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Effects of Fires and Roadkills on the Isolated Population of *Testudo hermanni* Gmelin, 1789 (Reptilia: Testudinidae) in Central Montenegro

Ana Vujović[1], Vuk Iković[2], Ana Golubović[3,4], Sonja Đorđević[3,4], Vladimir Pešić[5], Ljiljana Tomović[3,4,6]

[1] Natural History Association of Montenegro. 81410 Pažići, Danilovgrad, Montenegro; E-mail: hermanna85@gmail.com
[2] Montenegrin Ecologists' Society. Bulevar Sv. Petra Cetinjskog 73, 81110 Podgorica, Montenegro; E-mail: vukikovic@gmail.com
[3] University of Belgrade, Faculty of Biology, Institute of Zoology, Studentski trg 16, 11000 Belgrade, Serbia; E-mails: golubovic.ana@bio.bg.ac.rs, sonjadj@bio.bg.ac.rs, lili@bio.bg.ac.rs
[4] University of Belgrade, Institute for Biological Research "Siniša Stanković", Bulevar despota Stefana 142, 11000 Belgrade, Serbia
[5] University of Montenegro, Faculty of Natural Sciences and Mathematics, Department of Biology, Džordža Vašingtona bb, Podgorica, Montenegro; e-mail: vladopesic@gmail.com
[6] Serbian Herpetological Society "Milutin Radovanović", Bulevar despota Stefana 142, 11000 Belgrade, Serbia

Abstract: In general, fires and traffic impose serious threats to wildlife. Chelonians are also gravely affected by these two perils, both directly (many die in fires and on the roads) and indirectly (due to habitat fragmentation and degradation). In the Hermann's tortoise population subjected to capture-mark-recapture (CMR) study we analysed the impacts of fires and road kills. We found that the most vulnerable to both perils were the immatures and adult females, with most fatalities in summer. During the course of our research, along with the reconstruction and widening of a motorway, a rockslide-protective wire mesh was fixed along one section of the road under study. It reduced the number of road kills, but it also acted as a deadly trap for some of the tortoises trying to escape the fast-spreading fire. Here we presented the results of a short survey: in future more time and effort need to be devoted to the given population and its conservation needs.

Keywords: *Testudo hermanni boettgeri*, fires, road kills, Montenegro, Mediterranean habitats, conservation

Introduction

Fires are generally considered disastrous (Barnthouse *et al.* 2007). Worldwide, their frequency and intensity are high, even rising, often due to human influences (e.g. Esque *et al.* 2003, Moreira, Russo 2007, Pausas, Fernandez-Muñoz 2011). Fires pose huge direct and indirect impacts on vegetation and fauna, both short- and long-term, demographic as well as genetic (Cheylan, Poitevin 1998, Schrey *et al.* 2011). The intensity of these effects depends on the fire regime itself, vegetation cover composition and configuration of the terrain (Hailey 2000, Smith 2000, Boarman 2002, Baeza *et al.* 2011). Especially vulnerable to fires are "small, isolated or stressed" populations, and/or animals with limited locomotor/migratory abilities, such as tortoises (e.g. Kiss, Magnin 2006; Hossack, Pilliod 2011).

Roads and motorways are among the anthropogenic constructions with serious negative effects on wildlife. Direct mortality is the most obvious impact of traffic (Lodé 2000, Ashley *et al.* 2007; Litvaitis,

*Corresponding author: lili@bio.bg.ac.rs

Tash 2008, Jackson, Fahrig 2011, Iosif *et al.* 2013). In the long-term sense, roads often impose fragmentation, i.e. isolation of (sub)populations of many species (including reptiles), by cross-cutting their habitats (e.g. Boarman 2002, Boarman, Sazaki, 2006). This can lead to alterations in age structure, sex ratios and genetic structure of populations (Rodriguez *et al.* 1996, Marchand, Litvaitis 2004, Steen, Gibbs 2004), especially in species with low reproductive rates (Ruby *et al.* 1994, Kline, Swann 1998, Gibbs, Shriver 2002), small home ranges and limited dispersal abilities (e.g. tortoises – Boarman, Sazaki 1996). The severity of road impacts on ecosystems depends on the density of road network, frequency of traffic and structure of habitats along the roads (Vos, Chardon 1998, Lebboroni, Corti 2006, Iosif *et al.* 2013, Iković 2012).

