Non-lethal injury in Hermann's tortoise, *Testudo hermanni*, in Croatia and Montenegro

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ABSTRACT – Field injuries in Hermann's tortoise (*Testudo hermanni*) from Croatia and Montenegro are described. In Montenegro, male damage scores were significantly lower than those of females, but in Croatia the difference was not significant. Regression analysis indicated that damage score in Croatia was associated with growth ring number (i.e. age) but in Montenegro with body mass, although only in females. Based on mark-release/recapture data, damaged tortoises were recaptured more frequently than undamaged tortoises. No significant differences were found between body mass levels or body temperatures of damaged or undamaged tortoises.

THE ability of animals to survive injury in field populations is a key factor in population ecology. Survival from injury may enhance reproductive effort (Harris, 1989) and contribute to the evolution of anti-predator strategies (Vermeij, 1982). Reptiles show several physiological characteristics that pre-adapt their survival from injury. For example, blood loss from a wound is much less than in an endotherm and reptilian nerve tissue is resilient and may remain viable for long periods without oxygen (Close *et al*., 1996; 1997). However, other than direct physiological impacts from injury, including secondary infection, there may be ecological costs. These include a reduction in locomotory capacity, the ability to secure food items, reduced growth, delayed maturation and also injured animals could fare poorly in intra-sexual conflicts (Gregory & Isaac, 2005). Therefore the ability to tolerate sub-lethal injury, either through contact with predators or accidental contact with objects in the environment, has broad evolutionary and ecological consequences.

A well known form of non-lethal injury in reptiles is autotomy. Found mostly in lizards and to a lesser extent in snakes and salamanders (e.g. McConnachie & Whiting, 2003; Bernando & Agosta, 2005; Gregory & Isaac, 2005) autotomy has evolved as a defence mechanism, but its employment may necessitate behaviour changes or have ecological costs (e.g. Brown *et al*., 1995; Downes & Shine, 2001). Non pre-adapted wounds, which could be more debilitating, may also have behavioural and/or physiological consequences, but most studies of reptilian field injuries have focused on the squamates, species that usually have relatively short life spans and fast generation times (e.g. Schoener & Schoener, 1980; Gregory & Isaac, 2005). The chelonians on the other hand are typically long-lived and although known to sustain damage from a variety of sources, have been less studied in this respect. For instance, Hailey, (1990) has indicated that successful repeated annual reproduction in Hermann's tortoise *Testudo hermanni* is a critical component of lifetime reproductive success, since although it is mature for an estimated 42 – 56% of its lifespan with egg production approximately 1-6 eggs per clutch, hatching success may be low (Swingland & Stubbs, 1985) with mean annual survivorship in the region of 3–5% (Meek, 1985, 1989; Stubbs & Swingland, 1985). Under such reproductive constraints, it might be expected that by necessity, there would be a trend towards the evolution of high tolerance of field injuries, at least in the adult state.. This paper examines this possibility using information on injuries or damages gathered during field studies of *T. hermanni* in Montenegro and Croatia where both population ecology (Meek, 1985; 1989) and thermoregulation (Meek, 1984; 1988a; 1988b) was studied.

METHODS

The data used in this paper is based on tortoises measured during fieldwork undertaken in

Montenegro during 1983 and 1986 and Croatia in 1986. There were three study sites in total, all typical Mediterranean mixed scrub, situated on the Adriatic coast. However, because of the small sample sizes of damaged individuals and the fact that the Montenegrin populations were living in more rocky environments alongside agricultural activities, the data from these populations were pooled. Typical habitat types are shown in photographs in Meek (1989) and Meek & Inskeep (1981). Field methodology was described in the original papers (Meek, 1985; 1989) so only brief descriptions are given here. The results are derived from 127 (18 damaged) tortoises from Croatia (20 kilometres south of Dubrovnik) and in total 168 animals in Montenegro (18 damaged; 8 from Sutomore - Meek, 1989) and 10 from Budva (Meek, 1985).

