



universität
wien

MASTERARBEIT / MASTER'S THESIS

Titel der Masterarbeit / Title of the Master's Thesis

The Importance of Various Habitat Characteristics
reflected by Population density in Dalmatian Tortoises
(*Testudo hermanni hercegovinensis*)

verfasst von / submitted by

Karoline Bürger, BSc

angestrebter akademischer Grad / in partial fulfilment of the requirements for the degree of

Master of Science (MSc)

Wien, 2021 / Vienna, 2021

Studienkennzahl lt. Studienblatt /
degree programme code as it appears on
the student record sheet:

UA 066 879

Studienrichtung lt. Studienblatt /
degree programme as it appears on
the student record sheet:

Naturschutz und
Biodiversitätsmanagement

Betreut von / Supervisor:

emer. o. Univ.- Prof. Dr. Hannes F. Paulus

Danksagung

Danke an Prof. Walter Hödl, Dr. Herbert Hoi und Dr. Katharina Mahr, die mir dieses Projekt anvertraut und ermöglicht haben. Danke für euer Vertrauen, eure Unterstützung und eure Geduld!

Der größte Dank gebührt meinen Eltern Regina und Hans Bürger, die mir dieses Studium durch ihre großzügige persönliche und finanzielle Unterstützung überhaupt erst ermöglicht haben! Mein Interesse an Biologie und speziell meine Liebe zu Tieren ist aus eurem respektvollen und interessierten Umgang mit der Natur gewachsen. Die Überzeugung, dass jedes Lebewesen Teil eines großen Ganzen und dadurch wertvoll ist, habt ihr mir immer vorgelebt und so an mich weitergegeben. Danke, dass ihr mich immer darin unterstützt habt, diesem Interesse nachzugehen. Angefangen beim ersten Tierlexikon, als ich noch kaum lesen konnte, bis hin zu den wöchentlichen Universum-Folgen, meinen zahlreichen, geliebten Haustieren, die ich hegen und pflegen durfte und den unzähligen Ausflügen in den Linzer Tierpark. Die vielen, vielen Stunden, die ich in der Natur verbringen und diese erkunden durfte, sind meine am höchsten geschätzten Erinnerungen an meine Kindheit.

Danke an Roland und Heidi Sasse, die mir von Beginn an tatkräftig zur Seite gestanden und mir gezeigt haben, wie man die Theorie in die Praxis umsetzt! Nur durch euer tolles Engagement, euer Wissen und unseren gemeinsamen Ehrgeiz konnten wir diese unglaublich hohe Anzahl an Schildkröten überhaupt finden!

Danke an Wolfgang Pegler, der bereit war einen Ausflug in die Herpetologie zu machen, obwohl sein Steckenpferd die Ornithologie ist. Es hat sich herausgestellt, dass es sich durchaus lohnt, den Blick von den großen Höhen auch mal auf den Boden zu richten, denn im Schildkröten entdecken bist du spitze :) Danke für deine Unterstützung und Freundschaft, die Dank einer Musikkassette in meinem Auto begonnen hat!

Danke an Peter Rößl, meinen Mann, der mich immer in allen meinen Vorhaben unterstützt und sein Leben mit mir teilt. Ein spezielles, riesiges Danke gebührt dir für deine zeitaufwändige Hilfe beim Verarbeiten der unzähligen GPS-Punkte und der Gestaltung des Rasters! Ohne dich wäre ich nie fertig geworden!

Danke an Cornelia Amon, die mich so tatkräftig wochenlang unterstützt hat und mir mit ihrer gewinnenden Art und immer guten Laune, mit Funkgeräten und unzähligen gemeinsamen Lachanfällen den Aufenthalt versüßt hat. Živjeli!

Danke an Veronika Harrer, die treueste Seele von allen, die jedes Jahr eine lange Zug- und Busfahrt auf sich genommen hat, um mich bei meiner Arbeit zu unterstützen und mir Gesellschaft zu leisten! Danke für deine Freundschaft!

Danke an Johannes Hill und Rudolf Klepsch, die mir durch umfangreiches Wissen und fachliche Unterstützung bei der Umsetzung des Projekts geholfen haben.

Table of Contents

Zusammenfassung	1
Abstract	2
Introduction	3
Material and Methods	5
Study species	5
Study site and habitat	6
Data sampled and data processing	8
Results	9
Discussion	15
References	19

Zusammenfassung

Nachhaltige Schutzmaßnahmen erfordern umfangreiche Kenntnisse über die Populationsdynamik und ökologischen Ansprüche (z.B. Habitatpräferenzen) einer Art. Um bei Bedarf effektive in-situ Schutzmaßnahmen und Managementpläne gestalten zu können, sind Studien an wildlebenden Populationen von Vorteil. Daher konzentriert sich unsere Studie auf die gefährdete Spezies *Testudo hermanni hercegovinensis*, welche in einem $\pm 1,5 \text{ km}^2$ großen Untersuchungsgebiet in der Nähe von Dazlina/Kroatien durchgeführt wurde. Das Geschlechterverhältnis lag bei 2,06 Weibchen pro Männchen, was auf eine abnehmende Populationsgröße hindeuten könnte. Es konnten keine signifikanten geschlechts- oder altersspezifischen Unterschiede bei der Habitatwahl festgestellt werden. *T. hermanni hercegovinensis* bevorzugt Wiesenflächen mit Gebüsch oder Hecken und Habitate, welche eine moderate Diversität, jedoch eine hohe Heterogenität aufweisen. Monotone Landschaften wie Weingärten, Olivenplantagen, Felder oder die Mediterrane Macchie werden in den meisten Fällen gemieden. Zusätzlich wurde der gesundheitliche Zustand der Tiere überprüft sowie das Vorhandensein von sichtbaren Ektoparasiten oder Verletzungen, welche von Räubern oder landwirtschaftlicher Tätigkeit verursacht wurden. Während keine Ektoparasiten gefunden wurden, zeigten 61 % der 500 markierten Individuen Verletzungen wie Bisswunden, Schnitte im Carapax, fehlende oder verstümmelte Extremitäten sowie beschädigte Schilder.

Abstract

Sustainable conservation measures require considerable knowledge about the population dynamics and ecological needs, e.g. habitat preferences of a species. In order to establish effective in-situ conservation management plans if necessary, populations of (endangered) species should preferably be studied in their natural habitat. Therefore, our study of the threatened species *Testudo hermanni hercegovinensis* was conducted near Dazlina/Croatia within a $\pm 1.5 \text{ km}^2$ sized area characterized by extensive farming, grazing and partly unmanaged landscapes. We observed, that the sex ratio was strongly female biased (2.06), which might indicate a declining population. No significant sex or age specific differences in habitat use were found and the tortoises mostly preferred grassland with shrubs or hedges and habitats showing moderate diversity, but high heterogeneity. Vineyards, olive groves, agricultural fields or the Mediterranean Macchia were usually avoided. We were also interested in the presence of ectoparasites and injuries due to predators or farming activity. While no visible ectoparasites were found, 61 % of 500 marked individuals showed injuries like bite marks, cuts, missing or mutilated limbs, or damaged scutes.

Introduction

Environment on earth is constantly changing. Some of these changes are predictable, such as seasonal or annual changes, but others are unpredictable (Jacobs, 1997). Under the current human influence, environmental changes are dramatically increasing and occur much faster (Sih et al., 2011). Species try to either adapt to the changing environment, search for a different and more suitable environment or might face extinction. The options may vary between individuals and species and depend on individual- or species specific characteristics, like mobility, life span and experience, foraging specialization, niche breadth, reproductive strategy (e.g. k- or r-selectionists), body size or metabolism (Pierotti, 1982) (Danchin et al, 1998). Habitat choice can be even more influential on an individual's life and consequently on its fitness and survival chances (Stamps, 1990).

