Turtles on the Brink in Madagascar: Proceedings of Two Workshops on the Status, Conservation, and Biology of Malagasy Tortoises and Freshwater Turtles
Christina M. Castellano, Anders G.J. Rhodin, Michael Ogle, Russell A. Mittermeier, Herilala Randriamahazo, Rick Hudson, and Richard E. Lewis, Eds.
Chelonian Research Monographs (ISSN 1088-7105) No. 6, doi: 10.3854/crm.6.a20p119 • © 2013 by Chelonian Research Foundation, Lunenburg, MA, USA • Published 30 October 2013

Do Bigger Females Produce Bigger Eggs? The Influence of Female Body Mass on Egg Mass in *Astrochelys radiata*

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Abstract. – A possible correlation of egg mass with female body mass was examined in *Astrochelys radiata*, a tortoise species endemic to Madagascar. Data from a wild population were compared with observations from captive individuals. Nesting data and egg mass were related to female body mass. Females from the captive population were significantly larger than those in the wild. They also produced significantly larger eggs with larger mean egg mass. Regular feeding and access to a water supply in captivity may have enabled these females to be highly productive during the breeding season.

Key Words. – Reptilia, Testudines, Testudinidae, captive breeding, Madagascar, Radiated Tortoise, reproductive biology

Through the development of shelled eggs, reptiles became able to live independently from water bodies, about 300 million years ago, and colonize terrestrial habitats (Benton and Donoghue 2007). Over time these animals have developed further adaptations towards terrestrial living conditions. Today, several species occur in dry areas with unpredictable climatic conditions and prolonged dry periods, such as the southwestern part of Madagascar (Gould et al. 1999; Dewar and Richard 2007). One of these species endemic to this subarid region is the Radiated Tortoise, *Astrochelys radiata*.

At present, this species is at high risk of extinction in the wild due to poaching and the illegal pet trade (Nussbaum and Raxworthy 2000; O'Brien et al. 2003; Leuteritz et al. 2005), and may as well be predated from other animals, such as bushpigs. Population densities of *A. radiata* are declining in the wild (Lewis 1995; O'Brien et al. 2003) and at the same time the species population growth is limited by slow reproductive rates, which is similar to other tortoise species (Hailey and Willemsen 2003; Inman et al. 2009).

This underscores the importance to produce hatchlings that are fit enough to survive in the wild to help ensure the species survival. In this context, egg mass might be an important factor to hatchling development. Tortoise eggs incubate for several months enduring the dry season when food availability is limited. Larger eggs contain more fluids providing nutrition for the embryos during the incubation period and therefore impart greater offspring survival (Tucker 2000; Valenzuela 2001). Larger eggs in tortoises are also believed to result in larger hatchlings (Loehr et al. 2004).

This study examined egg mass and nesting frequencies of two tortoise populations that were exposed to different living conditions. Data from a wild population of *A. radiata* was compared to a population held in enclosures under natural climatic conditions, but which was provided with food and drinking water on a regular basis. More specifically, this study addressed the following questions. Do big females produce bigger clutch sizes and bigger eggs than small females? And what factors determine egg mass?

METHODS

Two sites in southwestern Madagascar were surveyed for tortoise breeding activity. The sites were chosen for their differences in the living conditions of the tortoises in order to allow a comparison to be made between female *A. radiata* held in enclosures and those from the wild.

Ifaty-Mangily. — The first study site was the Village des Tortues in Ifaty-Mangily, which maintains tortoises that were confiscated from the illegal trade by authorities. The animals live in enclosures that are built into the natural forest. This forest is located within the original geographic distribution of *A. radiata*; therefore, these animals remain exposed to their natural climatic conditions. Due to the limited space and availability of food resources in the enclosures, additional food and water is supplied, with food plants every two days and drinking water twice per week. Two enclosures with both male and female *A. radiata* were surveyed. These individuals arrived as two different groups in January and February 2008. The first group was returned to Madagascar after being confiscated in Mayotte (Razafindrakoto 2008) while the second group was confiscated in the southeast of Madagascar (WWF Toliara, pers. comm.). Each of the two groups was kept in an enclosure as a mixed-sex group, but separated from other tortoises. These groups were formed haphazardly and do not represent natural tortoise populations. Nesting data were recorded from 2008 to 2010. In total, 21 female *A.*

radiata were found to be nesting during the study period. These animals from Ifaty-Mangily are referred to as the captive population (CP) throughout the remainder of this document.

