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Local and Invasive Species of Freshwater Turtles (Reptilia: Emydidae, Geoemydidae) in the Eastern Part of Strandzha Nature Park (Bulgaria) - Distribution and Populations Assessment

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Abstract. The current study presents data on the distribution, abundance, density, sex and age ratio of the populations of two freshwater turtle species - *Emys orbicularis* (Linnaeus, 1758) and *Mauremys rivulata* (Valenciennes,1833) in selected wetlands in the eastern part of "Strandzha" Nature Park. During the research period were recorded a total of 142 ind. of *E. orbicularis* and 65 ind. of *M. rivulata*. Two single localities of the invasive species *Trachemys scripta elegans* (Wied-Neuwied, 1839) have been documented as well. Morphometric characteristics and body condition index of the captured individuals is also presented. Unmanned aerial vehicles (drones) were used for video recording of some sections of the studied transects, testing their possible usage for monitoring purposes. The current conservation status and threats for the studied species are also discussed.

Key words: *Emys orbicularis, Mauremys rivulata, Trachemys scripta elegans*, UAVs monitoring, "Strandzha" Nature Park, South-East Bulgaria.

Introduction

There are two species of freshwater turtles native to Bulgaria - the European Pond Turtle *Emys orbicularis* (Linnaeus, 1758) and the Balkan Pond Turtle *Mauremys rivulata* (Valenciennes, 1833), but the invasive species *Trachemys scripta* is also present with three subspecies already established in the country: *T. s. elegans* (Wied-Neuwied, 1839), *T. s. scripta* (Schoepff, 1792) and *T. s. troostii* (Holbrook, 1836) (Biserkov et al., 2007; Rhodin et al., 2017). The European Pond Turtle in Bulgaria is widespread throughout the country, missing only in the highlands (Biserkov et al., 2007) - up to 1221 m a.s.l. (Kornilev et al., 2017). The habitats that *E. orbicularis* uses are: swamps, rivers, limans, micro-dams, fishponds, irrigation and drainage canals, thermal springs, standing and slow-flowing reservoirs (Biserkov et al., 2007; Kornilev et al., 2017). *M. rivulata* is found only in the southernmost, warmest and lowest habitats in Bulgaria, representing the northernmost limit of distribution of the species (Beshkov, 1987; 1993; Biserkov et al., 2007; Petrov, 2007).

The maximum altitude at which it is registered is 474 m. (Kornilev et al., 2017). The distribution of the two native species of freshwater turtles partially overlaps only in the southern parts of the country. The Eastern part of the "Strandzha" Nature Park is one of the few places in Bulgaria where the two native species can be observed together.

There are relatively few studies on the ecology of freshwater turtles in the country. The data from this study will help to prepare an assessment of the state for their populations in the eastern part of "Strandzha" Nature Park and possibly to predict their future development.

Material and Methods

Study area

The study was conducted in the period June - August 2021 on the territory of "Strandzha" Nature Park, which overlaps with NATURA2000 protected area - "1007 Strandzha", with code BG0001007. The mouths of the rivers Silistar and Veleka were studied, as well as several smaller rivers flowing into the Black Sea - north and south of the town of Ahtopol. Also, the studied areas fall within the boundaries of several protected areas: Protected Area "Estuary of the Veleka River", Protected Area "Silistar" and Nature monument "Nakovo Kladenche". These are important habitats for local species of freshwater turtles, included in the National System for Monitoring the State of Biological Diversity (Tsankov et al., 2016).

Field surveys

The turtles were identified visually using binoculars "MINOX BF 10x25 BR" (MINOX GmbH Walter Zapp.). The field guides by Bannikov (1977) and Biserkov et al. (2007) were used for determine the native species and the CITES Identification Guide - Turtles & Tortoises (Charette, 1999) and the Global Invasive Species Database (ISSG, 2021) for determine the invasive species.

In the present work, the density and the abundance of freshwater turtle populations were studied by the linear transect method (Sutherland, 2000), following the appropriate habitat types (river banks, nearby small standing water basins). The transects we used are given in Fig. 1: Transect №1 - from the river mouth to the bridge over Veleka River; Transect №2 (upstream from the bridge of Veleka River - covered by boat; Transect №3 (Silistar River); Transect №4 (NM "Nakovo kladenche") and Transect №5 (Ahtopol Town).

The transects for Veleka and Silistar Rivers have a fixed width of 50 m (25 m on each side of the medial line of observation), and for the smaller rivers near the town of Ahtopol they have a fixed width of 10 m (5 m on each side of the medial line of observation). The density was calculated by the following formula:

$$D_e = \frac{n}{2rl} \times 10000,$$

where:

D_e - population density (number of individuals per ha);

n - number of observed individuals;

r - transect width (in meters);

l - transect length (in meters).

