
FACING HABITAT REDUCTION IN YOUR OWN SHELL: PATTERNS OF NON-LETHAL INJURIES IN THE ENDANGERED TORTOISE *TESTUDO HERMANNI* IN ITALY

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Abstract.—Shell damage occurs frequently in tortoises, and represents evidence of non-lethal injuries of both natural and anthropogenic origins. By analyzing the occurrence of non-lethal injuries in 130 free ranging Hermann's Tortoises (*Testudo hermanni*) in Italy, we observed that significantly more damage was present (nearly 90% of the sampled tortoises) at sites < 1 km from cultivated areas. Further, we found an increased prevalence of wounds and enhanced severity of damage to individuals (considering both the carapace and plastron) at these sites. Interestingly, near agricultural activities, most injuries were concentrated on the rear of the carapace. This pattern of injuries is compatible with threat sources coming from above, such as agricultural machinery. As expected, the carapace was the most injured shell region, showing different kinds of damage: abrasions (superficial or deep enough to expose the bony layer), indentations, fractures, and fire damage. The incidence of individuals with abrasions and minor indentations (the most common injuries) did not vary between sexes or by body size; thus, injuries were seemingly independent from the rates of activity and the distances traveled by tortoises, which usually vary with sex and age. We found, however, shell deformation due to deep fractures and fire damage more frequently in older (bigger) tortoises. Given the progressive reduction and fragmentation of *T. hermanni* habitat due to the expansion of human activities, the presence of uncultivated buffers and the protection of natural and semi-natural areas inside agricultural landscapes should be considered as a key target for *T. hermanni* protection.

Key Words.—agriculture; carapace; conservation; habitat fragmentation; Hermann's Tortoise; plastron

INTRODUCTION

The impacts of human activities, including habitat destruction and fragmentation, can be particularly severe for long-lived reptiles, such as chelonians, which generally show delayed sexual maturity and low reproductive rates (Congdon and Van Loben Sels 1991; Congdon et al. 1993). In turtles and tortoises, adults are the critical component in population dynamics (Congdon et al. 1993; Doak et al. 1994) and, consequently, long-term survival of sexually mature individuals is essential for population persistence. Of approximately 356 species of turtles and tortoises in the world, 50.3% are listed as Critically Endangered, Endangered, or Vulnerable by the International Union for Conservation of Nature (IUCN), and 10.7% are Near Threatened (Turtle Taxonomy Working Group 2017).

Throughout the Mediterranean area, *Testudo* populations are exposed to a number of threats, among which are illegal collection, urbanization, fire, habitat destruction, and habitat alteration, mainly due to rapid expansion of intensive cultivation and abandonment of traditional agricultural practices (Cheylan 1984; Lambert 1984; Hailey et al. 1988; Hailey 2000b; Pérez et al. 2004). The extensive human-induced changes

in land use are particularly critical for the persistence of *Testudo* populations because these tortoises have relatively limited dispersal ability compared to similar sized mammals (Hailey 1989) and require well-defined habitat types (Rozyłowicz and Popescu 2013). Among the *Testudo* species present in southern Europe, the Hermann's Tortoise (*T. hermanni*) is most at risk, being classified as Near Threatened on the IUCN Red List (van Dijk et al. 2004). In Italy *T. hermanni* is classified as Endangered (Rondinini et al. 2013).

Damage to carapaces and plastrons can occur relatively frequently in chelonians due to both anthropogenic and natural factors (Dodd 2001; Meek 2007; Saumure et al. 2007). Predation attempts by wild animals and accidental falls are the main natural sources of shell injuries. However, there are many anthropogenic sources of shell damage, including motorized agricultural machinery and vehicles (Saumure and Bider 1998; Gibbons et al. 2001; Szerlag and McRobert 2006), fires set for managing or creating pastures and cultivation areas (Hailey 2000a), falls from infrastructure or artificial obstacles, such as walls (Meek and Inskeep 1981; Sos 2005), and predation attempts by animals whose presence is fostered by human activities (Dodd 2001; Draud et al. 2004). Injuries and



FIGURE 1. Regions in Italy in which surveys were performed for adult Hermann's Tortoise (*Testudo hermanni*) from 2012–2016. Numbers identify individual sites (see Table 1). Site localization is omitted for conservation reasons.

their physiological consequences can represent real ecological costs for individuals, possibly impairing locomotion, growth rates, or fitness (Werner and Anholt 1993; Gregory and Isaac 2005).

The study of injuries in reptiles can be used to infer the health status of wild populations and the pressures to which they are subjected (Borczyk 2004; Gregory and Isaac 2005; Fenner et al. 2008). Lizards are the most widely studied group in this field and the frequency of injuries in lizards, typically the presence of broken or regenerated tails, is usually analyzed to assess predation pressure within populations (Schall and Pianka 1980; Cooper et al. 2004 but see also Jaksić and Greene 1984; Medel et al. 1988), or mortality risk rates of individuals (Wilson 1992; Niewiarowski et al. 1997). In contrast, relatively few studies have focused on the occurrence of non-lethal injuries on turtles (e.g., Saumure and Bider 1998; Saumure et al. 2007) and tortoises (e.g., Meek 2007; Cecala et al. 2009; Buică et al. 2014), although some information is given in descriptive notes (Sos 2012) or as anecdotal observations in papers not specifically focused on the topic (e.g., Meek and Inskip 1981; Popgeorgiev 2008).

