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Diet patterns of water green frogs (*Pelophylax esculentus* complex) in mixed population systems in Serbia

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Abstract. Population systems in which all three taxa of the *Pelophylax esculentus* complex coexist are rare and in Serbia can only be found along the Danube. Although several studies describe the diet in populations with only one taxon, there is a lack of data from mixed populations. We studied the diet in mixed populations of edible frogs at three sites for three years. We collected gut contents from 221 adult frogs using the stomach flushing method and identified 1,477 prey items. The diet consisted mainly of insects (88%). For all three taxa, the most frequently consumed prey groups were Hymenoptera (28%), Coleoptera (18%), Lepidoptera (17%) and Diptera (11%). Larger prey (in terms of length and volume) was mainly consumed by *P. ridibundus*, followed by *P. esculentus*. The smallest prey were mainly consumed by *P. lessonae*. However, *P. ridibundus* consumed fewer prey items than *P. lessonae*. No significant difference was found between the taxa in the staple diet, while there were differences in the less abundant prey categories, especially between *P. ridibundus* and *P. lessonae*. The narrowest trophic niche width was observed in *P. lessonae*, followed by *P. esculentus*, and the widest in *P. ridibundus*. Our results suggest that the diet of the three taxa is diverse and consists of a large number of invertebrate groups. However, the diet was locality-specific, with the dominance of different prey groups in different localities depending on habitat characteristics. These results confirm our expectation that the frogs of the *Pelophylax esculentus* complex follow an opportunistic foraging strategy, as predicted by optimal foraging theory.

Keywords. European water frogs, feeding habits, diet composition, prey diversity, Danube, Serbia.

INTRODUCTION

Frogs have an important position in the trophic network by regulating the populations of invertebrates and other groups of organisms on which they feed (Hocking and Babbitt, 2014). Larval stages feed on algae and detritus and, as primary consumers, influence the ecosystem structure and function by altering algal communities, patterns of primary production, and organic matter dynamics in a variety of freshwater habitats (Kupferberg, 1997; Flecker et al., 1999). Within trophic webs, adult frogs occupy an intermediate position and are involved in aquatic and terrestrial food webs as both prey and predator of various taxa due to their complex life history (McCoy et al., 2009). Frogs are reported as prey for various vertebrates and invertebrates, but also as predators of invertebrates and some vertebrate species (e.g., small lizards, snakes, birds, mammals, and other anurans) (Toledo et al., 2007).

Green frogs are semi-aquatic ambush ("sit and wait") predators (Moore and Biewener, 2015) that feed both during the day and at night (Cogălniceanu et al., 2000). The success of this strategy depends on factors such as prey density, prey mobility, and energy requirements of the predator (Moore and Biewener, 2015). According to the requirements of optimal foraging theory, animals

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with such a strategy must be generalists across the spectrum and opportunists in prey selection to maximize energy intake per unit of time (Pianka, 2000; Glaudas et al., 2019).

Information about dietary habits and trophic interactions are the key determinants of the structure and dynamics of ecological niches in coexisting species (Lunghi et al., 2022). They are necessary for basic understanding of the life history of anurans and the fluctuation patterns of their populations (Anderson et al., 1999), for identifying environmental conditions and habitat changes (Batista et al., 2011), and for designing and implementing management and conservation strategies for species that require protection (Stuart et al., 2004). Although some species among anurans are considered specialists (Simon and Toft, 1991), frogs are mainly considered generalists and opportunists (e.g., ÇiÇek and Mermer, 2007; Almeida-Gomes et al., 2007). In generalist species, aspects such as phylogeny, foraging mode, prey availability and abundance, and morphological constraints to capture and ingest a particular type of prey, may be related to resource partitioning among species (Lima, 1998; Vignoli et al., 2009). In addition, community niche micro-differentiation of both frog taxa and prey may be influenced by anthropogenic activities that can affect ecological interactions (Albrech and Gotelli, 2001).