To compare the data on the numbers of the individuals of the populations of the two native species, we used the abundance parameter (Ab). Abundance is defined as the total number of individuals of a given species found in a given territory (Turpie, 1995; Sutherland, 2000). Due to the difference in the widths of the transects, the following formula was used for better comparison and analysis of the data from the present thesis:

$$Ab = \frac{n}{L}.1000,$$

where:

Ab - abundance (number of individuals per 1000 linear meters - l.m.);

n - number of observed individuals;

L - studied area in linear meters.

In some cases, the turtles are caught by hand or with the help of a fishing net, for more accurate determination; taking some morphometric parameters (SCL - straight carapace length, MPL - maximum plastron length, H - height of the shell and MCW - maximum carapace width (measured at the widest part) (following Mazzotti, 1995 and Alcayde, 2007), measured in millimeters (mm), with a caliper (with an accuracy of 0.1 mm - Fig. 2, as well as body weight (BW) in grams (g), measured with a digital scale "WeiHeng" with an accuracy of 10 g); determination of sex (where possible, following characteristics, indicated by Biserkov et al. (2007) and Zuffi & Gariboldi (1995) and age of the individuals, based on carapace length (individuals with carapace length of less than 110 mm were considered juveniles (according to Ayaz & Çiçek (2011); between 110 and 140 mm - subadults (according to Kotenko, 2000) and individuals over 140 mm were considered adults.

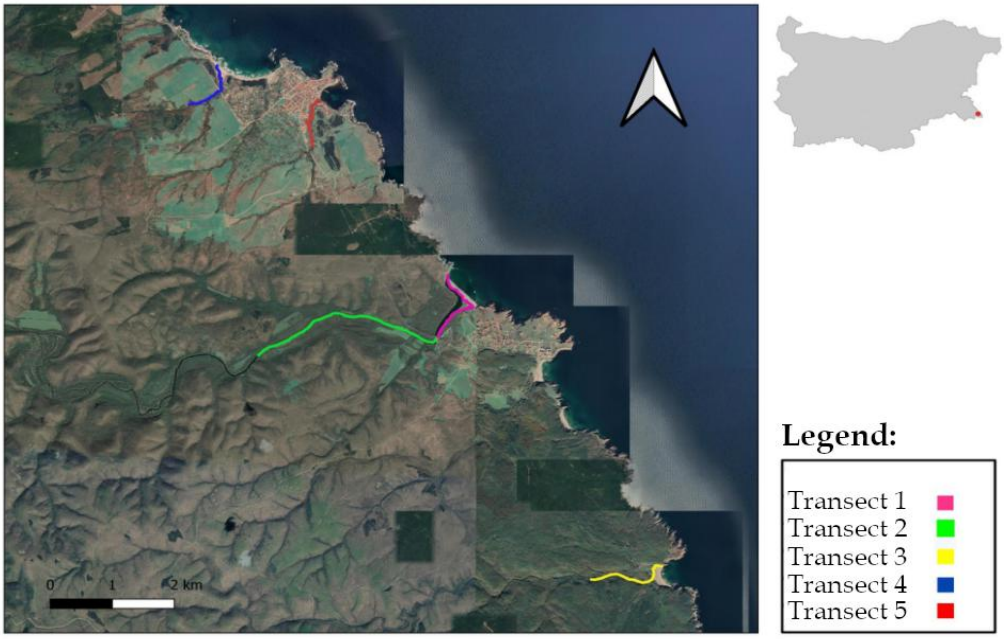

Fig. 1. Locations of the used transects for the field studies (explanations are in the text).

