

Ecological Study on the Populations of Two Sympatric Species of Tortoises – Testudo hermanni and T. graeca (Testudines: Testudinidae) in South-Western Bulgaria

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Abstract. Although studies on *Testudo hermanni boettgeri* and *T. graeca iberica* have been conducted for more than a century in Bulgaria, little or no data have been published on some aspects of their ecology. Moreover, most of the local populations have still not been examined. The purpose of the study was to examine two local populations of the Hermann’s and the Spur-thighed Tortoise occurring together. The study was carried out during two different periods from 2012 to 2023. Here we present data on the numbers, age structure, and sex ratio of the two populations. We also provide information on their home range and real and potential threats. The results indicated that *T. hermanni boettgeri* is much more numerous than *T. graeca iberica*. The age structure of both species was very similar – the adults predominated over subadults and juveniles. The sex ratio was slightly in favor of males in the Hermann’s Tortoise, whereas it was female-biased in the Spur-thighed Tortoise. The mean home range of the Hermann’s Tortoise was smaller compared to other populations of the species. Females had a larger home range than males. The mean home range of the Spur-thighed Tortoise was larger than that of the Hermann’s Tortoise and similar to the home range of other populations on a species level. The biggest threat identified was related to partial deforestation within the habitat of the tortoises. Overall the two species seemed to successfully coexist even in the presence of threats as their number did not decline during the study but rather was stable.

Key words: Balkans, ecology, threats, tortoises, Hermann’s Tortoise, Spur-thighed Tortoise.

Introduction

The genus *Testudo* Linnaeus, 1758 consists of five species – *T. hermanni*, *T. graeca*, *T. marginata*, *T. kleinmanni*, and *T. horsfieldii*. Their general distribution includes parts of Europe, Africa, and Asia (Rhodin et al., 2021). Two of the species *T. hermanni* Gmelin, 1789 and *T. graeca* Linnaeus, 1758 occur in Bulgaria and are represented by one subspecies – *T. hermanni boettgeri* Mojsisovics, 1889 and *T.*

graeca iberica Pallas, 1814, respectively. They are distributed in most parts of the country and usually occur sympatrically (Beshkov & Nanev, 2002; Stojanov et al., 2011).

Historically, in Bulgaria, most of the studies on the two species have been primarily focused on determining their distribution (Beshkov, 2015a, 2015b; Buresch & Zonkow, 1933; Kovachev, 1912; Stojanov et al., 2011). Information on the number, age

structure, and sex ratio of certain local populations can be found in the works of Ivanchev (2007), Zhivkov et al. (2007), Popgeorgiev (2008), Pulev & Sakelarieva (2011), Popgeorgiev et al. (2016), Malakova et al. (2018).

Data on the age structure (Corti & Zuffi, 2003; Hailey et al., 1984; Loy et al., 2007; Mazzotti, 2004; Meek, 1985; Meek & Inskip, 1980; Stojadinović et al., 2017b; Vujović et al., 2015) and sex ratio (Budó et al., 2003; Corti & Zuffi, 2003; Duro et al., 2021; Hailey, 1988, 1990; Hailey & Willemssen 2000; Kicaj et al., 2016; Loy et al., 2007; Mazzotti, 2004; Mazzotti et al., 2002; Meek 1985; Meek & Inskip 1980; Vujović et al., 2015) of *T. hermanni* are also available for other populations across its range. Information on the same population characteristics has also been published for some populations of *T. graeca* (Andreu et al., 2000; Kaddour et al., 2006; Keller et al., 1997; Rouag et al., 2007).

To the best of our knowledge, there are no results in the literature regarding the home range of both species in Bulgaria. Outside of Bulgaria, some authors have studied the home range of *T. hermanni boettgeri* (Hailey, 1989; Rozyłowicz & Popescu, 2012; Stojadinović et al., 2017a; Türkozan et al., 2018) and *T. hermanni hermanni* (Longepierre et al., 2001; Loy & Cianfrani, 2009; Luiselli et al., 2009; Mazzotti et al., 2002; Swingland et al., 1986). As far as we know, so far, no data on the home range of *T. graeca iberica* have been reported in the literature. On a species level, such data have been published by some authors (Attum et al., 2011; Bernheim et al., 2019; Giménez et al., 2004).