To our knowledge, there are no studies of combined effects of fires and traffic on reptile populations in Europe, and especially not in the west-central Balkans, where the basic tortoise population studies are also scarce. Hence, the goals of this report were: a) to compare the effects of fires and road kills on a single, isolated population of *Testudo hermanni*, b) to consider conservation measures, and c) to compare body sizes of tortoises from continental regions with those from Mediterranean-influenced areas.

Material and Methods

Study species

Hermann's tortoise is still widely distributed, especially its eastern subspecies, *Testudo hermanni boettgeri*, in the Balkan Peninsula (e.g. Bertolero *et al*. 2011). Its adult body sizes are usually ≤20 cm (Vetter 2006, Bertolero *et al*. 2011), life span is long (over 60 years: Eendebak 2002), and home ranges entail 1.8 ha on average (Hailey 1989). Sex ratios are often male-biased, from 1.5:1 to 6:1 (Hailey, Willemsen 2000). The densities of its populations range from 3 to 77 (Hailey, Willemsen 2000; Rozylowicz, Dobre 2010). Average clutch size is 4.3; in general, larger females tend to produce larger clutches (Bertolero *et al*. 2007, 2011).

Study area

Our study site is located in central Montenegro, in the outskirts of Danilovgrad (42°32′47″ N, 19°05′50″E). The climate of this region is sub-Mediterranean, with warm summers: average annual temperature is 15°C, total annual precipitation 2,000 mm; altitude ranges from 35 m to 200 m a.s.l. (Burić 2000). The terrain is rocky and the vegetation cover consists mostly of scrub (or pseudomacchia) (Bešić 1978). The area selected for a CMR study and surveyed for the consequences of fire is a plot of app. 4.25 ha. In the northeast it is bordered by the motorway (surveyed for roadkills), the military camp is in the south, arable land in the southwest, and a settlement in the west (Fig. 1). The study area was patrolled by one to four researchers every two to three days during the tortoise activity season (August–October 2010, and June–October 2011; continued in 2012).

In most parts of Montenegro fires are quite regular, and are especially frequent between July and September. They are repeatedly lit by local inhabitants attempting to remove the shrub, and often spread beyond control (V. I. and A. V. pers. comm.). In the vicinity of the country capital (app. 20 km from Danilovgrad) as much as 6,467 fires "in the undergrowth and landfills" occurred between 2005 (792) and 2009 (1,174) (Anonymous 2010). According to the Hydrometeorological and Seismological Service of Montenegro, the entire 2011 was extremely dry and warm (average annual temperature in Podgorica was 17.5°C, total precipitation only 844 l/m^2). The Danilovgrad region is naturally predisposed to frequent fires: it is mostly covered with sparse, dry, low vegetation, which increases the risk of fires, especially during summer (Pavićević 2012). According to the city protection services, during 2011 Danilovgrad and its surroundings were struck by 435 fires (Anonymous 2011). The fire-fighting office in Danilovgrad reported as much as 50 fires in a single day (August 21: pers. comm. A. V.).

Capture-Mark-Recapture study

A capture-mark-recapture study in the given population of Hermann's tortoises was started in 2010. In the current study we included data collected during fieldtrips performed from August to October 2010, and from June to October 2011.

The animals were caught by hand, measured and marked in the field (modified from Stubbs *et al.* 1984), and released at exact places of capture. Where possible, their sex was determined on the basis of shell and tail shape (Willemsen, Hailey 2003). For assessing the sexual maturity status of these animals we used literature threshold values for straight carapace length (SCL): 130 mm for males and 150 mm for females (Willemsen, Hailey 1999); tortoises smaller than 130 mm SCL were considered immature. The tortoises in the Danilovgrad population are on average smaller than in other places we study this species; consequently, some individuals we re-