In our study we examined the population structure and social structure, habitat choice and population dynamics of a wild reptile species. We focused on *Testudo hermanni hercegovinensis*, a subspecies of the Hermann's Tortoise (*Testudo hermanni*) on its northernmost border of distribution, which can be found along the coast of Bosnia and Herzegovina, Croatia and Montenegro (Vetter, 2006).

Given that habitat choice can also be based on previous reproductive success (Switzer, 1997) and habitat requirements do not only differ between species, but may also show intraspecific differences (Ebenman, 1987) (Rogner, 2012), for example age or size cohorts as well as sex specific differences, we additionally focused on possible sex and age specific differences.

In tortoises, our study species included, females typically need appropriate sites for egg deposition (Vetter, 2006) (Celse et al., 2014) or particular food for egg production and therefore might show a different habitat preference than males.

For many species, living in groups is advantageous in terms of reduced predator pressure on individuals (diluting effect), higher alertness of many eyes, thermoregulation, etc. (Hoogland & Sherman, 1976). Reptiles, especially turtles and tortoises are not necessarily known for being very social (Sovrano et al., 2017) (Vetter, 2006). Thus, based on their solitary lifestyle (Celse et al, 2014), we expected a rather low abundance for our study area.

Due to wildlife trade between the 1950s and 1980s of about 2 million individuals (Ljubisavljević et al, 2011), 400.000 just in 1971 (Windolf, 1980), and illegal collection as well as habitat loss and/or fragmentation (Longpierre et al., 2001) (Celse et al., 2014), the population of this species, which naturally shows a small range, significantly decreased in the past decades (Vetter, 2006) (Wegehaupt, 2005) (Zenboudji et al., 2016) and can be considered as endangered in many areas (Rogner, 2012) (Windolf, 1980). *T. hermanni* is listed in Appendix II of the Washington Convention (Windolf, 1980) and the Bern Convention, Appendices II and IV of the Fauna and Flora Habitats Directive (Berardo et al., 2015) and is considered as near threatened (NT) and strictly protected in Croatia (Celse et al., 2014). Increasing road traffic, heavy use of pesticides (Willemsen & Hailey, 2001), the decline of traditional, extensive farming practices (Celse et al., 2014) and predator

pressure (wild boars, dogs, weasels) also continue to represent significant threats (Guyot & Clobert, 1996) (Schweiger, 2006) (Celse et al., 2014). Farmers still consider tortoises as food competitors and a pest, which is reason enough for them to kill them in great numbers (Vetter, 2006) (Celse et al., 2014).

In contrast, Rugiero & Luiselli (2006) and Stojadinović et al. (2017) could demonstrate that artificial structures or landscapes might not necessarily be an obstacle or are even preferred for populating an area, yet fields and other manmade landscapes like vineyards showed the lowest occurrence of *T. hermanni* in a study by Couturier et al. (2014). Since natural habitats without any human impact are hard or even impossible to find in the coastal region of Croatia, our study will also include landscapes, where extensive farming takes place with variable sized patches of unmanaged land in between.

Since with farming also mowing and ploughing activities along in most areas, which represent a further threat (Hailey, 2000), we were interested in the significance of injuries related to farming activity as well as predators.

Furthermore, since it is known, that tick infestation depends on the area (Qviller et al., 2013) and not all subspecies of *T. hermanni* are affected in the same intensity (Široký et al., 2006), we were also interested in the frequency of ectoparasites and its importance as indicators for the health condition of an organism (Tschirren et al, 2007).

The aim of this study was to investigate important requirements of *T. hermanni hercegovinensis* in order to be able to preserve and effectively protect this species in its natural habitat. For that reason the study area was divided into subsets (50m x 50m grids, for details see methods), where density of tortoises (dependent variable) and habitat parameters (independent parameters) were quantified to evaluate their importance for habitat choice in *T. hermanni hercegovinensis*.

Material and Methods

Study species

Since Testudines show phenotypic plasticity, their morphology as well as their ecological demands can strongly vary between populations of a species (Duro et al., 2021).

The Dalmatian Tortoise (*Testudo hermanni hercegovinensis*) inhabits dry or slightly humid mediterranean areas, extensively managed landscapes (Couturier et al., 2014), groves or shrubs (preferably blackberries) with surrounding grassland, hedges, but also rocky areas, embankments or Macchia (Rogner, 2012) (van Dijk et al., 2004) but can also be found in urban parks, despite being isolated from outer populations, such as documented in Rome/Italy (Rugiero & Luiselli, 2006). The chosen habitats can strongly vary between as well as within populations, indicating this species to be quite flexible in its lifestyle and requirements (Berardo et al. 2015) (Rogner, 2012) (Zuffi & Corti, 2003). While tortoises in Sardinia and Sicily inhabit dry, sandy, quite bleak landscapes, the populations in eastern Europe prefer more diverse and vegetated environments (Rogner, 2012) such as landscapes with various tree species, e.g. olives or oaks (van Dijk et al., 2004) (Celse et al., 2014) (Vetter, 2006). Dense forests, swamps and open, monotonous landscapes are usually avoided (Celsi et al., 2014) (Couturier et al., 2014). Despite their obvious adaptability, some requirements should be basic. Tortoises need the possibility to bask, hide, forage and reproduce within a rather small area (Türkozan et al., 2018) (Calzolari & Chelazzi, 1991) (Cheylan, 2001) and under mostly mild temperatures (Celse et al., 2014), preferably ranging from about 20 to 34 °C (Vetter, 2006), despite their limits being much lower (-2 °C) or higher (44 °C) (Rogner, 2012). This makes the mediterranean area appropriate as a living environment but the conditions to become more adverse the farther north they live.

In France and Spain, it is quite common that populations of *T. hermanni* consist of more females than males (Vetter, 2006). In France, 1.7 females per male, in Spain 2.6 females per male were observed. According to Celse et al. (2014) a female biased sex ratio is an indication for a declining population, whereas healthy populations show a balanced sex ratio. In Italy, Montenegro (Wallace & Wallace, 1985) and Romania (Cruce, 1978), the sex ratio is more or less balanced, whereas in Greece (Hailey, 1990) and in Croatia, the sex ratio is reversed. In the northeast of Greece, 3.45 males per female were found and in Croatia, 1.33 males per female were recorded (Vetter, 2006). Based on the literature, we therefore suspected a male biased sex ratio of *T. hermanni hercegovinensis* in our study area.

Study site and habitat

In May 2014 and 2015, data on tortoise density and habitat parameters were collected in a ± 1.5 km² sized area near Dazlina, a small village near Tisno (Croatia) (Fig.1). Many parts of the area are used as farmland, some parts are more natural (unmanaged).



Fig.1: Study area in Croatia. Every individual was given a number and its exact position was determined with a GPS device. Five pathways were used for orientation.

The area is characterized by a typical Mediterranean climate with hot and dry summers. The most active phase, the breeding season, of *T. hermanni hercegovinensis* starts as soon as the amount of rain decreases in spring (Fig.2a).

