SALAMANDRA **58**(2): 151–156
15 May 2022 | ISSN 0036–3375

SALAMANDRA
German Journal of Herpetology

The effects of handling on natural populations of the Critically Endangered Pancake Tortoise, *Malacochersus tornieri* (Testudines: Testudinidae)

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Manuscript received: 5 October 2021
Accepted: 10 April 2022 by Edgar Lehr

Abstract. Despite its low population densities and restricted geographical distribution in the eastern and southern African countries of Kenya, Tanzania, and Zambia, the Pancake Tortoise, *Malacochersus tornieri* (Siebenrock 1903), has been the most exploited species for the international pet trade. Whilst being extracted from their hiding rock crevices, and on handling, these tortoises have frequently been observed to void faeces, presumably as a response to handling stress. However, the potential effects of handling on these animals are unknown and have never been evaluated. The present study was conducted at two sites outside the Tarangire National Park, Tanzania, with two objectives: i) to establish the proportion of individual Pancake Tortoises that respond to handling by humans with faecal elimination, and ii) to evaluate the amount of water lost through faecal elimination. Thirty-one Pancake Tortoises were encountered and handled, and 22 (70.97%) excreted faeces within an average of five minutes of handling. The mean percentage of water contained in the excreted faecal wet mass was 82% (SD ± 10, N = 22), suggesting that their faecal mass was essentially water. Whereas handling stress alone may not have a lasting negative impact, the physiological impact of lost body fluid may constitute a more significant problem.

Key words. Critically Endangered, dehydration, faecal elimination, *Malacochersus tornieri*, semi-arid.

Introduction

The Pancake Tortoise, *Malacochersus tornieri* (Siebenrock 1903), is a morphologically distinctive terrestrial chelonian, historically considered endemic to the East African countries of Kenya (Malonza 2003) and Tanzania (Klemens & Moll 1995, Mwaya et al. 2018). Recently, Chansa & Wagner (2006) have reported a further population of Pancake Tortoise in Zambia's northern province of Nakonde. However, Eustace et al. (2021), in their modelling of the distribution of the Pancake Tortoise under current and future climate scenarios, did not find climatically suitable habitats for this species in Zambia. *Malacochersus tornieri* has a maximum straight-line carapace length (CL) of 17.8 cm and rarely weighs more than 500 g, even though Moll & Klemens (1996) have reported a Pancake Tortoise weighing 600 g from Tanzania. The shell is dorsoventrally flattened and very pliable (Siebenrock 1903, Mautner et al. 2017). This is an adaptation to making use of rock crevices, which provide ideal hiding places from generalist predators and retreats from thermal stress in the semi-arid Maasai steppe (Ngana 1992, Kangalawe 2003). Daily temperature oscillations in semi-arid environments can exceed the thermal optima for these tortoises.

Since the species is morphologically unique, endemic to eastern and southern Africa, and being relatively little studied, field ecologists are working towards increasing the information base necessary for designing and implementing specific conservation strategies. Apart from the collection of Pancake Tortoises for the international pet trade, field studies have likewise involved extracting and handling of these tortoises (e.g., Klemens & Moll 1995, Chansa & Wagner 2006, Mwaya et al. 2021). The extraction of the tortoises from their rock crevices can be laborious (Fig. 1), and subsequent handling appears to inflict considerable stress on the animals.

Generally, wild animal capture, handling, transport, restraint, and release into new environments have been reported to cause acute and sometimes chronic stress (Canabac & Bernieri 2000, Fazio et al. 2014, Currylow et al. 2017). The commonly used endogenous indicator of stress in reptiles has been the measurement of the plasma corticosteroid (CORT) hormone. Indeed, some authorities have even considered plasma CORT as a "biomarker" of an altered physiological state (Drake et al. 2012). Effects associated with the handling response have been observed in some tortoises (Canabac & Bernieri 2000) and include stress fever and tachycardia, as has been reported from *Clemmys insculpta* LeConte, 1830.

