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Nothing Else Eaten! The Fish Diet of *Natrix tessellata* in the Danube Gorge, Romania

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Abstract.—**The Dice Snake (*Natrix tessellata*) is a water snake that feeds primarily on fish and regionally on amphibians. In 2020, we studied the food composition of 452 *N. tessellata* in the Danube Gorge in Romania. Of these, only 97 individuals yielded stomach contents representing 115 fish items of 10 taxa. Dice Snakes had ingested usually only one fish, rarely two or three, with the most common prey species being the Round Goby (*Neogobius melanostomus*; 52.17%), the Monkey Goby (*N. fluviatilis*; 22.61%), and the Bighead Goby (*Ponticola kessleri*; 9.57%). Dice Snakes consumed only few primarily benthic species (88.69%) of the numerous fish species present in the Danube. The most common prey, the invasive *N. melanostomus*, colonized the Danube Gorge only about 20 y ago. Thus, *N. tessellata* seems capable of rapidly shifting its diet, probably depending on prey accessibility and abundance. Both gobies and Dice Snakes occupy a human-made habitat formed after the construction of Iron Gates I Dam, which consists of the Danube banks consolidated with numerous large stones. This artificial habitat benefits both the prey and the predator, facilitating their contact. Therefore, the diet of Dice Snakes reflects the changes in environmental conditions both in the region and in the habitats they occupy.**

Key Words.—Dice Snake; diet shift; feeding; gobies; invasive species; prey taxa

Introduction

Snakes are predatory reptiles, with various feeding behaviors (Ananjeva and Orlov 1982; Voris and Voris 1983; Glaudas et al. 2019). Many snakes associated with aquatic environments have a fish-based diet (Mushinsky and Hebrard 1977; Voris and Murphy 2002; Jones et al. 2009; de Carvalho Teixeira et al. 2017), including the Dice Snake, *Natrix tessellata* (Filippi et al. 1996; Luiselli et al. 2007; Metzger et al. 2011). Although it is mainly piscivorous, adding amphibians where fish are less abundant or missing, the Dice Snake is relatively generalist in terms of prey species within its two preferred animal orders, which in part explains the capacity of the species to colonize diverse areas (Weiperth et al. 2014a).

One area known for its Dice Snake population is the Danube Gorge in Romania (Iftime 2005; Strugariu et al. 2011; Cogălniceanu et al. 2013), where numerous individuals are present on the slopes near the Danube River (Iftime 2005; Covaciu-Marcov et al. 2009). Except for a single observation on the consumption of two goby species of fish (Iftime 2005), however, there are no data regarding their diet in the Danube Gorge. In Romania, studies regarding the diet of Dice Snakes have been mainly focused on the southeastern regions of the country (Băcesco 1934; Sloboda et al. 2010; Carlsson et al. 2011). In the gorge, the Danube shelters numerous fish species, including invasive gobies (Bănărescu et al. 1975; Bănăduc et al. 2014, 2016; Danalache et al. 2020). Therefore, Dice Snakes found in this region could benefit from this diversity of potential prey.

We questioned to what extent gobies are found in the diet of Dice Snakes because this snake generally feed on gobies in areas where such fish occur (Ahmadzadeh et al. 2011; Hutinec and Mebert 2011; Tuniyev et al. 2011), including the lower Danube Basin (Weiperth et al. 2014a, b) where several goby species have recently moved upstream on the Danube (Harka and Bíró 2007; Roche et al. 2013; Bănăduc et al. 2014). The presence of an invasive goby species can affect diet composition of snakes and even have been shown to induce reproductive changes in another water snake species (King et al. 2008). We studied the feeding habits of Dice Snakes in the Danube Gorge to: (1) establish the food composition of Dice Snakes in the Danube Gorge; (2) establish the proportion of non-native prey items in the diet of the Dice Snake; (3) compare diet composition of the Dice Snake with the literature on fish assemblages in the Danube Gorge (Bănărescu et al. 1975; Bănăduc et al. 2014, 2016; Danalache et al. 2020); and (4) determine the direction of prey ingestion of the Dice Snake.

