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Biological and Ecological Characteristics of *Testudo hermanni* Gmelin, 1789 and *T. graeca* Linnaeus, 1758 (Testudines: Testudinidae) in the Northwestern Foothills of the Pirin Mountains, Bulgaria

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Abstract: A long-term study (2012–2021) on the populations of two species of tortoises (*Testudo hermanni* and *T. graeca*) in the vicinity of the village of Rakitna, the northwestern foothills of Pirin Mountains, was conducted. The study aimed at determining some species traits and population characteristics in connection with the long-term survival of the two species in an area of particular conservation interest. The specimens have been captured, marked, released and recaptured. The analysis and comparison of the morphometric data showed that *T. graeca* is bigger and heavier than *T. hermanni*. This was also true for females of both species, which surpassed males in size and weight. It was found that the specimens have diverse feeding habits. The total number of specimens marked was 97, with a clear prevalence of *T. hermanni* (80 individuals, 82.47%). The ratio between the numbers of specimens of both species (*c.* 5:1) has been reported previously in other studies. The age structure of the populations of both species was relatively identical and was characterised by a predominance of adult specimens over juveniles and subadults. In both species, the sex ratio in adults was female-biased.

Key words: morphometry, diet, movement, population ecology, age distribution, sex ratio

Introduction

The genus *Testudo* Linnaeus, 1758 consist of five species (*T. hermanni*, *T. graeca*, *T. marginata*, *T. kleinmanni* and *T. horsfieldii*) (Rhodin et al. 2017, Uetz et al. 2021). Their general distribution includes parts of Europe, Africa and Asia (Rhodin et al. 2017). In Bulgaria, two of the species occur, represented by the subspecies *T. hermanni boettgeri* (Mojsisovics, 1889) and *T. graeca ibera* (Pallas, 1814) (Stojanov et al. 2011). They inhabit most parts of the country and are found together almost everywhere (Buresch & Zonkow 1933, Beshkov & Nanev 2002, Petrov 2007). The vertical distribution of the Hermann's tortoise in Bulgaria reaches 1400–1450 m a. s. l. (Buresch & Zonkow 1933, Beshkov & Nanev 2002) and, of the Spur-thighed tortoise, about 1300 m a. s. l. (Beshkov 1961, Beshkov & Nanev 2002).

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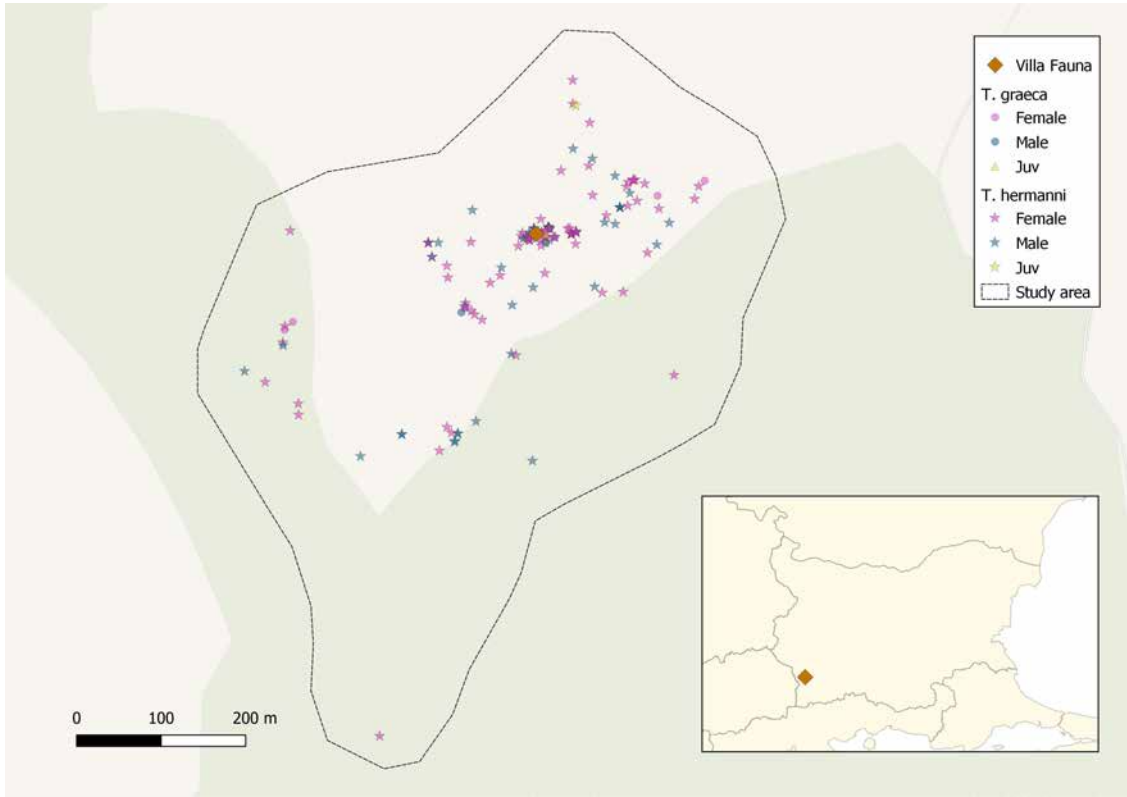