Threats to tortoises in Bulgaria have been documented since the end of the 19th century (Hristovitsch, 1892). But Beshkov (1984, 1993) was the first to investigate the threats to *T. hermanni boettgeri* and *T. graeca iberica* at a national scale in detail. He found that habitat loss and degradation along with the illegal collection of individuals were the main factors leading to the extirpation or decline of many local populations throughout the country. Because of this, the two species are protected by the Biological Diversity Act of Bulgaria (Appendices II and III) (Biological Diversity

Act, 2002). They are also listed in the Red Data Book of the Republic of Bulgaria, category “Endangered” (Beshkov, 2015a,b).

In our previous paper (Mitrevichin et al., 2023), we provided some preliminary data on the populations of the two species in the study area (Fig. 1). The present study gives more comprehensive information about some ecological aspects of the two species and provides some new data, e.g. on their home range.

Materials and Methods

Study area

The study area is located in the vicinity of the village of Rakitna, Blagoevgrad Province, Bulgaria at about 700 m a.s.l. (Fig. 1). It lies within the Continental-Mediterranean climatic region, which is characterized by warm summers and mild winters (Velev, 2002b). Direct solar radiation typically varies from 5000 to 6000 MJ/m² for the whole year, and its intensity is the highest in July – up to 570 MJ/m² (Velev, 2002a). The mean annual air temperature is 11°C, and the mean air temperature is positive during all winter months. The warmest month is July with an average air temperature of 21.3°C (Kyuchukova, 1983). The average annual rainfall is approximately 755–760 mm – 197 mm in spring, 172 mm in summer, 195 mm in autumn, and 193 mm in winter (Stoyanov & Ilieva, 2007).

The study area is located almost exclusively within the xerothermic oak belt. The forest communities are dominated by *Quercus pubescens*, *Q. frainetto*, *Q. cerris*, *Fraxinus ornus*, and *Acer monspessulanum* (Bondev, 1991; Gruev & Kuzmanov, 1994). Large areas are also occupied by secondary vegetation, which is mainly composed of grasses and shrubs, and to a lesser extent by trees. *Paliurus spinachristi*, *Juniperus oxycedrus*, and *Carpinus orientalis* are typical representatives of the secondary vegetation (Bondev, 1991).

Field surveys

Two local populations – one of the Hermann’s Tortoise and another of the Spur-thighed Tortoise – occurring together, were studied during two different periods. The first period was from 2012 to 2015, and the second one was from 2021 to 2023. No intentional

research was carried out between 2016 and 2020. Nevertheless, some tortoises were found when we were visiting the area, and the data concerning these observations were gathered. The data obtained from the two periods as well as those obtained from 2016 to 2020 were combined and analyzed together.

During the first period, the tortoises were located by random walking almost exclusively around the Nature Conservation Center Villa Fauna (N41°50'48.6" E23°10'29.0") of the Fund for Wild Flora and Fauna. The handling of the animals was carried out in accordance with Permit No. 842/27.05.2020 issued by the Ministry of Environment and Water of Bulgaria. The individuals captured were marked using aluminum plates with a unique identification number. The plates were glued to the tenth or eleventh marginal scute with epoxy resin.

During the second period, the boundaries of the study area were expanded, and the whole area was searched for tortoises uniformly. Newly found individuals, i.e. those that had not been marked during the first study period, were marked by filing the marginal scutes

with a fine-toothed hacksaw blade (Stubbs et al., 1984). This method was preferred during the second period as it saves time and thus allowed us to have more time for searching. After the tortoises had been marked they were released at the place of capture.

Both in the first and second period, tortoises smaller than 10 cm carapace length in *T. hermanni* and 12 cm in *T. graeca* were considered juveniles. The larger individuals were considered subadults and adults (Hailey, 1990; Hailey et al., 1988). The distinction between the two species as well as the sexes within each species was made according to the identification features described by Stojanov et al. (2011).

Home range estimation

To calculate the home range, we used the minimum convex polygon method, which is probably the most reliable method for calculating home ranges in reptiles (Row & Blouin-Demers, 2006). The calculation was made using QGIS 3.30.1. Only the individuals that were recaptured at least four times were included in the calculation.