Carapace length was taken as a straight-line measurement between the leading edges of the nuchal and supracaudal scutes and body mass by suspending the animals in a cloth bag using a spring balance. Hermann's tortoise produces one growth ring annually at least until maturity of 13-14 years (Castanet, 1985) and age estimates were made using these counts (e.g. Meek, 1985; 1989, Stubbs & Swingland, 1985). Body temperatures recorded were cloacal and recorded with mercury bulb thermometers. They were spot measurements of animals when they were located and not continuous on any one animal (Meek, 1984; 1988a).

Information of physical injury was collected in both written and photographic form and examples are shown in Fig 1. A score system was employed reflecting the degree of the injury sustained. This was based on estimates of 1) the estimated impact of the injury at the time it occurred and 2) on the estimated effect throughout its life. Other factors taken into account was the likely physiological effort the animal would have needed to recover from the wound, based partly on known veterinary treatments for wounds in captive tortoises. A high score (5) was applied to animals with jawbone breaks, limb loss or major shell damage (Figure 1) on the assumption that the injury would probably have been a major threat to life and indeed continued to act as a handicap to normal activity. Middle scores (2–4) were applied to animals exhibiting large or small dents in the shells with these scores generally reflecting the extent of injury; Figure 1D for example would be scored as 2 and Figure 1E scored as 3). So generally lesser damages were given progressively lower scores with a score of 1 applied to animals showing minor shell damage, for example around the shell margins.

Means of body masses, growth ring numbers, damage scores and body temperatures have been given with standard deviations. Standard errors have been attached to the regression coefficients in equations (1) and (2) and the allometric exponents.

Table 1. Mean growth ring counts with standard deviations of damaged and undamaged tortoises. Values of *p* from ANOVA tests for comparisons of damaged and undamaged data sets are also given.

	Damaged	Undamaged	*p*
Croatia males	22.4 5.3	21.1 5.2	0.36
Croatia females	18.0 2.3	20.2 3.6	0.18
Montenegro males	23.2 3.0	22.6 4.9	0.79
Montenegro females	20.9 3.1	20.2 2.8	0.59

RESULTS

Size comparison of damaged and undamaged tortoises. Figure 2 shows the ranges of damaged tortoises in relation to the ranges of the undamaged sections of the population. Undamaged animals in the graphs include juveniles and/or immatures but these were not included in the statistical analysis unless stated, as they could not be sexed. There was no significant difference (ANOVA, $p > 0.05$) between the mean size (determined here as a function of body mass but excluding unsexed juveniles) of undamaged or damaged males in the samples (Croatia damaged *mean* = 605.7±72.3, not damaged *mean* = 608.1±128.3; Montenegro damaged *mean* = 824.9±123.4, not damaged *mean* = 835.9± 147.6) or between females (Croatia damaged *mean* = 864.2±180.1, not damaged *mean* = 968.4± 211.9; Montenegro damaged *mean* = 1114.2±279.0, not damaged *mean* = 1097.0±251.7). Excluding unsexed juveniles or those showing less than 14 growth rings, Levine's tests were applied for homogeneity of variance between damaged and undamaged samples. The results indicated no significant differences between body mass variances (Levine's test statistics from 0.07 – 2.97 and p values from 0.78 – 0.09). These results suggest that although the ranges of injured/damaged tortoise were smaller than non-damaged individuals, tortoises are just as likely to sustain some sort of injury once they reach maturity – no juvenile was found with any damage.