Almost immediately after extraction from a crevice, Pancake Tortoises habitually defecate. This behaviour is most likely a sign of handling stress. However, neither the relationship between defecation and handling nor its physiological effects on the species have as yet been elucidated. The direct stress caused by handling, subsequent faecal elimination, and likely the loss of the water contained in the voided faeces may have a negative impact on the fitness of the handled tortoise.

In desert and semi-desert habitats, chelonians survive the limited availability of water through a combination of adaptations (Abere & Oguzor 2011). For example, the American Desert Tortoise, *Gopherus agassizii* Cooper, 1863, and the African Spurred Tortoise, *Geochelone sulcuta* Miller, 1779, shelter inside subterraneous burrows or in rock piles (Averill-Murray 2002). The Pancake Tortoise employs a similar strategy and has even become dependent upon rock crevices that will keep them shielded from extreme temperatures as well as from attacks by generalist predators (Klemens & Moll 1995). As a further adaptation to avoid thermal stress and water loss, the species may avoid activity outside crevices during the hot days of the dry season even though they have been observed to be active any time of the day (Moll & Klemens 1996, Schmidt 2002). However, this might be the case only during the wet season when temperatures are more tolerable for these tortoises (Malonza 2003). In this manner, they may avoid the extreme and therefore unfavourable day temperatures of the African semi-arid climate (Ngana 1992, Kangalawe 2003). Being a species of dry land, most of its water requirements is sourced from its herbaceous diet, and this is especially true during the long dry season. Water conservation is therefore one of the ecological challenges facing Pancake Tortoises.

Figure 1. A Pancake Tortoise hiding inside a rock crevice. Photo by R. Mwaya.

While faecal elimination in response to extraction from a shelter crevice may affect body water homeostasis, the improper transportation of live individuals can amplify the effects even further (Luiijf 1997, Fazio et al. 2014). Unfortunately, there is no study quantifying the effects of handling and transportation stress. Understanding these effects and the associated water loss is important to all Pancake Tortoise handlers including captive breeders and field researchers. This knowledge would allow the formulation of handling management strategies to alleviate stress and its associated consequences. Thus, this study was carried out specifically to; i) establish the proportion of sampled individuals that respond with faecal elimination to handling stress, and ii) to quantify the amount of water lost by tortoises in the process.

Materials and methods

Description of the study area

Our study was conducted within the Tarangire-Manyara ecosystem, beyond the northwestern boundary of the Tarangire National Park. The whole ecosystem lies between latitudes 3°40'–5°35' S and longitude 35°45'–37° E at an altitude between 1200 and 1600 metres above sea level (Msoffe 2003). Specifically, the study area falls within the Kwakuchinja Wildlife Corridor, which connects the Tarangire and Lake Manyara National Parks, and this area lies within the graben of the eastern arm of the Great African Rift Valley. Two sites (names withheld for conservation reasons) outside Tarangire National Park were chosen for our study because of convenient accessibility.

The Tarangire-Manyara ecosystem is part of the semi-arid Maasai Steppe, receiving an average of 650 mm of rain annually (Ngana 1992, Kangalawe 2003, Msoffe 2003). The rain is bimodal with a longer rainy phase from March through May, and a shorter rainy period in November and December. It is especially hot between November and the beginning of January, when temperatures reach up to 29°C. While daytime temperatures rarely fall below 25°C, the nights are considerably cooler during the long dry season.

The vegetation of the Tarangire-Manyara ecosystem comprises mostly dry, open woodlands, including thorny acacia thickets and some stands of flat-topped acacia, *Vachellia tortilis* (Forsk.) dotted with *Adansonia digitata* L. on the high ground. In areas with a better water supply, *Vachellia xanthophloea* Benth. and occasional stands of palm trees, *Borassus* spp. L, are common.