Serbia is populated by all three taxa of the *Pelophylax esculentus* complex: *Pelophylax ridibundus* (*P. rid*), *Pelophylax lessonae* (*P. les*), and their hybrid *Pelophylax esculentus* (*P. esc*). Throughout its range, mixed populations in which all three taxa of the *P. esculentus* complex coexist in the same locality are generally rare ("REL population systems", named after the initial letters of the taxa that compose them) (Suriadna et al., 2020). In Serbia, these population systems are detected in a few areas along the Danube (Krizmanić and Ivanović, 2010). We believe that REL population systems provide the opportunity to study all three taxa living in syntopy and sharing the same resources. Although they are likely to have similar ecological needs, they should have measurable differences in resource use that reduce the possibility and/or extent of competition between taxa and thus promote coexistence (Costa-Pereira et al., 2019). Before the clarification of the taxonomic status of green frogs (Berger, 1973) and the advent of molecular identification techniques, field identification of taxa in this complex was difficult (see Breka et al., 2020). We note that most trophic studies treated green frogs as a single unit without considering possible dietary differentiation within the complex (e.g., Lőw and Török 1998; Cogălniceanu et al., 2000). Trophic studies on green frogs have mostly been reported for populations with a single taxon (e.g., Šimić et al., 1992; Mollov, 2008; Sas et al, 2009), while only a few have reported on diet in mixed populations where at least one parental species coexists with the hybrid taxon - LE or RE population systems (Sas et al., 2007; Ferenti et al., 2009), and a few on diet in REL systems (Popović et al., 1992; Paunović et al., 2010; Karaica et al., 2016). More recently, field studies on green frogs have been conducted on population systems based on inter-taxon differences, especially when considering implications for effective conservation and management of altered aquatic and wetland habitats (Jośko and Pabijan, 2020).

The aim of the present study was to (1) provide the necessary data for an overview of the diet composition of the *P. esculentus* complex in Serbia in REL population systems; (2) determine the dietary pattern and niche breadth for each taxon at three sites with different habitat quality and anthropogenic pressure; (3) determine whether there are niche shifts in the composition of the staple diet and/or dietary partitioning between three taxa in syntopic population systems of green frogs.

MATERIALS AND METHODS

Study area

Frogs were collected in three consecutive years (2016-2018) at three sites in the South Banat district, Serbia (Fig. 1). The sampling sites were selected according to the confirmed presence of the REL system (Krizmanić and Ivanović, 2010) and represent typical habitats for water frogs, but with different levels of preserved natural features and anthropogenic activities and pressures: 1) Stevanove ravnice (STR) (44°49'57.8"N 21°18'33.1"E) is an alluvial plain of the Danube within the special nature reserve "Deliblatska peščara" (Deliblato Sands). Here, underground waters form semi-permanent pools along the sandy plains. During high water or floods, these pools merge into larger water bodies that are connected to the Danube. The pools are overgrown with helophytes, which form extensive reed belts. The area is traditionally used as pastureland, but has retained its characteristic structure and composition of a mosaic landscape characterised by an alternation of steppe and hygrophyte vegetation depending on the microtopography and water level. The frogs were collected in the wet meadows between the ponds. 2) The Danube-Tisa-Danube Canal (DTD) (44°51'14.4"N 21°18'17.8"E) is an artificial, man-made structure in which natural aquatic vegetation only grows in a very narrow belt along the shoreline. Frogs have been collected along the canal in the macrophyte beds and the canal embankment. The embankment is a popular picnic, recreation, camping, and fishing spot for locals