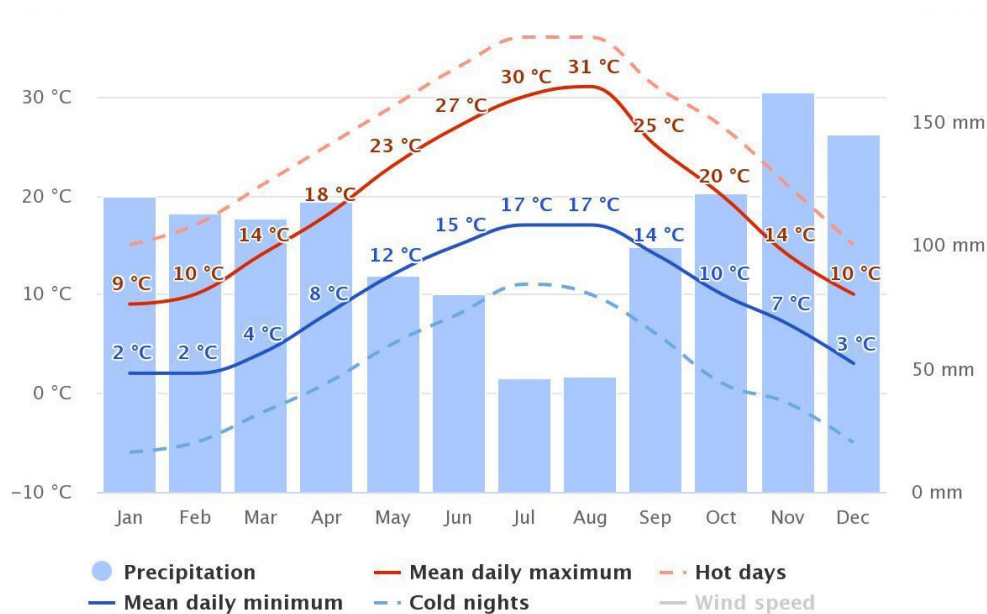


Fig.2a: Graph reflecting different climate parameters for the study area based on a 30-year global history derived from the global NEMS weather model at 30 km resolution. ©www.meteoblue.com

The small-scaled landscape mostly contains grassland with shrubs and hedges, agricultural fields, macchia, olive groves and vineyards (Fig.2b). Non-organic farming takes place with often heavy use of pesticides in some places.

Unsealed field paths can be found throughout the study area, whereas five of them were used for orientation during our study (Fig.1). Occasionally, the grassland is grazed by small herds of sheep and goats.



Fig.2b: Most parts of the study area were characterized by fields, grassland with shrubs, macchia and olive groves. © Karoline Bürger

For our investigation, the study site was separated into same sized grids á 50 x 50 m (Fig.1). Each grid was searched for the same amount of time for tortoises. Depending on the weather conditions, data collection began at 9 am and kept going until the animals stopped being active around 6 pm.

When a tortoise was found, the exact position was registered and noted with a GPS device. Furthermore, sex and body size were ascertained. The sex was only possible to determine for individuals showing a SCL of 10 cm or more. Smaller individuals (<10 cm) were considered to be juvenile.

In general, males were smaller than females and showed a different habitus, such as broader marginal scutes and a longer tail. Additionally, the following parameters were noted: date, time, weight, condition and possible injuries, the habitat type in which the animal was found, activity and position (e.g. exposure to sun) of the individual when found, as well as weather conditions and temperature.

The body size (SCL and CCL) and weight were measured with callipers and electronic scales. As mentioned above, every individual was checked for possible injuries (e.g. mowing vehicles, natural enemies) and its general condition (e.g. parasites, physical fitness, damaged or missing scutes or extremities).

The dorsal and ventral side of each tortoise was photographed, after being marked with a continuous number. Since the possibility was given that the marked number faded over time, a collection with dorsal and ventral pictures of all individuals was established and helped individual identification due to visual differences in shell pattern. Together with the numbers provided on the shell, all individuals could be identified.

To determine the exact distribution of the individuals, their habitat preferences, as well as the sex/size-specific distribution (males, females, juveniles), GPS data were transferred to an accurate map of the study area.

Data sampled and data processing

For the analyses the statistical package SPSS 22.0 was used to determine habitat preferences of the recorded individuals of the population as well as the distribution of the population within the study area. Based on the demographic study and the conditions of the individuals, conclusions about the necessity of conservation measurements can be made.

The number of all tortoises found per grid or separately for males, females and juveniles was used as dependent parameters respectively. The percentage of a grid covered with the six most important habitat types was determined and used as independent variables. The six parameters include i) Macchia, ii) trees, iii) meadow (grass), iv) gravel roads/path, v) fields and vi) shrubs. Based on them we additionally determined habitat diversity and heterogeneity.

To determine habitat diversity we used the Shannon Wiener index based on the quantity of the habitats per grid. To determine which habitat parameters influence individual abundance, stepwise multiple regression analyses were used with overall (= total number of tortoises per grid), male, female or juvenile abundance per grid as dependent variable respectively and the different habitat parameters as independent variables.

To determine heterogeneity, we counted the number of changes in habitat type per grid from left to right.

Stepwise multiple discriminant function analysis was used to detect which habitat parameters might be important for *T. hermanni hercegovinensis*. Therefore grids with tortoises were compared with grids without tortoises as group variables and the habitat parameters as independent variables. The same analyses were used to determine whether there are differences in habitat requirements between the sexes (grids with only males or females) and between adults and juveniles (grids with only adults or juveniles) tortoises. In a further attempt also grids with both (males and females or adults and juveniles), were run respectively.

Results

As shown in Fig.3 in May 2014 and 2015, 500 individuals were found and marked altogether. 302 tortoises in 2014 and 198 in 2015 with 64 recaptures (= individuals found more than once) (51 individuals) in the first, and 103 recaptures (69 individuals) in the second year, whereas 52 individuals recaptured in 2015 were also marked in 2014. Of these 500 tortoises, 262 (52%) were female, 127 (26%) male and 111 (22%) were considered juvenile, which leads to a female biased sex ratio of 2.06 (for details see also Bürger in prep).

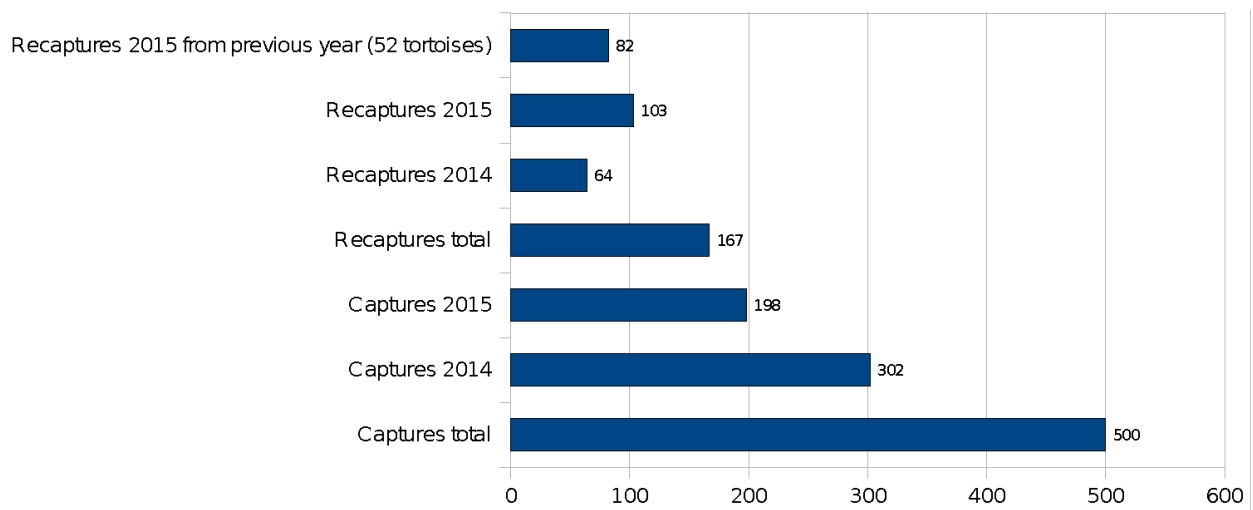


Fig.3: Altogether, 500 individuals were marked within the study area. 302 in 2014 and 198 in 2015.