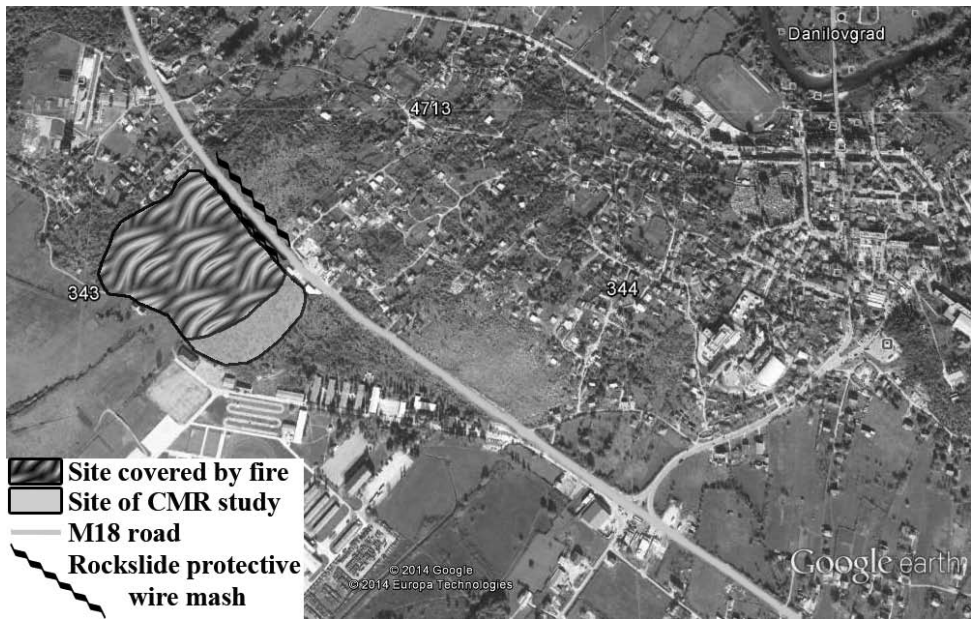

Fig. 1. Position of the surveyed locality near Danilovgrad, Montenegro, with precise location of the CMR study site and area covered by fire

garded as sub-adults may in fact be sexually mature. However, until we obtain precise data, in order to provide comparability of information from various localities, we used the same threshold values in all studied populations.

Road kills survey

The study on amphibian and reptilian road mortality near Danilovgrad was started in 2009 (Iković 2012). In search for casualties, a stretch of 2,000 m of the high-frequency M18 motorway (running along the CMR study site, Fig. 1) was patrolled on a regular basis. In 2011, a section of the road included in the study was reconstructed. The works involved road widening, and fitting of a rockslide-protective wire mesh over steep road sides, including 500 m of the surveyed road part (Fig. 1). We wanted to compare the incidence of road-kills in various seasons, as well as before and after the mesh was fixed.

Road surveys were performed throughout the year, once a week on average; surveillance periods and times of day were adjusted to environmental temperatures, levels of tortoise activity, and to the intensity of traffic (details in Iković 2012). The highest traffic frequency was recorded in summer (corresponding to the peak of tourist season): 13,500 vehicles per day, compared to 10,300 vehicles/day in other seasons (Iković 2012). All dead and injured animals were recorded and removed from the road, to prevent data duplication. Whenever possible, the sex and age of road-killed tortoises were recorded.

Impacts of fire

In August and September 2011, several huge fires occurred around Danilovgrad. One of the larger took place on September 2nd 2011 (A. V. pers. comm.). The exact area of our study was severely damaged (Fig. 1). After the fire, one of the researchers (A. V.) visited the study area every second day (20 visits in total), searching for tortoises and their remains. All new and recaptured individuals were processed, and the animals with injuries and burn marks were recorded. We assessed the presence and frequency of adults vs. immatures and males vs. females, before and after the fire, in order to check the influence of fire on population structure. The records were divided into three categories: animals alive before the fire, animals alive after the fire, and those that died in the fire. Into the "alive before the fire" category we included all living marked animals and the ones that were found dead after the fire (both marked and new). As "alive after the fire" we considered recaptured and newly encountered animals found in the study area after the fire.

For the analyses of differences in frequencies of road fatalities between two years of the study, we used χ^2 tests, with Yates' correction. The same tests were used for analyses of differences in frequencies of live animals before the fire and those that died in the fire. Descriptive statistics and nonparametric Mann-Whitney tests were used for comparison of body size between live and burned individuals. For all statistical analyses the Statistica 5.1 package was used.