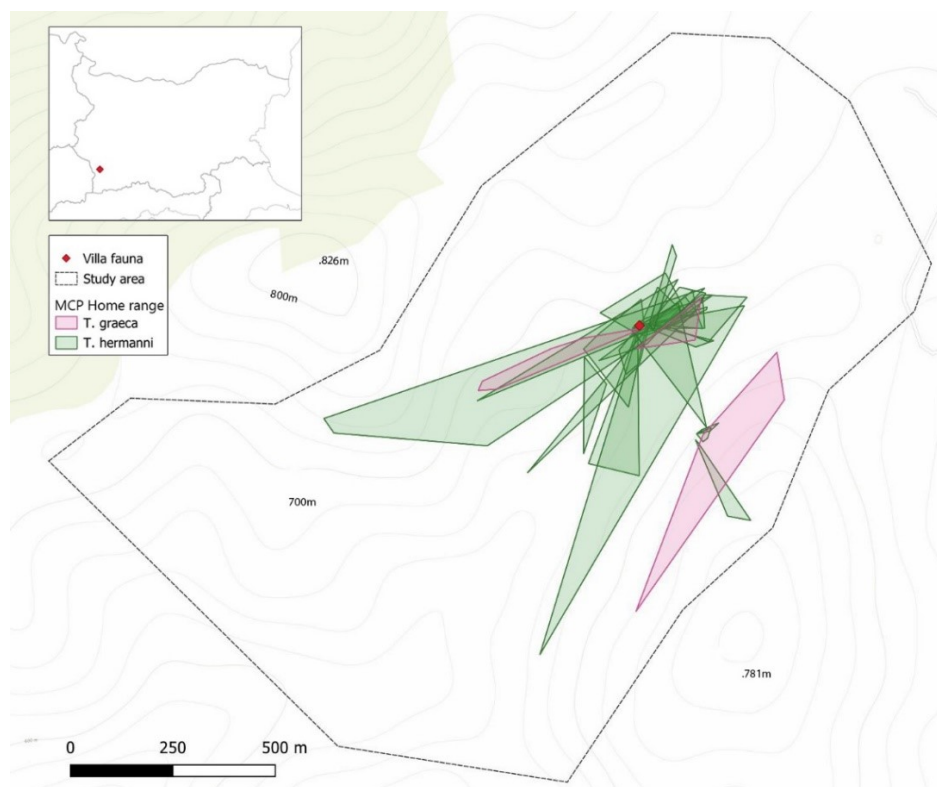


Fig. 1. The study area and the home ranges of individuals of *T. hermanni boettgeri* and *T. graeca iberica* within it.

Results

Number of individuals

Two hundred and thirty-six Hermann's Tortoises were captured at least once, and the total number of all the captures was 431. Of the 236 individuals, 88 were captured more than once, which is equivalent to 37.2%. Forty-five Spur-thighed Tortoises were captured at least once, and the total number of all the captures was 65. Of the 45 specimens, 12 were captured more than once, or 26.6%. Thus, the ratio between the specimens of the two species was 5.2:1 in favor of the Hermann's Tortoise.

Age structure

The age structure of the population of *T. hermanni boettgeri* was characterized by high dominance of the adults over subadults and juveniles (Fig. 2). In fact, 88% of all the tortoises registered were adults, with only 5.8% subadults and 6.3% juveniles, respectively. The age structure of *T. graeca iberica* was very similar to that of the Hermann's Tortoise (Fig. 2). The adults comprised 84% of all the individuals found, with only 12.5% subadults and 4.6% juveniles, respectively. A slight difference between the two species was found in the percentage of the subadults and juveniles recorded.