Tsimanampetsotsa. — The second study site was located in the northwestern part of Tsimanampetsotsa National Park, which is located approximately 100 km south of Ifaty-Mangily. A total of 26 females from a wild population at this site were studied during two consecutive breeding seasons from February to June in 2009 and 2010. The tortoises were equipped with radio transmitters and surveys were conducted daily in order to record breeding activities and locate their nests through direct observation. In the first year, 19 tortoises were surveyed and during the second year 16 females were provided with radio-tags. Nine of these individuals were surveyed during both years. The tortoises from the National Park are referred to as the wild population (WP).

Body mass of *A. radiata* was recorded either with a spring scale (Pesola Macro-line, accuracy 100 g) or a digital scale (MyWeigh Ultraship 55, accuracy 10 g). The number of eggs per clutch was recorded for each nest. Each egg was weighed on a digital scale (MyWeight Pointscale PT-500, accuracy of 0.1 g). Prior to handling, all eggs were marked with a pencil to avoid a change in their orientation during data acquisition. The number of clutches per female was also recorded. Mean egg mass per clutch was determined and used in the data analysis. To avoid bias from highly productive tortoises, those females that laid more than one clutch within the study period were included only once. The mean egg mass was then determined for all their clutches laid within the study period. All mean values are indicated with their standard deviation.

The analysis of tortoise data was carried out using Excel. A linear regression was performed to examine the relationship of female body mass to mean egg mass per clutch. As graphical analyses indicated regular variations, the residuals of both study groups were tested for differences. A Pearson correlation was performed to describe the relation of female body mass and total clutch mass. All statistical testing was performed using SPSS 13.0 (Bühl 2006). Only data from female tortoises that were observed during egg deposition were considered for analyses.

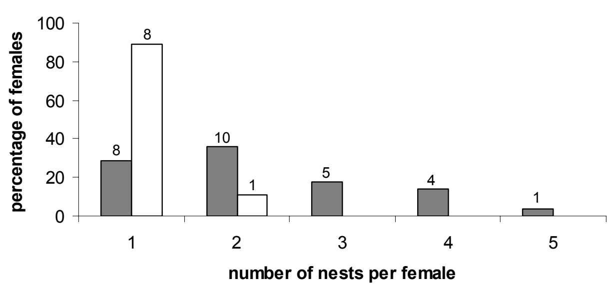

Figure 1. The number of nests per season per female *A. radiata* for the two survey areas: CP (n = 64) is shown in gray bars, WP (n = 10) is displayed as white bars. Numbers above the bars indicate the number of nesting females. WP = wild population in Tsimanampetsotsa National Park, CP = captive population in the Village des Tortues, Ifaty-Mangily.

Table 1. The total number of female *A. radiata* observed in this study, the number of breeding females that were observed during egg deposition, as well as the total number of nests and eggs. The data are divided by study site and survey year. WP = wild population in Tsimanampetsotsa National Park, CP = captive population in the Village des Tortues, Ifaty-Mangily.

	Year	Total Females	Breeding Females	No. of Nests	No. of Eggs
WP	2009	19	6	7	18
	2010	16	3	3	7
CP	2008	40	15	40	134
	2009	40	6	14	45
	2010	40	7	10	39

RESULTS

The two surveyed tortoise populations differed greatly in body mass. The CP tortoises were significantly heavier than the WP tortoises (Mann-Whitney-U-Test, z = -2.2, p = 0.03, n = 30). CP tortoises showed a mean body mass of 7.1 ± 2.2 kg (mean ± 1 S.D.; range = 4.3–12.3 kg), while mean body mass in WP tortoises was 5.3 ± 0.7 kg (range = 3.9–6.3 kg).