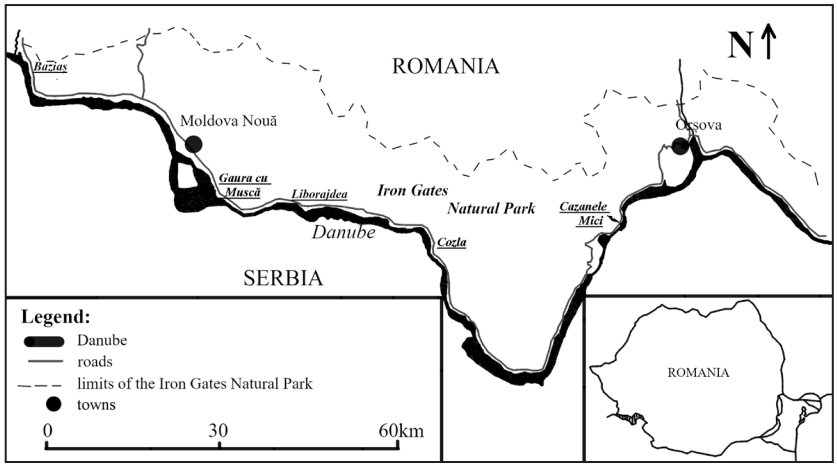

Figure 1. Map of the study area in the Danube Gorge, Iron Gates Natural Park, southern Romania.

Materials and Methods

We studied Dice Snakes in southwestern Romania, in the Danube Gorge in Iron Gates Natural Park (IGNP; Fig. 1). We focused on three study sites within this area: (1) Cazanele Mici; (2) Liborajdea; and (3) Gaura cu Muscă Cave (near Coronini). Cazanele Mici is situated at the eastern end of the gorge, in Mehedinți County. Liborajdea and Gaura cu Muscă are situated in the western area of the gorge in Caraș Severin County. We also, occasionally, encountered Dice Snakes in other sites within the IGNP (e.g., Cozla and Baziaș). At Liborajdea and Gaura cu Muscă, the Danube bank consists of large rocks, which are situated on a 2–4 m strip between the water and a road, and snakes were frequently encountered on those stones. In Cazanele Mici, the Danube bank is steeper, with almost vertical limestone slopes. We collected snakes at this location from a gentler slope, situated approximately 10 m above the water level.

We spent 21 d in the field between May and November 2020. We walked the sampling areas in the first part of the day. We hand-captured 452 Dice Snakes over the course of the study. We palpated each snake to probe for the presence of stomach contents and we immediately released snakes without obvious stomach contents. Some snakes regurgitated spontaneously, immediately after capture, whereas others had to be palpated gently along the body until they regurgitated the prey. This method has been used successfully in other studies (Filippi et al. 1996; Metzger et al. 2011; Bissattini et al. 2021). After the prey was regurgitated, we immediately released all snakes. Each stomach content was put in a different jar, labeled with the date and locality, preserved in a formaldehyde solution, and brought to the lab. We recorded the orientation in which the prey was ingested as the snake regurgitated the prey items. Prey orientation also was verifiable in the laboratory based on the different stages of digestion of the two extremities of prey items. We identified stomach contents using keys to fishes of Romania (Bănărescu 1964, Oțel 2007), in most cases to a species level depending on their degree of decomposition as a result of the digestion. We calculated the percentage abundance of each prey taxon, and the frequency of occurrence of each taxon in the studied months.

Results

Of the 452 Dice Snakes we captured, 97 (21.46%) had collectible stomach contents, which included 115 prey items of 10 species (Table 1). The number of prey items per snake varied between one and three. We were able to identify almost all prey items to species. Snakes regurgitated both intact prey and partial prey samples in different stages of digestion. We found three gobies in an advanced stage of digestion so species could not be determined. Also, another four highly digested fish could not be identified to a lower taxonomic level. We identified 12 prey taxa ingested in various numbers by Dice Snakes at our study sites (Table 1). The Round Goby (*Neogobius melanostomus*) had the highest percentage abundance (52.17%), followed by the Monkey Goby (*N. fluviatilis*; 22.61%) and by the Bighead Goby (*Ponticola kessleri*; 9.57%; Fig. 2). The prey species found in most months over the study duration were the Round Goby (6 of 7 mo) and the Monkey Goby (occurred in 5 of 7 mo; Table 1).

