PRELIMINARY DATA ON FAUNA RAILWAY MORTALITY IN DOBRUJA (ROMANIA) INDICATE A POSSIBLE IMPACT ON SPUR-THIGHED TORTOISE, *Testudo graeca*

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Abstract. Spur-thighed tortoises (*Testudo graeca*) are protected in Romania, where they are at the limit of their distribution range. Because tortoises are negatively affected by transportation networks, we supposed that this impact would also be obvious in an area from Romania where they are present. For these, we chose to study the fauna mortality on three railway segments from Dobruja, south-eastern Romania, during the 2020 summer. We identified 75 animals killed on railways, belonging to 20 taxa. Among them, four were tortoises (*Testudo graeca*). Dead tortoises were identified both between the rails, but also outside them. Thus, it is difficult to know if they were victims of the railway traffic (directly or indirectly) or died from other causes near the railway. We encountered significant railway mortality differences between the studied sectors, probably influenced by the aspect of surrounding habitats. The survey season (summer) and region have a prominent influence on railway wildlife mortality. Many victims were killed long before the study, thus the railway impact upon the fauna may be less severe.

Keywords: protected species, tortoise, railway ecology, human impact, habitats, region.

Rezumat. Date preliminare asupra mortalității feroviare a faunei în Dobrogea (România) indică un posibil impact asupra țestoasei de uscat dobrogeană, *Testudo graeca*. Țestoasa de uscat dobrogeană (*Testudo graeca*) este protejată în România, unde se află la limita arealului. Deoarece țestoasele sunt afectate negativ de rețelele de transport, am presupus că acest impact ar fi evident într-o zonă a României unde țestoasele sunt prezente. Din această cauză am ales să studiem mortalitatea faunei pe trei segmente de cale ferată din Dobrogea, sud-estul României, în vara anului 2020. Am identificat 75 de animale ucise pe calea ferată, aparținând la 20 de taxoni. Dintre victime, patru au fost țestoase (*Testudo graeca*). Țestoasele moarte au fost identificate atât între linii, cât și în afara lor. Astfel, este dificil de stabilit dacă acestea au fost victime ale traficului feroviar (în mod direct sau indirect) sau au murit din alte cauze în vecinătatea liniei de cale ferată. Am identificat diferențe semnificative ale mortalității feroviare între sectoarele studiate, diferențe influențate probabil de aspectul habitatelor înconjurătoare. Sezonul în care a avut loc studiul (vara) și regiunea au avut o influență evidentă asupra mortalității feroviare a faunei. Multe victime au fost ucise cu mult înaintea studiului, astfel încât impactul traficului feroviar asupra faunei ar putea fi mai puțin sever.

Cuvinte cheie: specii protejate, țestoasa de uscat, ecologie feroviară, impact uman, habitate, regiune.

INTRODUCTION

Dobruja is a region in Romania with a diverse herpetofauna; some species, like the spur-thighed tortoise (*Testudo graeca* Linnaeus 1758), are present in the country only in this region (e.g., COGĂLNICENU et al., 2013; FUHN & VANCEA, 1961). Although this species was identified initially in more natural areas of Dobruja (e.g., COVACIU-MARCOV et al., 2006; FUHN & VANCEA, 1961), recent data proved that it is more widespread, as it was also recorded in anthropic areas, like agricultural fields (MORARU et al., 2016). Nevertheless, in altered habitats, *T. graeca* presents a remarkably high rate of shell injuries (MORARU et al., 2018). A high rate of blows and scars in human-altered habitats was recorded in other tortoise species (BIAGGINI & CORTI, 2018), as well as snakes (ILE et al., 2020). A type of human impact upon the tortoise population is mortality caused by transportation networks. Among these, mostly roads are known to affect various tortoise species (e.g., ASHLEY & ROBINSON, 1996; LOEHR, 2012; SOUZA et al., 2015; SANTORI et al., 2018; BAXTER-GILBERT & RILEY, 2019), including from the Testudo genus (e.g., TOK et al., 2011; VUJOVIĆ et al., 2015). Although studies on road mortality are relatively numerous in Romania (e.g., CICORT-LUCACIU et al., 2012, 2016; CIOLAN et al., 2017; COVACIU-MARCOV et al., 2017, 2020; POPOVICI et al., 2018), they only covered regions outside *T. graeca* distribution range. Besides road mortality, tortoises are also affected by railway traffic. Still, data on this topic is scarce. Many refer to a possible indirect railway impact upon these reptiles, as the tortoises often remain captive between the rails and die even without being hit by trains (KORNILEV et al., 2006; ENGEMAN et al., 2007; RAUTSAW et al., 2018). Railway mortality studies are generally few (e.g., BUDZIK & BUDZIK, 2014; DORNAS et al., 2019; HESKE, 2015; KUŠTA et al., 2011; ST. CLAIR et al., 2020), and only one such study has been conducted in Romania so far (POP et al., 2020). Thus, we hypothesized that if tortoises are negatively impacted by railroads (KORNILEV et al., 2006; ENGEMAN et al., 2007) they will also be negatively affected in a region where they are present in Romania. Consequently, we investigated a railway in Dobruja, to establish its impact upon tortoises and upon the fauna in general. Thus, we had the following objectives: **1.** to identify the animals killed on the railway including tortoises, and **2.** to establish the potential peculiarities of the railway mortality induced by the region`s particularities.