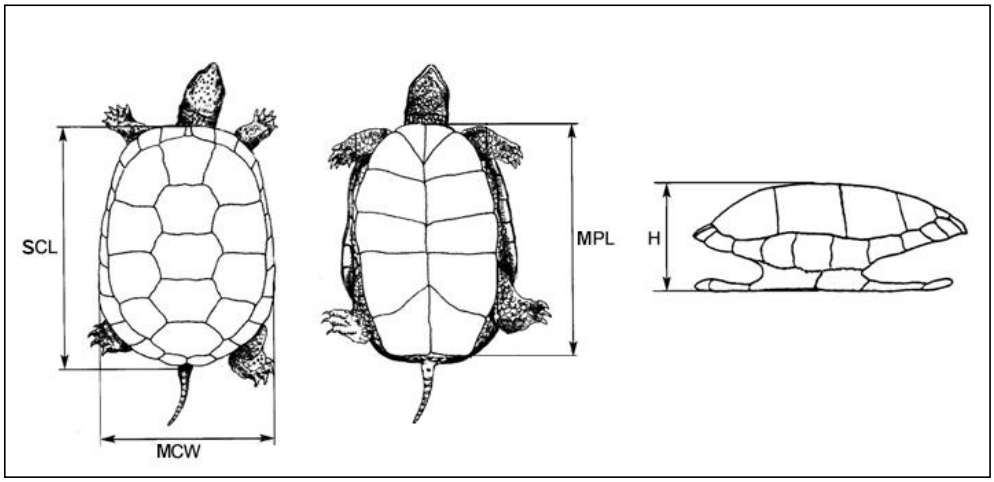

Fig. 2. Measured morphometric parameters (abbreviations according to Zuffi & Gariboldi (1995), with changes): SCL - straight carapace length, MCW - maximum carapace width, MPL - maximum plastron length, H - height of the shell (after Alcayde, 2007).

The body condition index (BCI) is used to assess the health status of individuals, which is widely used in conservation ecology, as it provides indirect data on the quality of habitat (Stevenson & Woods, 2006). The body condition index (BCI) used in the present work is defined in the sense of Willemsen & Hailey (2002), by the formula:

$$BCI=\log_{10} BW/BW',$$

where: BCI – body condition index;
BW – the actual weight of the individual;
BW' – the expected weight of the individual as a function of size.

The expected weight of the individuals is obtained according to the modified ellipsoid volume formula (after Loehr et al., 2004 and Alcayde, 2007):

$$BW' = \frac{\pi \times SCL \times H \times MCW}{6000}.$$

A BCI value = 0 indicates that the observed weight is equal to the expected weight; values above "0" indicate good health of the individual due to good environmental conditions, while negative values indicate poor physical condition as a result of poor nutrition or some kind of stress (Stevenson & Woods, 2006; Alcayde, 2007).

The team conducting the field research has an up-to-date permit issued by the MOEW (№ 870 / 29.04.2021) pursuant to Ordinance № 8 (Promulgated State Gazette No. 4 / 16.01.2004) on the terms and conditions for issuing permits for exemptions from the prohibitions introduced by the Law on the Biological Diversity of Animal and Plant Species from Annexes № 2, 3 and 4.

QGIS 3.16.6-Hannover (QGIS.org, 2021) and Garmin BaseCamp (Garmin Ltd., 1996-2021) were used for geographical visualization of the collected data. The statistical package PAST v. 4.0 was used for the statistical processing of the data and their graphic presentation (Hammer et al., 2001). The data were analyzed by descriptive statistics and presented graphically by Box & Whiskers plots. A Shapiro-Wilk test was used for testing the normal distribution of the data (Shapiro & Wilk, 1965). When comparing or looking for differences between individual variables, nonparametric tests (χ^2-test, Mann-Whitney U-test for independent pairs) were applied, in the absence of a normal distribution of data (Fowler et al. 1998). Differences with $p<0.05$ [α=5%] were considered statistically significant.

Results

Spatial distribution

The distribution of the three registered species of freshwater turtles (*E. orbicularis, M. rivulata and T. s. elegans*) in the study area is presented in Fig. 3, 4 and 5.

Fig. 3. Distribution of the European Pond Turtle (*Emys orbicularis*) in the eastern part of NP "Strandzha".

Fig. 4. Distribution of the Balkan Pond Turtle (*Mauremys rivulata*) in the eastern part of NP "Strandzha".

Fig. 5. Distribution of the Red-eared slider (*Trachemys scripta elegans*) in the eastern part of NP "Strandzha".

In total, in all studied areas, for the period of the study, we recorded 142 ind. *E. orbicularis,* 65 ind. *M. rivulata* and 2 ind. *T. s. elegans.* For Veleka River - a total of 89 ind. *E. orbicularis* and 16 ind. *M. rivulata,* indicating that *E. orbicularis* is the predominant species. The results of the study at the mouth of the Silistar River show a sympatric distribution of the two local species, with a total of 45 ind. *E. orbicularis* and 44 ind. *M. rivulata.* From the invasive species *T. s. elegans,* we recorded 2 ind. along the Veleka River (upstream above the bridge and in a small spillway near the river mouth).

Fig. 6 and 7 show the spatial distribution of *E. orbicularis* and *M. rivulata* along the rivers Veleka and Silistar - the two rivers with the most registered individuals of the target species. The distance from each recorded point of an observed individual or group of individuals of each species to the river mouth is measured by the actual river kilometers, and the graphs show the mean values and the standard deviation.