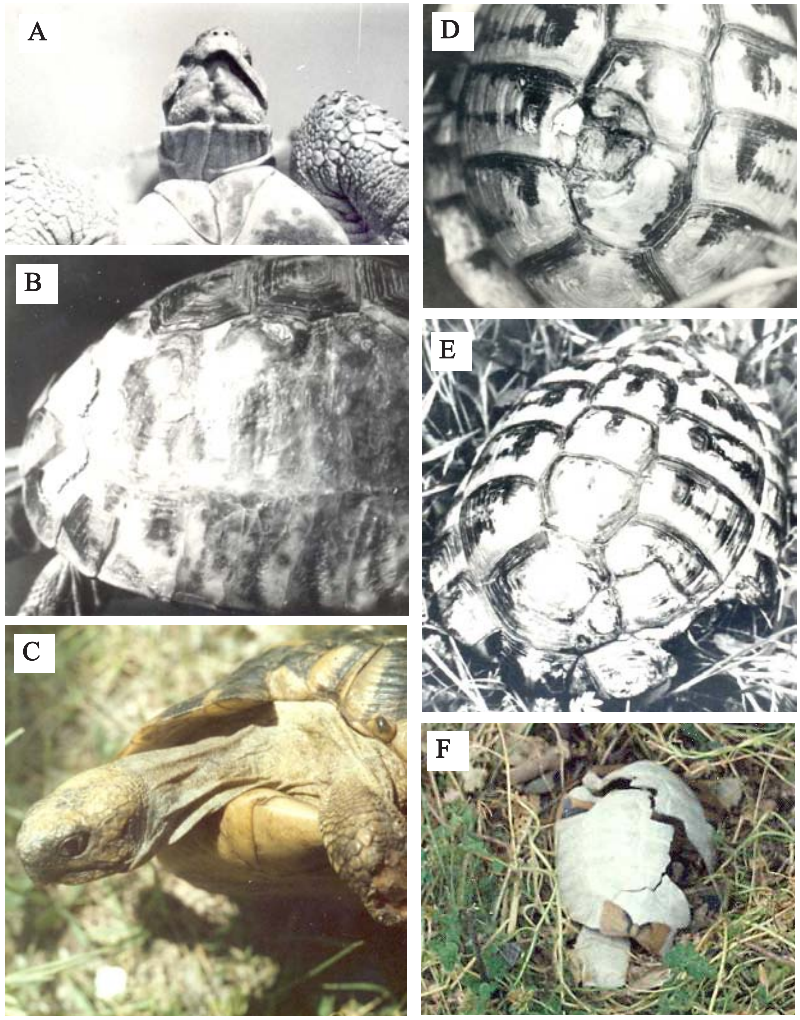

Figure 1. Examples of injuries and mortality in *T. hermanni*. **A**: male with broken lower jaw at the anterior region of the dentary bones (Croatia). **B**: female with heavy scute damage probably from fire (Croatia). **C**: female with front limb and shell damage possibly from farm machinery (Montenegro). Photographs **D** and **E** show tortoises (Montenegro) with dents and shell breakages. These were the common injuries and most probably caused by falls. Photograph **F** shows the remains of a dead tortoise (Montenegro).

Comparison of growth ring numbers. There was no significant difference between growth ring counts of either males or females that had sustained injury against males and females that had no injuries in either population (ANOVA, all tests at p = 0.05). There was also no inter-population differences between damaged male or damaged female growth ring counts (ANOVA). Table 1 shows the basic data sets on which the statistical testing was applied.

Comparison of damage scores. Croatian males with injuries (*mean damage score* = 1.7±1.3, *range* = 1 – 5, n = 13) had slightly lower means than females (*mean damage score* = 1.8±1.1, *range* = 1 – 3, n = 5) with the difference not significant (Mann Whitney U-test, w = 137.5, p = 0.85). In Montenegro, injured males (*mean damage score* = 1.6±1.1, *range* = 1 – 4, n = 8) also scored lower than females (*mean damage score* = 3.2±1.3, *range* =1 – 5, n = 10) with the difference significant (w = 38.5, p = 0.024). Male damage scores were not significantly different between populations (w = 157.5, p = 0.82) but female damage scores in Montenegro were significantly higher than damaged females in Croatia (w = 15.0, p = 0.03).