Chelonians are arguably an optimal study system for this approach because of their ability to heal from extreme injuries (Andrei 2002; Meek 2007; Sos 2012), which mean they often carry evidence of the original

injury throughout their life. The traces of non-lethal injuries can be interpreted as evidence of the trauma and accidents tortoises have experienced. In this paper, we analyzed the occurrence of non-lethal injuries in *T. hermanni* in Italy to determine if the proximity of human activities correlates with increases in shell damage and if shell injuries have a differential occurrence in relation to tortoise sex and size. We expected to find that a higher frequency of injured tortoises occurs near human activities, that individuals accumulate injury traces with age, and that males and females may be differently subject to injuries due to different activity rates and movement patterns throughout the year (e.g., Corti and Zuffi 2003; Cheylan et al. 2010 and references therein).

MATERIALS AND METHODS

Field sampling.—We collected data from 130 free ranging tortoises (51 males, 79 females) at 12 sites distributed along the Italian Peninsula and in Sardinia (Table 1, Fig. 1). We classified sites by proximity to the nearest human land use and considered sites natural (Nat) if they were > 1 km away from the nearest area of human use, or agricultural (Agr) if they were < 1 km to the nearest area of human use (Table 1). The nearest human use always consisted of agricultural activities in this study. We chose the 1 km distance threshold because daily movements of both sexes rarely exceed 1 km (Chelazzi and Francisci 1979; Stubbs and Swingland 1985).

We gathered data from 2012 to 2016. We chose sites to represent a wide geographical range and a variety of environments. At two sites (sites 1 and 12 in Table 1, Fig. 1), we performed multiple sampling sessions in spring 2012 and 2016, as part of a separate monitoring program. At these sites we avoided recaptures within seasons by temporarily marking each tortoise on the rear scutes with nail polish, and between years by photo-recognition of the carapace patterns. For each tortoise we captured, we recorded position (using a GPS device), sex, straight midline carapace length (CL), carapace height (CH), and mass. We also took five photographs of the carapace (lateral left, lateral right, dorsal, anterior, and posterior) and one of the plastron. We took all measurements and photographs in the field and released tortoises at the place of capture within 15 min of initial handling. We considered only adults (CL > 10 cm, Stubbs et al. 1984) for analysis because of the low number of juveniles detected (n = 8).

Indices.—To quantify the extent and severity of shell injuries, we created an index of carapace injuries (Carapace I), similar to Saumure et al. (2007) but with some modifications. The carapace was analyzed using four quadrants defined by a vertical line passing

TABLE 1. Number of adult Hermann's Tortoises (*Testudo hermanni*) and habitat types at 12 study sites across the Italian Peninsula and Sardinia from 2012–2016. Study sites were classified as natural if more than 1 km from nearest human land use and agricultural if less than 1 km from nearest human land use. Numbers in parentheses indicate males (M) and females (F). When available in literature, we indicated *T. hermanni* population density at the surveyed sites, or at sites in the same geographical area and with similar habitat features.

Site	n (M, F)	Site category	Main habitat features	Population density
1	40 (19, 21)	Agricultural	Contact zone between oak woods (<i>Quercus ilex</i>) and anthropogenic habitats: after-blaze garigue (<i>Cistus</i> spp., <i>Ampelodesmos mauritanicus</i>), small rushes and ecotonal boundaries adjacent to arable fields. Marginal areas are managed through mowing and fire. Surveyed area: about 3.8 ha.	5.85 n/ha (Biaggini et al. 2018)
2	13 (5, 8)	Agricultural	Mosaic of arable fields, pastures, and olive orchards, separated by ecotonal boundaries and broadleaf woodlots. Surveyed area: about 6 ha.	0.083 ± 0.041 sightings/hours × researcher (Luiselli et al. 2014), same geographical area
3	3 (2, 1)	Agricultural	Dismantled quarry with unmanaged herbaceous vegetation and small rushes, next to cultivated areas (vineyards, olive orchards, arable fields). Surveyed area: about 1 ha.	3.03 n/ha (Biaggini et al. 2018), same geographical area
4	6 (4, 2)	Agricultural	Garigue dominated by <i>Rosmarinus officinalis</i> on sandy dunes. Arable fields are present nearby. Surveyed area: about 4.5 ha.	46 n/ha (Ventrella et al. 2007)
5	6 (1,5)	Agricultural	Coastal area with typical sand dune vegetation (dominated by <i>Pistacia lentiscus</i>) and a pine forest (<i>Pinus pinea</i>). Just behind the dunes, a road and a very wide zone of arable fields are present. Surveyed area: about 4 ha.	Not available
6	2 (1, 1)	Agricultural	Cultivated areas (arable fields, vineyards, olive orchards) alternated with ecotonal boundaries (mainly <i>Rubus</i> sp.). Surveyed area: about 0.5 ha	Not available
7	2 (1, 1)	Agricultural	Alternation between agricultural areas (mainly arable fields and pastures) and sparse maquis. Surveyed area: about 1 ha.	Not available
8	1 (1, 0)	Agricultural	Wooded area made up of broadleaf trees and conifers. Pastures and olive orchards are present nearby. Surveyed area: about 2.4 ha.	Not available
9	4 (2, 2)	Agricultural	A mosaic made up of abandoned olive orchards, now used as pastures, semi-natural vegetation and woodlots (<i>Quercus suber</i>) situated next to a built-up area. Surveyed area: about 2.5 ha.	Not available
10	15 (5, 10)	Natural	Mainly garigue (dominated by <i>Euphorbia arborea</i> , <i>Astragalus terraccianoii</i> , <i>Centaurea horrida</i>), alternated with meadows and maquis. Surveyed area: about 14 ha	5 n/ha (Corti and Zuffi 2003)
11	2 (1, 1)	Natural	Mainly garigue, natural meadows and glassworts with emerging rocks. Surveyed area: about 6 ha.	Not available
12	36 (9, 27)	Natural	Mosaic of Mediterranean maquis, open grassland and small garigue patches within a cultivated pine forest (<i>Pinus pinea</i>). Surveyed area: about 2 ha.	31.29 n/ha (Biaggini et al. 2018)