Field methods

Field data collection was conducted towards the end of the wet season from 25 to 31 May. Three field-experienced persons were involved in the microhabitat search for the tortoises and spent a total of 21 field man-days. Searches were specifically focused upon among rocks on two rocky hillsides. When a suitable crevice was discovered, a batterypowered flashlight was used to check out the dark interior. If it sheltered a tortoise it was retrieved using a modified iron bar, about 100 cm long, slightly bent at its distal end to form a hook.

Our field protocol regarding the extraction of tortoises from their crevices was limited to a maximum of three consecutive attempts with the iron bar. If the tortoise could not be recovered it was left for the day. The three attempts were furthermore limited to less than a half a minute in duration. Our field experience showed that the more time it takes to retrieve a tortoise, the more firmly it would wedge itself in, and the more force it would require to loosen it. Indeed, the tortoise may end up being injured.

The person handling a tortoise wore disposable surgical gloves to avoid possible contaminations. Body mass was measured using a portable solar-powered scale (MAUL tronic), with an accuracy of ± 1 g. Individuals were sexed on the basis of tail shape. Tortoises with long and slender tails were categorized as males whereas females were considered those with short and stumpy tails (Moll & Klemens 1996).

A stopwatch was used to record the time from the point when the tortoise was picked up by hand and held to the time it defecated, if it indeed did. It excluded the time taken to work the tortoise out of its crevice. The faeces were allowed to drop into a drug prescription plastic bag, which was immediately sealed to avoid moisture loss. Since the faeces were relatively small, the MAUL tronic scale proved too insensitive for precise weighing. Therefore, a more accurate electronic scale with an accuracy of 0.1 g was employed later the same day at the base camp.

To avoid recapturing and subjecting an already processed tortoise to unnecessary repeated stress, individuals were temporarily marked using nail vanish dabbed on the tapering back of the carapace to avoid being easily scraped off against the ceiling of a crevice. Five days later, at Mweka Wildlife College, faecal samples were unpacked from the plastic bags into the petri-dishes, re-labelled, and placed in an oven. The samples were then dried at a constant temperature of 50°C, and their weights were monitored until no further weight loss was notable. This took approximately eight hours. The differences between wet and dry weights were recorded as the amounts of water originally contained in the faeces.

The field data were transferred to a Microsoft Excel spreadsheet and used for descriptive tabulation, bar charts, and scatter graphs. A chi-square test of independence determined whether the frequency of faecal elimination differed between sexes. The Student's t-test for paired data was used to compare between mean wet and dry faecal weights, as well as between wet faecal weights versus the weight of the water content in the faeces. We furthermore tested for the correlation coefficient (r) between a tortoise's body mass and its excreted faecal mass as well as between wet faecal mass and mass of water content alone. All inferential statistics were considered significant at $p < 0.05$ (Zar 1984, Hassard 1991).