Dice Snakes consumed different fish species in different months (Table 1). The highest number of total prey was consumed in June, and the highest number of prey taxa in July (seven prey taxa). In the autumn months snakes consumed fewer prey and prey taxa. In May, the Dice Snakes fed exclusively on gobies.

Table 1. Number of prey items consumed by the Dice Snake (*Natrix tessellata*) in the Danube Gorge, Romania, 2020, by month over the study period. The acronym f% = frequency of occurrence in the study months, and — = no prey of this species observed.

Species	May	June	July	August	September	October	November	Total	f%
Cyprinidae									
Carassius gibelio (Gibel Carp)	—	—	1	1	1	—	—	3	42.85
Ballerus sapa (White-eye Bream)	—	1	—	—	—	—	—	1	14.28
Blicca bjoerkna (White Bream)	—	1	—	—	—	—	—	1	14.28
Perchidae									
Perca fluviatilis (European Perch)	—	—	—	—	—	1	1	2	28.57
Sander lucioperca (Pikeperch)	—	—	2	—	—	—	—	2	14.28
Gobiidae									
Ponticola kessleri (Bighead Goby)	10	—	1	—	—	—	—	11	28.57
Ponticola eurycephalus (Mushroom Goby)	1	—	—	—	—	—	—	1	14.28
Babka gymnotrachelus (Racer Goby)	1	—	—	—	—	—	—	1	14.28
Neogobius fluviatilis (Monkey Goby)	13	7	3	2	1	—	—	26	71.42
Neogobius melanostomus (Round Goby)	9	32	12	4	—	1	2	60	85.71
undetermined	—	—	1	2	—	—	—	3	28.57
Unidentifiable fish	—	—	2	1	1	—	—	4	42.85
Total prey taxa	5	4	7	5	3	2	2	115	

Most of the prey were ingested headfirst (88.69%), and few were ingested tail first (10.43%). In one case (0.86%), a Round Goby was ingested sideways, being folded in half. The largest prey was a 21 cm Pikeperch (*Sander lucioperca*) consumed by a female Dice Snake of approximately 1 m length. Nevertheless, the anterior third of the fish was completely digested (thus the prey probably exceeded 30 cm length). The gobies were generally 10 cm long, but sometimes we encountered larger individuals up to 15 cm long.

Discussion

Although the fish fauna in the Danube Gorge is rich and diverse, with more than 60 species (Bănărescu et al. 1975; Bănăduc et al. 2014), Dice Snakes fed on only 10 species. Moreover, only two fish species were important for the snakes. Among these, the Round Goby is an invasive species with a large distribution (Kottelat and Freyhof 2007; Brownscombe and Fox 2012; Kornis et al. 2012). This goby was not recorded in the area before the construction of the Iron Gates I Dam (Bănărescu et al. 1975), and it was first recorded only in 1997 downstream at the Iron Gates II Dam (Simonović et al. 1998). Afterwards it advanced upstream rapidly (Harka and Bíró 2007; Roche et al. 2013). Thus, the Round Goby has been present in the Danube Gorge for only about 20 y and already has populations large enough to become the primary food of Dice Snakes. This indicates not only that the generalist fish diet of Dice Snakes allows them to consume novel prey, but also that the Round Goby occupies the most favorable trophic niche for the Dice Snake. A similar situation was documented for another water snake (the Lake Erie Watersnake, *Nerodia sipedon insularum*) shortly after the Round Goby was introduced (King et al. 2006). Thus, the introduction of these gobies seems to cause similar changes in the diet of two separate water snake species, but which probably hunt in the same niche. Although non-native fish species had a positive effect on the Dice Snake, in other cases (Dubey et al. 2015) the consumption of invasive prey species could also have negative effects on Dice Snakes, resulting in individuals in poor condition and reduced populations (Stellati et al. 2019; Bissattini et al. 2021). Poor condition and reduced populations are probably a consequence of the