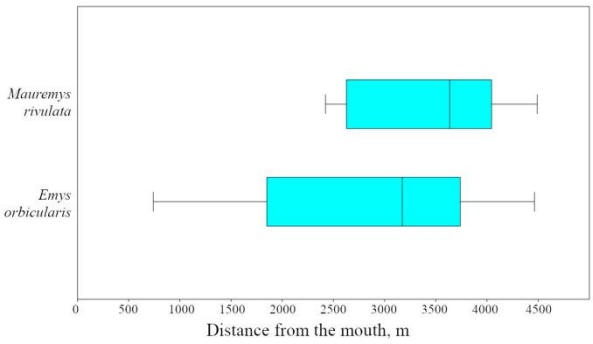

Fig. 6. Box & whiskers plots of the spatial distribution of the two native freshwater turtle species at Veleka River.

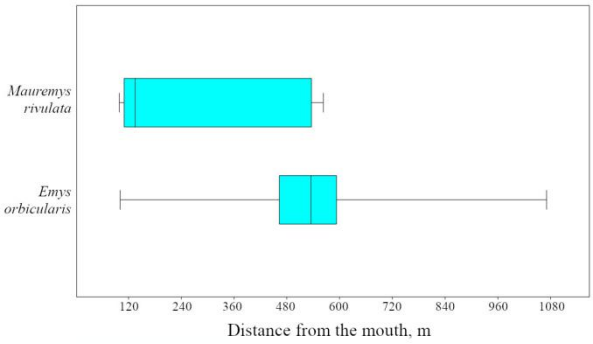

Fig. 7. Box & whiskers plots of the spatial distribution of the two native freshwater turtle species at Silistar River.

In the area of the Veleka River *E. orbicularis* is distributed mainly in the lower to middle parts of the studied area, while *M. rivulata* is observed mostly in the middle to the upper parts (Fig. 6). Although we did not find a statistically significant difference in the calculated distances from the estuary between the two species (Man-Whitney test: U=166.00, z=-1.44, p=0.15), the minimum distances are quite different (741 m for *E. orbicularis* and 2422 m for *M. rivulata*), while the maximums are similar (4463 m vs. 4491 m). Most likely, this result is due to the strong degree of disturbance by people from the estuary to about 2000 m upstream.

Similar to our results on the spatial distribution of the two species of native aquatic turtles at Veleka River from the bridge to 4.5 km upstream were reported by Popgeorgiev et al. (2017), who survey the river by boat using a slightly different methodology. However, the results they obtained also showed the prevalence of *M. rivulata* in the middle and upper parts of the study area, while *E. orbicularis* was found mainly in the lower and middle parts.

Along the Silistar River, the opposite trend was observed in the spatial distribution of the two species, as *M. rivulata* was recorded mainly closer to the river mouth, where there is a strong degree of disturbance by humans (Fig. 7). We did not record a statistically significant difference in the measured distances from the estuary to the individuals (Man-Whitney test: U=37.00, z=-1.48, p=0.14), although there is a visible difference in maximum values (1071 m for *E. orbicularis* and 563 m for *M. rivulata*), at very close minimum values. During our field work we observed a large accumulation of freshwater turtles near the bridge at the beach, at the river mouth of Silistar. The reason for this was the tourists in the area who threw food (bread) into the river, which may be the probable reason for the observed trend in the spatial distribution there.

Abundance and density of the populations

Data on the numbers, abundance and density of the studied five transects are presented in Table 1.

Table. 1. Numbers, abundance and density of the recorded species of turtles in Transect №1 (from the mouth of the river to the bridge over Veleka River). *Legend:* N - numbers, Ab - abundance (ind./1000 l.m.), D - density (ind./ha), ± - standard deviation, * - individuals registered with a drone.

Species	June			July			August			Total		
	N	Ab	D	N	Ab	D	N	Ab	D	N	Ab	D
Emys orbicularis	19	11.88	1.19	1	0.63	0.06	1	0.63	0.06	21	13.13±6.50	1.31±0.65
*Emys orbicularis**	-	-	-	0	0.00	0.00	1	0.63	0.06	1	0.63	0.06
Trachemys scripta	1	0.63	0.06	0	0.00	0.00	0	0.00	0.00	1	0.63	0.06

Table 2. Numbers, abundance and density of the recorded species of turtles in Transect №2 (Veleka River). *Legend:* N - numbers, Ab - abundance (ind./1000 l.m.), D - density (ind./ha), ± - standard deviation.