in the middle of the vertebral scutes and a horizontal line passing in the middle of the third vertebral scute down between the second and the third coastal scutes (Fig. 2). For each quadrant, we recorded the highest level of injury using the following scale: 0 = intact, no damage, or limited superficial abrasions of the keratin scute layer only; 1 = minor, restricted areas of deep shell abrasions (up to 1 cm², corresponding to about 2% of one quadrant coverage, based on average CL = 153.48 ± 23.60 mm and CH = 74.82 ± 9.31 mm) and/or indentations on the marginal scutes (< 0.5 cm deep); 2 = moderate, larger areas of deep shell abrasions (> 1 cm²) and/or larger indentations (> 0.5 cm deep); 3 = severe, shell deformations including deep indentations

or fractures in inner shell regions (i.e., due to predation or strong mechanical impacts), fire damage, or limb amputations (attributed to the corresponding quadrant). Deep abrasions are injuries that reveal the bony layer underlying the scutes (see Fig. 3 for examples of injuries). For each tortoise, we summed the score of the four quadrants and divided by 12 (the maximum achievable total score) to obtain an index ranging from 0 to 1 (Saumure et al. 2007). We also calculated the index scores relative to anterior and posterior sides of the carapace (dividing by six, the maximum achievable score in two quadrants). Tail damage (observed twice) was attributed to only one of the posterior quadrants, while we never observed injuries to the head. Analogously,

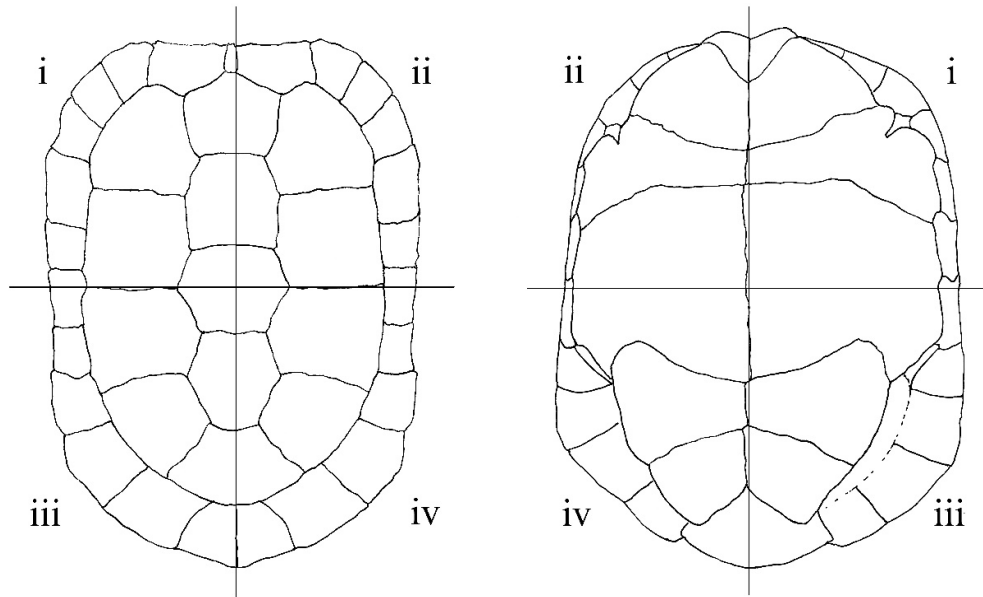


FIGURE 2. Quadrants of the carapace (left) and plastron (right) of adult Hermann's Tortoise (*Testudo hermanni*) into which injury extent and severity scores were partitioned for the calculation of the indices Carapace I and Plastron I at 12 sites in the Italian Peninsula and Sardinia from 2012–2016.

we computed the index of plastron injuries (Plastron I), dividing the plastron into four quadrants corresponding to those of the carapace (Fig. 2). While computing the indices we also recorded the kinds of injuries observed, distinguishing among deep abrasions, indentations (on marginal shell regions), deformations, and amputations.