Regression analysis has been used to test for the probability of tortoises sustaining greater damage score with increases in either growth ring count, body mass or carapace length. Damage score has

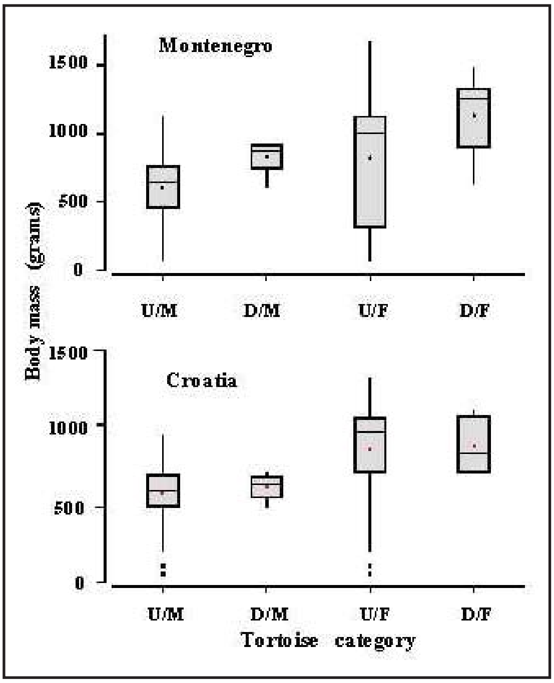

Figure 2. Box plots of body mass distributions of damaged and undamaged *T. hermanni*. The keys are U/M = undamaged males, D/M = damaged males, U/F = undamaged females and D/F = damaged females. The boxes represent the interquartile ranges with the means indicated as solid circles and medians as horizontal bars. The vertical lines either side of the interquartile ranges represent the general ranges of the data. Solid squares represent outliers – data that are between 1.5 to 3 times from the interquartile ranges.

been treated as the dependent variable y and the other variables as independent (x). The equations have the form:

$$\text{damage score(y)} = mx + b$$

where m is the regression coefficient and b the y-intercept. In theory, damage score will be seen to increase as a function of any independent variable when $m > 0$ and no relationship when $m = 0$. To test for significant departures from 0 or between regression coefficients, t-tests at n-2 degrees of freedom have been applied.

The results showed that in Croatian tortoises the probability of sustaining injury was more closely associated with growth ring number (i.e. age). The regression coefficients for males (m = 0.35) and females (m = 0.40) were not significantly different (t = 0.28, p>0.05) and the data sets were pooled. This gave,

$$\text{Damage score} = 0.39 \pm 0.1 \text{growth ring number} - 5.49,\ r^2 = 50.0\%\ (1)$$

with the departure from 0 significant, t = 3.87, *d.f.* = 16, p = 0.001. In Montenegro, the only association was found with increasing body mass in females and gave,

$$\text{Damage score} = 0.003 \pm 0.001 \text{ body mass} + 0.94,\ r^2 = 39.0\%\ (2)$$

with the regression significantly different from 0, t = 2.66, *d.f.* = 10 p = 0.02.

Comparisons of body mass. To test for the possibility that injury may have a long-term impact on body mass condition, Model 1 allometric equations were calculated after transforming the data into logarithmic form. Body mass has been treated as the dependent variable y and straight line carapace length the independent variable x. Model 1 equations have the form,

$$y = ax^b$$

where b is the exponent and a the y- intercept. Juveniles were included into each of the sub-sets to give a true indication of the y – intercepts. Tortoises with lower body masses will therefore have lower exponents and were compared using a t-test at n-2 degrees of freedom.

The results for the y-intercepts were in good agreement between all groups ranging from 0.0002 to 0.0003 in Montenegro and from 0.0002 to 0.007 in Croatia. The exponents for damaged and undamaged tortoises were virtually identical in all data sets and gave, with undamaged values first; Montenegro males b = 3.05±0.09 and 3.1±0.13, females both b = 2.99 with standard errors of 0.08 for undamaged and 0.11 for damaged; Croatia females b = 3.1±0.07 and 3.2±0.12, males b = 2.73±0.11 and 2.82±0.10. The latter exponents for damaged and undamaged males in Croatia showed the greatest departures but the difference was not significant, t = 0.08, p >0.05. Hence no differences in body mass status between damaged and non-damaged animals could be found in any population.