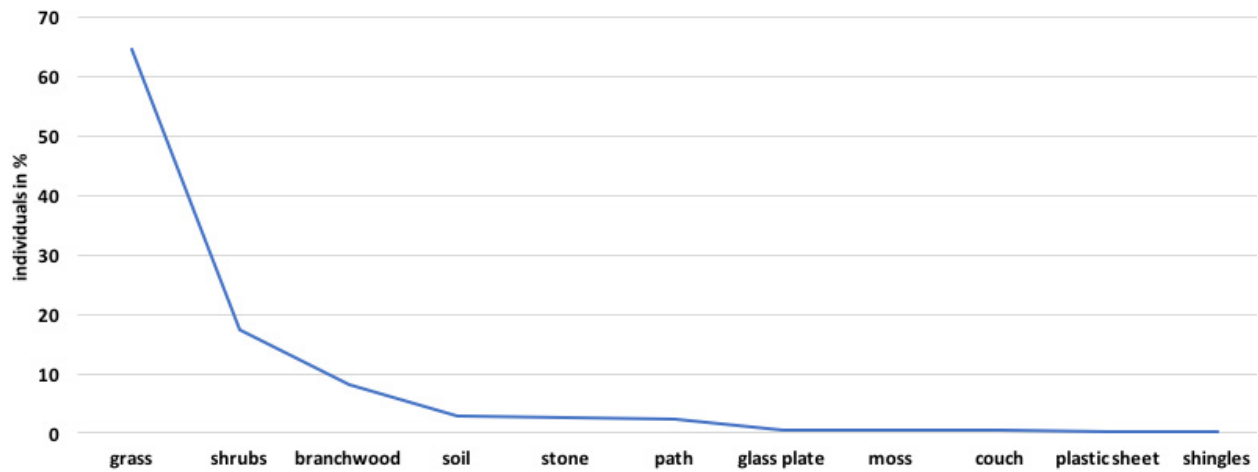


Fig.4: Different habitat types used by *Testudo hermanni hercegovinensis* (in %). Grassland with shrubs and branchwood were most popular, but artificial structures were also used. (N=500)

A detailed examination suggests that grassland turned out to be most frequently used (Fig.4). Mostly, tortoises were found in short grass near shrubs or hedges. Some individuals also used human artefacts such as glass plates, shingles, an old sofa or plastic sheets, that were dumped in the area.

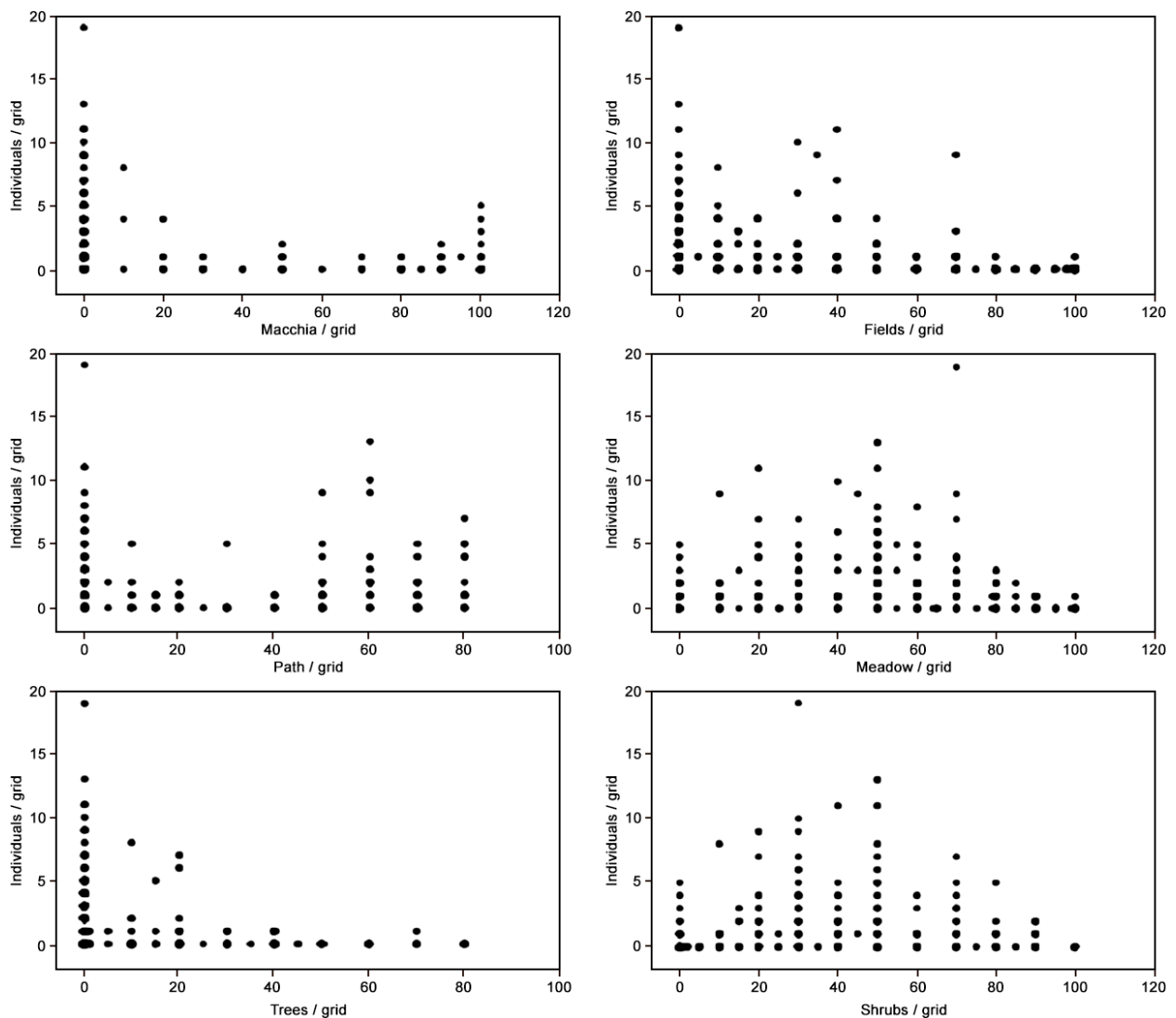


Fig.5: Relationship between individual density of *Testudo hermanni hercegovinensis* per grid and proportion of i) Macchia, ii) trees, iii) meadow, iv) gravel roads/path, v) fields and vi) shrubs. N=500

Our results suggested that grassland is the most important habitat type for Dalmatian tortoises, but there is an optimal amount of grassland cover (Fig.5). The relationship is inversely U-shaped and individual density was highest when about 50% of a grid was covered with grass, whereas individual density clearly decreased when grass cover was above or below 50% (Fig.5).

Shrub cover was similarly important and our results revealed the same distribution pattern with highest individual densities around 50% coverage (Fig.5). The typical Mediterranean Macchia however, was not very popular among the tortoises, indicated by the negative relationship between individual densities and Macchia cover/grid, although some individuals were found in pure Macchia stands. A clear negative relationship was also found between individual density and tree as well as field cover (Fig.5).

No clear pattern could be detected for the existence of paths/grid (Fig.5). Paths are not avoided, but also not a necessary prerequisite for the existence of individuals (Fig.5).