In the WP, seven female *A. radiata* were observed laying eggs during the study period (see Table 1) with one female laying two clutches in 2009 and two females were found nesting during both survey years. In the CP most females produced more than one clutch per breeding season (Fig. 1). Nesting activities during consecutive years were observed in several tortoises in the CP: two tortoises were recorded to nest during all three survey years, one

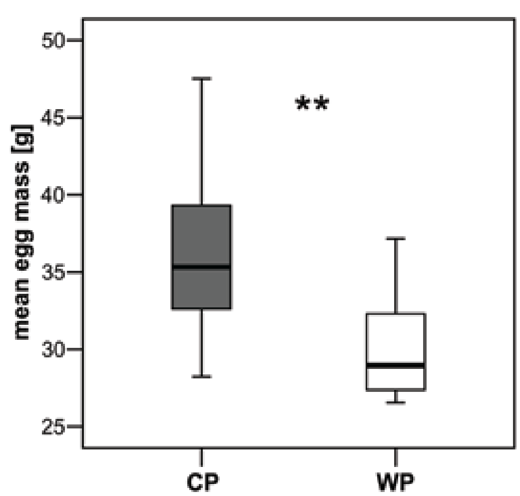

Figure 2. Comparison of mean egg masses per clutch in captive (CP) and wild (WP) populations of *A. radiata*. Both populations differed significantly (T-Test: t = -2.95, p = 0.006, n = 30).

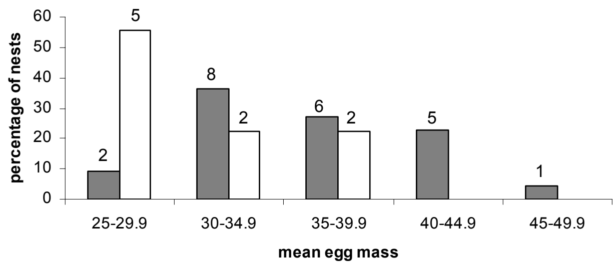

Figure 3. Distribution of mean egg masses per clutch in *A. radiata* for both study sites: CP (captive population, n = 21) in gray bars, WP (wild population, n = 9) shown in white bars. Numbers on top of the bars indicate the number of clutches. Concerning females that laid several clutches, the mean egg mass was determined for all clutches within the whole study period.

female nested during the first two years of data recording and two females were observed to nest during the first and last survey year. All of these females produced at least three nests within one of the surveyed years.

While in the WP only one female was observed laying eggs twice within the same breeding season, the CP females produced up to five clutches per nesting season (Fig. 1) with 71% of the nesting females laying more than one clutch. The internesting interval ranged from 11 to 148 days (mean = 51 ± 25 days, n = 34).

The CP tortoises laid bigger eggs than the WP females. Mean egg mass in CP females ranged from 28.2 to 47.5 g with a mean of 36.3 ± 5.2 g, while the WP females produced eggs ranging from 26.6 to 37.2 g with a mean of 30.6 ± 3.8 g. The two populations differed significantly in mean egg mass (Fig. 2; T-Test: t = -2.95, p = 0.006, n = 30). In the WP no mean egg mass larger than 40 g was detected, while about 25% of all CP clutches had mean egg masses larger than 40 g (Fig. 3).

There was a significant difference in the relationship of mean egg mass and female body mass between CP and WP. The bigger CP tortoises produced bigger eggs than the smaller females from the WP. The residuals gained from linear regression tended to have positive values in CP, while in WP the residuals had more negative values (Fig. 4; T-Test: t = -2.1, p = 0.045, N = 30).

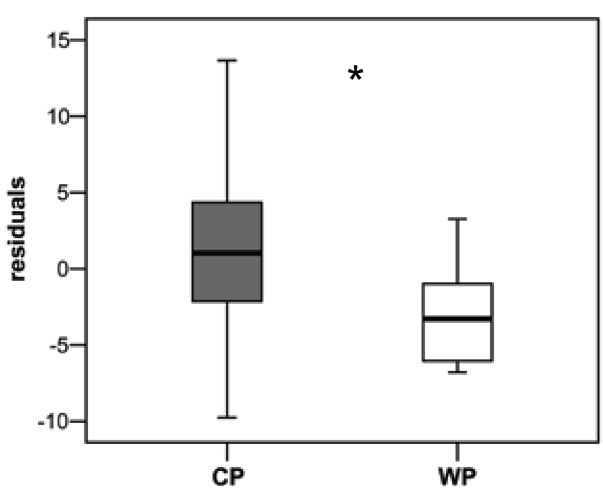

Figure 4. Residuals from the regression between tortoise body mass and mean egg mass per female *A. radiata*. WP = wild population in Tsimanampetsotsa National Park, CP = captive population in Village des Tortues, Ifaty-Mangily.