Fig. 1. Study area and location of the specimens captured during the study period.

In recent decades, there has been a sharp decrease in the number and density of most of the species populations throughout their range. This is due to a combination of reasons, the most important being fragmentation, degradation and destruction of their habitats, illegal collection for consumption and pet trade, wildfires, etc. (Beshkov 1960, 1984, 1993, Lambert, 1984, Stubbs et al. 1985, Petrov et al. 2004, Popgeorgiev 2008, Bertolero et al. 2011, Graciá et al., 2020). In this regard, a number of measures have been taken concerning their conservation and protection. Both species are included in the Red Data Book of the Republic of Bulgaria under the category Endangered. They are listed in Annexes II and III of the Biodiversity Protection Act of Bulgaria, Annexes II and IV of the Council Directive 92/43/EEC, Appendix II of the Bern Convention, Appendix II of CITES (Beshkov 2015a, b), the IUCN Red List of Threatened Species under the categories Vulnerable (for *T. graeca*) (Tortoise & Freshwater Turtle Specialist Group 1996) and Near Threatened (for *T. hermanni*) (van Dijk et al. 2004). According to Graciá et al. (2020), however, the status of the populations of both species needs to be reassessed. This can lead to a shift into categories with a higher level of threat.

Historically, most of the studies on tortoises in Bulgaria have been primarily focused on filling the knowledge gaps about their distribution (Kowatscheff 1912, Buresch & Zonkow 1933, Stojanov et al. 2011, Beshkov 2015a, b). More detailed information on some local populations can be found in the works of Lazarkevich-Stancheva (1997), Beshkov (1984), Ivanchev (2007), Zhivkov et al. (2007, 2009), Malakova et al. (2018). Data on morphometric characteristics are found in the publications of Corti & Zuffi (2003), Turkozan et al. (2003), Carretero et al. (2005), Ljubisavljević et al. (2012), Kicaj et al. (2016), Duro et al. (2021). In general, there is still a need to supplement data on the local populations and their status.

The study aimed at determining some species traits (morphometric features, diet and movement) and some population characteristics in connection with the long-term survival of the two species in an area of particular conservation interest.

Materials and Methods

The study was conducted in the period 2012–2021 during work on projects related to the reintroduction and conservation of griffon vulture (*Gyps fulvus*) in the area. An intentional search for tortoises to capture, mark, register their sizes and weight was carried out on certain dates from 2012 to 2015. During this period, the tortoises were located by random walk-

Table 1. Morphometric data of *Testudo graeca*. SCL, MPL, SH, MCW in mm, and BM in g. SD: standard deviation. For other abbreviations, see Materials and Methods.