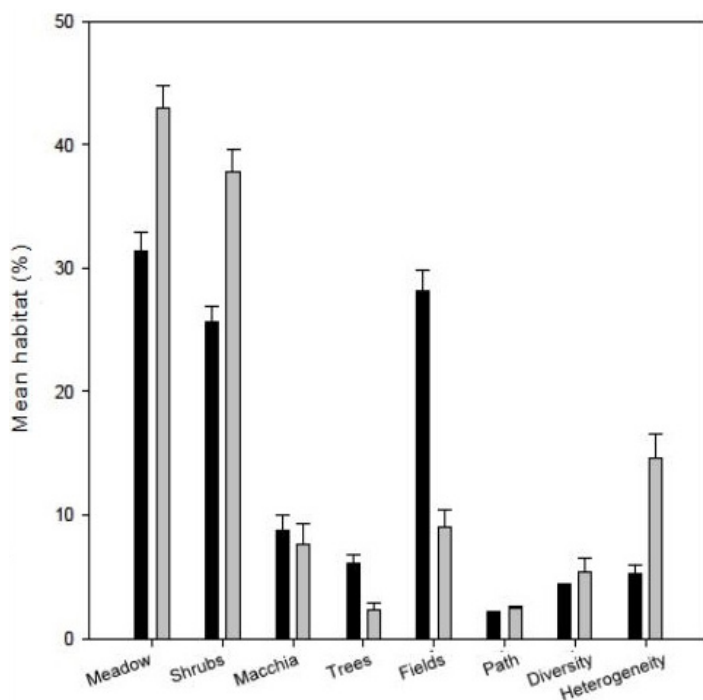


Fig.6: Proportion of different habitat types, habitat diversity and heterogeneity for occupied (grey) and unoccupied (black) grids

In order to evaluate the importance of habitat parameters, occupied and unoccupied grids were compared by a stepwise regression model. Four different habitat variables were entered to explain overall density per grid. Based on a stepwise regression analysis a significant model could be derived ($R = 0.333$, $df = 4$, 704 , $F = 21.771$, $p < 0.0001$). Habitat diversity, tree density, road and fields entered the model. The partial regression coefficient suggested a positive relationship with habitat diversity: 0.217 , $p < 0.0001$; road 0.129 , $p < 0.001$, and a negative relationship with fields: -0.117 , $p < 0.001$ and tree density: -0.178 , $p < 0.001$.

Using a stepwise discriminant function analysis to separate populated habitats (grids) from unpopulated ones, based on the habitat variables determined, it was possible to derive a significant model (Wilks Lambda = 0.845 , $df = 1, 4$, $1, 703$; $p < 0.0001$) and 100% of the variance could be explained. The variables field, habitat diversity, trees and paths entered the model and were

identified to be important, whereas field and trees were on the opposite side than habitat diversity and paths.

Based on these results, the amount of trees, agricultural fields, gravel roads (paths) and habitat diversity is sufficient to define and predict the distribution and abundance of the population (Fig.6) (Table X). In this context, fields, trees (e.g. olive groves) were clearly avoided, whereas grassland, shrubs and paths were necessary for habitat occupation in most cases (Fig.6).

Table X: Trees, fields, paths and habitat diversity statistically define habitat choice								
	Wilks-Lambda							
					exact F			
	statistics	df1	df2	df3	statistics	df1	df2	significance
agricultural fields	0,942	1	1	703,0	43,016	1	703,0	0,000
habitat diversity	0,900	2	1	703,0	38,798	2	702,0	0,000
trees	0,855	3	1	703,0	39,706	3	701,0	0,000
gravel roads (paths)	0,845	4	1	703,0	32,205	4	700,0	0,000

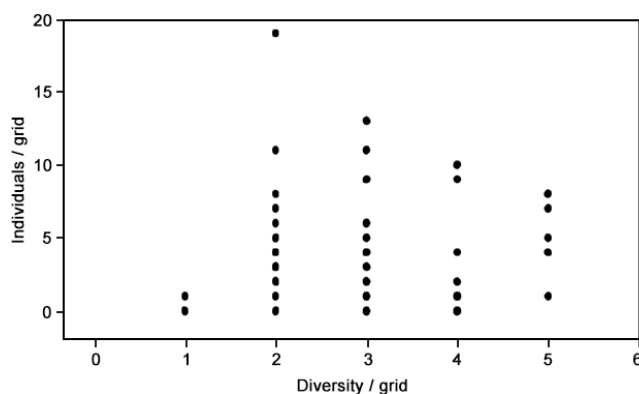


Fig.7: Relationship between individual density and habitat diversity (left).

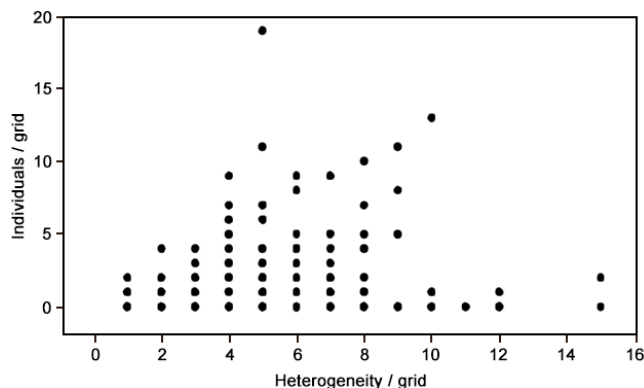


Fig.8: Relationship between individual density and heterogeneity (right).

Habitat diversity was not linearly related to tortoise density and was therefore not crucial to explain density. Results however suggest that there is an optimal, but only moderate level of diversity for Dalmatian tortoises. Thus, their preferred habitat does not need to be too diverse (Fig.7). Our study showed that individual density increased with habitat heterogeneity (Fig.8).

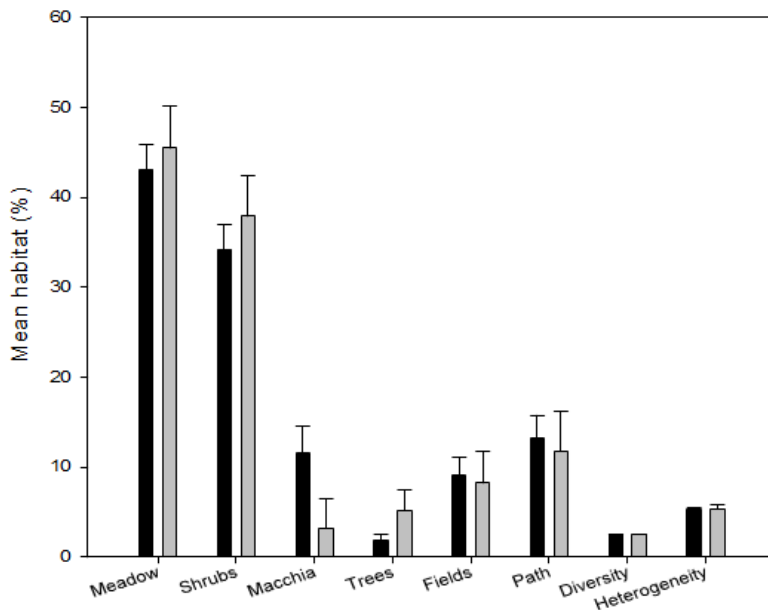


Fig.9: Differences in habitat use between males (grey) and females (black). N=389

For a stepwise regression model, five different habitat variables were entered to explain female habitat choice per grid (Fig.9). Based on a stepwise regression analyses, a significant model could be derived ($R = 0.281$, $df = 5$, 704 , $F = 11.994$, $p < 0.0001$), whereby five variables entered the model, namely paths, grass, heterogeneity, trees and habitat diversity. The partial regression coefficient suggested a positive relationship with road: 0.118 , $p < 0.002$; meadow: 0.109 , $p < 0.004$; heterogeneity: 0.083 , $p < 0.028$; and habitat diversity: 0.078 , $p < 0.04$ and a negative relationship with tree density: -0.123 , $p < 0.001$.

Based on the stepwise regression analysis, a significant model could be derived ($R = 0.278$, $df = 5$, 704 , $F = 14.619$, $p < 0.0001$) to explain male habitat choice (Fig.9), whereby four variables entered the model, namely paths, grass, shrubs and habitat diversity. Partial regression coefficients suggest a positive relationship for all (partial r for road: 0.164 , $p < 0.0001$; meadow: 0.115 , $p < 0.002$; bush: 0.1 , $p < 0.008$; habitat diversity: 0.089 , $p < 0.019$).