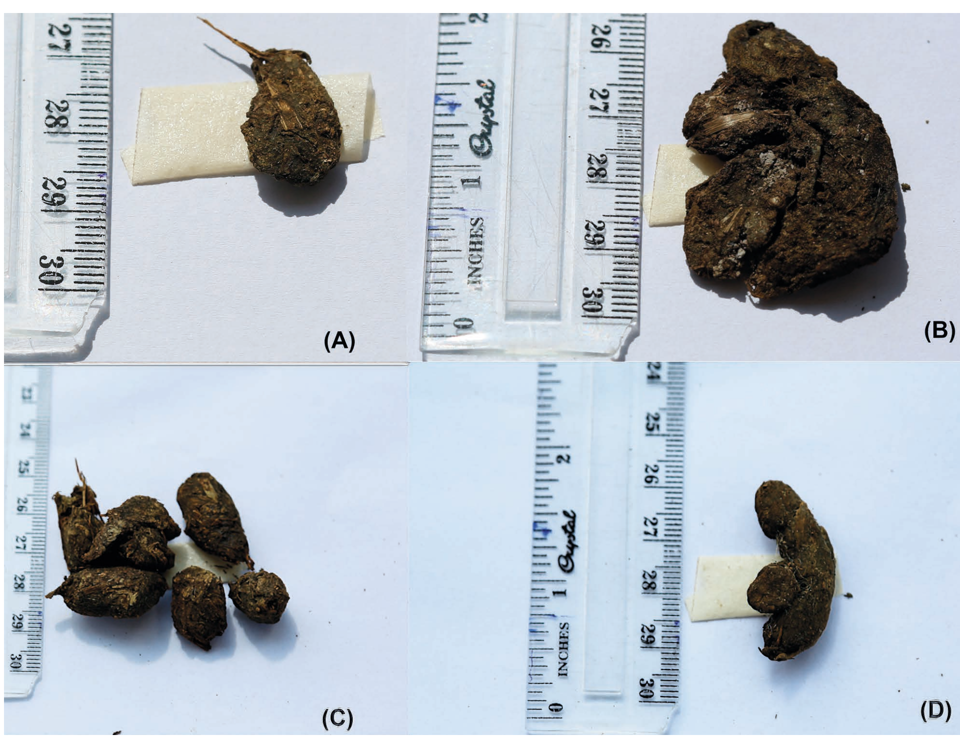

Figure 2. Different shapes of dried faeces from the Pancake Tortoise. (A) a small single faecal pellet voided by an immature tortoise, (B) a lumped faecal mass, (C) fragmented faeces, (D) constricted piece of faeces. Photos by S. Kiondo.

Results

A total of 31 Pancake Tortoises were located and extracted from their crevices. Twenty-eight were sexed and yielded an equal representation of 14 (45.27%) males and 14 (45.27%) females. Three (9.7%) were immature tortoises. Out of the 31 temporarily collected tortoises, 22 (70.97%) voided faeces within an average of 5 minutes (range: 1–9 minutes, N = 22) of handling, and nine (20.3%) did not.

Tortoises with faecal elimination (N = 22, 70.97%) in response to handling were significantly greater in number than those without elimination (N = 9, 20.3%) ($\chi^2 = 5.4$, df = 1, $p < 0.05$). Out of those tortoises that voided faeces, 11 (39.29%) were males, nine (32.14%) females, and two (18.57%) were immature. We found no influence of sex on faecal elimination following handling ($\chi^2 = 0.70$, df = 1, $p < 0.05$). The faeces voided by the Pancake Tortoises were quite amorphous. Whereas some faecal masses came out in fragments of two or more unequal chunks, often voided by adults, the three immature tortoises all voided smaller but coherent faeces (Fig. 2).

The mean mass of wet faeces was 6.68 g (SD ± 3.91, N = 22) while the mean mass of dry faeces was 1.16 g (SD ± 0.74, N = 22). The mean percentage of water contained in the faecal wet mass was 82% (SD ± 10, N = 22) (Fig. 3). Applying a paired t-test between mean wet and dry faecal masses revealed a statistical significance ($t = 3.4$, df = 20, $p < 0.05$). However, there was no statistical difference between wet faecal mass and mass of water content in the faeces ($t = 2.04$, df = 20, $p > 0.05$) (Fig. 4).

There was a weak but significant positive correlation ($r^2 = 0.255$, $y = 0.019x + 0.579$) between tortoise body mass and wet faecal mass ($r = 0.505$, df = 20, $p > 0.05$) (Fig. 5). Furthermore, there was a high positive correlation ($r^2 = 0.99$, $y = 0.848x + 0.177$) between wet faecal mass and mass of water content in the faeces (Fig. 6) and this was highly significant ($r = 0.994$, df = 20, $p > 0.05$).