Results

During two years of the CMR study in the vicinity of Danilovgrad we processed 200 Hermann's tortoises: 104 (52%) adult males, 74 (37%) adult females, and 22 (11%) immature animals. In this population tortoises are smaller than in several other populations we study in the Balkans (Table 3; A. V. unpublished). The density of tortoises observed in the study plot was 47 ind./ha, and adult sex ratio was slightly male-biased (1.4 : 1.0).

Road fatalities

Considering the entire road section under survey (2,000 m), most road kills occurred in summer (Fig. 2). For a relatively large number of adults the sex could not be determined due to bad condition of carcases. Among the tortoises killed by vehicles included in this analysis there we no marked individuals from the CMR study plot.

Comparative analysis of road fatalities along the 1,500 m of the road without the wire mesh in 2009 (N = 6: 4 adult females, 1 adult male, 1 indeterminable) and 2011 (N = 5: two immatures and three adult females) showed no differences in numbers of tortoises killed by vehicles (χ^2=0.04, df=1, P=0.842 – Table 1, Fig. 2).

In the spring of 2011, a wire mesh was set over steep road sides, including the 500 m of the surveyed road section, in order to prevent rockslides. After this intervention no dead tortoises were found in the given portion of the road – which is a serious improvement (χ^2=8.17, df=1, P=0.04) compared to 16 animals ran over by cars in the same road portion during 2009: 12 adult females, 3 adult males and 1 indeterminable individual.

Effect of fire

During the CMR study preceding the fire of September 2nd 2011 we marked 121 animals (64 adult males, 39 adult females and 18 immatures). After the fire, we recorded 79 live animals (44 adult males, 29 adult females and 6 immatures). A large proportion of surviving animals (34.18%, i.e. 27 of 79) had burn marks on their shells (13 adult males, 12 adult females and two immatures). We recorded 22 animals killed by the fire: six adult males (5.77% compared to marked males), seven adult females (9.46%) and nine immatures (40.11%) (Table 1).

Of all immatures found in the surveyed area (live before the fire, live after the fire, dead in the fire), the ones killed by the fire comprised 37.5%. The same calculation performed on adult females and males revealed 10.3% and 5.6%, respectively.

Sex ratio before the fire in this population was biased toward males – 1.6 : 1.0; but after the fire, the ratio of males decreased slightly (1.4 : 1.0).

We analysed the differences in frequencies (age groups and sexes) of two subsets of tortoises: 1) live animals before the fire, and 2) tortoises that died in the fire. We found no differences in gender ratios of adults alive before the fire and those killed by the fire (χ^2=1.51, df=1, P=0.219). However, we found more dead immatures after the fire than we had found alive before (χ^2=12.90, df=1, P=0.000). Importantly, three of the nine immature tortoises burned to death were found under the rockslide-protective mesh. For now, we could not establish clear connection between the

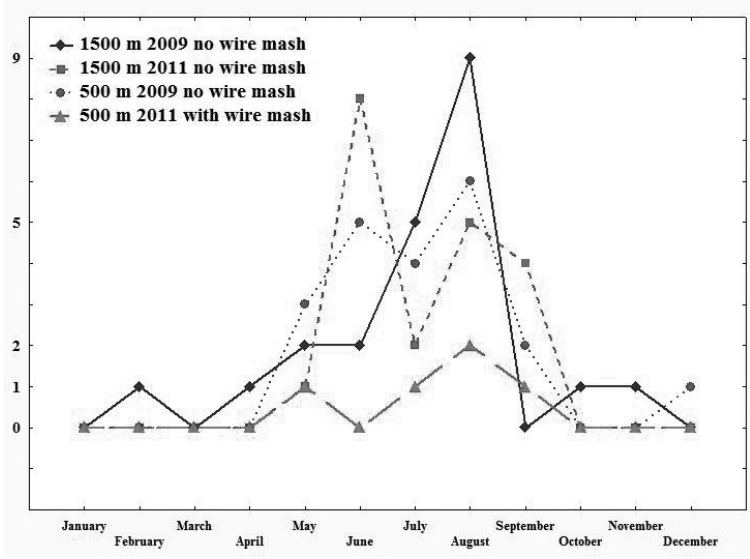

Fig. 2. Comparative number of road kills in 2009 and 2011 along the 500 m section of the road with and without the wire mesh and the 1,500 m section of the road without landslide protective mesh