MATERIAL AND METHODS

The study took place in central and northern Dobruja, a region situated in south-eastern Romania (MÂNDRUȚ, 2006). The survey was carried out on 26 and 31 July 2020. The studied railway (line 804, Medgidia - Tulcea Oraș) crosses

Dobruja from south to north (C.F.R., 2020). It is a single track, with Diesel traction, with a low number of passenger trains (four or six / day, depending on the period - C.F.R., 2020) and approximately the same number of freight trains. Because of the high length of the railway (144 km - C.F.R., 2020) and the summer heat, it was impossible to walk the entire railway. Thus, we chose to study two railway segments: one in northern Dobruja, between the Tulcea (Tulcea Freights station) and Cataloi localities, and an additional segment in central Dobruja, eastwards from Târgușor Dobruja (between the Târgușor Dobruja station and the Casian viaduct). The first segment is approximately 8 km long and crosses the Tulcea Hills, with the top of the hill slope situated in the middle of the segment. Therefore, this sector was further divided into two zones, each of approximately 4 km. The first zone, situated between Tulcea and the top of the hill slope, has a more pronounced gradient. Thus, the line has numerous curves, unlike the second zone, located between the top of the hill slope and Cataloi, where the line is almost straight. In the first zone, the railway tracks are surrounded by bushes, vineyards, but also by patches of forest recoveries and plantations. Because of the curves, the train speed is reduced (approximately 40 km / hour). In the second zone, agricultural areas and pastures surround the tracks. The second segment (Târgușor Dobruja) has a length of roughly 4 km. The line is surrounded by open grassy areas characteristic of central Dobruja, but there are small forest fragments in the vicinity. Both sectors were surveyed in the first part of the day, like in other studies (POP et al., 2020). All animal corpses identified on the railway were determined directly on the field at the lowest taxonomic level possible. We calculated the relative abundance of each taxon, both for the total railway tracks surveyed and for each railway sector, but also for the two zones of the Tulcea - Cataloi sector. Finally, we compared three railway sectors approximately 4 km long each but surrounded by different habitats, in terms of the killed taxa, killed individuals and diversity (calculated with the Shannon index). Also, we estimated the significance of differences between the studied railway sectors using the Kruskal Wallis index, and the Mann-Whitney index as post-hoc test, all calculated in the Past software (HAMMER et al., 2001).

RESULTS

On the 12 km of studied railway in Dobruja, we identified 75 animal corpses. Among them, only nine were vertebrates, and the others were invertebrates. The railway killed animals belong to 20 taxa (five vertebrates and 15 invertebrates). Only two railway killed taxa could be determined to the species level; both were vertebrates – tortoises and a dog (Table 1).

Table 1. The relative abundance and diversity of animals killed on railways in Dobruja according to the surveyed 4 km long zones.

	Tulcea – Cataloi		**Târgușor**	**Total**
	Tulcea	Cataloi		
Gastropoda with shell	61.53	50	6.25	37.33
Chilopoda Scutigeridae	-	-	3.12	1.33
Orthoptera	12.82	50	28.12	21.33
Dermaptera	-	-	3.12	1.33
Coleoptera Scarabeidae	-	-	3.12	1.33
Coleoptera Chrysomelidae	-	-	3.12	1.33
Coleoptera - undeterminable	2.56	-	3.12	2.66
Trichoptera	-	-	3.12	1.33
Lepidoptera	5.12	-	3.12	4
Diptera Brachycera	2.56	-	15.62	8
Hymenoptera Vespidae	2.56	-	-	1.33
Hymenoptera Apidae	-	-	3.12	1.33
Hymenoptera Formicidae	-	-	6.25	2.66
Hymenoptera - undeterminable	2.56	-	-	1.33
Insecta - undeterminable	-	-	3.12	1.33
Amphibia - undeterminable	2.56	-	3.12	2.66
Reptilia *Testudo graeca*	5.12	-	6.25	5.33
Aves - undeterminable	-	-	3.12	1.33
Mammalia Rodentia	-	-	3.12	1.33
Mammalia *Canis familiaris*	2.56	-	-	1.33
Total killed individuals	39	4	32	75
Total killed taxa	10	2	17	20
Diversity	1.43	0.69	2.46	2.16