Species	June			July			August			Total		
	N	Ab	D	N	Ab	D	N	Ab	D	N	Ab	D
Emys orbicularis	30	9.09	0.91	22	6.67	0.67	16	4.85	0.48	68	20.61±2.13	2.06±0.21
Mauremys rivulata	1	0.30	0.03	1	0.30	0.30	14	4.24	0.42	16	4.85±2.27	0.48±0.23
Trachemys scripta	0	0.00	0.00	1	0.30	0.30	0	0.00	0.00	1	0.30	0.03

Table 3. Numbers, abundance and density of the recorded species of turtles in Transect №3 (Silistar River). *Legend:* N - numbers, Ab - abundance (ind./1000 l.m.), D - density (ind./ha), ± - standard deviation, * - individuals registered with a drone.

Species	June			July			August			Total		
	N	Ab	D	N	Ab	D	N	Ab	D	N	Ab	D
Emys orbicularis	2	1.43	0.06	11	7.86	0.33	32	22.86	0.97	45	32.14±11.00	1.36±0.47
*Emys orbicularis**	-	-	-	3	2.14	0.09	5	3.57	0.15	8	5.71±1.01	0.24±0.04
Mauremys rivulata	0	0.00	0.00	12	8.57	0.36	32	22.86	0.97	44	31.43±11.55	1.33±0.49
*Mauremys rivulata**	-	-	-	0	0.00	0.00	1	0.71	0.03	1	0.71	0.03

Table 4. Numbers, abundance and density of the recorded species of turtles in Transect №4 (NM "Nakovo kladenche"). *Legend:* N - numbers, Ab - abundance (ind./1000 l.m.), D - density (ind./ha), ± - standard deviation, * - individuals registered with a drone.

Species	June			July			August			Total		
	N	Ab	D	N	Ab	D	N	Ab	D	N	Ab	D
Emys orbicularis	0	0.00	0.00	1	0.91	0.45	4	3.64	1.82	5	4.55±1.89	2.27±0.95
*Emys orbicularis**	-	-	-	1	0.91	0.45	0	0.00	0.00	1	0.91	0.45
Mauremys rivulata	0	0.00	0.00	0	0.00	0.00	5	4.55	2.27	5	4.55±2.62	2.27±1.31

Table 5. Numbers, abundance and density of the recorded species of turtles in Transect №5 (Ahtopol Town). *Legend:* N - numbers, Ab - abundance (ind./1000 l.m.), D - density (ind./ha), ± - standard deviation, * - individuals registered with a drone.

Species	June			July			August			Total		
	N	Ab	D	N	Ab	D	N	Ab	D	N	Ab	D
Emys orbicularis	2	1.43	0.71	0	0.00	0.00	1	0.71	0.36	3	2.14±0.71	1.07±0.36
*Emys orbicularis**	-	-	-	-	-	-	1	0.71	0.36	1	0.71	0.36

According to Beshkov (1987), along the lower reaches of the Veleka River (up to 4 km from the mouth) "the numbers of *M. rivulata* is at least a few dozen individuals". In our study at Veleka River a total of 89 ind. *E. orbicularis* and 16 ind. *M. rivulata.* According to the same author "along the river flowing into the sea on the northern beach near Ahtopol" are registered 3 ind. *M. rivulata* and 10 ind. *E. orbicularis,* later Beshkov (1993) reported that the populations of both species from this locality were

completely destroyed. Unfortunately, we also did not find any aquatic turtles at this locality during our study.

In a study by Popgeorgiev (2008) on the negative effects of fires in the Eastern Rhodopes and Sakar Mts., the author reports the following values for abundance (Ab) of the populations of *E. orbicularis* from several studied areas during the period 2004-2006. A total of 64 individuals have been identified near the village of Rogozinovo in the control sample (K) (Ab = 2.94 ind./1000 l.m.), near the village of Kolets, a total of 12 ind. (Ab = 0.11 ind./1000 l.m.) in the control sample (K), near the village of Ostar Kamak, a total of 64 ind. (Ab = 0.60 ind./1000 l.m.) in control sample (K). In the area of the village of Gorno Lukovo 1 individual of the species *M. rivulata* (Ab = 0.06 ind./1000 l.m.) was recorded in the control sample (K) and in a sample from the burned area (P), and near the village of Gorno Bryastovo *E. orbicularis* was recorded only in the dam in the burned area (P), with 7 ind. (Ab = 0.39 ind./1000 l.m.).

Mollov (2019) calculates the abundance of the *E. orbicularis* population along Maritsa River, in the city of Plovdiv (0.692 ind./1000 l.m.) and the abundance of the population of the same species from an irrigation canal in the northern part of the city (0.454 ind./1000 l.m).