Table 1. Numbers and percentages of age classes and sexes affected by the fire and roadkills in the studied population of *Testudo hermanni*

Age class / Sex	Live animals before the fire	Live animals after the fire	Died in the fire 2011	Roadkills 2009	Roadkills 2011
Immatures	18 (14.88%)	6 (7.59%)	9 (40.91%)	13 (61.90%)	2 (40.00%)
Adult males	64 (52.89%)	44 (55.70%)	6 (27.27%)	1 (4.76%)	0 (0%)
Adult females	39 (32.23%)	29 (36.71%)	7 (31.82%)	7 (33.33%)	3 (60.00%)
Total	121	79	22	21	5

probability of death in the fire and body size: Mann-Whitney tests (Table 2) showed no statistically significant differences between the sizes in three categories (immatures, adult males, and adult females).

Comparison of the frequencies of animals that died in the fire and those found among road kills showed that both perils had similar effects (χ^2=1.90, df=1, P=0.169) on age classes, i.e. immatures were the most vulnerable category concerning both impacts. Concerning the effects of these two impacts on sexes we found that no statistically significant differences between adult males and females were (χ^2=2.52, df=1, P=0.112: Table 1).

Discussion

Preliminary findings of the CMR study

Our short-term CMR records showed that density and structure of the Hermann's tortoise population near Danilovgrad correspond to previously published findings (Hailey, Willemsen 2000; Rozylowicz, Dobre 2010). However, maximum adult body sizes (SCL) in this population are smaller than in Balkan populations of *T. hermanni* sampled deeper in the continent (see Table 3).

Traffic fatalities

Our investigation of the impact of traffic on *T. hermanni* along the 2,000 m of a motorway showed that road kills occurred from February to December, with the peaks of casualties in June and August, probably due to coinciding increases in activity of tortoises and traffic intensity (Iković 2012). The same road-kill dynamics was found for various reptilian species in other parts of the world (Bernardino, Dalrymple 1992, Bonnet *et al.* 1999, Enge, Wood 2002, Smith, Dodd 2003).

Results of the current study show that immature individuals were the category most severely affected by traffic. One of the reasons for high road mortality of immatures could be their size: small animals need more time to cross the road, and they are less easily visible to the drivers, hence they are at higher risk to be run over by cars (Gibbs, Shriver 2002, Enge, Wood 2002, Smith, Dodd 2003). Immature tortoises often exhibit higher dispersion rates compared to adult animals, in search for territory or resources (Bonnet *et al.* 1999, Enge, Wood 2002, Aresco 2003, Smith, Dodd 2003). Also, in both years of our study, adult females were more susceptible to road kills compared to adult males (Table 1). Differences of daily movements between the sexes and/or larger home ranges in females could be the reason for such result (Hailey 1989, Mazzotti *et al.* 2002, Lagarde *et al.* 2003). In addition to food and shelter, female tortoises must find suitable nest locations. In a rough environment, additionally devastated by the motorway, the egg-laying period can become increasingly dangerous for females (e.g. Aresco 2003).

Concerning the impacts of roads on chelonians, numerous studies were dedicated to semi-aquatic species, mostly in the USA (Aresco 2003, MacKinnon *et al.* 2005, Beaudry *et al.* 2008, DeWoody *et al.* 2010, McCallum 2011). Data on terrestrial species are limited (Guyot, Clobert 1997, Boarman, Sazaki 1996, 2006). To our knowledge, the only scientific studies of road mortalities of terrestrial tortoises in Europe and the Mediterranean were conducted in France (*T. hermanni* – Guyot, Clobert 1997), Turkey (*T. graeca* – Tok *et al.* 2011), and Romania (*T. h. boettgeri* – Iosif *et al.* 2013). Therefore, we need to continue and intensify our traffic impacts study.

Influences of wire mesh

The effectiveness of various types of intentionally installed structures (e.g. fences, culverts, underpasses) in road mortality reduction was previously demonstrated for several reptile species (Boarman, Sazaki 1996, Guyot, Clobert 1997, Aresco 2003, Dodd *et al.* 2004). In our study, we had the opportunity to observe the incidental effect of road infrastructure. In contrast to preventive effect concerning exposure to traffic, horizontally laid wire mesh was shown to act as a trap for some animals trying to escape the fast-spreading fire. Namely, the adults are too large to go under or through the mesh, while the immatures of certain body sizes are small enough to go under the mesh, but too large to pass through it.

