A subsample of 33 individuals, 22 individuals were recaptured across consecutive sampling seasons (e.g., first captured in 2015 and recaptured in 2016). Between consecutive sampling seasons, we recorded individuals with changes in recorded annuli of $\mathrm{C_0}^3$ ($n = 1$), $\mathrm{C_0}^2$ ($n = 1$), 0 ($n = 6$), 1 ($n = 6$), 2 ($n = 6$), and 3 ($n = 2$; Fig. 2). We also recaptured 15 individuals across 2 sampling seasons (i.e., first captured in 2015 and recaptured in 2017). Across 2 sampling seasons, we recorded individuals with changes in recorded annuli of $\mathrm{C_0}^2$ ($n = 1$), 0 ($n = 4$), 1 ($n = 1$), 2 ($n = 5$), 3 ($n = 3$), and 4 ($n = 1$; Fig. 2). There was no correlation between the change in the number of counted rings and the number of annuli counted on the individual at recapture ($n = 22$; slope = 0.355; $r^2 = 0.034$; $F_{1,21} = 0.666$; $p = 0.42$).

Thirteen individuals were captured early in the active season (February or March) and then again later toward the end of the active season (May or June). Hypothetically, this may have provided an estimate of the number of rings added per growing season. Within these 13 individuals, 5 had no change in the number of rings, 4 added 1 ring, 1 added 2 rings, and 3 added 3 rings. Furthermore, 12 individuals were captured within 1 mo (30 d) of each other. We assumed that the number of growth rings should have remained constant during this time, allowing for an estimation of our error rate due to sampling error. The average difference between counts within the same month was $0.417 \pm 0.515$ SD (range 0–1).

The linear regression of the change in the number of scute rings on number of winters between captures was not statistically significant ($n = 33$; slope = 0.0048; $r^2 = 0.0022$; $F_{1,32} = 0.07044$; $p = 0.79$). However, there were significant relationships between the estimated AIR value (average AIR = 1.357) and the number of annuli counted on a turtle ($n = 33$; slope = 0.288; $r^2 = 0.178$; $F_{1,32} = 6.93$; $p = 0.012$) as well as between straight-line carapace length and number of annuli counted ($n = 104$; slope = 2.3; $r^2 = 0.58$; $F_{1,103} = 142.89$; $p < 0.0001$; Fig. 3).

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Discussion. — Our analysis supports the results of Litzgus and Brooks (1998a) and demonstrates that even in habitat 700 km south of their study site and with longer active growing seasons, growth annuli are ineffective for aging spotted turtles. As Wilson et al. (2003) noted, it is imperative that researchers provide evidence that this method is accurate at their site and ensure that they include measures of sampling error within their analysis and discussion. The nonsignificant relationship between change in number of growth rings and the number of years between captures demonstrates that growth rings are too unreliable, especially in shorter-term studies, to be used as an effective aging method. Additionally, our data corroborate the view that growth annuli are a function of increasing body size and may not follow the 1-ring/yr hypothesis (Fig. 2; Tracy and Tracy 1995; Berry 2002; Wilson et al. 2003). Our average AIR value was greater than 1, suggesting that older individuals added more than 1
ring/yr. It is unlikely that older individuals are adding more than 1 ring/yr, however; instead, inflated AIR values likely represent the increased sampling error associated with aging older individuals with smaller gaps between annuli that are more difficult to count. The difficulty associated with counting scute rings accurately and repeatedly in adult turtles led to high recorded rates of observer error (average change of 0.417 rings within 1 mo) and prevented us from accurately aging individuals during the present study.

Because there is currently a dearth of information on long-term population viability in declining freshwater turtle populations and spotted turtles are under review for federal listing under the Endangered Species Act (US FWS 2015), the ability to produce accurate demographic models is critical. Although growth annuli can play a role in helping to create multistate demographic models, verification of the relationship between age and the number of annuli laid down each year remains a critical step in the use of annuli. Future studies should address the accuracy of this method for aging juvenile spotted turtles, which will be critical to gathering important demographic parameters necessary for producing more accurate demographic models.

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