Our data on the abundance of populations of the two native species of freshwater turtles from the eastern part of "Strandzha" Natural Park greatly exceed those reported from the Eastern Rhodopes and Sakar by Popgeorgiev (2008) and those from the Plovdiv City (Mollov, 2019), which is an expected result, given the status of the protected area. This is an indication of the high abundance and density of the populations of *E. orbicularis* and *M. rivulata* in "Strandzha" Nature Park.

Various authors estimate of the density of *E. orbicularis* populations from some countries in Europe: in Bardello, Italy - 7.2 ind./ha (Mazzotti, 1995); for Lake Yayla, Turkey - 81 ind./ha. (Ayaz et al., 2008); from the area to Pazaragac (Turkey) - 83 ind./ha. Ayaz et al. (2007a); in Lake Sulyuklu (Manisa, Turkey) - 83 ind./ha. (Ayaz & Çiçek, 2011a); Tisza River in Southern Hungary - 142-228 ind./ha. (Balázs & Györffy, 2006), but all are based on the Capture-Recapture method and do not allow comparison with our data. Güçlü & Türkozan (2010) report a population density of *M. rivulata* - 434 ind./ha in Izmir province, western Turkey, using the same method.

The values established by us for the density of the populations of *E. orbicularis* in the studied area, using the transect method vary from 1.07 ind./ha to 2.27 ind./ha., and for *M. rivulata* - from 0.48 ind./ha to 2.27 ind./ha.

In the present study, an attempt was made to use unmanned aerial vehicles (UAV, drones) to travel the distance along the constructed transects and photograph and video record the turtles, in order to determine and count them in laboratory conditions. This is the second study in Bulgaria that uses UAVs in the study of freshwater turtles, after the pilot study of Biserkov & Lukanov (2017) in Sofia City. Our experience has shown that the use of UAVs for monitoring freshwater turtles is possible only over water basins with less vegetation, which allows unimpeded flight over the river or reservoir. In some cases during the field work this was not possible due to the dense and low tree vegetation along the river banks - e.g. Silistar River (Transect №3). This transect was explored by drone only for the first 500 m from the river mouth, as the passage of the drone upwards was impossible due to the low branches of the trees and the loss of connection with the remote control. This is the reason for the significantly smaller number of registered turtles with a drone than those recorded when walking the line transect. It was also impossible to study Transect №2 with UAVs, due to the curves that the river makes in this section and the loss of connection with the

drone. For this reason, and the densely overgrown shores, this transect was completely traversed only by boat.

Despite the indisputable advantages that UAVs gives in the monitoring of freshwater turtles, they do not provide 100% imaging of the entire transect and registration of all turtles (Fig. 8 and 9).

Improvements in the technology of UAVs (drones) and in particular the cameras (higher resolution) will allow the capture of smaller species of herpetofauna, mainly terrestrial representatives (lizards, frogs, turtles, etc.). The study by Huerta et al. (2020) assesses whether UAV technology can be used as a method for passive study of herpetofauna species, in addition to traditional research methods. It is possible that this method will be the main tool for future detection and monitoring of some species of the herpetofauna.

The UAVs equipment used in our study is a Phantom 4 Pro drone equipped with a 2.5 cm, 20-megapixel sensor capable of shooting 4K / 60 fps (ie 4000 horizontal pixel resolution at 60 fps), video with a flight time of 30 minutes (DJI Technology Inc. Shenzhen, China). Large (> 20 cm) and brightly colored objects can be recognized by video and photos taken up to 10 m altitude by the drone. Small objects (<10 cm) are indistinguishable up to about 5 m altitude. Although the UAVs recognition method may not be a significant improvement in determining the presence or relative abundance of herpetofauna in the study areas, it has the advantage of providing the researcher with a video of the present species, which can be used later.

Morphometric characteristics

The morphometric parameters measured by us for *E. orbicularis* and *M. rivulata* from the Veleka and Silistar rivers are presented in Table. 6 and 7. Only *E. orbicularis* individuals were caught from the Veleka River during the study.

The values of the main morphometric characteristics for both species of freshwater turtles presented by us fall within the ranges reported by other authors for these species from other European countries (Mazzotti, 1995; Ayaz & Çiçek, 2011a; Ayaz & Budak, 2008). However, due to the small number of individuals caught by us, we cannot make comparisons between the sexes and the two populations.

Fig. 8. *E. orbicularis,* captured with "Phantom 4 Pro" drone from 5 m altitude at Silistar River.

Fig. 9. *E. orbicularis,* captured with "MavicPro" drone from 5 m altitude at Silistar River.

The body condition index (BCI), calculated by us for *E. orbicularis* and *M. rivulata* from the two studied locations has positive values, although close to zero (Tables 6 and 7), which means that the weight of the studied aquatic turtles is close to expected one and the quality of the habitats is "good".