Higher vegetation, such as olive groves, influenced males less than females during our investigation period, since no negative relationship to this habitat type was found.

A stepwise regression analysis revealed that two variables are important to describe juvenile density (Fig.10). Habitat diversity and tree cover are sufficient to explain juvenile density per grid. These two variables entered a stepwise regression analysis resulted in a significant model ($R = 0.23$, $F = 19.541$, $p < 0.0001$, $df = 2$, 704). The partial regression coefficient (0.224 , $p < 0.0001$) suggested a positive relationship between juvenile abundance and habitat diversity, and the partial correlation coefficient (-0.128 , $p < 0.001$) a negative relation with tree cover (Fig.10).

However trying to separate male and female grids did not reveal a significant model (Wilks Lambda 0.939 , $p > 0.476$). Thus sex specific difference did not appear to be obvious regarding the observed habitat variables also not when including only grids with many females or many males ($p > 0.5$), or including grids, where both sexes occurred ($p > 0.61$). Finally, the same analysis revealed no significant difference between adults and juveniles ($p > 0.16$).

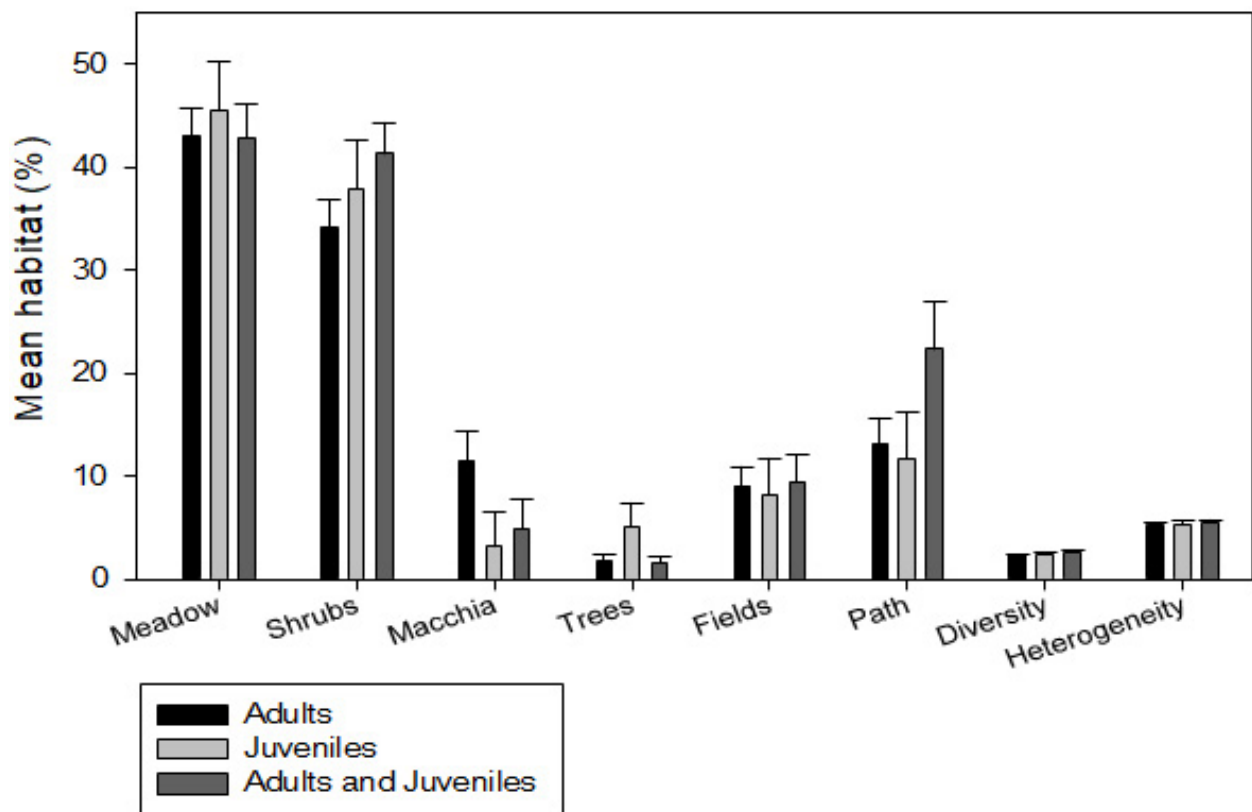


Fig.10: Differences in habitat use between adults and juveniles. (N=500)

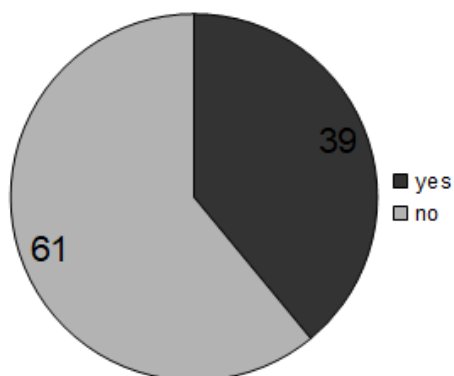


Fig.11: 61% of all marked individuals showed no signs of injuries, whereas 39% did. (N=500)

The majority (61%) of all marked individuals showed no signs of injuries, whereas 39% did (Fig.11). An individual was considered as injured, when bite marks or cuts from mowing activities were identified on its shell and some individuals also had mutilated or missing extremities, which could also be due to predators. Interestingly, not a single individual was infested by visible ectoparasites.

Discussion

Within two seasons (May 2014 and 2015) 500 individuals of the species *Testudo hermanni hercegovinensis* were found and marked. 120 of the 500 individuals were recovered and released once again or more often (up to four times) (Fig.3).

In contrary to results found in previous studies (Vetter, 2006), where more males than females were found in Croatia, the sex ratio in our study area was female biased. Similar results as recorded in Spain were calculated, namely 2.06 females per male, which could be an indication for a declining population our study area, since sex ratios in healthy populations are usually balanced (Celse et al., 2014) (Bürger in prep, 2021).

Another possible reason for the female biased sex ratio could be climatic conditions in this area (Cheylan et al. 2007) (for details see Bürger in prep.). Climate change (Simac & Vitale, 2012) might also influence sex ratios, since temperature plays a crucial role in embryonic development and sex determination in reptiles (Eendebak, 1995) (Bull, 1980) (Lambert, 1982).

Another factor influencing the sex ratio could be the higher mobility of males, which results in males being more exposed and therefore an easier target for predators and other risks. This however would mean that a biased sex ratio is due to differential predation rather than an original biased ratio due to sex determination.

Given that twice as many females than males were found, it is possible that competition is higher between females than males (Bürger in prep.). Since females have larger home ranges (Celse et al., 2014) (Fasola et al., 2002) and tend to be more stationary than males (Rogner, 2012) (Chelazzi & Francisci, 1979), especially during mating season, when males are very mobile (Celse et al., 2014), the competition might mainly occur over habitats and food resources. On the other hand, Türkozan et al. (2018) found no significant sex or age (size) specific differences in home range sizes and *T. hermanni* is considered to be a non-territorial species, therefore it is very common that two or more females and/or males share one territory (Celse et al., 2014). Individuals were observed sitting right next to each other, regardless of sex or age. Juveniles were often found right next to adult females or males.

Most of the time, the tortoises were located in the grass near shrubs or hedges, but some individuals also hidden under artificial structures such as glass plates, shingles, an old couch or plastic sheets (Fig.4). This was not surprising, given that this species is known to be very flexible in their habitat choice (Berardo et al. 2015) (Rogner, 2012) (Zuffi & Corti, 2003) and seems to be able to cope with different, even euhemerobic environmental conditions (Rugiero & Luiselli, 2006). The majority of all individuals observed preferred inhabiting well-structured habitat types (Fig.5), such as grassland with scattered shrubs or hedges. They tended to choose habitats, where they had the possibility to bask, hide, exploit food resources and find mating partners within a small home range, which matches previous observations (Vetter, 2006) (Türkozan et al., 2018) (Celse et al., 2014) (Calzolari & Chelazzi, 1991) (Cheylan 2001).