In both sectors, we identified two *T. graeca* corpses. They were old carrions, namely old shells without soft tissues. Nevertheless, we also encountered an alive individual, which moved between the tracks without any intention to escape (we put it outside the tracks). The highest number of taxa, 17, were killed at Târgușor. At Tulcea, we identified only 10 taxa. The differences between the two zones from the Tulcea sector were noticeable. Between Tulcea and the top of the hill slope 10 taxa were killed, but between the top of the hill slope and Cataloi only two taxa were killed, and those two were common with the other zone. The highest number of individuals, 43, was killed at Tulcea (Table 1). At Târgușor, 32 animals were killed. Snails registered the highest relative abundance, as they represented 37.33% of the victims. Snails were followed by Orthoptera, with a relative abundance of 21.33%. There were differences in relative abundance between the taxa killed in the three different zones. At Tulcea, snails were the most abundant, but at

Târgușor Orthoptera had the highest relative abundance (Table 1). The diversity also differed between the three zones; the highest value was registered at Târgușor (Table 1). Gastropoda and Orthoptera were killed at every railway zone. The differences in railway mortality between the studied zones were significant according to Kruskal-Wallis test ($p<0.0001$). The differences were also significant between: Târgușor and Tulcea ($p=0.005$), Târgușor and Cataloi ($p<0.0001$), and the two zones from Tulcea ($p=0.016$).

DISCUSSIONS

The railway from Dobruja has a negative impact on numerous animal groups, just like other railways (POP et al., 2020). The peculiarities of the region are obvious in the case of railway mortality. Thus, on the studied railway, animals that were not identified in other Romanian regions (POP et al., 2020), were killed. This is the case of *T. graeca*, which in Romania is present only in Dobruja (COGĂLNICEANU et al., 2013), and of *Scutigera*, which is also a southern, Mediterranean, element (e.g., DRUGESCU & GEACU, 2004). Although a total of four *T. graeca* corpses is not numerous, neither the studied railway length is high compared to the total length of railways from Dobruja (C.F.R., 2020). Moreover, in two of the three studied railway zones, we encountered railway-killed tortoises. Thus, if the 144 km railway length between Medgidia and Tulcea is considered, the number of railway-killed tortoises may be much higher.

The *T. graeca* corpses encountered on the railway were not fresh; the animals were killed long before the study (judging on the corpse's condition, they were probably killed at least a few months ago). Therefore, the impact on the tortoise's population in the area might be less severe. On the road, corpses disappear quickly, because of the traffic itself, which repeatedly crushes the victims (e.g., CABRERA-CASAS et al., 2020; ENGE & WOOD, 2002; RATTON et al., 2014). As opposed, on a railway, this could not happen because the contact between the train wheels and the rails is small, thus the tortoises that died between the rails are rarely crushed by the wheels. As previously reported (POP et al., 2020), the number of dead animals on the railway may represent a long period of accumulation of dead animals, as recent data indicated for some mammals a persistence time of almost a year (DASOLER et al., 2020). In the current study two *T. graeca* corpses were identified between the railway tracks, and two outside the lines. They were both intact and broken shells.

