FEEDING IN COLD WEATHER: FOOD COMPOSITION OF A *Salamandra salamandra* (AMPHIBIA) POPULATION FROM THE IRON GATES NATURAL PARK, ROMANIA, IN EARLY MARCH

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Abstract. The food composition of a *Salamandra salamandra* population from the Iron Gates Natural Park was studied on 22 individuals, using the stomach flushing method. The field work was done on 8 March 2020, on a cold rainy day. All *S. salamandra* individuals had stomach contents; they consumed 208 preys from 20 invertebrate taxa. In addition, they consumed inorganic elements, vegetal remains and shed skin fragments. The most important prey taxa were Gastropods and Diplopods. Despite the cold weather registered at the beginning of March, the feeding intensity of this population was higher in comparison with other studies made in Romania. Moreover, the prey taxa consumed in early spring are the same with those consumed in other periods by this species. The high feeding intensity indicates that *S. salamandra* is not affected by the low temperatures, having and optimal feeding even in the beginning of spring. Also, the increased feeding intensity indicates that the habitat has appropriate trophic resources and thus optimal conditions for *S. salamandra.*

Keywords: feeding, fire salamander, prey taxa, temperatures, cold weather, early spring.

Rezumat. Hrănire în vreme rece: compoziția hranei unei populații de *Salamandra salamandra* (Amphibia) din Parcul Natural Porțile de Fier, România, la început de martie. Compoziția hranei unei populații de *Salamandra salamandra* din Parcul Natural Porțile de Fier a fost studiată pe 22 de indivizi, utilizând metoda spălăturii stomacale. Activitatea pe teren a fost realizată în 8 martie 2020, într-o zi rece și ploioasă. Toți indivizii de *S. salamandra* au avut conținut stomacal, consumând 208 prăzi care au aparținut la 20 de taxoni de nevertebrate. Totodată, au mai consumat elemente anorganice, resturi vegetale și fragmente de exuvie. Cei mai importanți taxoni pradă au fost Gastropodele și Diplopodele. În ciuda temperaturilor reduse înregistrate la început de martie, intensitatea hrănirii acestei populații a fost mai ridicată în comparație cu cea menționată în alte studii din România. Mai mult, taxonii pradă consumați la începutul primăverii au fost identici cu cei consumați în alte perioade de către această specie. Intesitatea ridicată a hrănirii indică faptul că *S. salamandra* nu este afectată de temperaturile scăzute, având o hrănire optimă chiar și la începutul primăverii. Totodată, intensitatea ridicată a hrănirii sugerează că habitatul are resurse trofice corespunzătoare, asigurând astfel condiții optime pentru *S. salamandra***.**

Cuvinte cheie: hrănire, salamandra, taxon pradă, temperatură, vreme rece, primăvară timpurie.

INTRODUCTION

Knowing the activity of a species on all temperature levels it tolerates is important, especially in the case of species which tolerate low temperatures (CATENAZZI, 2016). Such species is *Salamandra salamandra* (Linnaeus, 1758), which maintains a higher metabolic rate when active at low temperatures, therefore it needs a higher amount of food (CATENAZZI, 2016). For this reason, *S. salamandra* is considered vulnerable to global warming, which could affect its life cycle (CATENAZZI, 2016). Probably, these can already be seen at this moment. Thus, in Bulgaria an active *S. salamandra* individual was reported at the end of February, at the temperature of 12.5ºC (PULEV et al., 2016). Because the species is vulnerable to climate change, and individuals active at low temperatures need larger amount of food (CATENAZZI, 2016), studies made on their food composition are important. Nevertheless, it seems that the species does not consume animal prey in the winter, but sometimes eats fragments of shed skin (BALAGOVÁ et al., 2015). At least in the Carpathian Mountains, there are studies on the food composition of this species (see a review in: BALAGOVÁ et al., 2015). Nevertheless, in some regions of the Carpathians like Romania, feeding studies on this species were performed only in the warm season, either in April - May (COVACIU-MARCOV et al., 2002; CICORT-LUCACIU, 2009; CICORT-LUCACIU et al., 2007; FERENȚI et al., 2010a, b; LEZAU et al., 2010), or in September (FERENȚI et al., 2008). Thus, we hypothesized that *S. salamandra* will consume a high amount of food even in early spring when the weather is cold. In this context, we started a study about the food composition of *S. salamandra* in the Iron Gates Natural Park - IGNP from southern Romania, in a cold beginning of March. The Danube Gorge is the region were the species reaches its lowest altitudes in Romania (COVACIU-MARCOV et al. 2009; 2017). Our objectives were: 1. To establish the food composition of a *S. salamandra* population from the IGNP, 2. To compare the species' food composition with studies on this topic undertaken in other periods.