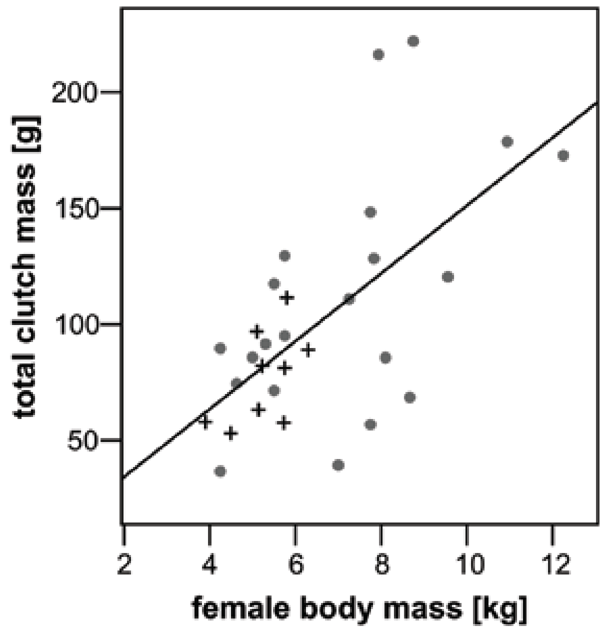

Figure 5. The relation of total clutch mass and tortoise body mass for female *A. radiata*. WP-data (n = 9) are shown as black crosses, CP-data (n = 21) are displayed as gray dots. The equation for total fit line is: $y = 14.6\,x + 5.03$; $R^2 = 0.39$.

Clutch sizes of both populations differed considerably with CP tortoises producing 1 to 7 eggs per clutch (total clutch mass: 36.7 to 221.9 g) and WP clutches contained 2 to 3 eggs (total clutch mass: 53.1 to 111.5 g). Total clutch mass was positively correlated with female body mass (Fig. 5; Pearson: r = 0.63, p < 0.001, n = 30) and mean total clutch mass (CP: 111.4 ± 52.3 g; WP: 76.9 ± 20.2 g) was significantly higher in CP (T-Test: t = -2.6, p = 0.015, n = 30).

DISCUSSION

The surveyed tortoise populations of *A. radiata* differed significantly in body mass and mean egg mass. Captive tortoises were larger and produced heavier eggs as well as larger total clutch masses than animals from the wild population. Positive correlations between female body mass and egg mass are known from other tortoise species (Hailey and Loumbourdis 1988; Averill-Murray and Klug 2000; Loehr et al. 2004; Bertolero et al. 2007) and in freshwater turtles (Congdon and Gibbons 1985). Big females may contain more or bigger eggs than small individuals. Still, captive tortoises were also observed to produce several clutches within the same reproductive season and during consecutive years. O'Brien (2002) reported captive female *A. radiata* producing 3 clutches per year on average, with two females nesting within ten consecutive years. In the wild population in the present study, nesting activities during consecutive years were observed in two individuals and only one female produced two clutches within the same breeding season.

Wild populations of European tortoises are commonly observed nesting multiple times within the same breeding season (Hailey and Loumbourdis 1988), while the North American desert tortoise (*Gopherus agassizii*) does not appear to produce more than one clutch per year and most individuals skip reproduction for at least one year after nesting (Averill-Murray and Klug 2000). Leuteritz (2002) found individual females *A. radiata* of a wild population at Cap Sainte Marie to be producing up to three clutches within the same season. Furthermore, mean egg mass recorded by Leuteritz (2002) in the Cap Sainte Marie population (39.0 g; range, 28.0–55.0 g; n = 56) was higher than records from the wild population from Tsimanampetsotsa in the present study. Site differences surely influence living conditions of *A. radiata* at both sites, with the Reserve Cap Sainte Marie lying further to the south and possibly representing a more productive habitat with less human impact.

Living conditions and food availability play a major role in the ability of animals to breed. Due to their herbivorous diet tortoises can take a long time to accumulate enough energy for laying eggs (Hailey and Loumbourdis 1988). In the wild, *A. radiata* is exposed to harsh living conditions with less than 400 mm of rainfall per year (Pedrono and Smith 2003) resulting in scarce food resources during the dry season. Within this study, wild female *A. radiata* may not have been able to build up enough energy to produce several clutches per year, nor able to produce eggs larger than 40 g as the captive females.