Nevertheless, also in the case of broken shells, it is difficult to establish if the tortoises died because of collision with a train or the cracks have occurred after the animal's death. Thus, the empty and lighter shells could be moved by the airflow produced by moving trains and broken, just like in snails (POP et al., 2020). At the same time, it is possible that empty shells were accidentally stepped on by large mammals (cattle) or smashed by different railway types of equipment. Probably the two *T. graeca* recorded between the rails arrived there accidentally, could not escape, and finally died from overheating and dehydration, as similar situations were recorded in other tortoises (KORNILEV et al., 2006; ENGEMAN et al., 2007; RAUTSAW et al., 2018) or snails (POP et al., 2020). If other tortoise species have great difficulties escaping from between the rails (KORNILEV et al., 2006; RAUTSAW et al., 2018) this could also be the case of *T. graeca*. Thus, tortoises are collateral victims of railways tracks since those are barriers that the tortoises have difficulty to climb and therefore get stuck in between the rails. Even if the above-mentioned is probably true for the tortoise corpses between the tracks, they are difficult to apply in corpses identified outside the railway tracks. Thus, they could not remain captive between the lines, and neither seems to be hit by trains. Although probably their death was indirectly caused by the railway, we could not exclude an accidental death from other reasons near the railway. If their death is due to the trains, a possible cause could be the air pressure generated by the heavy freight trains, a fact previously mentioned for amphibians (DORNAS et al., 2019). Nevertheless, because we encountered only old corpses, this fact is impossible to verify. Thus, it is difficult to establish if the tortoises were killed, even indirectly, by trains, or they just died accidentally near the railway.

The high percentage abundance of Orthoptera highlights the effect of the season on railway mortality. They had the second percentage abundance, contrary to a study realized for a whole year, where they were much rarer (POP et al., 2020). Orthoptera were killed in high numbers in summer also on roads (e.g., CIOLAN et al., 2017; POPOVICI et al., 2018), as they are thermophilous insects related to steppe areas (RADU & RADU, 1967). Among amphibians, we identified only one individual in Tulcea sector. Still, even that was so old that we could not determine it, even if in other cases railway-killed amphibians were determined to the species level (e.g., BUDZIK & BUDZIK, 2014; HESKE, 2015; POP et al., 2020). Nevertheless, the amphibian was intact, and just as the tortoises, probably reached accidentally between the tracks and were indirectly killed by the railway (KORNILEV et al., 2006; ENGEMAN et al., 2007; RAUTSAW et al., 2018). Although snails were also in Dobruja the taxa with the most railway killed individuals, they were nevertheless less affected than in the previously published survey (POP et al., 2020). This is, in our opinion, a consequence of the fact that Dobruja is a region with a warmer and drier climate compared to other regions in Romania (MÂNDRUȚ, 2006), which probably had a negative influence on snails. Unlike Dobruja, there was a river in the previous case close to the rails (POP et al., 2020). Although snails were killed in each of the three zones, they were more abundant at Tulcea. Moreover, most snails (20 from 26) were registered at the top of the hill slope, near and under the bridge through which the road to Tulcea crosses the railway. In that area the railway is surrounded by vertical concrete walls with gutters, it is shaded, and there is a forest plantation in the vicinity. Thus, the higher humidity and relative protection from the sun are advantageous for the snails.

The differences between the three rail sectors were noticeable. The region surrounded by agricultural area has the lowest railway mortality, unlike the areas covered by more natural habitats which have a higher victim diversity. The highest taxa number and diversity were registered in the more natural area from central Dobruja. Differences determined by the habitat's aspects were also noted in the case of road mortality on previous studies (e.g., COVACIU-MARCOV et al., 2017; GARRAH et al., 2015; KEILSOHN et al., 2018; POPOVICI et al., 2018; TEODOR et al., 2019). Although in the case of mammals, the railway sectors with curves seem to increase the victim numbers (ST. CLAIR et al., 2020), in Dobruja, the taxa number was the highest in the region where the line was straight. This is probably a consequence of the taxa differences (if a large mammal can avoid the train when sees it, in contrast a tortoise cannot do the same), but also a speed difference. Thus, the speed is much slower in the curved area than at Târgușor, where the line is straighter. At Târgușor we encountered the only railway-killed bird. Although there are numerous snakes in Dobruja (FUHN & VANCEA, 1960; COVACIU-MARCOV et al., 2006; COGĂLNICEANU et al., 2013) and in other areas, they were killed on railways (HESKE, 2015; POP et al., 2020), in this survey we did not register any victim. Probably snakes are too fast for slower trains. Moreover, as they have numerous available habitats, they do not have to use railway edges like in other regions (e.g., GRAITSON et al., 2020).

Because of the conservative status of *T. graeca* (O.U.G. 57/2007), dead individuals on the railway are concerning. Nevertheless, the number of railway-killed animals seems smaller than the one recorded on roads of the same length or even shorter (e.g., YAMADA et al., 2010; CICORT-LUCACIU et al., 2016; CIOLAN et al., 2017; POPOVICI et al., 2018). Even if the data are too brief and preliminary to draw clear conclusions, they are enough to recommend that in areas with terrestrial tortoises, their relationship with railways should be studied appropriately.

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