Females						Males					
	n	**Min.**	**Max.**	**Mean**	**SD**		**n**	**Min.**	**Max.**	**Mean**	**SD**
SCL	11	142	230	200.54	34.21	**SCL**	4	162	200	180	18.38
MPL	11	130	209	176.31	30.21	**MPL**	4	135	158	146.25	10.59
SH	10	72	116	103.5	16.49	**SH**	3	84	115	94.33	17.89
MCW	11	114	187	159.22	27.98	**MCW**	3	133	161	143	15.62
BM	3	735	2400	1719	872.87	**BM**	2	860	1930	1395	756.60
Juveniles						**Overall**					
	n	**Min.**	**Max.**	**Mean**	**SD**		**n**	**Min.**	**Max.**	**Mean**	**SD**
SCL	2	46	50	48	2.82	**SCL**	17	46	230	177.76	57.08
MPL	2	43	45	44	1.41	**MPL**	17	43	209	153.67	49.61
SH	2	25	27	26	1.41	**SH**	15	25	116	91.33	30.63
MCW	2	39	41	40	1.41	**MCW**	16	39	187	141.28	46.46
BM						**BM**	5	735	2400	1589.4	745.36

Table 2. Morphometric data of *Testudo hermanni*. SCL, MPL, SH, MCW in mm, and BM in g. SD: standard deviation. For other abbreviations, see Materials and Methods.

Females						Males					
	n	**Min.**	**Max.**	**Mean**	**SD**		**n**	**Min.**	**Max.**	**Mean**	**SD**
SCL	39	133	220	181.82	15.24	**SCL**	32	118.5	176	153.39	11.96
MPL	40	118	179	151.9	11.03	**MPL**	32	92	132	114.17	7.56
SH	35	74	110	92.24	7.43	**SH**	29	58	87	77.65	6.21
MCW	40	109	163	140.45	9.55	**MCW**	32	88	153	129.85	13.19
BM	13	1007	1885	1316.69	281.97	**BM**	12	420	950	766.25	156.61
Juveniles						**Overall**					
	n	**Min.**	**Max.**	**Mean**	**SD**		**n**	**Min.**	**Max.**	**Mean**	**SD**
SCL	8	39	92	57.93	21.77	**SCL**	79	39	220	157.75	39.10
MPL	8	35	79	50.8	18.15	**MPL**	80	35	179	126.69	32.86
SH	7	19	48	31.32	12.26	**SH**	71	19	110	80.27	19.21
MCW	8	30.5	77	48.37	18.17	**MCW**	80	30.5	163	127.00	29.38
BM	1	140	140	140		**BM**	26	140	1885	1017.38	395.73

ing almost exclusively in the area around the Nature Conservation Center Villa Fauna (41.846841, 23.174714; Fig. 1) of Fund for Wild Flora and Fauna. From 2016 to 2021, a purposeful search for tortoises was not implemented. Specimens were found accidentally during activities related to the conservation of griffon vulture.

Initially, the tortoises were marked with green oil paint. Roman numerals were painted on the carapace of the adults and subadults. Dots were painted on the carapace of the juvenile specimens. However, when the marked individuals were recaptured, it was found that the paint mark was illegible. For this reason, a new method for marking was chosen. Aluminium plates with a unique identification number were used. First, a small hole was drilled at the edge of the tenth or eleventh marginal scute and then a plate was attached with a rivet. After a certain number of specimens were marked in this way, we decided to apply a more practical method, analogous to the previous one but the plates were glued with epoxy resin.

The following measurements were recorded: straight carapace length (SCL), midline plastron length (MPL), maximal carapace width (MCW), shell height (SH), and body mass (BM). The measurements were taken with a calliper and an electronic scale. Individuals smaller than 10 cm carapace

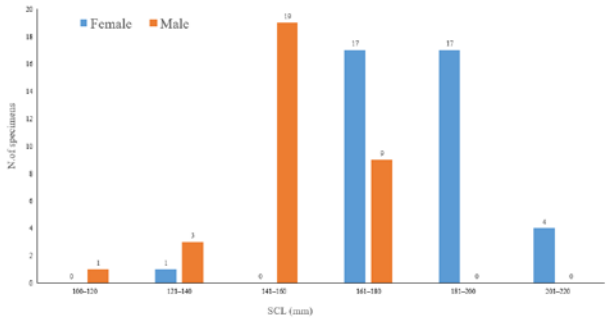

Fig. 2. Frequency distribution of straight carapace length of female and male *Testudo hermanni*.