Trapped under the mesh, tortoises can suffer from dehydration, starvation, or fires. Given the shape and size of the cells, only juveniles are capable of passing through the mesh.

Effects of fire

Previous studies revealed that several large-scale fires can eliminate up to 85% of the tortoises in a single year (Cheylan 1984). Furthermore, it was found that the loss of 11% of adults from a population presents a catastrophe for a species with low reproductive output – which is characteristic for tortoises (Esque *et al.* 2003). Information on fire effects on herpetofauna, especially in Europe, is comparatively scarce (e.g. Stubbs *et al.* 1985, Cheylan, Poitevin 1998, Hailey 2000, Popgeorgiev, Kornilev 2009). To our knowledge, the effects of fires on *T. hermanni* were previously assessed in France (Cheylan 1984, Couturier *et al.* 2011), Spain (Fèlix 1999, Fèlix *et al.* 1989), Greece (Stubbs *et al.* 1985, Hailey 2000), and Bulgaria (Popgeorgiev 2008). In our study population, fire caused the greatest mortality (37.5%) among the immatures. This finding is similar to 55.7% and 44% mortality of juveniles in populations of *T. hermanni* in the Eastern Rhodopes in Bulgaria (Popgeorgiev 2008), and Alyki in Greece (Stubbs *et al.* 1985), respectively. Because immature tortoises, in general, are hard to be observed in the field (Diemer 1992, Keller *et al.* 1997, but see Fernández-Chacón *et al.* 2011), it is possible that the number of juveniles present at the study site and killed by the fire was much higher than actually recorded. Due to their small size and thin shell, juveniles can be burned completely (Popgeorgiev 2008), precluding the detection of their remains.

Among the burned corpses, we found similar numbers of males (6) and females (7). However, because the sex ratio in this population is slightly male-biased (1.4 : 1.0), per cent loss of individuals per gender was unequal. Higher mortality of females caused by the fire was also found in Bulgarian Eastern Rhodopes population (73.5% vs. 58.4%, in females and males, respectively – Popgeorgiev 2008). Imbalanced mortality of the two sexes could result from sex-related differences in overall activity: in *T. hermanni* and related species, males are more active and move faster than females (Mazzotti *et al.* 2002), but females travel longer distances (Longepierre *et al*. 2001; Shine, Wall 2005). Behaviours related to fast and successful reaction to suboptimal environmental conditions, such as fire, could be highly significant for individual survival (Lyon *et al.* 1978). Thus, more active individuals might be expected to easily escape the approaching disaster (Stubbs 1981).

Table 2. Descriptive statistics and ANOVA tests of straight carapace length (SCL) of live and burned (measurable) individuals

Age class / Sex		x	n	SD	F	P
Immatures	Live	115.1	18	22.10	1.7	0.205
	Dead	102.8	8	22.96		
Adult males	Live	146.2	70	7.75	1.2	0.283
	Dead	141.3	3	7.95		
Adult females	Live	170.2	38	7.95	1.7	0.204
	Dead	165.5	5	4.90		

Contributions of environmental circumstances to attainable body sizes

According to the results of the present study, our data collected in other populations in the Balkans,

Table 3. Mean values of straight carapace length (SCL) in adult males and females from different Mediterranean and Continental regions

Region	Locality	SCL males	SCL females	Source
Greece - Mediterranean	Alyki main heath	155.0	167.0	Willemsen, Haley 1999
Greece - Mediterranean	Kilkis	154.0	165.0	Willemsen, Haley 1999
Montenegro - Mediterranean	Danilovgrad	146.2	170.2	This study
Montenegro - Mediterranean	Limljani	157.1	169.2	Ljubisavljević et al. unpubl. data
Greece - Continental	Deskati	195.0	214.0	Willemsen, Haley 1999
Greece - Continental	Kastoria	177.0	197.0	Willemsen, Haley 1999
Romania - Continental	Parcul Natural Porţile De Fier	170.9	207.1	Rozylowicz, Pătroescu 2004
Macedonia - Continental	Konjsko village	171.4	195.2	Unpublished data
Serbia - Continental	Pčinja valley	179.2	202.7	Unpublished data
Serbia - Continental	Prolom banja	181.6	214.0	Unpublished data
Serbia - Continental	Trstenik	167.1	199.2	Djordjević et al. 2011