Statistical analyses.—We first tested both Carapace I and Plastron I for spatial autocorrelation, using Moran's I values obtained at two distance intervals (range of distances among sites: 3–775 km, between site 10 and site 11 and between site 7 and site 11, respectively); Bonferroni corrections for multiple comparisons were applied to the resulting *P* values. Based on the results of Moran's I, we combined tortoises at all sites within site categories in subsequent analyses. We performed a Spearman rank correlation to investigate whether there were associations between Carapace I and Plastron I at agricultural sites and at natural sites (neither variable was normally distributed, Kolmogorov-Smirnov test for normality: Carapace I, $d = 0.289$, $P < 0.010$; Plastron I, $d = 0.699$, $P < 0.010$). We used CL as a proxy for tortoise size in all analyses, after investigating the relation between CL and CH by a Pearson correlation (CL and CH were normally distributed, Kolmogorov-Smirnov test for normality: CL, $d = 0.063$, $P > 0.050$; CH, $d = 0.067$, $P > 0.050$). We calculated body mass condition as the residuals from the linear regression between the natural logarithms of weight and CL (Hailey 2000a; Lagarde et al. 2001; Willemsen and Hailey 2002). To test if either index varied by site

category (natural or agricultural), sex (male or female), CL, body mass condition, and an interaction between site category and sex, we ranked models using Akaike Information Criterion (AIC) to identify the model with the best fit given the data. In order not to neglect possible important explanatory variables, when a single predictor model got the best AIC rank, we selected the model with the second-best AIC rank and $\Delta AIC < 2$ (indicated as substantial support for suboptimal models by Burnham and Anderson 2002). Next, we used generalized linear models to analyze either index in relation to the predictors in the selected model.

To determine if different portions of the carapace were differently injured, we compared index scores of injuries between anterior (quadrants i and ii) and posterior sides (quadrants iii and iv) of the carapace using a Wilcoxon Test. We performed the same comparisons of injury scores for anterior and posterior sides of the plastron. Because Carapace I and Plastron I varied between agricultural and natural sites (see Results), we ran separate comparisons for each site category. We focused on the occurrence rates of tortoises with different types of injuries (deep abrasions, indentations, or deformations while amputations were discarded from analyses due to a low sample size for this type of injury, $n = 7$). We analyzed only carapace injuries because of the high uniformity of injury type observed on plastrons, which were almost all abrasions. To explore whether site category, CL, sex, and the interaction between site category and sex influenced injury occurrence patterns, with the presence of each injury type included as