Body temperatures. Hermann's tortoise is a heliotherm regulating body temperature by shuttling between sunlit and shaded areas (Meek, 1984; 1988a). To test the assumption that damages, perhaps due to impaired locomotory capacities, may have influenced thermoregulation, the data were examined for differences in means (here shown with their standard deviations) and variances in body temperature. These data include autumn body temperatures, which were lower than in summer, but within season body temperatures of males and females were not significantly different, so were pooled. No differences were detected in body temperature means in any of the samples; Croatia in summer, non-damaged *mean* = 30.8±1.9°C versus damaged *mean* = 31.0±1.4°C; Croatia in autumn, non-damaged *mean* = 24.4±4.8°C versus damaged *mean* = 24.3±5.0°C; Montenegro in summer, non-damaged *mean* = 29.7±3.0°C versus damaged *mean* = 30.4±3.0°C (ANOVA, *p* values from 0.43 to 0.94). To test for influences of damages on an ability to precisely thermoregulate, it was assumed that body temperature variances around the means were indicators of thermoregulatory precision. The results, using variance ratio tests, showed no differences between damaged and non-damaged tortoises in any of the data sets; *F*-values from 0.91 to 1.8, *p* from 0.52 to 0.91. These results generally indicate that damages had no thermoregulatory effect.

Recapture rates. Field sampling was carried out by making routine patrols of regular routes with each tortoise encountered marked and released. If injury or damage does not influence mobility or behaviour, then it is reasonable to assume that all marked tortoises had equal chance of being recaptured. To test this, the percentage differences of recapture frequencies between injured and non-injured tortoises were compared using *h*-tests which compares percentage differences after calculating their corresponding *x*-values. The results (males and females pooled) showed that in both populations tortoises that had sustained some sort of injury were recaptured more frequently than those that had not, with the differences significant; Croatia, damaged = 16.7%, non-damaged = 7.3%, *h* = 0.49, 38.4 *d.f.*, $p < 0.05$; Montenegro, damaged = 53.3%, non - damaged = 17.6%, *h* = 0.78, 23.2 *d.f.*, $p < 0.01$. Figure 3 shows the daily percentage recapture rates for both damaged and undamaged tortoises in both areas.

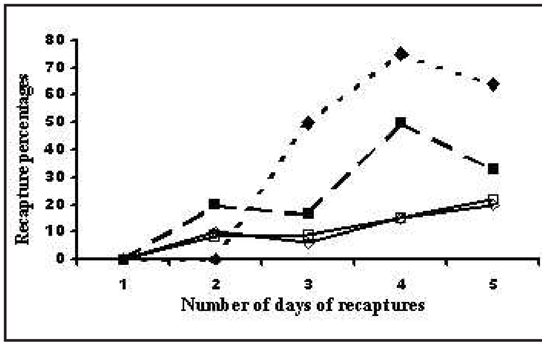

Figure 3. Graph showing percentage recapture frequencies of damaged and undamaged tortoises. Closed diamonds represent damaged Montenegrin tortoises, closed squares damaged Croatian tortoises, open squares undamaged Croatian tortoises and open diamonds undamaged Montenegrin tortoises.