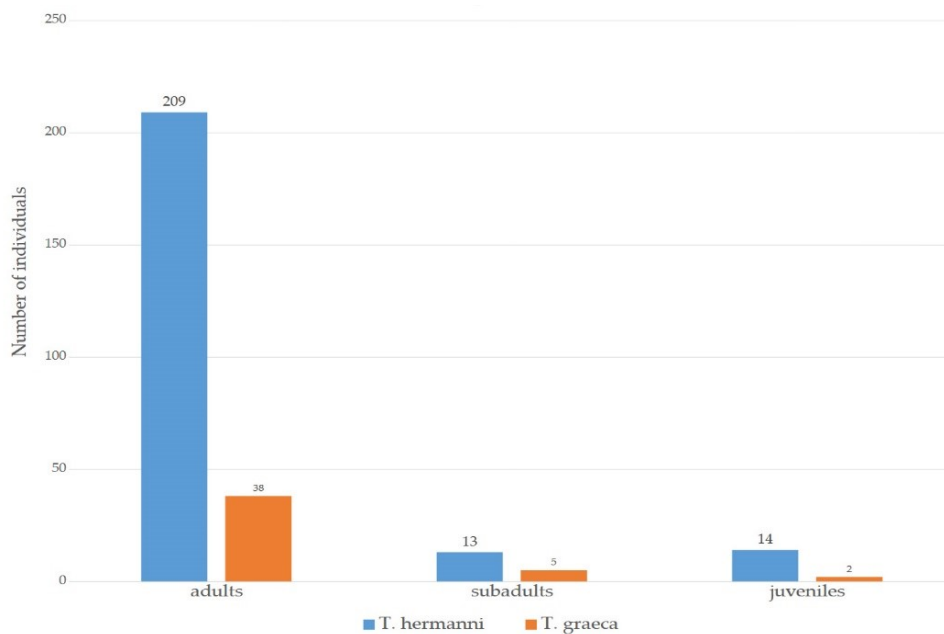


Fig. 2. Number of individuals of both species by age groups.

Sex ratio

The sex ratio in the Hermann's Tortoise was slightly in favor of males - 1.09:1. Conversely, it was in favor of females in the Spur-thighed Tortoise - 0.72:1.

Home range

The average home range area of *T. hermanni boettgeri* was 0.86 ± 1.50 ha (range 0.008–5.99). Females had a larger average home range area than males: 1.33 ± 2.06 ha (range 0.13–5.99); 0.43 ± 0.43 ha (range 0.008–1.22), respectively. The average home range area of *T. graeca iberica* was 1.25 ± 1.23 ha (range 0.4–2.67). The home range area of females and

males was not estimated separately as only three individuals - two females and one male - were included in the calculation (Fig. 1). This is because they were the only tortoises that were captured at least four times.

Real and potential threats

The real and potential threats identified were the same for both species. The real ones were related to human activity within the study area. Some residents often deforested in the habitat of the tortoises. Also, once a tortoise was collected and transported illegally to another part of the country. The potential threats were related to the constant presence

of domestic dogs and cats as well as a herd of cows within the study area. Another potential threat would be the possible construction of a highway, part of which can fragment the habitat.

Discussion

Number of individuals

The significant difference in the number of the individuals of the two species is not unusual. A similar ratio has already been found in other local populations in south-western Bulgaria (Malakova et al., 2018; Manolev et al., 2019; Popgeorgiev et al., 2016; Pulev & Sakelarieva, 2011). However, none of these authors suggested possible reasons for this difference. This discrepancy can be partly explained by the position of the range of *T. hermanni boettgeri* and *T. graeca iberica* within this part of the country. The middle of the range of *Testudo hermanni boettgeri* is located close to south-western Bulgaria, whereas the peripheries of the range of *T. graeca iberica* are near it (Rhodin et al., 2021).

Age structure

It seems that the lower percentage of juveniles in our study is not surprising. Many other studies on local populations of *T. hermanni boettgeri* also found that the number of juveniles was much smaller than the number of adults (Hailey et al., 1984; Ivanchev, 2007; Malakova et al., 2018; Meek, 1985; Meek & Inskoop, 1980; Popgeorgiev, 2008; Stojadinović et al., 2017b; Zhivkov et al., 2007). The same appears to be true also for the western subspecies – *T. hermanni hermanni* (Corti & Zuffi, 2003; Loy et al., 2007; Mazzotti, 2004).