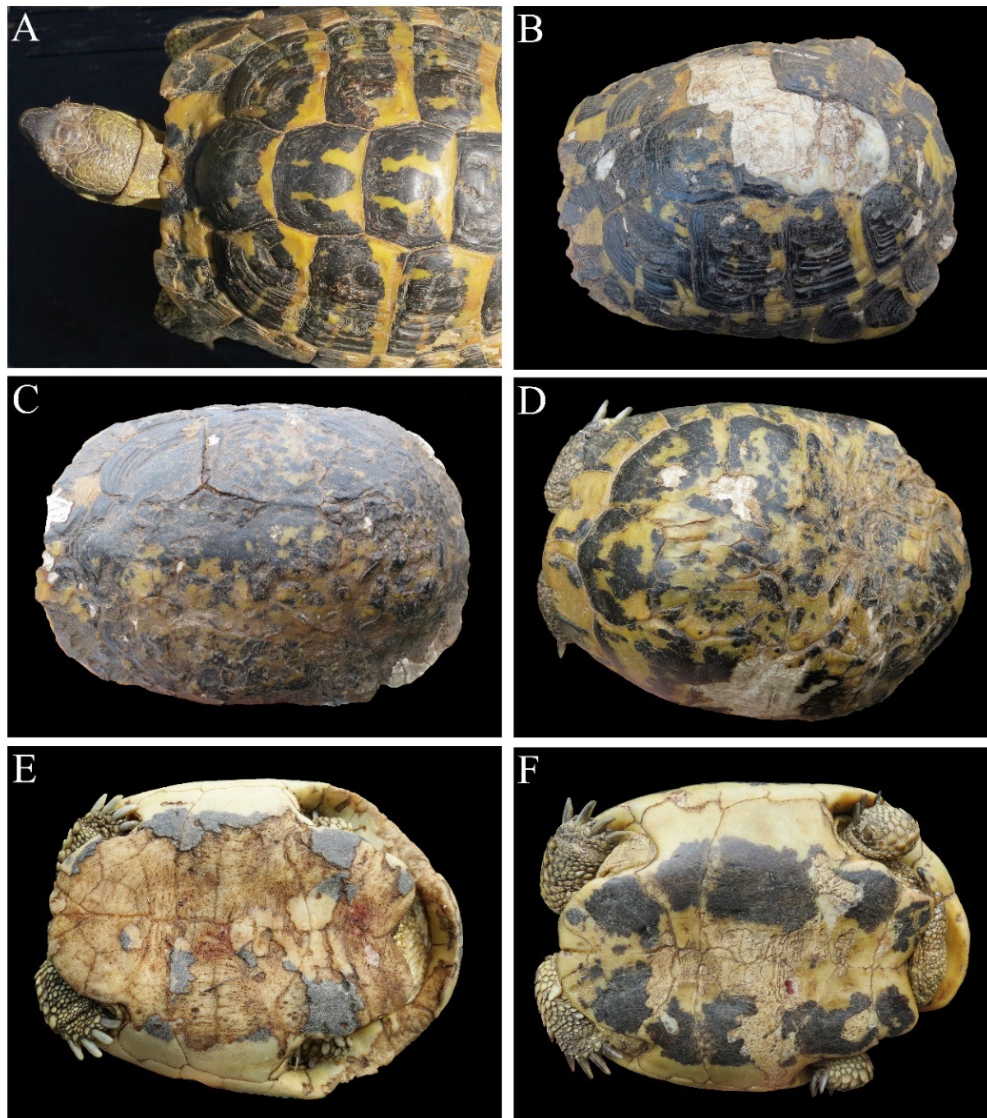


FIGURE 3. Adult Hermann's Tortoises (*Testudo hermanni*) showing indentations and superficial abrasions (A), indentations and deep abrasions (B, D) and deformations (C, D) to the carapace and adult *T. hermanni* showing abrasions on the plastron (E, F) observed at agricultural sites (< 1 km distance from nearest human land use) in the Italian Peninsula from 2012-2016. (Photographed by Marta Biaggini. Black background was digitally created).

binomial dependent variable, we first ranked models using AIC to select the best predictors. For each injury type, we used generalized linear models with the selected predictors. The a-priori critical α value was 0.05 for all analyses. We used PAST 2.17b (Hammer et al. 2001) for Moran's I, and for all other analyses we used Statistica 10.0 (TIBCO Software Inc., Palo Alto, California, USA).

RESULTS

Almost 90% of the tortoises living near human activities (in agricultural sites) had at least some carapace injury more serious than superficial abrasions, while

70% had more serious carapace injuries in natural sites (Fig. 4, 5). In both natural and agricultural sites, deep abrasions were the most common injuries, followed by indentations, carapace deformations, and amputations (Fig. 4). Injuries were not spatially autocorrelated (Carapace I: $I = 0.002$, $P = 0.074$ at average distance of 21.4 km; $I = -0.003$, $P = 0.058$ at average distance of 241.7 km. Plastron I: $I = -0.0003$, $P = 0.070$ at 21.4 km; $I = -0.0003$, $P = 0.054$ at of 241.7 km). Carapace I and Plastron I were positively correlated at agricultural sites ($n = 77$; $r_s = 0.467$; $P < 0.001$) but were not correlated at natural sites ($n = 53$; $r_s = 0.109$; $P = 0.438$). The traits CL and CH were positively correlated ($r = 0.881$, $t = 20.94$; $df = 128$; $P < 0.010$). The best model to explain

TABLE 2. Akaike Information Criterion (AIC) in the selection of the best model fitting the patterns of the indexes of injury to carapace and plastron (Carapace I and Plastron I), and of the occurrence rate of tortoises with deep abrasions, indentations and deformations to the carapace observed in adult Hermann's Tortoise (*Testudo hermanni*; n = 130) at 12 agricultural (< 1 km distance from nearest human land use) and natural (> 1 km from nearest human land use) sites in the Italian Peninsula and Sardinia from 2012–2016. We reported AIC, Δ AIC values and model weight of the best selected model (with at least two predictors and Δ AIC < 2) and AIC values of the overall model including straight midline carapace length (CL), body mass condition, site category (natural or agricultural), sex (male or female), and the interaction between site category and sex.

Response variables			Predictors			AIC	Δ AIC	Model weight
Carapace I			Site category	Sex		2.844	1.064	0.110
	CL	Body mass condition	Site category	Sex	Site category \times sex	6.864	5.083	0.015
Plastron I			Site category	Sex	Site category \times sex	-111.993	0	0.102
	CL	Body mass condition	Site category	Sex	Site category \times sex	-109.051	2.942	0.023
Occurrence of tortoises with deep abrasions			Site category		Site category \times sex	154.567	1.760	0.143
	CL	NA	Site category	Sex	Site category \times sex	158.228	5.421	0.023
Occurrence of tortoises with indentations			Site category		Site category \times sex	174.218	1.760	0.131
	CL	NA	Site category	Sex	Site category \times sex	178.201	5.742	0.018
Occurrence of tortoises with deformations	CL		Site category	Sex		111.831	0	0.215
	CL	NA	Site category	Sex	Site category \times sex	113.789	1.957	0.081

Carapace I variability included site category and sex (Table 2). Carapace I was higher at agricultural than at natural sites (Carapace $I_{Agr} = 0.410$, 95% CI 0.030–0.470; Carapace $I_{Nat} = 0.193$, 95% CI 0.029–0.136), but did not vary in relation to sex (Table 3, Fig. 5). The best model selected for Plastron I included site category, sex and the interaction between site category and sex (Table 2). Plastron I was higher at agricultural than at natural sites (Plastron $I_{Nat} = 0.060$, 95% CI 0.030–0.089; Plastron $I_{Agr} = 0.124$, 95% CI 0.076–0.172), but did not vary by sex, or an interaction between site category and sex (Table 3, Fig. 5).