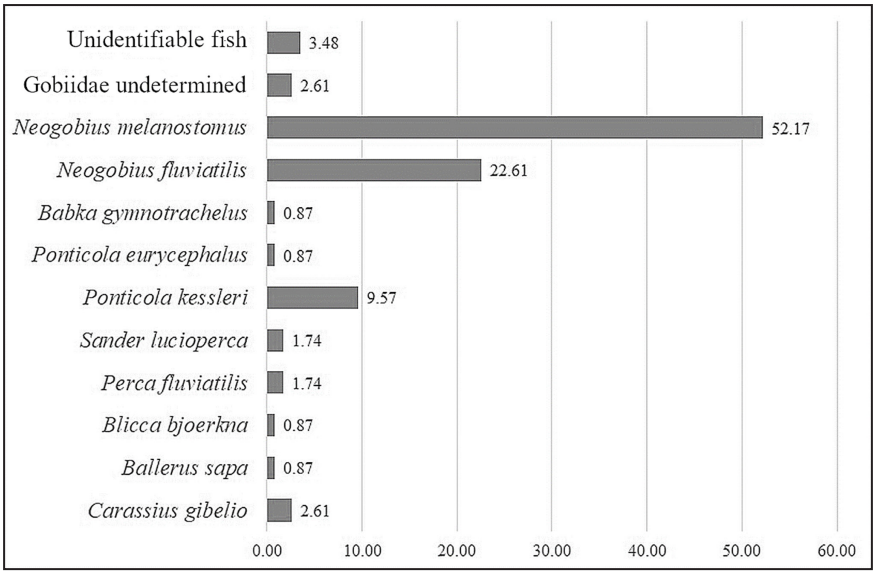

Figure 2. Percentage of fish species consumed by the Dice Snake, *Natrix tessellata*, in the Danube Gorge, Romania, 2020. Common names of each species can be found in Table 1.

ability of some invasive fish species to injure snakes with their spiny fins, as they perforated the body of the individuals that ingested them (e.g., Šukalo et al. 2014; Emmons et al. 2016; Stellati et al. 2019). Nonetheless, despite most prey in the IGNP being non-native, Dice Snake populations were large, a fact reflected by the high number of individuals killed on the road parallel to the Danube (Covaciu-Marcov et al. 2022). Gobies are probably easy prey for Dice Snakes because that have soft fins (Bănărescu 1964), which would not injure the snakes.

Although other studies have found this snake to feed on amphibians, insects, gastropods, crustaceans, or even reptiles in addition to fish (Filippi et al. 1996; Bakiev et al. 2011; Göçmen et al. 2011), we found that the Dice Snake consumed only fish in the Danube Gorge. Exclusive consumption of fish may be a consequence of the high number of fish species in the gorge (Bănărescu et al. 1975; Bănăduc et al. 2014, 2016; Danalache et al. 2020). Fish also are probably easier to capture compared to prey from terrestrial environments, as the increased visual acuity of this snake in the water allows it to prey on fish (Schaeffel and Mathis 1991). There are other cases in which the Dice Snake consumed exclusively fish (Metzger et al. 2009; Hutinec and Mebert 2011; Ajtić et al. 2013), even numerous gobies (Hutinec and Mebert 2011). Nevertheless, as at the IGNP site, Dice Snakes at other sites used large aquatic habitats (Metzger et al. 2009; Hutinec and Mebert 2011; Ajtić et al. 2013), whereas the habitats where snakes consumed different trophic resources were in mountainous or arid areas (Weiperth et al. 2014a).