Discussion

Faecal elimination response to human handling appears to be common in wild Pancake Tortoises, at least during

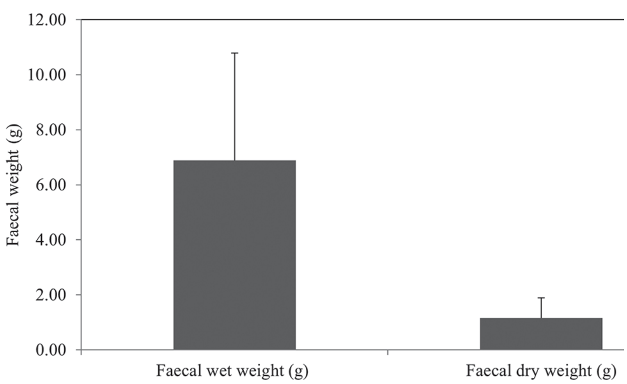

Figure 3. Mean faecal wet and dry weights (g) from twenty-one tortoises were statistically significant ($t = 3.4$, df = 20, $p < 0.05$). Error bars are standard deviations (SD).

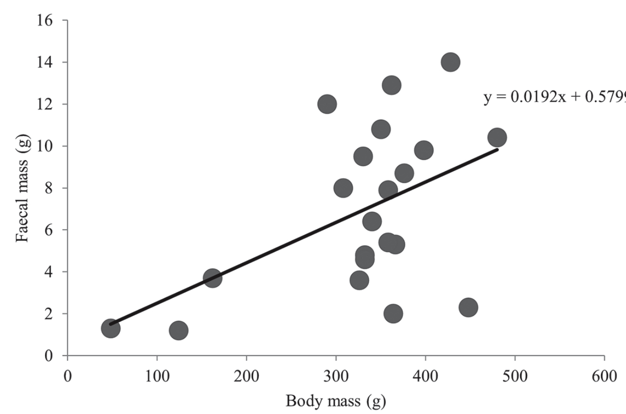

Figure 5. A weak, but significant, correlation ($r^2 = 0.255$, $y = 0.019x + 0.579$) between body mass and wet faecal mass ($r = 0.505$, df = 20, $p < 0.05$).

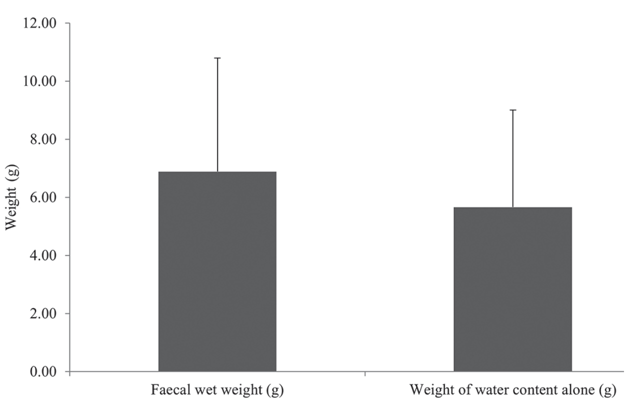

Figure 4. Mean faecal weight (g) versus weight of water content (g) in the faeces were not statistically significant ($t = 2.04$, df = 20, $p > 0.05$). Error bars are standard deviations (SD).

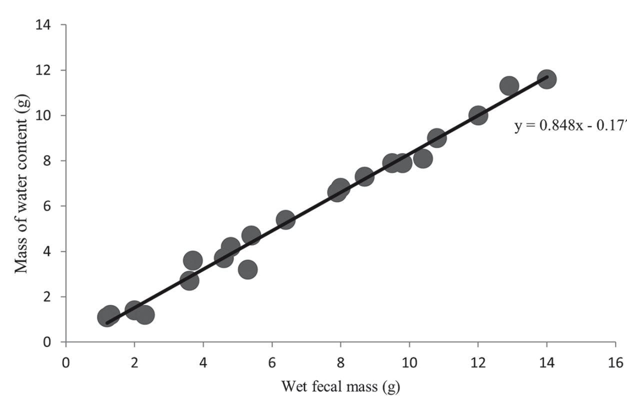

Figure 6. A significant correlation ($r^2 = 0.99$, $y = 0.848x - 0.177$) between wet faecal mass and water content ($r = 0.994$, df = 20, $p < 0.05$).

the wet season. We do not know for sure whether faecal elimination is an external manifestation of the effects of handling stress or not. There is no literature on chelonians in general, and Pancake Tortoises in particular, that has linked with scientific accurateness of faecal elimination to stress caused by handling. However, Averill-Murray (2002) has reported urination as a response to handling in the American Desert Tortoise, *Gopherus agassizzii*. Stress has also been associated with increased temperature in a lizard, *Callopistes maculates* Gravenhorst, 1838 (Canabac & Gosselin 1993), and tachycardia in a tortoise *Clemmys insculpta* (Canabac & Bernieri 2000).