Sex and age ratio of the populations

The sex ratio of the populations of *Emys orbicularis* was studied at Veleka River (Transect №1 and 2) and Silistar River (Transect №3), and that of *Mauremys rivulata* only at Silistar River, due to the low number of captured individuals at Veleka River.

The population of *E. orbicularis* at Veleka River, showed a male-female ratio of 1:0.82 (n=20), and we did not record a statistically significant difference from the normal distribution - 1:1 (χ^2=0.10025, df=1, p=0.75). We recorded a similar sex ratio at Silistar River - 1:0.7 (n=17), again without a statistically significant difference from the normal expected distribution - 1:1 (χ^2=0.26, df=1, p=0.61).

Table 6. Measured morphometric parameters in *E. orbicularis* from the mouth of the Veleka River. Mean values ± standard deviation (SD) are presented. The abbreviations are indicated in the chapter "Material and Methods".

Parameter	Males (n=2)	Females (n=2)	Juveniles (n=1)
Body weight (BW), g	572.50±123.73	495±28.28	60
SCL, mm	146.50±9.19	132.5±1.41	65
MPL, mm	140.50±0.19	127.5±3.53	60
H, mm	62.5±9.19	55.5±0.71	28.1
MCW, mm	110.50±2.69	108.00±16.97	56
SCL/MCW	1.32±0.049	1.24±0.18	1.16
SCL/BW	0.26±0.042	0.27±0.014	1.08
BCI	0.0052±0.0007	0.0065±0.00099	0.03

Table 7. Measured morphometric parameters in *E. orbicularis* and *M. rivulata* from Silistar River. Mean values ± standard deviation (SD) are presented. The abbreviations are indicated in the chapter "Material and Methods".

Parameter	*Emys orbicularis*			*Mauremys rivulata*		
	Males (n=7)	**Females (n=3)**	**Juveniles (n=7)**	**Males (n=3)**	**Females (n=5)**	**Juveniles (n=8)**
Body weight (BW), g	382.43±86.47	346.67±102.75	131.25±17.02	515.00±265.56	494.00±137.13	134.37±36.20
SCL, mm	134.03±6.68	128.67±12.66	91.05±6.49	163.37±41.30	149.50±12.76	103.76±9.55
MPL, mm	119.10±9.03	118.33±15.57	86.27±6.15	133.90±26.42	135.50±11.14	85.47±8.57
H, mm	52.48±6.47	39.90±10.65	34.95±1.63	52.53±26.42	60.10±7.15	34.20±3.37
MCW, mm	103.34±5.22	101.00±8.89	74.17±5.38	114.83±21.03	109.10±7.60	76.09±6.58
SCL/MCW	1.30±0.030	1.27±0.023	1.23±0.015	1.41±0.11	1.37±0.077	1.36±0.033
SCL/BW	0.36±0.059	0.39±0.086	0.70±0.056	0.36±0.12	0.31±0.061	0.81±0.15
BCI	0.0069±0.0012	0.0096±0.0026	0.017±0.0022	0.0059±0.0031	0.005±0.001	0.015±0.0033

The sex ratio of the population of *M. rivulata* at Silistar River showed a male-female ratio of 1:1.25 (n=16), but again without a statistically significant difference from the expected normal distribution of 1:1 (χ^2=0.12, df=1, p=0.72).

According to Mazzotti (1995), the ratio between males and females ind. of *E. orbicularis* population in Bardello, Italy is 1:2. In a population studied by Balázs & Györffy (2006) in southern Hungary, the sex ratio of *E. orbicularis* was approximately 1:1, slightly in favor of females. According to Ayaz et al. (2007a) near Pazaragac (Turkey) the sex ratio in adult *E. orbicularis* individuals was significantly in favor of males (2.02♂:1♀; p<0.001). Ayaz et al. (2008) reported a ratio of 54% males, 42% females, and 4% juveniles for *E. orbicularis* population, and overall sex ratio of 1.31:1 (659♂:504♀, p<0.001). Güçlü & Türkozan (2010) studied a population of *M. rivulata* in Izmir province, western Turkey and reported a sex structure (females:males:juveniles) in favor of the females (3.01:1.17:1).

Rifai & Amr (2004) calculate the ratio between males and females for *M. rivulata* (1:1.3), as their data are similar to ours from Silistar River - 1:1,25, in favor for the females.