At agricultural sites, the posterior side of carapaces had a higher Carapace I value than the anterior side (n = 77, $Z = 3.100$, $P = 0.002$) while in natural sites we found no differences in the index values between posterior and anterior sides (n = 53, $Z = 0.188$, $P = 0.851$). In agricultural sites we found no difference in Plastron I values between anterior and posterior (n = 77, $Z = 0.012$, $P = 0.990$) plastron sides; in natural sites, the anterior side of the plastron showed higher Plastron I values than the posterior side (n = 53, $Z = 2.045$, $P = 0.041$).

Site category and the interaction between site category and sex were the best predictors for the occurrence rate of tortoises with deep abrasions and with indentations to the carapace (Table 2). Tortoises with deep abrasions to the carapace occurred 27.69% more frequently at agricultural sites but their frequency did not vary by the interaction between site category and sex

(Table 4). Tortoises with indentations to the carapace occurred 22.35% more frequently at agricultural sites and their occurrence showed no relationship with an interaction between site category and sex (Table 4). The best model to predict the occurrence of tortoises with deformations to the carapace included CL, site category and sex (Table 2). The occurrence of tortoises with deformations was 10.05% higher at agricultural sites, increased with tortoise size using CL ($\beta = 0.050$, 95% CI = 0.003–0.098), but it did not vary by sex (Table 4).

TABLE 3. Logistic regressions of the effects of site category and sex on the index of injuries to the carapace (Carapace I) and of the effects of site category, sex and the interaction between site category and sex on the index of injuries to the plastron (Plastron I) of Hermann's Tortoise (*Testudo hermanni*; n = 130) at 12 sites in the Italian Peninsula and Sardinia from 2012–2016. Sites were classified as natural or agricultural based on proximity to nearest human land use (> 1 km distance, < 1 km distance, respectively).

Response variable	df	Wald Stat	P-value
Carapace I			
Intercept	1	186.943	< 0.001
Site category	1	15.859	< 0.001
Sex	1	0.941	0.332
Plastron I			
Intercept	1	44.499	< 0.001
Site category	1	5.228	0.022
Sex	1	2.960	0.085
Site category \times sex	1	2.733	0.098

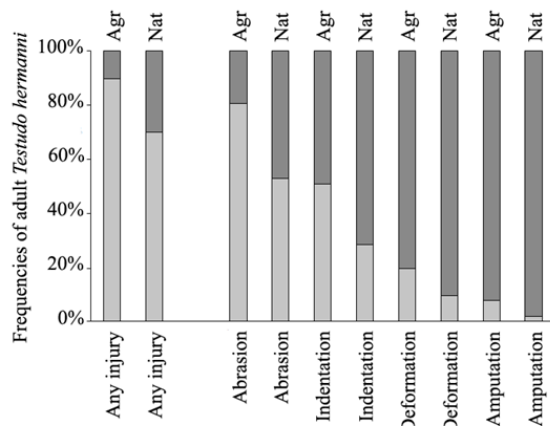


FIGURE 4. Observed frequencies of adult Hermann’s Tortoises (*Testudo hermanni*) with any injury and of adult *T. hermanni* displaying different injury types to their carapaces (light gray: present; dark gray: absent) at 12 sites in the Italian Peninsula and Sardinia from 2012–2016. Sites were classified as natural (Nat) or agricultural (Agr) based on proximity to nearest human land use (> 1 km distance, < 1 km distance, respectively).

DISCUSSION

The occurrence and severity of non-lethal injuries in free ranging *Testudo hermanni* exhibited different patterns of variability in sites that were near human activities (< 1 km) and in sites surrounded by wider areas of natural or semi-natural habitats. In particular, tortoises in the proximity of cultivated areas had a higher rate of injury than in sites that were farther from cultivated areas, not only in terms of frequency of damaged individuals, but also in terms of the extent of injured areas and severity of damage on the carapace. Moreover, at sites adjacent to agricultural activities, injuries occurred more often to the posterior side of the carapace than to the anterior side. The method we used to assess and record injuries to tortoises involved a quick procedure in the field that required minimal manipulation of tortoises, and could be useful for future monitoring efforts, as the lack of standardized methods has been criticized in previous research (Meek 2007).

Our results suggest that the higher injury scores to the carapace that were observed near human activities occurred without evident differences between sexes or among sizes. This implies that both the total amount of damage per individual and the occurrence of the different types of injuries were seemingly independent from the rates of activity and the distances covered by tortoises, which usually differ between sexes and change with age (Cheylan et al. 2010). Similarly, previous studies indicated similar injury rates between males and females in Testudines (Meek 2007; Buică et al. 2014) and in other chelonians (Saumure and Bider 1998; Saumure et al. 2007; Cecala et al. 2009). In terms of body size, a higher occurrence of injuries might

TABLE 4. Logistic regressions of the effects of site category and the interaction between site category and sex on the occurrence of tortoises with deep abrasions and indentations to the carapace, and logistic regression of the effects of straight midline carapace length (CL), site category and sex on the occurrence of tortoises with deformations to the carapace on 130 adult Hermann’s Tortoises (*Testudo hermanni*) at 12 agricultural (< 1 km distance from nearest human land use) and natural (> 1 km from nearest human land use) sites across the Italian Peninsula and Sardinia from 2012–2016.