MATERIALS AND METHODS

The study was made in 2020. The studied population was identified in the Slătinicu Mare valley, near the eastern limit of IGNP, in the Mehedinți County. The valley is situated in south-western Romania, approximately 10 km from Orșova town, near the Iron Gates I dam. Alongside the valley there is an abandoned forest road, with overgrown vegetation. The region is covered with oak, hornbeam, and beech forests, which in the past were affected by forestry activities. The forest road is situated approximately 10 m higher than the stream. Therefore, the individuals were not present near the water, but were collected from the woods, at a distance of at least 10-15 m from the valley. The stomach contents were collected from 22 *S. salamandra* individuals, on 8 March, on a rainy day, with a temperature of only 7-8° C in daytime (between 12:00-13:00). The stomach contents were collected using the stomach flushing method (SOLÉ et al., 2005). This method, appropriate for analyzing food composition without harming the individuals, was used in numerous studies on *S. salamandra* (e.g. ANDREONE et al., 1999; COVACIU-MARCOV et al., 2002; CICORT-LUCACIU, 2009; FERENȚI et al., 2010a, b). This method was successfully used even in the case of *S. salamandra* larvae (COSTA et al., 2017). The samples were kept in test tubes with alcohol. After the stomach contents were collected, the individuals were released in the area where they were captured. The stomach contents were analyzed in the laboratory, using Petri dishes and a stereomicroscope, like in other studies (e.g. CICORT-LUCACIU et al., 2007; LEZAU et al., 2010; BALAGOVÁ et al., 2015). Preys were identified on a high taxa level (class, order, family), just like in other studies (FERENȚI et al., 2008, 2010a, b; LEZAU et al., 2010; ÇIÇEK et al., 2017; BALAGOVÁ et al., 2015). Only in two cases the prey was determined to the species level. We calculated the average number of preys/individuals, the percentage abundance of prey from each taxon and the frequency of occurrence of each consumed taxon. Also, we calculated the diversity of the preys using the Shannon index, and the homogeneity using the Pielou index.

RESULTS

All *S. salamandra* individuals from the Slătinicu Mare valley had stomach contents. They consumed 208 preys, from 20 different taxa of invertebrates (Table 1). In two cases we managed to determine the prey species (*Euscorpius carpathicus* (Linnaeus, 1767) and *Ligidium hypnorum* (Cuvier, 1792). At the same time, salamanders also ingested inorganic materials, vegetable elements and shed skin fragments (Table 1). Shed skin fragments were found in 50% of the individuals. The highest percentage was registered by Gastropods (47.11%) and Diplopods (14.90%). Unlike this, seven taxa had a percentage of only 0.48%. In terms of frequency, the highest value was seen for Gastropoda Limacidae (95.45%) and Diplopoda (54.54%). They were followed by Lumbricidae and Gastropoda with shell, both with the same frequency (36.36%). The maximum number of preys/individuals of *S. salamandra* was 23, and the minimum was 2. The average number of preys/individuals was 9.45. The Shannon diversity of food consumed by the *S. salamandra* population from the Slătinicu Mare valley was H=2.46, and the homogeneity 0.46.

Table 1. Percentage abundance and frequency of occurrence of the prey consumed by *Salamandra salamandra* population from the Slătinicu Mare valley, IGNP.

	Percentage abundance %	Frequency of occurrence %
Inorganic elements	-	68.18
Vegetal Remains	-	90.90
Shed Skin	-	50
Lumbricidae	7.21	36.36
Gastropoda – with shell	8.17	36.36
Gastropoda – Limacidae	47.11	95.45
Scorpionidae – *Euscorpius carpathicus*	0.96	9.09
Araneida	7.69	31.81
Opilionida	0.48	4.54
Terrestrial Isopoda – *Ligidium hypnorum*	1.92	18.18
Diplopoda	14.90	54.54
Chilopoda	2.40	18.18
Thysanura	0.96	4.54
Orthoptera	1.44	9.09
Coleoptera Lampiridae	0.96	9.09
Coleoptera Chryzomelidae	0.48	4.54
Coleoptera undetermined	0.48	4.54
Neuroptera	0.48	4.54
Lepidoptera larvae	1.92	13.63
Insecta larvae	0.48	4.54
Diptera Brachicera	0.96	4.54
Diptera Nematocera	0.48	4.54
Formicidae	0.48	4.54