Increasing mobility leads to increased vulnerability (Celse et al., 2014), which seems to be avoided most of the year, except during mating season (Rogner, 2012). Bigger patches of open space such as grassland without hiding possibilities, fields, vineyards and olive groves, where there is a high risk of exposure to predators, were usually avoided (Fig.5) as found by Celse et al. (2014) and Stojadinović et al. (2017) (Couturier et al., 2014).

Since the use of pesticides is common in areas used for agriculture and poses a threat to *Testudo hermanni* (Willemssen & Hailey, 2001), it might also be the case that the tortoises avoided the polluted areas, even though hiding places and/or feeding opportunities were given in some cases, especially in vineyards.

Since tortoises are not very flexible in their movements due to their shell, they were observed using “comfortable” and familiar ways, as found by Chelazzi & Francisci (1979), such as man made gravel roads or pathways without any obstacles (Fig.5), whereas hedges along these pathways were used to hide and consequently populated.

Based on our results, trees, fields, paths and habitat diversity were selected to be sufficient to define individual distribution based on grid abundance (Table X). As found by Celse et al., 2014, our observations showed that *T. hermanni hercegovinensis* avoided trees and fields (Fig.5) (Fig.6), but preferred moderately diverse habitats (Fig.7) next to pathways, although latter were not identified to be important for settlement (Fig.5) (Fig.6).

Since many farmers still kill tortoises, because they consider them as crop pests (Vetter, 2006) (Celse et al., 2014), it is important at this point, to mention that any monotonous landscapes, such as fields or meadows, are avoided by the majority of the individuals, since they strongly prefer more diverse areas with a higher heterogeneity (Fig.6). Celse et al. (2014) even considered heterogeneity as the most important variable in habitat choice.

Most of the time, the individuals were found in the grass with shrubs, hedges or branchwood not far away, in case they felt the need to hide. The analysis revealed that the ideal grid has about half of the grid covered with grassland in their habitat (Fig.5) and the other half with options to hide (Fig.5).

The typical Mediterranean Macchia seems to be not very appropriate for this species (Fig.5). The ground is uneven and full with obstacles like stones of various sizes and the vegetation is dense and thorny. From the few tortoises found in the macchia, many had grazed and damaged shells. Even very small and young individuals already had these typical „Macchia-shells“.

As already pointed out, different results of habitat diversity (Fig.7) and heterogeneity (Fig.8) were achieved. It turned out that tortoises preferred habitats of moderate diversity, more precisely about three habitat types within a 50m x 50m grid (Fig.7). This matched our observations well, that grassland with shrubs, hedges or branchwood was by far the most common and popular habitat type. Considering habitat heterogeneity, it showed that the higher it is, the better (Fig.8), since tortoises do not like to move too far, a high heterogeneity allows them to inhabit different habitat types within their territory. Celse et al. (2014) even considered heterogeneity as the most important parameter in habitat choice.

In this area, *T. hermanni hercegovinensis* does not seem to inhabit dense vegetation as suggested by Rogner (2012), van Dijk et al. (2004) and Vetter (2006), but prefers a well-mixed landscape, where all ecological needs of this species are met within a small patch of land.

There were no significant differences between males and females regarding habitat choice, but the analysis showed that the crucial parameters to inhabit an area (grid) were for both sexes grassland, pathways and habitat diversity. For females the absence of trees and a high habitat heterogeneity were additional parameters identified and for males the amount of shrubs or hedges (Fig.9). An explanation for sex specific differences could be, that females are more stationary (Rogner, 2012) (Chelazzi & Francisci, 1979) and therefore might pay more attention to the quality of their habitat. Males on the other hand are more mobile in search for mates and only need a short term shelter, e.g. in shrubs, before moving on. Since males are highly mobile during the mating season, they might also more likely risk walking through areas, where they are more exposed (e.g. olive groves), resulting in the absence of trees not being a crucial parameter for males. Since investigation was restricted to mating season, results may change over the year.

Macchia seemed to be avoided by juveniles even more than by adult individuals (Fig.10). This habitat type is likely not suitable for egg deposition as the ground is covered in stones. Therefore, it is obvious that not many, especially recently hatched individuals could be found there, since juveniles tend to stay near their place of birth for a few years (Celse et al., 2014). Tree accumulations on the other hand were less avoided by juveniles than by adult tortoises (Fig.10). An explanation for that most likely is that females chose this habitat for egg deposition and the hatched juveniles stayed in the area.

This study allowed a major insight in life history and habitat choice as well as possible threats to the population. As shown in Fig.11, most individuals did not show any signs of injuries. Not one individual was found with visible ectoparasites, such as ticks or mites, even though dogs in the area were often covered with ticks. This might be an indicator for a high physical fitness of the tortoises or for a high abundance of tick species that do not consider tortoises as hosts (Široký et al., 2006) (Tschirren et al, 2007).

39% of the individuals did show signs of injuries such as bite marks, most likely caused by foxes or wild boars. Many of the animals had bite marks or cuts on their shells, but also individuals with mutilated legs, broken marginals or missing eyes were found. Damaged marginals are often the result of intraspecific interactions (Rogner, 2012). However, mutilated legs, bite marks or cuts are probably caused by mowing vehicles, wild boars, dogs or other mammals (Celse et al., 2014). In order to act against dangerously high decimation in the future caused by these factors, effective conservation measures would have to be taken in time and long-term monitoring needs to take place. Since wild boars are quite common due to feeding by hunters, their abundance is probably unnaturally high in this area.

So far, our analyses imply an unexpectedly high population density based on the high number of individuals in relation to our search effort, yet the strongly female biased sex ratio (2.06) might indicate a declining population (Celse et al., 2014). We suggest that as long as long-term

monitoring takes place, the extensive farming keeps maintained to ensure well-structured, diverse habitats with a high heterogeneity and grazing by farm animals continues (Celse et al., 2014), the population in this area can be protected effectively.

Within the next few years, we are planning to widen our research on this population with studies about their migration behaviour, observations for a longer period of time and we are also interested in finding out about the genetic relationship within the population, since hardly any DNA sampling of eastern populations of *T. hermanni* took place so far (Roberto et. al. 2018).

A promising study, which demonstrates the importance of genetic analyses of an endangered species was conducted by Roberto et al. (2018). The geographic origin of confiscated individuals was identified via microsatellite markers, which enables a reintroduction of illegally owned tortoises into their natural habitat.

