Short note

Testing the reliability of ring counts for age determination in the Egyptian tortoise (*Testudo kleinmanni*)

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Counting shell growth rings is a common method of determining the ages of young tortoises, but the accuracy must be validated for each species. The objective of this study was to test if the age of Egyptian tortoises *Testudo kleinmanni* can be reliably determined by counting the growth rings on their shells. Our results suggest that as individuals become larger and older, age is more difficult to determine. Seventy-five percent of individuals below 90 mm carapace length (n=24) exhibited a one ring per year relationship, which was the case for only 6% of the 16 individuals with a carapace length above 90 mm. Ring counts were relatively reliable for determining ages of tortoises five years or younger, as 76.2% of these individuals exhibited a one ring per year relationship. The threshold age for the reduced reliability of ring counts is around six years of age, as only 16% of the tortoises six years or older exhibited a one ring per year relationship.

Key words: annuli, chelonians, growth rings

The counting of shell growth rings is the most widely used method for estimating the age of young turtles and tortoises, but many studies employing this method have failed to test its accuracy (Wilson et al., 2003). To assure the reliability of this method, the periodicity of deposition must be tested for each species, and accuracy may be affected by local environmental conditions and/or phylogeny (Galbraith & Brooks, 1987, 1989; Germano, 1988, 1998; Wilson et al., 2003). Growth rings on tortoise shells are formed by the successive deposition of layers of epithelial tissue during periods of intensive growth, followed by grooves generated during seasonal periods when growth slows or ceases, which often occur during hibernation or aestivation (Germano, 1988, 1998; Bertolero et al., 2005). These easily identifiable rings become unreadable or may cease being deposited altogether as turtles become mature, and growth slows dramatically. Ring counts are often unreliable in determining the age of captive animals or members of a population that do not experience the seasonal cessation of growth (Tracy & Tracy, 1995; Germano, 1988, 1998; Mitrus 2009). Counting the lines or rings deposited on the shell scutes is a convenient, non-destructive method of age estimation in young individuals (Wilson et al., 2003; Germano & Bury, 2009; Bury et al., 2010), although rings are not as useful for determining the age of older chelonians (Germano & Bury, 1998; Bury et al., 2010).

Age estimation and knowledge of a population's age structure are important for population modelling, which incorporates age-dependent survival and mortality rates. Population modelling has the potential to assist in the successful management and conservation of the Egyptian tortoise, *Testudo kleinmanni*. The Egyptian tortoise is one of the smallest, most endangered and least studied tortoise species in the world, having the most restricted range of all tortoises in the Mediterranean Basin (Baha El Din et al., 2003). Habitat loss, fragmentation and the illegal pet trade have led to dramatic declines in wild populations over the last few decades. In Egypt, much of the Mediterranean coast has been altered by urban development and large-scale agriculture. Suitable habitat now exists only in a few protected areas (Baha El Din et al., 2003).

The purpose of this study was to determine if ring counts from photographs can be used to reliably estimate the age of Egyptian tortoises. We specifically tested if one ring was deposited per year (Wilson et al., 2003). This study took place in Zaranik Protected Area (ZPA) in North Sinai, Egypt. ZPA is located 30 km west of the town of El Arish (31º05'N, 33º25'E), occupies 250 km^2, has an altitude range of sea level to 30 m, is characterized by stable and unstable sand dunes, and receives 50–100 mm of rainfall per year. The vegetation within ZPA mostly consists of 5–10% coverage and is dominated by an *Artemisia monosperma–Stipagrostis scoparia* plant community.We photographed wild tortoises between 2006 and 2010 to create a graphic database. Tortoises were identified from filed notches on the marginal scutes as part of a larger mark–recapture programme. We did not photograph all of the tortoises encountered, as the photographic equipment was not always available. We photographed individuals once yearly during a survey period of approximately ten days that occurred around December each year. The lateral sides of each individual were photographed using a variety of digital SLR cameras, using standard kit zoom lenses with a focal range of 28–80 mm. We photographed tortoises under natural lighting conditions at time of capture. We also measured the straight carapace length of each individual.

We used Adobe Photoshop Elements 8.0 to view the photographs and the number rings on the shells. We counted growth rings on the 1st, 2nd, 3rd and 4th costal scutes on the carapace because the carapace receives less wear than the plastron and the costal scutes seemed to have the most visible growth rings. We distinguished between

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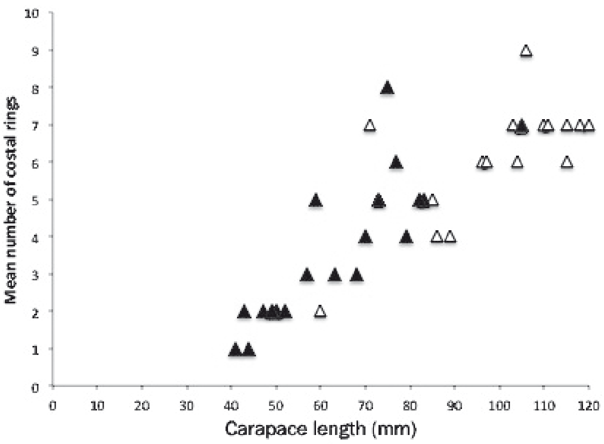