Some authors (Ivanchev, 2007; Zhivkov et al., 2007) proposed that the small number of juveniles in the population they studied indicated a deteriorated condition. This suggestion can be valid when an anthropogenic factor has been identified as the main reason for the poor representatives of juveniles (Stubbs & Swingland, 1985).

Several factors can contribute to the lower number of juveniles in most local populations when no apparent reason has been found. Firstly, due to the smaller sizes of juveniles, it is difficult for observers to locate

them (Meek, 1985; Meek & Inskoop, 1980; Stubbs et al., 1985). Secondly, to remain hidden from potential predators, juveniles stay close to their nesting sites for several years after hatching (Stubbs & Swingland, 1985). Thirdly, the duration of their daily activity is less than that of adults (Hailey et al., 1984).

Fourthly, we hypothesize that the much longer duration of the adult stage of tortoises than their juvenile and subadult stage can be another important factor leading to skewed age structure in most populations. Finally, when tortoises reach a subadult stage and their shell hardens, the number of their natural predators is smaller compared to their juvenile stage. Thus, the probability of finding juveniles should be greatly reduced. Consequently, the different ratio registered between the age groups of most populations probably does not reflect their real structure. A lower percentage of juveniles in most populations can thus be expected and this should not always be interpreted as a sign of declining population.

Similar to the Hermann's Tortoise, the age structure of the Spur-thighed Tortoise in our study was characterized by the predominance of adults over subadults and juveniles. This does not appear to be unusual since other studies on populations of *T. graeca iberica* have indicated analogous results (Buică et al., 2013; Malakova et al., 2018; Moraru et al., 2018; Popgeorgiev, 2008). A similar ratio between different age groups was also observed in other subspecies of *T. graeca* (Kaddour et al., 2006; Rouag et al., 2007). The reasons for the smaller number of juveniles are probably the same (Andreu et al., 2000; Keller et al., 1997; Lambert, 1981) as in the Hermann's Tortoise. Thus, the conclusions made for the smaller number of juveniles in *T. hermanni* populations must be relevant for *T. graeca* populations as well.

Sex ratio

Our results that the sex ratio in *T. hermanni boettgeri* is normally in favor of males confirm those of many other authors (Hailey, 1988, 1990; Hailey & Willemsen, 2000; Ivanchev, 2007; Malakova et al., 2018; Meek 1985; Popgeorgiev, 2008; Stojanov et al., 2011;

Vujović et al., 2015; Zhivkov et al., 2007). However, according to other publications, this ratio can also be in favor of females (Duro et al., 2021; Popgeorgiev, 2008) or at parity (Hailey, 1988; Meek & Inskeep, 1980). In the western subspecies – *T. hermanni hermanni* – the sex ratio has also varied among populations (Budo et al., 2003; Corti & Zuffi, 2003; Loy et al., 2007; Kicaj et al., 2016; Mazzotti, 2004; Mazzotti et al., 2002). However, it is more often female- than male-biased (Budo et al., 2003; Kicaj et al., 2016; Mazzotti, 2004; Mazzotti et al., 2002).

These differences can be attributed to at least a few factors. For example, males and females tend to have different patterns of activity during the year (Ivanchev, 2007; Willemsen & Hailey, 2000). This can lead to sampling bias if the search effort is focused mainly on the period when one of the two sexes is more active (Willemsen & Hailey, 2000). The search effort in our study was comparatively equal during the periods of the highest activity of the two sexes. Thus, a sampling bias should not be among the reasons determining the difference in the sex ratio.

The sex determination in *T. hermanni* is temperature-dependent (Eendebak, 1995), which can cause an unbalanced sex ratio. However, this factor probably does not play a key role in determining uneven sex ratios among *T. hermanni* populations (Hailey & Willemsen, 2000). Different mortality rates of males and females can also explain the uneven sex ratio in some local populations (Corti & Zuffi, 2003; Hailey, 1990; Hailey & Willemsen, 2000). Hailey & Willemsen (2000) commented that the higher mortality rates of the females in the populations they studied were strongly associated with the unusually high density of the males. This reason can thus contribute to an uneven sex ratio mostly in populations in which the density of males is unnaturally high.