DISCUSSIONS

The *S. salamandra* population from the Slătinicu Mare valley presented an increased feeding intensity, even if the study was done on a cold spring day. Although, regarding the food composition of *S. salamandra,* this study was undertaken at the earliest time of a year in Romania (COVACIU-MARCOV et al., 2002; CICORT-LUCACIU, 2009; CICORT-LUCACIU et al., 2007; FERENȚI et al., 2008; 2010a, b; LEZAU et al., 2010), the average number of 9.45 prey/individual was higher than mentioned before (COVACIU-MARCOV et al., 2002; CICORT-LUCACIU, 2009; CICORT-LUCACIU et al., 2007; FERENȚI et al., 2010a). The average number of prey/individuals was almost double compared to the one registered in September in the Jiu Gorge (FERENȚI et al., 2008), or in comparison to other species from this genus (ÇIÇEK et al., 2017). This number was larger, even compared to the one previously recorded in the only study from IGNP, which was conducted in mid-April (FERENȚI et al., 2010b). Also, the maximum number of prey/individuals was larger than in other cases (COVACIU-MARCOV et al., 2002; CICORT-LUCACIU, 2009; FERENȚI et al., 2010a, b). In only one case, the number of prey/individuals was higher than in the Slătinicu Mare valley (LEZAU et al., 2010). Also, all salamanders from the Slătinicu Mare valley were fed, while in some of the previous studies, carried out in warmer periods of the year, there were cases in which a small number of individuals had empty stomachs (COVACIU-MARCOV et al., 2002; FERENȚI et al., 2010a, b). Thus, these data prove that the feeding of the *S. salamandra* population in the Slătinicu Mare valley was intense, although the temperature was low, and the study was conducted in early spring. This confirms both the species' tolerance to low temperatures and its higher energetic requirements, which determines an increased food intake in these conditions (CATENAZZI, 2016). It is possible that some of the previous studies carried out during warmer periods of the year have recorded lower values of feeding intensity, because higher temperatures have reduced the salamanders' trophic requirements.

All preys consumed by *S. salamandra* individuals from the Slătinicu Mare valley were terrestrial animals, although cases of salamanders consuming few aquatic preys were previously recorded (COVACIU-MARCOV et al., 2002; FERENȚI et al., 2008; 2010b). The exclusive consumption of terrestrial prey is a consequence of the fact that salamanders were identified at a distance from the watercourse and thus they were not able to come into contact with aquatic animals. Although we searched for salamanders in the vicinity of the stream, we did not identify any individuals there. Probably, as salamanders had already laid their larvae in the water, they had no reason to stay next to the stream.

Despite the high feeding intensity, the taxa consumed by salamanders from the Slătinicu Mare valley were approximately the same as those consumed in other cases by *S. salamandra* (e.g. KUZMIN, 1990; LEZAU et al., 2010; FERENȚI et al., 2010b; CICORT-LUCACIU et al., 2007; CICORT-LUCACIU, 2009) or congener species (ÇIÇEK et al., 2017). The two taxa with the highest percentage abundance (Gastropoda Limacidae and Diplopoda) had high percentage abundance in other cases (e.g. CICORT-LUCACIU et al., 2007; FERENȚI et al., 2010a; LEZAU et al., 2010), being important preys due to their low mobility and large size (BALAGOVÁ et al., 2015). Thus, not only the high intensity of feeding, but also the fact that the species consumes approximately the same prey in a cold period as in other periods, shows that it is not affected by low temperatures. However, there are also differences between the food of the *S. salamandra* population from the IGNP, and that of other populations. Thus, for example, in May, caterpillars were the most consumed group (CICORT-LUCACIU, 2009), but in the IGNP they had low values, probably due to the extremely poor vegetation from the beginning of spring. At the same time, in IGNP beetles registered low percentage abundance (0.48%), but they had much higher abundance in the case of other congener species food (GUERRERO et al., 1990; ANDREONE et al., 1999; ÇIÇEK et al., 2017). This is surprising, given that beetles are the most numerous group of insects (e.g. NIELSEN & MOUND, 1999), being frequently consumed by other amphibian species (ÇIÇEK, 2011; COVACIU-MARCOV et al., 2012; NAJIBZADEH et al., 2016; ORTEGA et al., 2016; PAFILIS et al., 2019). Probably because many beetles are fast, they are more difficult to catch by salamanders, which have other slower prey at their disposal. However, salamanders also consumed fast, even venomous preys such as *Euscorpius carpathicus*, a taxa previously reported in the food of populations from Danube Gorge and Jiu Gorge (FERENȚI et al., 2010b; LEZAU et al., 2010). In fact, it would have been difficult for the species to consume scorpions elsewhere, because they are present only in a small part of Romania (BUNESCU, 1959; GHERGHEL et al., 2016). The consumption of two scorpions is somewhat difficult to explain, because normally during the day they are hiding under different shelters (COVACIU-MARCOV & FERENȚI, 2019). Also, as in other cases, Diplopods were consumed in greater numbers than Chilopods, their reduced mobility making them more accessible (LEZAU et al., 2010).