Fig. 1. Relationship between mean number of costal rings and carapace length (mm). Filled triangles: individuals adding one ring per successive year (based on photographic assessment); open triangles: individuals not adding one ring per successive year. Carapace length and number of rings are based on the last recapture.

sub-annular and annular, with annual rings being delineated by a distinct groove that forms during the cessation of growth (Germano, 1998; Zug, 1991). In contrast, subannular rings are shallow and incomplete (Stone & Barb, 2005). We used the mean number of rings of the four costal scutes as an index of age to account for any potential minor variation of ring counts between scutes. We compared the mean number of costal rings from successive years to test if one ring is deposited per year. The number of rings used in the analysis was based on the last recapture. We used two separate logistic regressions (α=0.05) to predict the relationship of carapace length and number of rings (independent variables) with the response variable (one ring deposited per year valid or invalid).

We determined the age of 105 individuals over the course of the study. Hatchlings did not have any rings and the maximum ring count was nine. We did not capture and photograph all tortoises in consecutive years, and most recaptured individuals were only recaptured once. We recaptured 40 tortoises (males n=8; females n=21; juveniles n=11), which we used in the analysis to test if one ring was deposited per year.

The logistic regression correctly predicted 85.0% of the outcome for the relationship between carapace length and reliability of the one ring per year relationship (χ^2=26.92, df=1, P<0.001), with this relationship becoming less reliable as individuals became larger (Wald=11.62, B=0.10$\pm$0.030 SE, P=0.001). The size threshold at which ring counts are no longer accurate appears to be around 90 mm, as 75% of 24 individuals exhibited a one ring per year relationship (Fig. 1). In contrast, only 6% of the 16 individuals with a carapace length greater than 90 mm exhibited a one ring per year relationship (Fig. 1).

The logistic regression also correctly predicted 75.0% of the outcome for the relationship between the number of rings and reliability of the one ring per year relationship (χ^2=16.25, df=1, P<0.001) with this relationship becoming less reliable as individuals became older (Wald=10.39, B=0.74$\pm$0.23 SE, P=0.001). The threshold age for the reliability of ring counts appeared to be around six years of age, as only 16% of the tortoises six years or older (n=19) exhibited a one ring per year relationship (Fig. 1). Ring counts were relatively reliable for determining ages of tortoises five years or younger as 76.5% of the 21 individuals exhibited a one ring per year relationship (Fig. 1), with this relationship being 100% reliable within plus or minus one year.

Our results add to the growing evidence that counting growth rings to age chelonians is reliable for young and small individuals who have not reached sexual maturity, whereas ring counts become less reliable for older and larger individuals (Germano, 1988, 1998; Wilson et al., 2003; Stone & Barb, 2005; Bertolero et al., 2005). Ring counting is a reliable method for Egyptian tortoises that are five years of age or less or with a carapace length less than 90 mm, as most of these individuals have a 1:1 ratio between the number of growth rings and prolonged periods of reduced growth that occur each year (Fig. 1). The reduced growth occurs during the summer months from as early as May until October when tortoises undergo minimal activity or aestivate (Attum et al., 2007a,b, 2008).

Counting growth rings is unreliable for Egyptian tortoises that are six years or older or with a carapace length greater than 90 mm. It is believed that Egyptian tortoises start reaching sexual maturity between five and seven years of age (Baha El Din, pers. obs.). Chelonians usually experience sharp decreases in growth after sexual maturity, resulting in the deposition of growth rings that are too thin or close together to be visually distinguished and reliably counted (Galbraith & Brooks, 1989; Germano, 1988, 1998; Stone & Babb, 2005). For example, age of *T. hermanni* can be reliably determined until sexual maturity at roughly seven years of age, with the age of older, sexually mature individuals being difficult to determine with any accuracy (Castanet & Cheylan, 1979; Bertolero et al., 2005).

Ring count variation is the result of several factors, including the ability of individual observers to correctly distinguish between annual and subannual rings, environmental conditions that regulate growth, such as years of drought and available forage, and physical wear that reduces the distinctiveness of rings (Brooks et al., 1997; Galbraith & Brooks, 1987, 1989; Germano & Bury, 1998; Zug, 1991; Berry, 2002; Wilson et al., 2003). Although we were not able to examine the validity of sex-specific ring counts given our low recapture rate, our qualitative observations suggest that growth rings on males are less visually distinct than on females because males are smaller, and therefore their rings are shorter, closer together and more difficult to distinguish. In contrast, the rings are larger, more pronounced and easier to visually distinguish in females.

The use of photographic material for age counts is beneficial because a permanent record is maintained for future reference. However, we found that post-processing of images can potentially lead to errors when counting rings. For example, increasing the sharpness and/or colour contrast of the photographs beyond the camera's default setting in image-editing software has the potential to increase the distinctiveness of sub-rings, which could then be mistakenly counted as full rings. In addition, zooming in or enlarging images to 50% or more may also enlarge the appearance of sub-rings that could also be mistaken for full rings. An alternative method is to use dental alginate and dental casting plaster to preserve scute ring impressions for later study (Germano & Bury, 1998).

In conclusion, our study suggests that ring counting is a convenient and effective technique to determine the age of young Egyptian tortoises that are not sexually mature, but is unreliable for larger, sexually mature individuals.

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