Consequently, the factor responsible for the slight male-biased sex ratio in our study must differ from those mentioned. It is probably related to the greater recruitment of the adult males than that of females. This is because males reach maturity about two years earlier than females (Hailey, 1990). In fact,

Hailey (1990) calculated that the recruitment of the adult males in his study was 48% higher than that of adult females. This can cause a sex ratio up to approximately 1.5:1 in favor of males (Hailey, 1990; Hailey & Willemsen, 2000), especially when no other possible reasons have been identified.

Our results showed that the sex ratio in the Spur-thighed Tortoise was female-biased. This is in contrast with the results of many other studies on *T. graeca iberica*, which found that males tend to be more numerous than females (Arslan et al., 2021; Hailey, 1988; Malakova et al., 2018; Moraru et al., 2018; Popgeorgiev, 2008; Stojanov et al., 2011; Türkozan et al., 2023). Additionally, a male-biased sex ratio was also recorded in other subspecies of *T. graeca* (Kaddour et al., 2006; Rouag et al., 2007). This reinforces the impression that males generally predominate in most populations.

In our study, a sampling bias, environmental sex determination, and different mortality rates do not seem to be among the reasons for determining the female-biased sex ratio (see the explanations about *T. hermanni*). The greater recruitment of males can suggest a male-biased sex ratio as in *T. hermanni*. Yet, females were found to be more abundant than males. Other factors must be the reason for the greater number of females. In addition, our experience with *T. graeca iberica* over the years has indicated that the external sexual features of males and females may sometimes be similar and the two sexes can be wrongly identified. This can skew the sex ratio to some extent.

Home range

To our knowledge, so far, no results have been published on the home range of *T. hermanni boettgeri* in Bulgaria. The mean home range of the subspecies as well as of the males and females in our study was smaller than in other populations of *T. hermanni boettgeri* (Hailey, 1989; Rozyłowicz & Popescu, 2012; Stojadinović et al., 2017a; Türkozan et al., 2018). This was also true when compared to the western subspecies *T. hermanni hermanni* (Longepierre et al., 2001; Loy & Cianfrani, 2009; Luiselli et al., 2009; Mazzotti et al., 2002;

Swingland et al., 1986). This discrepancy can be a result of the different methods used.

Nevertheless, the small average home range of *T. hermanni boettgeri* in our study can have a reasonable explanation. The habitat is composed of grasslands, forests, forest edges, shrubs, dirt roads, and creeks. These habitat components provide different conditions and access to various resources. For example, grasslands are utilized for basking, feeding, and sheltering (Rozyłowicz & Popescu, 2012; Stojadinović et al., 2017b). Shrubs and forest edges are used for thermoregulation and hibernation (Rozyłowicz & Popescu, 2012). Forests and dense shrubs are preferred in summer when ambient temperature is high (Nikolić et al., 2020).

Consequently, the habitat heterogeneity within our study area probably reduces the need for long-distance movements in search of suitable conditions and resources throughout the year. In theory, this should explain the small home range. On the other hand, some resources (e.g. food) can be scarce in homogeneous habitats, thus triggering individuals to move longer distances to find them. This can result in unusually large home range sizes of the species (Mazzotti et al., 2002).

Our results are in agreement with many studies that indicated larger home range areas in females than in males (Loy & Cianfrani, 2009; Mazzotti et al., 2002; Stojadinović et al., 2017a; Swingland et al., 1986). However, other studies did not show significant differences between the home range of the two sexes (Hailey, 1989; Longepierre et al., 2001; Rozyłowicz & Popescu, 2012; Türkozan et al., 2018). Further, males occasionally have a larger home range than females (Luiselli et al., 2009).

Diverse habitat conditions are expected to lead to similar home range sizes in males and females as they have equal access to vital resources (Rozyłowicz & Popescu, 2012; Türkozan et al., 2018). Yet, in our study, females had a larger home range compared to males, although the habitat was complex and thus provided abundant resources. This suggests that other factors should be responsible for the observed difference. One area of future work will be dedicated to identifying possible reasons for this difference.