A difference between the food of the population of *S. salamandra* from IGNP and that of a related species (*Salamandra infraimmaculata* Martens, 1885) consists in the percentage abundance of consumed isopods (ÇIÇEK et al., 2017). While *S. salamandra* from IGNP has a low percentage abundance of isopod consumption, in the case of *S. infraimmaculata* (ÇIÇEK et al., 2017) their abundance was much higher. However, we were able to determine all four isopod individuals that were consumed as *Ligidium hypnorum*, a species previously recorded in the Danube Gorge, in the vicinity of the Slătinicu Mare valley (RADU & TOMESCU, 1975). Although terrestrial isopods were identified in other cases in *S. salamandra* food, the species have not been determined (COVACIU-MARCOV et al., 2002; CICORT-LUCACIU, 2009; FERENȚI et al., 2008; 2010b). However, isopods consumed by other amphibian species have been determined, providing useful information about their trophic activity (TOMESCU et al., 2010; COVACIU-MARCOV et al., 2012). This is also true in the IGNP, *Ligidium hypnorum* being a species related to forested and humid mountain areas (TOMESCU et al., 2011), and *S. salamandra* is present in places related to humidity and forests (FUHN, 1960). Thus, the consumption of *Ligidium hypnorum* by *S. salamandra* is a consequence of the fact that the two species have similar ecological requirements, a fact mentioned in the case of other isopod species consumed by amphibians (TOMESCU et al., 2011).

As in other cases, we consider that the fragments of shed skin are the salamanders' own skin, because individuals are present at relatively large distances from each other (e.g. LEZAU et al., 2010). In the Slătinicu Mare valley, individuals were generally located at several meters distance from each other. Thus, salamanders could not consume the skin of other individuals from the population, although this is possible in hibernation habitats with many individuals (BALAGOVÁ et al., 2015). The high feeding intensity of the *S. salamandra* population in IGNP shows that shed skin consumption is not only a solution in case of unfavorable feeding conditions (BALAGOVÁ et al. 2015), although this has been mentioned in other amphibian species (KOVACS et al., 2010). Our data indicates that shed skin belongs to the individual who consumed it, and that they are consumed regardless of the intensity and amount of food consumed, a fact mentioned also for newts (KOPECKÝ et al., 2011). At the same time, it is possible that inorganic elements were ingested with shed skins (BALAGOVÁ et al., 2015), although they were identified in more individuals than those who consumed shed skin. Thus, inorganic elements were probably ingested along with any type of prey, as in the case of other amphibians (KOVACS et al., 2010). Vegetal remains that were consumed by 20 of the 22 *S. salamandra* individuals were probably accidentally ingested. The consumption of vegetal remains by *S. salamandra* was mentioned before, but their frequency is higher in the Slătinicu Mare valley than in other cases (COVACIU-MARCOV et al., 2002; CICORT-LUCACIU et al., 2007; CICORT-LUCACIU, 2009; FERENȚI et al., 2010b). Plant fragments have also been consumed by congener species (ÇIÇEK et al., 2017).

Despite the fact that in Romania there are numerous studies on the trophic spectrum of *S. salamandra* (COVACIU-MARCOV et al., 2002; CICORT-LUCACIU et al., 2007; FERENȚI et al., 2008; LEZAU et al., 2010), this is the first study conducted in early spring in a cold period. However, feeding intensity was higher than in other cases, indicating the existence and extent of seasonal variations in species feeding. At the same time, local variations are obvious, as the trophic spectrum of the species is influenced by habitats (BALAGOVÁ et al., 2015). Thus, the presence of scorpions in the food shows the local note of the region. At the same time, the richness of the food is a consequence not only of the period but also of some habitats with rich trophic resources, because although the species would have high trophic needs in early spring, if it had nowhere to obtain food, it would not have anything to eat. Thus, the habitats in IGNP can provide a quantitatively rich trophic resource for the species. The conditions of maintaining this species even in the context of climate change is ensured in the park if the forested habitats will remain unaffected in the future.

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