The age structure of the population of *E. orbicularis* at Veleka River has a visible, statistically significant (χ^2=31.671, df=2, p=0.0000001) predominance of adult individuals (n=53), followed by subadults (n=14) and the lowest share of juveniles (n=2). The population at Silistar River is again dominated by adults (n=23), as the share of subadults (n=13) and juveniles (n=10) is almost equal, and here we did not find a statistically significant difference from the normal expected distribution 1:1:1 (χ^2=2.81, df=2, p=0.24).

For *M. rivulata,* the age structure of the Veleka River population is with a slight dominance of adult individuals (n=9), followed by subadults (n=5) and juveniles (n=4) are almost equal (χ^2=1.09, df=2, p=0.58), while the population at Silistar River showed dominance of adults (n=24), followed by juveniles (n=12) and subadults (n=8), without a statistically significant difference from the normal expected distribution 1:1:1 (χ^2=4.38, df=2, p=0.11).

The low proportion of juveniles in three of the studied four populations of the two species of freshwater turtles does not necessarily mean that these populations are decreasing. It should be noted that the juveniles are the most difficult to register due to their small size and more discrete way of life. In the area of Silistar River, at

coordinates N42° 01.366', E28° 00.506' we found 3 hatchlings of a freshwater turtle, as the number of hatched eggs in each varied from 7 to 9. Unfortunately, based only on the shells of the eggs we can not determine the species, but this is a possible proof that the two species of native freshwater turtles are breeding successfully and in all probability their populations in the area of the Silistar and Veleka rivers are stable.

Similar results were reported in study by Mollov (2019), using the same methodology, the age structure of the *E. orbicularis* population from Maritsa River in Plovdiv City, showed a ratio between the three age groups (Ad:Sub:Juv) 1:0.75.:0.5, with the largest proportion of adults (χ^2=0.67; df=2; p=0.72). In a study by Vamberger et al. (2017) in the largest swamp in Slovenia, 20% of the *E. orbicularis* caught were subadult. In a one-year observation of a population of *E. orbicularis* in the "Los Aribes del Duero" Nature Park (Zamora, Spain), Alarcos et al. (2008) calculated the percentage of adult individuals - 87.2%, using a transect method to study the population, in combination with other methods.

Conservation significance and threats

Both species of native freshwater turtles are of high importance for the conservation of the biodiversity, according to the National and European environmental legislation.

During the present study, we identified the following threats for the freshwater turtles in the study area:

- Disturbance from tourists, especially during the active summer season (July-August). A stream of tourists, using boats, kayaks and other vessels, enter the Veleka River and disturb the turtles. Near the mouth of Silistar River there is a well-developed camping area near the river itself, which also disturbs our two native species.

- Aquatic turtles are caught accidentally on the hooks of fishermen, and according to Beshkov (1993), most of them are often killed afterwards. On the Silistar River we captured a *M. rivulata* individual with a hook stuck in its mouth, as well as observations of numerous cases of illegal fishing in the mouth of Veleka River.

- We recorded two adult individuals of *Trachemys scripta elegans* - one at the mouth of Veleka River and one a little further upstream. The uncontrolled release of *T. scripta* and its subspecies into various water bodies is leading to the potential spread of this highly invasive species.

Conclusions

1. In the study area, for the period of the study, we found both native species of freshwater turtles, in all major rivers. In most of the researched localities the species are found sympatrically and *E. orbicularis* is the predominant species.

2. In the area of Veleka River *E. orbicularis* is found mainly in the lower to middle parts of the studied area, and *M. rivulata* is observed mostly in the middle to the upper sections, while in the area of the Silistar River *M. rivulata* is found mainly in the lower sections of the river, near the mouth, and *E. orbicularis* - higher upstream in the study area.

3. The populations of both species are characterized by medium to high values of abundance and density (compared to other regions in the country).

4. The sex ratio of the populations of the two species shows a ratio close to 1:1, with a slight predominance of the males. The age structure shows the predominance of adults from both species, with almost equal ratio of subadult and juvenile individuals.

5. The body condition index (BCI) shows values above zero in all studied individuals of both species, which is an indirect indication of good health status of the turtles and the good condition of the surveyed habitats.

6. Both species of freshwater turtles are characterized by a high conservation status, and the threats we recorded in the study area are disturbance by the tourist flow (especially along Veleka and Silistar rivers),

involuntary capture of individuals by fishermen, as well as the presence of the invasive species of red-eared slider (*Trachemys scripta elegans*), in the area of Veleka River. We propose to ban the import and sale in pet stores of *T. s. trostii* (*T. s. elegans* and *T. s. scripta* are already banned), as well as raising public awareness of the problem, building appropriate centers for the invasive turtle species, collected from the wild, as a more ethical and environmentally safe alternative for dealing with unwanted pets and eliminating established populations by removing individuals from the wild.

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