In our study, the home range of the two sexes in *T. graeca iberica* is not comparable due to the small sample size. Nonetheless, when their home range is combined, it can be compared to the home range of other populations. As far as we know, until now, no data on the home range of *T. graeca iberica* have been reported in the literature. On a species level, our results (mean home range 1.25 ± 1.23 ha) are similar to those of other authors (Bernheim et al., 2019; Giménez et al., 2004). Bernheim et al. (2019) calculated a mean home range of 1.489 ± 0.29 ha for *T. graeca* in Israel, and Giménez et al. (2004) estimated a mean home range of 2.56 ha for males and 1.15 ha for females in Spain. However, a larger mean home range area of 6.3 ± 1.3 ha was calculated for *T. graeca* in Jordan (Attum et al., 2011).

Our results indicated that *T. graeca iberica* had a larger average home range than *T. hermanni boettgeri*. The conditions of the habitat and the availability of the resources within it were the same for both species. This is because they coexist in the study area. Thus, the cause for the difference in the sizes of their home ranges should be sought elsewhere. Some differences in the biology of the two species are possibly the reason for this. It is assumed that *T. graeca iberica* is more mobile than *T. hermanni boettgeri*, which seems to exhibit a more sedentary lifestyle (Biserkov et al., 2007; authors' observations). This can be suggested as one of the factors determining the larger home range of *T. graeca iberica*.

Real and potential threats

The threats identified can be considered the same for the two species because they occur together in the study area and have similar biology and ecology. The partial deforestation observed leads to a reduction of the shaded areas, which are crucial for thermoregulation (Lambert, 1981; Meek, 1984; Meek & Jayes, 1982; Rozyłowicz & Popescu, 2012). Because of this, deforestation can have a serious negative effect on tortoises, especially during summer when ambient temperatures reach high levels. Forests are also a preferable place for hibernating (Rozyłowicz & Popescu, 2012) and sheltering during summer (Nikolić et al., 2020).

The illegal collection of tortoises was one of the biggest threats to *T. hermanni boettgeri* and *T. graeca iberica* in Bulgaria during the second half of the 20th century (Beshkov, 1984, 1993). Some of the residents told us that few people had visited the area to collect a tortoise, which they intended to transport to another part of the country. But this case can be seen as an exception. Presently, the collection of individuals does not appear to happen often at least in south-western Bulgaria. This is supported by the fact that we never witnessed such illegal practices in the area. Furthermore, conversations with several residents indicated that local people do not collect tortoises nowadays, although they regularly did it three to four decades ago.

Eggs and juveniles can be vulnerable to predation by dogs and cats (Cheylan, 2001; Hailey et al., 1988; Petrov et al., 2004; Stojanov et al., 2011). Their presence is related to a herd of cows and flocks of sheep and goats, which often graze within the study area. Nonetheless, we did not observe a case of predation by dogs and cats on eggs or juveniles. According to Petrov et al. (2004), herds of cows are a potential threat to juveniles as they can be trampled by the cows. However, such a situation was not observed during our fieldwork.

The possible construction of a highway, part of which can fragment the habitat of the tortoises, would cause serious negative effects on them (Iosif et al., 2013; Vujović et al., 2015). In fact, mortality rates between 5 and 30% can be expected to occur on roads with high intensity of traffic (Iosif et al., 2013). This prediction should be true as our ongoing research on the effect of the traffic on the main road E79 on *T. hermanni boettgeri* and *T. graeca iberica* in Kresna Gorge has indicated mortality rates of 7.59% (n = 395) and 15.48% (n = 168), respectively (authors' unpublished data).

Conclusions

Testudo hermanni boettgeri and *T. graeca iberica* had similar ecology in the study area. Data on the home range of both species have been reported for the first time for Bulgaria. The two species appeared to successfully co-exist even in the presence of threats as no decline was registered in their number during

the study period. Our findings can be applied in different situations. For example, when a new action plan for conserving the two species needs to be prepared and implemented. One area of future research will be to study and analyze the morphometric characteristics of the two species. Another future work will be to provide data on the herpetofauna in the area. We recommend that studies of populations of *T. hermanni boettgeri* and *T. graeca iberica* be given priority. This is important due to the overall lack of information on some aspects of their ecology. Further, the new data obtained can help conservationists to more accurately assess the current conservation status of both species.

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Received: 02.11.2023

Accepted: 05.12.2023