Response variable	df	Wald Stat	P-value
Deep Abrasions			
Intercept	1	14.502	< 0.001
Site category	1	9.129	0.003
Site category × sex	1	0.240	0.624
Indentations			
Intercept	1	5.490	0.019
Site category	1	6.783	0.009
Site category × sex	1	0.239	0.625
Deformations			
Intercept	1	6.513	0.011
CL	1	4.401	0.034
Site category	1	6.642	0.010
Sex	1	2.445	0.118

be expected in larger (older) tortoises due to injury accumulation over time. We verified such prediction only for deformations to the carapace (fractures or fire damage), a type of damage leaving permanent traces on the shell. On the contrary, we observed that the most widespread damage type, namely deep abrasions that leave the bony layer uncovered, can heal over time, thus reducing the incidence of accumulation. In four individuals that possessed deep abrasions in 2012 and that were recaptured in 2016 at Site 1, we observed a complete scute regeneration and the presence of deep abrasions was visible as a superficial abrasion (category 0 in our classification of damages). In essence, due to this healing ability, we effectively considered only relatively recent deep abrasions, not yet repaired. Following Sos (2012), new osteoderms can take over one year to cover the horny layer again after being damaged. The regeneration process can also involve the bony layer, when damaged, in spans from 1–2 y (Sos 2012) to 5–6 y (Martínez-Silvestre and Soler-Massana 2000). The data available on the relationship between shell damage and tortoise size (a proxy for age) are extremely variable, probably due to the scarce number of studies on the topic, and also to the inconsistent classifications used to record shell damage. Concordant with our observations, Buică et al. (2014) observed that some injuries increased with age class in the Spur-thighed Tortoise (*T. graeca*), while Meek (2007) did not find differences in the sizes of injured and not-injured *T. hermanni*; Cecala et al. (2009) and Saumure et al. (2007) recorded an increase

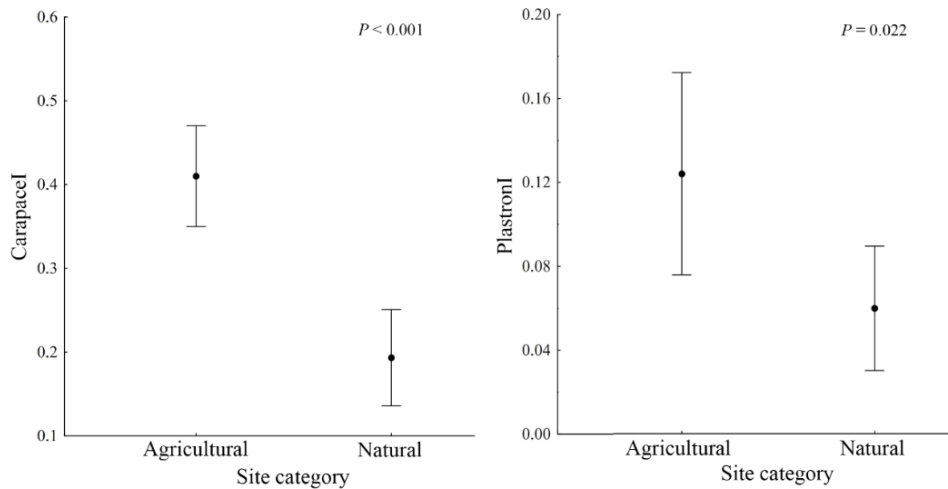


FIGURE 5. Effect of site category on injury index scores for the carapace (Carapace I; left) and plastron (Plastron I; right) of the Hermann's Tortoise (*Testudo hermanni*; n = 130) at 12 sites in the Italian Peninsula and Sardinia from 2012–2016. Parameter estimates and 95% confidence intervals are shown. Sites were classified as natural or agricultural based on proximity to nearest human land use (> 1 km distance, < 1 km distance, respectively). Note the different range of the y-axes.

of injury rates with size in the Diamond-backed Terrapin (*Malaclemys terrapin*) and the Wood Turtle (*Glyptemys insculpta*), respectively.

Predictably, the carapace was the most injured region of the shell, showing different kinds of damage: abrasions (superficial or deep enough to let the bony layer uncovered), indentations, fractures, and fire damage. Interestingly, in sites near agricultural activities, the index of carapace injuries was higher in the back portion of the carapace. This arrangement of shell damage in individuals could be explained by possible injury dynamics. During resting, tortoises typically tuck into grass soil cover, brambles, or heaps of dry vegetation, often leaving just the back of their carapaces partially exposed. The same behavior can be seen when tortoises perceive danger signals (Micheli et al. 2014). Given these considerations, the pattern of injuries occurring more frequently to the rear of the carapace could be compatible with wounding during both resting and refuge behaviors, at least during the seasons of tortoise activity (during brumation most individuals burrow, even if not deep), or with threat sources coming from the above such as agricultural machinery, directly observed near some of the study sites.