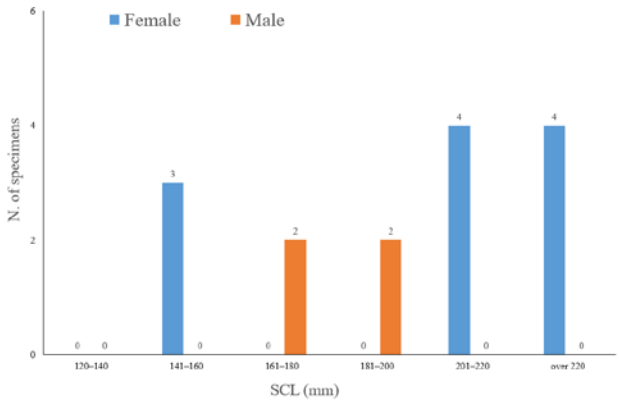

Fig. 3. Frequency distribution of straight carapace length of female and male *Testudo graeca*.

length for *T. hermanni* and 12 cm for *T. graeca* were considered juveniles. Individuals up to 13 cm for males and up to 15 cm for females of *T. hermanni* were considered to be subadults. For *T. graeca* as subadults were treated, males up to 14cm and females up to 16cm. In both species, individuals with carapace length larger than those mentioned were treated as adults (Hailey et al. 1988, Hailey 1990). 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). After measurements were recorded, all the animals were released at the place of capture.

Results

The analysis and comparison of the morphometric data showed that *T. graeca* is bigger than *T. hermanni*. The mean carapace length was 177.76 ± 57.08 mm (n = 17) (Table 1) and 157.75 ± 39.10 mm (n = 79) (Table 2), respectively. Therefore, *T. graeca* was also the heavier species with an average weight of 1589.4 ± 745.36 g (n = 5) (Table 1), while for *T. hermanni* it was 1017.38 ± 395.73 g (n = 26) (Table 2).

Females of both species were bigger and heavier than males. For *T. graeca*, the mean carapace length of females was 200.54 ± 34.21 mm (n = 11) and that of males 180 ± 18.38 mm (n = 4). The mean weight was 1719 ± 872.87 g (n = 3) and 1395 ± 756.60 g (n = 2), respectively. The largest and heaviest specimen was a female with a body length of 230 mm and a weight of approximately 2400 g (Table 1). For *T. hermanni*, the mean carapace length of females was 181.82 ± 15.24 mm (n = 39) and that of males 153.39 ± 11.96 mm (n = 32). The mean weight was 1316.69 ± 281.97 g (n = 13) and 766.25 ± 156.61 g (n = 12), respectively. The largest and heaviest specimen found was a female with a body length of 220 mm and a weight of about 1885 g (Table 2).

The peak frequency distribution of carapace length of *T. hermanni* ranged between 161–180 mm (43.58%) and 181–200 mm (43.58%) for females and 141–160 mm (59.37%) for males (Fig. 2). The peak frequency distribution of carapace length of *T. graeca* ranged between 201–220 mm (36.36%) and over 220 mm (36.36%) for females and 161–180 mm (50%) and 181–200 mm (50%) for males (Fig. 3).

The analysis of the data related to the movement model showed that between the first and the last capture the female Hermann's tortoises travelled a longer average distance (259.03 ± 251.33m, n = 21) than the males (193.94 ± 193.12m, n = 15). The longest distance travelled was about 946.48m, and the shortest one was approximately 5.56 m. The information about distances travelled by juveniles is very scarce. The only distance recorded was 23 m. For the Spur-thighed Tortoise, information was available for only one juvenile and one female individual. The first one was found only 2.23 m away from the place of the first registration and the second specimen had realized a movement of 325 m.

During the study period, we found that in addition to herbaceous plants, which are the major component of the food spectrum of both species, they often consume fallen fruits such as mulberries and sometimes figs. Furthermore, we have noticed that certain individuals tend to feed on dog faeces.

The total number of specimens marked was 97. Eighty of them (82.47%) belong to the species *T. hermanni*. Therefore, the ratio between the individuals of both species was approximately 5:1 in favour of *T. hermanni*. More than the half (53.75%) of the marked specimens of *T. hermanni* and about one-third (29.41%) of the individuals of *T. graeca* were recaptured at least once. The total number of subsequent captures of *T. hermanni* was 100 and of *T. graeca* only 7.