DISCUSSION

The results of this study support the view that *T. hermanni* is able to survive injuries in the field with only apparent negligible ecological effects. These include, broken jaw, major shell damage, limb loss and fire damage. The ability to survive such injuries must be adaptive given the low annual recruitment and necessity for reproduction over a relatively long time period in *T. hermanni* (Hailey, 1990). Effects of injury, where they were indicated, appeared to be largely confined to recapture rates, which is interesting and potentially important, but it should also be noted that sample sizes for damaged individuals within the populations were relatively small and any conclusions regarded as tentative. For example, application of the Sequential Bonferroni Procedure suggested that the significant results could have arisen by chance and $p < 0.0016$ the appropriate test statistic for significance. Using this criterion the differences in recapture frequencies between damaged versus undamaged animals would not be significant. However, behavioural shifts in reptiles as a result of injury are known, for instance tail loss influences the behaviour of lizards (e.g. Downes & Shine, 2001) and hence there is the possibility that damaged tortoises had indeed altered / restricted activity levels and / or departures in behaviour rendering them liable to higher encounter frequency.

Absent limbs must handicap locomotory performance, but apparently not enough to influence thermoregulation or body mass condition - even tortoises with broken jaws had normal body mass. Presumably, the effects of limb loss on armoured reptilian herbivores are less critical than in non-armoured reptiles, as mobility is key in the latter. Similarly, shuttling heliothermy may be less affected if the distances between shaded and sunlit areas are small and less locomotory effort required. The damage score results are problematical, as the criteria in which they were applied were subjective and may not truly represent a real or significant ecological impact and hence some standard criteria for score counts should be determined for future studies. However, damage score differences, if approximate to reality, could be explained by size and an associated loss of agility in the larger females from Montenegro (Meek, 1985; 1989, Meek & Inskeep, 1981). For instance, the body masses of Montenegrin damaged females (*mean* = 1114.2.2±279g) were greater than Croatian damaged females (*mean* = 864.2±180.1g). This difference, although not significant (ANOVA, p = 0.09) may nevertheless represent a potential for injury during activity in the rockier Montenegrin environment – tortoises are frequent climbers and females may migrate to nesting areas (Stubbs and Swingland, 1985). The increase in damage with age seen in Croatian tortoises may simply be a consequence of living longer and hence higher probability of accident, as suggested for the Grass snake *Natrix natrix* (Gregory & Isaac, 2005). General dents in the carapace and jawbone breakages were likely the result of falls whilst climbing in all three populations.

Hailey (1990) has proposed that damage and survivorship for females in field populations could involve male courtship attempts, although based on the observations of the extensive damages survived by tortoises in the present study, these would surely need to be major injuries. Remains of dead *T. hermanni* were found in somewhat limited numbers and formed only 2.3–3.05% of the total field samples (Meek, 1989). All appeared to have been older individuals with 20–25 annuli (see example in Figure 1F) with largely intact shells and did not appear to have suffered violent deaths. Certain tortoises showed what appeared to be injury from fire damage (see Figure 1B) although this was found only in large tortoises and, as suggested in previous studies (e.g. Lambert, 1982), may indicate that old/large *T. hermanni* survive fire better than juveniles.

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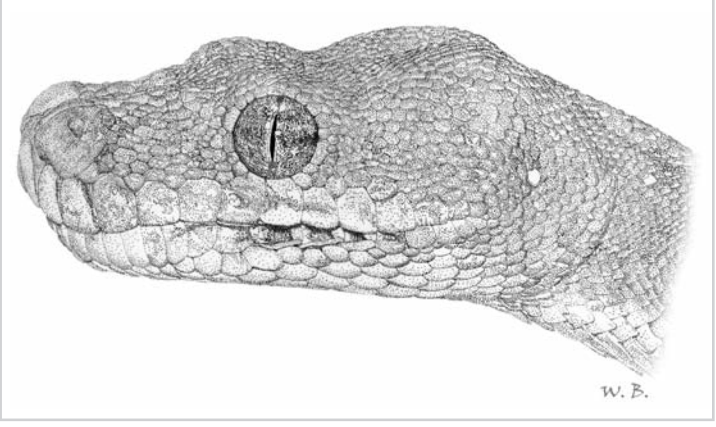

Morelia viridis (Green tree python). Pen and ink illustration by Will Brown. www//blueridgebiological.com