and available literature, we assume that there is a correlation between the average SCL in Hermann's tortoise populations and different climate and habitat characteristics (Mediterranean – macchia and pseudomacchia, vs. moderate-continental climate – thermophilous oak forests; undisturbed vs. disturbed habitats; frequently burnt vs. unburned habitats). Apparently, in Mediterranean parts of the distribution range of this species, adult tortoises attain smaller body sizes (SCL) compared to those studied deeper in the continent (Table 3). This is probably a result of insufficient resources (see e.g. Fritz *et al*. 2005) – a situation worsened by frequent fires. It is possible that in harsh environments certain life history traits are somewhat modified compared to favourable surroundings. It was shown that small tortoises are highly vulnerable to fires (Popgeorgiev 2008); on the other hand, it was suggested that juvenile tortoises in areas under frequent fires grow at higher rates: although fires destroy vegetation cover, soon after the fire "nutritional buds" start to occur (Stubbs *et al.* 1985), enabling fast growth of surviving immature tortoises. High growth rates lead to maturation at smaller body sizes (Stubbs *et al.* 1985). Because the Mediterranean region is notorious for high frequency of disastrous fires, increased growth rate and lower size at maturity could reflect the indirect effect of habitat type on body size (see Table 3). In our population sample the youngest male regarded as adult was an animal of 137.4 mm SCL, and with only 7 countable growth rings. The same number of annuli was counted in a juvenile of 88.8 mm SCL. The smallest adult male had a SCL of 133.0 mm, and 6+4 years (until maturation and afterwards, respectively). Also, two adult females of identical SCL (156.4 mm) were assessed to have 10 (9+1) and over 25 years, respectively. These observations support the hypothesis of highly variable growth rates in environments subjected to drastic oscillations in quality and quantity of resources (e.g. Dunham 1978). Our observations also support previous findings on high mortality of large adults in fires: a 100% mortality of animals ranging from 180 to 200 mm was found in Bulgaria (Popgeorgiev 2008). It is possible that larger individuals are eliminated from populations due to their inability, i.e. lack of opportunities to escape/hide from fast-spreading fires. This depends on individuals' performance, but also on the configuration of habitat (soil depth, amount and quality of hiding places, etc.). Therefore, it is possible that in perturbing environments the animals of average body sizes are favoured. In a long-term sense, smaller average body sizes of adult females may result in smaller clutches and smaller hatchlings, with numerous further effects on individuals and entire populations. In order to obtain accurate data on tortoise growth rates, maturity attainment and reproductive success near Danilovgrad, we must continue our CMR study, and also collect precise data on oscillations in climatic parameters and vegetation cover, as well as frequencies of fires and road fatalities.

Fig. 3. Photo of the burned immature individual under the wire mesh (Photo: A. Vujović)

Conservation considerations

In the tortoise population we study, most severely affected by both fires and traffic are immature individuals and adult females, categories crucial for the persistence of tortoise population (e.g. Hailey, Willemsen 2000, Jonsson, Ebenman 2001). The effects of fires and road kills are most pronounced in late summer (August–September), due to seasonal shift in animals behaviour, coinciding with an increase in traffic (tourist season) and fire frequency. Therefore, conservation efforts should be focused on this period of the year.

We need to consider specific and effective protective measures for this region. Side effects of a rockslide-protective mesh on tortoises are twofold: positive regarding the prevention of road kills, but negative in a sense of trapping the animals. Relatively cheap road-killing prevention structure –

a vertical fence – could be set along the critical parts of the road, e.g. those with high traffic frequencies and/or with dense tortoise populations. The existing culverts, or special tunnels designed for small vertebrates, should be built to allow bi-directional movements of animals under the road (e.g. Boarman, Sazaki 1996, Aresco 2003, Kaye *et al*. 2005). Installing of appropriate protective structures and warning signs in the field has to be accompanied by educational campaign: the drivers have to become aware of the existence of protected species along the roads they use. Also, measures must be taken to lower the occurrence of human-induced fires in the given region. Because fires often escape control and approach houses and other properties/infrastructure, we suppose that their prevention shall be supported by local authorities and inhabitants.

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