References

- Berardo, F., et al. (2015). Seasonal habitat preference by the flagship species *Testudo hermanni*: Implications for the conservation of coastal dunes. *Comptes rendus biologies*, 338(5), 343-350.
- Bull, J. J. (1980). Sex determination in reptiles. *The Quarterly Review of Biology*, 55(1), 3-21.
- Calzolari, R., & Chelazzi, G. (1991). Habitat use in a central Italy population of *Testudo hermanni* Gmelin (Reptilia Testudinidae). *Ethology ecology & evolution*, 3(2), 153-166.
- Celse, J., et al (2014). *Management Guide of Populations and Habitats of the Hermann's Tortoise.* Life, 8.
- Chelazzi, G., & Francisci, F. (1979). Movement patterns and homing behaviour of *Testudo hermanni* Gmelin (Reptilia Testudinidae). *Monitore Zoologico Italiano-Italian Journal of Zoology*, 13(2-3), 105-127.
- Cheyland, M. (2001). *Testudo hermanni* Gmelin, 1789–Griechische Landschildkröte. *Handbuch der reptilien und amphibien Europas*, 3, 179-289.
- Cheyland, M. et al. (2007). Breeding traits of Hermann's tortoise *Testudo hermanni hermanni* in two western populations. *Amphibia-Reptilia*, 28(1), 77-85.
- Couturier, T., (2014). Factors determining the abundance and occurrence of Hermann's tortoise *Testudo hermanni* in France and Spain: fire regime and landscape changes as the main drivers. *Biological Conservation*, 170, 177-187.
- Cruce (1978) „Structure et dynamique d'une population de *Testudo hermanni* (Reptilia), - Trav. Mus. Hist. Nat. Bukarest, 19:325-328
- Danchin, E. et al (1998) „Conspecific Reproductive Success And Breeding Habitat Selection: Implications For The Study Of Coloniality, *Ecology*, 79(7), 1998, Pp. 2415–2428
- Q 1998 By The Ecological Society Of America
- Duro, S., et al. (2021). Morphological Divergence of Hermann's Tortoise (*Testudo hermanni boettgeri* Mojsisovits, 1889) in Albania. *Animals*, 11(1), 134.
- Ebenman, B. (1987). Niche differences between age classes and intraspecific competition in age-structured populations. *Journal of theoretical biology*, 124(1), 25-33.
- Eendebak, B. T. (1995). Incubation period and sex ratio of Hermann's tortoise, *Testudo hermanni boettgeri*. *Chelonian Conservation and Biology*, 1(3), 227-231.
- Fasola, M. et al. (2002). Activity and home range of *Testudo hermanni* in Northern Italy. *Amphibia-Reptilia*, 23(3), 305-312.
- Guyot G. & Clobert J. (1996) „Conservation Measures for a population of Hermann's tortoise *Testudo hermanni* in southern france bisected by a major highway“ *Biological Conservation*, 79 (1997) 251-256
- Hailey, A. (1990) „Adult survival and recruitment and the explanation of uneven sex ratio in a tortoise population“, *Ca. J. Zool.* 68: 547-555
- Hailey, A. (2000). The effects of fire and mechanical habitat destruction on survival of the tortoise *Testudo hermanni* in northern Greece. *Biological conservation*, 92(3), 321-333.
- Hailey, A. & Willemsen R.E. (1999) „Population density and adult sex ratio of the tortoise *Testudo hermanni* in Greece: evidence for intrinsic population regulation“, *J. Zool., Lond.* (2000) 251, 325-338
- Hoogland J.L. & Sherman P.W. (1976) „Advantages and Disadvantages of Bank Swallow (*Riparia riparia*) Coloniality“, *Ecological Monographs*, Vol. 46, No. 1 (Winter, 1976), pp. 33-58

- Jacobs, J. D. (1997). Regulation of life history strategies within individuals in predictable and unpredictable environments.
- Lambert, M. R. (1982). Studies on the growth, structure and abundance of the Mediterranean spur-thighed tortoise, *Testudo graeca* in field populations. *Journal of Zoology*, 196(2), 165-189.
- Ljubisavljević, K. et al. (2011). The commercial export of the land tortoises (*Testudo* spp.) from the territory of the former Yugoslavia: a historical review and the impact of overharvesting on wild populations. *North-Western Journal of Zoology*, 7(2).
- Longepierre, S. et al. (2001). Home range area in the tortoise *Testudo hermanni* in relation to habitat complexity: implications for conservation of biodiversity. *Biodiversity & Conservation*, 10(7), 1131-1140.
- Pierotti R. (1982) „Habitat Selection and Its Effect on Reproductive Output in the Herring Gull in Newfoundland, *Ecology*, Volume 63, Issue 3, Pages 854-868
- Qviller, L. et al. (2013). Landscape level variation in tick abundance relative to seasonal migration in red deer. *PLoS One*, 8(8), e71299.
- Roberto, B., et al. (2018). Who's who in the western Hermann's tortoise conservation: a STR toolkit and reference database for wildlife forensic genetic analyses. *BioRxiv*, 484030.
- Rogner M. (2012) „Griechische Landschildkröten – Lebensweise, Haltung, Nachzucht“ *Natur und Tier – Verlag GmbH, Münster*
- Rugiero L. & Luiselli L. (2006) „Ecological modelling of habitat use and the annual activity patterns in an urban population of the tortoise, *Testudo hermanni*, *Italian Journal of Zoology*, 73:3, 219-225
- Schweiger M. (2006) „Die Dalmatinische Landschildkröte, *Testudo hermanni hercegovinsensis*“, *Marginata* 24
- Sih, A., Ferrari, M. C., & Harris, D. J. (2011). Evolution and behavioural responses to human-induced rapid environmental change. *Evolutionary applications*, 4(2), 367-387.
- Široký, P., Petrželková, K. J., Kamler, M., Mihalca, A. D., & Modrý, D. (2006). *Hyalomma aegyptium* as dominant tick in tortoises of the genus *Testudo* in Balkan countries, with notes on its host preferences. *Experimental & applied acarology*, 40(3-4), 279-290.
- Sorvano V.A. et al (2017) „Tortoises in front of mirrors: Brain asymmetries and lateralized behaviour in the tortoise (*Testudo hermanni*), *Behavioural Brain Research* 352 (2018) 183–186
- Stojadinović, D., et al. (2017) „Activity patterns and habitat preference of eastern Hermann's tortoise (*Testudo hermanni boettgeri*) in Serbia.“ *Turkish Journal of Zoology* 41.6: 1036-1044.
- Stamps, J. A. (1991). The effect of conspecifics on habitat selection in territorial species. *Behavioral Ecology and Sociobiology*, 28(1), 29-36.
- Switzer P.V. (1996) „Past reproductive success affects future habitat selection“, *Behav Ecol Sociobiol* (1997)40: 307-312
- Tschirren B. et al. (2007) „Host condition and host immunity affect parasite fitness in
- Türkdoğan, O. et al. (2019). Daily movements and home range of Eastern Hermann's Tortoise, *Testudo hermanni boettgeri* (Reptilia: Testudines). *Zoology in the Middle East*, 65(1), 28-34.
- a bird–ectoparasite system“, *Functional Ecology* 2007 21, 372–378
- Vetter H. (2006) „Griechische, Dalmatinische und Italienische Landschildkröte“ *Chimaira*
- Buchhandels-gesellschaft mbH, Verleger Andreas S. Brahm, Frankfurt am Main

Wallace & Wallace (1985) *Observations of a Population of Hermann's Tortoise (Testudo hermanni) in Southern Yugoslavia – Testudo 2 (3): 15-25*

Wegehaupt (2005) *Kroatien-Exkursion, Die Dalmatinische Landschildkröte, Testudo hermanni (hercegovinensis), Werner 1899*

Windolf (1980): *Zur Biologie, Ökologie und zum Artenschutz der Griechischen Landschildkröte (Testudo hermanni h.) in Jugoslawien – ÖKO.L Zeitschrift für Ökologie, Natur- und Umweltschutz – 1980_4: 14 - 20.*

Willemsen, R. E., & Hailey, A. (2001). *Effects of spraying the herbicides 2, 4-D and 2, 4, 5-T on a population of the tortoise Testudo hermanni in southern Greece. Environmental pollution, 113(1), 71-78.*

van Dijk, P. P., et al. (2004). *Testudo hermanni. IUCN 2010. IUCN Red List of Threatened Species.*

Zenboudji, S. et al. (2016). *Conservation of the endangered Mediterranean tortoise Testudo hermanni hermanni: The contribution of population genetics and historical demography. Biological Conservation, 195, 279-291.*

Zuffi, M., & Corti, C. (2003). *Aspects of population ecology of Testudo hermanni hermanni from Asinara Island, NW Sardinia (Italy, Western Mediterranean Sea): preliminary data. Amphibia-Reptilia, 24(4), 441-447.*

https://www.meteoblue.com/de/wetter/historyclimate/climatemodelled/dazlina_kroatien_3202169