We recorded fewer scars on plastrons but, analogously to the pattern of injuries on the carapace, the injury score for plastrons was higher at sites near human activities. Plastron scars were almost all abrasions (we observed only one individual with fire damage on the plastron, and another one with a fracture) likely due to predation attempts. Indeed, at agricultural sites, we often observed distinct gnawing signs especially on the marginal zones of the plastron, and in one case fractures caused by deep bites into the inner plastron

regions. Moreover, in our sample, six of the seven tortoises with amputations occurred at agricultural sites. Limb amputation in tortoises and turtles can be caused by agricultural mowers (Saumure et al. 2007), but it is generally attributed to predation attempts, primarily by carnivores (Harding 1985; Farrell and Graham 1991; Boyer 1998; Saumure and Bider 1998). Boars, foxes, rats, and dogs (both domestic and feral), together with different species of birds, including birds of prey, are all potential tortoise predators (Cheylan et al. 2010) and frequently occur in agricultural areas where their presence can be fostered by human activities (Hailey et al. 1988; Gloor et al. 2001; Cardador et al. 2011).

Injured tortoises seemed to cope quite well even with high damage rates, based on the absence of relationship between body mass condition and amount of injuries to both carapace and plastron (similarly to Meek 2007). However, it could also be possible that body condition is not a suitable index to reveal possible differences in the health status of damaged and undamaged tortoises. The body mass condition in tortoises is mainly indicative of the level of body hydration and of the fullness of the gut (Willemsen and Hailey 2002), factors which could outweigh more subtle mass differences due to atrophied muscles or lack of fat stores (U.S. Fish and Wildlife Service 2015). In addition, differences in habitat features and food availability among sites, or differences in activity rates of individuals could further complicate the interpretation of the pattern of variation of body mass condition (Jacobson et al. 1993; Willemsen and Hailey 2002). At a deeper level of analysis, damaged individuals could show altered behaviors, such as in intraspecific conflicts, time spent foraging, and ability to escape danger, which could impair reproductive

fitness or longevity, as seen in other reptiles (Werner and Anholt 1993; Downes and Shine 2001; Gregory and Isaac 2005).

We did not estimate survivorship at our sites. Our analyses of injuries were necessarily restricted to only those animals that survived the direct and indirect effects of injuries. To our knowledge, the correlation among injuries and mortality rates has not been thoroughly investigated in *Testudo* species. The few reports available indicate mortality rates (from 18% to 64%) and percentages of surviving individuals with damaged carapaces (from 21% to almost 23%) in *Testudo* populations after a fire (Félix et al. 1989; Popgeorgiev 2008). However, we could reasonably infer from our observations that indicated a 19.8% greater chance of occurrence of carapace injuries in sites near agricultural activities that those populations might experience increased mortality. In accordance with this hypothesis, Saumure et al. (2007) compared agricultural and forested areas and observed that agricultural activities resulted in at least an 11.5% increase of shell damage, and a 10–13% decrease in adult survival. Other studies report reduced survivorships in tortoise populations due to agricultural activities, mainly from injuries from machinery and fire (Cheylan 1984; Stubbs et al. 1985; Félix et al. 1989; Saumure and Bider 1998; Hailey 2000a). In particular, Hailey (2000a) and Popgeorgiev (2008) recorded up to over 60% decrease in *T. hermanni* populations due to mechanical habitat destruction and fire. In site 1 we also repeatedly observed pieces of tortoise shells, clearly sliced by agricultural machinery.

The remaining suitable habitats for *Testudo* in Italy are mainly concentrated inside or at the edge of agricultural landscapes and along coastal areas. In such environments, cultivated plots often cross, delimit or surround the areas inhabited by tortoises, placing tortoise populations at increased risk of injury. In areas with non-intensive farming and traditionally managed orchards, and where these plots are surrounded by ecotones or are adjacent to forests or maquis, suitable conditions for *T. hermanni* may be met. On the other hand, as we observed, the effects of managing nearby cultivations can manifest in tortoise populations even inside protected areas. This can happen when sites inhabited by tortoises are not surrounded by buffers of natural or semi-natural vegetation of adequate width to prevent a tortoise from easily moving into cultivated plots (> 1 km).

In a framework in which human activities continuously expand and habitat fragmentation represents one of the main threats for tortoises, increasing the presence of uncultivated surfaces and protecting natural and semi-natural areas occurring inside agricultural landscapes should be a key target for *T. hermanni* protection in Italy. Moreover, some management interventions should

be considered for agricultural areas inhabited by *T. hermanni* or adjacent to areas inhabited by the species, such as setting the blades of agricultural machinery higher (as discussed in Saumure et al. 2007), at least on the margin of crops. It would be helpful also if ecotone maintenance was strictly regulated by establishing permissible actions and their timing and banning the use of fire where not absolutely necessary or, at least, regulating its timing to lessen its detrimental effects on tortoises (Hailey 2000a).

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