The high percentage of gobies in the diet of Dice Snakes seems specific to the lower Danube Basin (Weiperth et al. 2014a, b), although gobies were found to be the main food for the Dice Snake in the Caucasus region as well (Tuniyev et al. 2011). Dice Snakes are benthic foragers (Hutinec and Mebert 2011; Metzger et al. 2011), where gobies are also present (Bănărescu 1964; Oţel 2007). In the IGNP, Dice Snakes occasionally fed on pelagic fish species, even large predators such as *S. lucioperca* and *P. fluviatilis* (Bănărescu 1964; Oţel 2007). Thus, the food preferred by *N. tessellata* and the easiest to capture consists of benthic fish, like gobies. It is possible that the numerous Dice Snake distribution records in the area (Strugariu et al. 2011; Cogălniceanu et al. 2013) and the high number of individuals is a consequence of the abundance of food (Iftime 2005). Gobies were initially marine fish that colonized the Danube and its tributaries (Harka and Bíró 2007; Kottelat and Freyhof 2007; Roche et al. 2013). They probably also benefited from the Iron Gates I Dam, which raised the water level (Mihai et al. 2016; Şelău 2018) and decreased its speed, affecting fish assemblages (Bănăduc et al. 2014). Perhaps this increase in prey availability led to an increase in the Dice Snake populations (Iftime 2005). A similar situation occurred in the case of the Lake Erie Water Snake from the Great Lakes area in North America, whose growth rates and population densities increased after the introduction of gobies (King et al. 2006; Jones et al. 2009).

Dice Snakes ingested fish mostly headfirst, some tail first, and only one fish sideways. In the laboratory, the Dice Snake captured fish from various angles, with no preference (Ghira et al. 2009; Metzger et al. 2011). Most fish were ingested headfirst in other cases (Metzger et al. 2009), but the IGNP had a higher percentage of fish ingested tail first compared to these. All fish ingested tail

first were gobies. The fish ingested sideways also were gobies, and this behavior seems to be unusual for this snake. Ingesting the prey from the side was probably a consequence of snakes usually grasping fish from the trunk, and then manipulating it to the right position for ingestion (Ghira et al. 2009). In this case, the ingestion behavior probably started immediately after the fish was captured, as ingestion time is important for Dice Snakes (Ghira et al. 2009). Ingesting fish tail first is considered difficult, because of the harsh fins and scales of fish (Metzger et al. 2011). Gobies, however, have soft fins (Bănărescu 1964) and a relatively uniform body; thus, they can be easily ingested from any direction. In other cases, the most consumed fish by the Dice Snake was the European Bullhead (*Cottus gobio*; Metzger et al. 2009, 2011), a benthic fish that shelters under rocks (e.g., Bănărescu 1964) similar to some gobies (e.g., Bănărescu 1964; Kottelat and Freyhof 2007; Oţel 2007). Thus, snakes do not select certain prey taxa, but a certain ecology of the prey, which corresponds with their behavior. As the Dice Snake searches for its prey under the stones (Metzger et al. 2011), the gobies presence in this habitat seems all that matters for snakes. At the same time, the gobies hidden under stones are probably more often grasped from the rear part of their body by the Dice Snakes.

The number of prey items collected per snake was typically one, occasionally two, as in other studies (Bakiev et al. 2011; Reshetnikov et al. 2013). There are instances when Dice Snakes consumed more prey items per individual, but those were small prey like tadpoles or newts (Brecko et al. 2011; Göçmen et al. 2011). In contrast, in the IGNP Dice Snakes consumed a small number of larger prey per individual. Our observation of the Pikeperch from Liborajdea seems to be one of the largest prey consumed by the Dice Snake (Vlček and Jablonski 2016).

Banks of the Danube River were consolidated with large rocks, which are optimal habitat both for the Dice Snake (Conelli et al. 2011; Ioannidis and Mebert 2011; Mebert 2011; Gezova and Jablonski 2018) and for some gobies (Oţel 2007; Brownscombe and Fox 2012; Kornis et al. 2012). Thus, the raised water level caused by the Iron Gates I Dam (Mihai et al. 2016; Şelău 2018) favored gobies, which colonized the rocky habitats on the banks. Those habitats also favored Dice Snakes, offering food, shelter, and basking habitats. Consequently, human structures increased both the prey and the predator populations, facilitating contact between them. The diet of Dice Snakes in the IGNP seems to be a consequence of an artificial situation and an example of positive impact from a non-native species on a native one, as previously observed for this species (Dubey et al. 2015). Although in other regions Dice Snakes also consumed other prey items (Filippi et al. 1996; Göçmen et al. 2011; Weiperth et al. 2014a, b), in the Danube Gorge they fed only on fish, because fish and especially gobies were so numerous and accessible that snakes seemingly no longer needed to consume anything else. Given the choice, *N. tessellata* will only eat fish.

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