The age structure of the populations of both species was relatively identical. The adults prevailed over juveniles and subadults. The total number of adult specimens of *T. hermanni* was 69 (the number of juveniles was eight and of subadults was three), and of *T. graeca* was 12 (the number of juveniles was two and of subadults was three).

In both species, the sex ratio in adults was female-biased. It was 0.8:1 for *T. hermanni* and 0.36:1 for *T. graeca*.

Discussion

In both species, the differences in weight and size found between the sexes (Tables 1 and 2) are not surprising, as many other studies have shown that females are bigger and heavier than males (Lazarkevich-Stancheva 1997, Willemsen & Hailey 1999, 2003, Corti & Zuffi 2003, Carretero et al. 2005, Ivanchev 2007, Zhivkov et al. 2007, Ljubisavljević et al. 2012, Turkozan et al. 2019). These differences are probably related to sexual selection to increase courtship success but also to natural selection for fecundity (Willemsen & Hailey 2003). In addition, the same authors have noted that males spend much more time courting females than battling each other. This indicates that size is not paramount in the context of access to more females and that the choice of breeding partner is likely to be made by the latter (Willemsen & Hailey 1999). Furthermore, Ivanchev (2007) found that when male *T. hermanni* compete for resources and access to females, the size is not the most important attribute, ensuring dominance over the opponent but rather the determination.

It is worth noting that the largest and heaviest specimens of both species have been found in Bulgaria. The first one was probably a female Hermann's Tortoise with a shell length of about 357 mm and the second one was a male Spur-thighed Tortoise with a shell length of about 389 mm and weight of approximately 6000 g (Beshkov 1997). At present, however, tortoises of similar sizes are extremely rare in the country. The main reason for this is rooted in the mass collection of specimens for various purposes, such as consumption, the use of different parts including blood to "cure" diverse diseases, etc. In this practice, collectors focus on larger specimens, as they are easier to find and bring more benefits than smaller ones (Beshkov 1984). Conversations with residents of the village of Rakitna confirmed that in the second half of the 20th century, people regularly collected tortoises in the area. However, this illegal practice does not seem to be taking place in the area anymore, because when the locals talked about it, they associated it with the past rather than the present. Moreover, we never witnessed such activity during the study period. According to the same author (Beshkov 1984), it is still possible to find very large individuals near the upper limit of their vertical distribution. These parts are not attractive to collectors because of their inaccessibility and the lower population density of the tortoises.

The results related to the distances travelled by the specimens could hardly be compared with those obtained in other studies. This is because we have not registered the daily distances travelled but only those between the place of the first and last capture of the individuals (Table 3). Nevertheless, the data indicate well enough that there are differences between the sexes and the age groups. The longer average distances travelled by females may be due to the active search for suitable nesting sites as well as the intensification of the feeding process after the breeding and nesting period, to restore energy reserves (Stubbs & Swingland 1985, Díaz-Paniagua et al. 1995). However, if the food sources in the habitat are abundant and in close proximity, the need for continual searching for food is likely to decrease (Rouag et al. 2017). Even though males were generally less active, some of them travelled relatively long distances. This is probably related to the breeding season when they look for females to copulate with (Díaz-Paniagua et al. 1995). Although the information about juvenile specimens is scarce, it is consistent with other studies recording that their mobility is greatly reduced (Keller et al. 1997), especially in the first few years after hatching (Stubbs & Swingland 1985). This behaviour is probably related to minimizing the possibility of predation. However, when they reach an approximate size of 80 mm and their shell hardens enough, the juveniles begin to exhibit exploratory behaviour (Stubbs & Swingland 1985). This inevitably leads to a significant change in their mobility, which directly affects the distances they travel.