In contrast, the regular feeding and water supply in Ifaty-Mangily allowed the captive tortoises to be highly productive, with over 70% of the breeding females laying more than one clutch within the same season and between consecutive years. The captive population benefited from unlimited food resources, which allowed them to overcome seasonal constraints, resulting in an extended breeding season. In Ifaty-Mangily nesting activities were still recorded in late September, while the females in the wild population appeared to go into estivation by the end of June (pers. obs.).

While populations in captivity that receive food regularly may easily produce bigger animals, the observed differences in the body mass of tortoises between the surveyed populations could not be related to their living conditions. The captive tortoises arrived only shortly before this study was carried out in Ifaty-Mangily, with former living conditions unknown. The largest female detected in the wild during this study had a body mass of 6.3 kg. In contrast, Leuteritz (2002) found an average female body mass of 6.3 kg (range, 4.3–8.4 kg) at Cap Sainte Marie. These numbers are still very low considering that this species can grow up to 13 kg (Pritchard 1979). However, adult females are of major interest to tortoise poachers as they might contain eggs that can be consumed in addition to their meat (Leuteritz et al. 2005). The wild population of *A. radiata* in Tsimanampetsotsa National Park is clearly influenced by human activities, as large individuals are rare (Goodman et al. 2002; Leuteritz 2002; Hammer and Ramilijaona 2009). Accessibility to this area can easily be achieved and fishing boats from Toliara are known to sail down south for tortoise collections (O'Brien 2002; SuLaMa 2011). Differences in body mass between the two populations of *A. radiata* in this study are therefore assumed to be related to differences in the age of the tortoises rather than in food availability or living conditions. A continuing depletion of wild populations of *A. radiata,* with a special demand for big females, will likely result in smaller individuals that produce smaller egg masses. These smaller eggs contain less nutritious egg yolk, which is essential for embryonic development during incubation, and this might ultimately lead to embryonic development failures during incubation, and produce underdeveloped hatchlings. Lower survival rates of hatchlings after emergence would be the expected outcome, especially with prolonged dry periods in a local climate as unpredictable as in Madagascar (Dewar and Richard 2007).

Acknowledgments. — This study was carried out under the Accord de Collaboration between ANGAP (now MNP: Madagascar National Parks), the Département de Biologie Animale, Université d'Antananarivo, and the Department of Biology, Hamburg University. I am grateful to the late Olga Ramilijaona, Daniel Rakotondravony, and Miguel Vences for their help at various stages of the study, and Jocelyn Rakotomalala and Domoina Rakotomalala (both MNP Toliara) and WWF Madagascar for their logistical support in the field. Part of this study was carried out in the Village des Tortues in Ifaty-Mangily where I would like to thank Jean Kala, Daniel Kotonirina Ramampiherika, and Bernard Devaux for their support. In Tsimanampetsotsa I would like to thank M. Edson and the Team Andranovao. The study was financed by grants from the German Academic Exchange Service (DAAD) to Jutta Hammer, DFG/BMZ (Ga 342/15-1), the European Association of Zoos and Aquaria (EAZA), and WWF Germany to Jörg Ganzhorn.

Résumé

Les tortues n'arrivent à maturité sexuelle qu'après de longues années. De ce fait, les populations sauvages peuvent s'éteindre lorsque les tentatives de reproduction échouent. La survie tout au long de l'incubation pourrait être influencée positivement par un plus grand volume des œufs. Des œufs plus gros constituent une source plus grande de nourriture pendant l'incubation et protège de la sécheresse pendant les périodes sans pluie. Une possible corrélation entre le volume des œufs et la masse de la femelle a été examinée chez *Astrochelys radiata*, une tortue endémique de Madagascar. Deux populations de tortues soumises à des conditions de vie différentes ont été étudiées. Des données provenant d'une population sauvage ont été comparées aux observations d'individus en captivité. Les données du nid et la taille des œufs ont été reliées à la taille de la femelle. Les femelles provenant

de la population en captivité étaient significativement plus grandes que celles sauvages. Elles ont produit également, de façon significative, des œufs plus gros et des couvées plus importantes. Une alimentation régulière et l'accès à de l'eau en captivité peuvent avoir permis à ces femelles d'être hautement productives pendant la période de reproduction.

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