The most common assessment of handling stress has been through measuring endogenous corticosterone (CORT) hormone concentrations (Grandin 1997, Von der Ohe & Servheen 2002). This has so far been done for a range of vertebrate groups, such as rats and mice (Briese & deQuijada 1970), a lizard (*Callopistes maculates*; Canabac & Gosselin 1993), and in chelonians (Canabac & Briese 1991, Gregory et al. 1996, Cash et al. 1997, Ott et al. 2000, Palme et al. 2005, Fazio et al. 2014), but neither Pancake Tortoises nor their allies – the Leopard Tortoise *Stigmochelys pardalis pardalis* Bell 1828, Speke's Hinged Tortoise *Kinixys spekii* Gray, 1863, and Bell's Hinge-back *K. belliana* Gray, 1830 have been studied to understand the effects of handling stress and its link to CORT levels.

Presumably, the faecal elimination by handled Pancake Tortoises in the wild is likely a reflex induced by handling stress. Our study on a wild population of *M. tornieri* did not take measurements of body temperature and heart rates. However, handled individuals struggled vigorously by kicking their legs and shaking their heads, presumably in an effort to free themselves. Undoubtedly, this behaviour use up considerable energy, and thus we hypothesize that it should go along with increased heart beats and body temperature and probably also activate the production of plasma CORT to restore body homeostasis. These suppositions, however, warrant further investigation.

The extent and persistence of the stress response depends on the severity of the stressor (Fazio et al. 2014). Using *Clemmys insculpta* as a study object, Canabac & Bernieri (2000) demonstrated that unrepeated short-term stressing, lasting for a few minutes, saw both emotional fever and tachycardia abate quickly. Long-term stressing, lasting for two hours, had the stress response maintained, on the other hand. One effect of sustained stress is the lowering of the body condition index (BCI), and subsequently increased susceptibility to compromised health (Fazio et al. 2014). This might have contributed to the mass dying in an illegal consignment of Pancake Tortoises that was intercepted at Schiphol Airport, Amsterdam, in 1991 after long hours of flight from Tanzania in badly packed conditions (Luiijf 1997).

The strong correlation between wet faecal mass and water content in the wet faeces implies that the eliminated faeces were essentially water. Indeed, this can be a significant loss of water for this dry-land species, and especially when stress-related. Water loss through faecal voiding could contribute to body fluid imbalance and dehydration, and can be lethal if not controlled. However, whereas short-term stress may not have a lasting negative impact (Canabac & Bernieri 2000, Currylow et al. 2017), the physiological impact in terms of the loss of water may be a more significant problem. It is therefore recommended: i) to minimize stress when studying Pancake Tortoises in the wild, field data collection involving physical intervention should be carefully planned, and only experienced researchers should be permitted to undertake procedures; ii) research associated with Pancake Tortoise handling should be limited, preferably, to the wet season when tortoises can more easily replenish their vital water reserves, and iii) further investigation, using the glucocorticoid hormone, is recommended in order to understand the link between faecal elimination and levels of plasma CORT in the Pancake Tortoise.

Acknowledgements

We thank the late H. Zwartepoorte who used to work with the Rotterdam Zoo, for providing material support that enabled the collection of field data. Thanks to J. Naibala and N. Kapinga for their assistance in Pancake Tortoise searching in the field. D. Moll and M. Jones helped with reviewing the manuscript, and their comments were invaluable.

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