Our observations on the feeding habits of both species are consistent with those of other authors. The predominance of the plant component in the diet of both species is a well-known fact (Cobo & Andreu 1988, Lazarkevich-Stancheva 1997, Beshkov & Nanev 2002, Iftime & Iftime 2012). If possible, individuals supplement their food spectrum with fallen fruits and excrement of other animals (Beshkov & Nanev 2002, Stojanov et al. 2011). In addition, sometimes tortoises prey on small invertebrates (Beshkov & Nanev 2002, Petrov et al. 2004,

Gagno et al. 2012) and can feed even on carrion (Stojanov et al. 2011, Iftime & Iftime 2012). Geophagy also seems to play an important role in the life of tortoises (Gagno & Alotto 2010, Iftime & Iftime 2012). Based on the above, it can be concluded that in the availability of different food sources in the habitat of the species, the specimens are able to exploit them efficiently.

The greater number of recaptures of the Hermann's tortoises compared to the Spur-thighed tortoises can be explained by the higher population density of the first species within the study area. The relatively high percentage of recaptures in *T. hermanni* during the study period to some extent indicates that its population is in good condition. This is also supported by the fact that the number of dead individuals found was too low. The percentage of subsequent recaptures of *T. graeca* was not high. Nevertheless, almost a third of the individuals were recaptured at least once. This and the lack of dead individuals gives us a reason to believe, that the conclusions drawn for the other species are relevant for this one as well.

The ratio between the specimens showed a strong predominance of *T. hermanni* over *T. graeca*. This is not unusual, because the same or close to this ratio has already been found in some populations in the south-western part of the country (Pulev & Sakelarieva 2011, Popgeorgiev et al. 2016, Malakova et al. 2018, Manolev et al. 2019). Moreover, studies on local populations from other parts of the country have also found similar results. For example, in eastern Bulgaria (Eminska Mountain), Ivanchev (2007) registered 57 specimens of *T. hermanni* and only one specimen of *Testudo graeca*. However, it should be noted that there are some populations in which the opposite trend was observed. Undjian (2000) has found that *T. graeca* is more abundant than *T. hermanni* in the central-northern and north-eastern parts of Bulgaria. Furthermore, some of these areas are inhabited only by *T. graeca* (Beshkov & Nanev 2002).

A similar ratio between the different age groups was found in other populations in the country (Lazarkevich-Stancheva 1997, Ivanchev 2007, Zhivkov et al. 2007, Popgeorgiev 2008, Malakova et al. 2018) but also in other parts of the species ranges (Stubbs & Swingland 1985, Corti & Zuffi 2003, Kaddour et al. 2006, Loy et al. 2007, Rouag et al. 2007, Buică 2011, Buică et al. 2013, Stojadinović et al. 2017). During the first few years after hatching, the juveniles stick close to their nesting sites, where they can remain hidden and safe (Stubbs & Swingland 1985) and the period of their daily activity is more limited than in adults (Lambert 1981, Hailey et al. 1984). Moreover, their small size makes them quieter and harder to see than adults. Therefore, it can be concluded that the probability of detecting juvenile specimens is significantly lower (Henry et al. 1998, Andreu et al. 2000). These facts indicate that in many cases the different ratio between the age groups does not reflect the real picture. Hence, the lower percentage of juveniles does not necessarily mean that a certain local population is in decline.

Although studies on other populations of both species in Bulgaria have found that the sex ratio is in favours of males (Lazarkevich-Stancheva 1997, Ivanchev 2007, Zhivkov et al. 2007, Stojanov et al. 2011, Malakova et al. 2018), this is not constant and the opposite trend can be observed in other populations, as shown by our results. Studies carried out outside of Bulgaria have also demonstrated that the ratio between the sexes was in favour of females (Budó et al. 2003, Kicaj et al. 2016, Duro et al. 2021). Sometimes the sex ratio can be in parity or close to it (Meek & Inskeep 1980, Hailey, 1988, Mazzotti et al. 2002, Mazzotti 2004, Loy et al. 2007). It is a dynamic feature that could be a result of differential activity patterns of females and males during the year (Díaz-Paniagua et al. 1995, Hailey & Willemsen 2000), mortality (Hailey 1990, Hailey & Willemsen 2000, Corti & Zuffi 2003), and recruitment (Hailey, 1990, Hailey & Willemsen 2000). Moreover, Hailey et al. (1988) discovered that male *T. hermanni* tend to inhabit more open places than females, which makes them easier to detect.

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