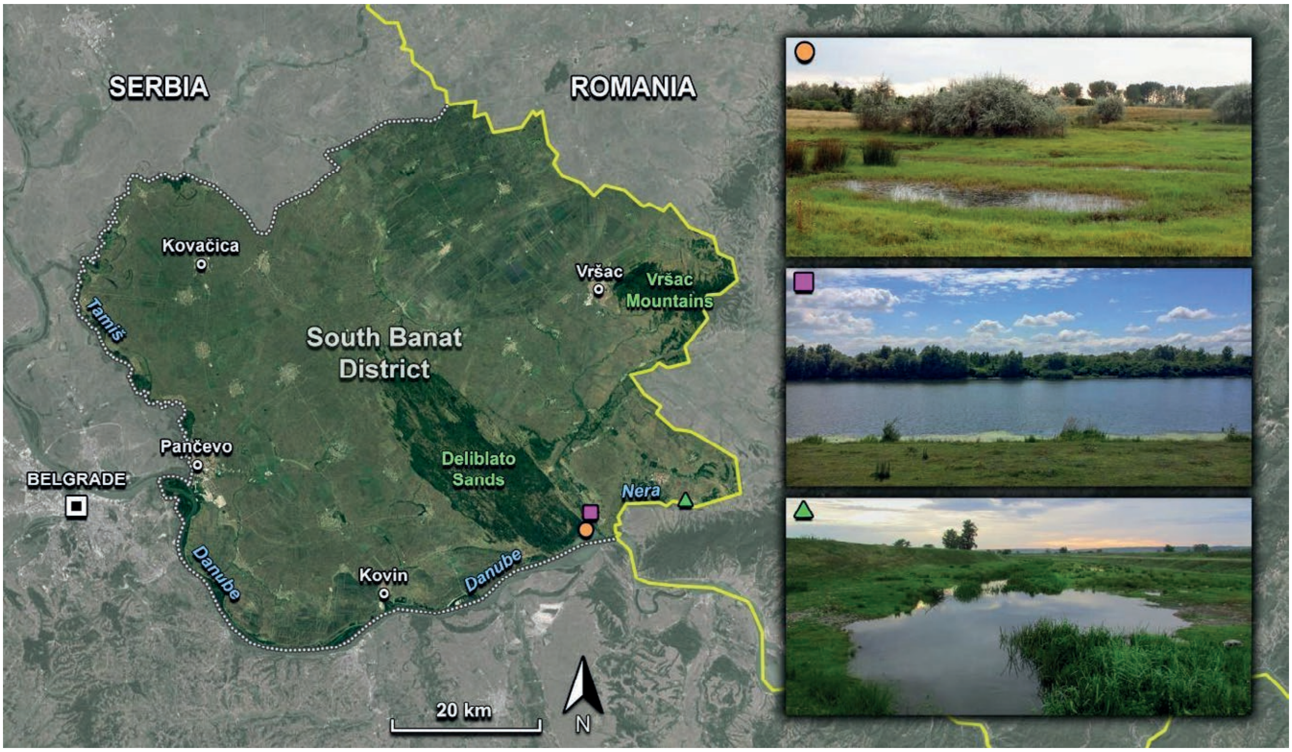

Fig. 1. Map of the South Banat district (dotted border) in northern Serbia showing three sites where green frogs (*P. esculentus* complex) were sampled: STR (orange circle), DTD (pink square) and JRG (green triangle).

and tourists with planned management activities. 3) Jaruga (JR) is a canal on the edge of the outstanding natural landscape area "Karaš-Nera" near the village of Kusić (44°52'30.8"N 21°28'16.0"E). It was built to regulate the flooding of the Nera River and to ameliorate the surrounding agricultural land, but it has lost its main purpose and is no longer used. The canal is surrounded on one side by a mosaic of agricultural fields and orchards and on the other by floodplain vegetation and abandoned gravel pits. When the Nera is at low water, which is the case for several months of the year, the canal has a low water flow. This allows the formation of dense macrophyte beds, which consist mainly of underwater plants. Frogs were collected within the canal where access was possible and in the floodplain meadows along the canal.

Data collection

Frogs were sampled at night after the breeding season from May to October using dip nets and then transported to a field laboratory for further processing. Frogs were identified to taxon level according to Breka et al., (2020). Individuals with a snout-vent length < 55 mm (*P. ridibundus*) or < 45 mm (*P. esculentus, P. lessonae*) were considered juveniles and not included in further analyses (Mikulíček et al., 2014).

The prey samples were extracted from the stomach contents using the stomach flushing method (Solé and Rödder, 2010), which allows to obtain residuals of the last foraging activity without sacrificing the individual. Due to its ability to digest food very quickly, the stomach of each frog was flushed immediately (within one hour) after capture to minimise the time between frog capture and prey extraction. Stomach contents were stored individually and preserved in vials containing 70% ethanol. Individual prey items were identified to the lowest taxonomic level based on their digestive status. After stomach flushing, all frogs were released near their capture site and checked to ensure they behaved normally.

The identification of prey taxa was carried out at the Institute of Zoology, University of Belgrade - Faculty of Biology, Serbia, using published key references (Nilsson, 1996; Nartshuk, 2003; Horsák et al., 2013; Brock, 2017; Araneae, version 03.2022) and adopting the current nomenclature (Fauna Europaea version 2.4, 2011). The length and width of intact prey were measured using a digital calliper, while the lengths of incomplete prey were estimated using known proportional ratios of body parts (Chinery, 1993). Prey volume was estimated from the volume of a prolate spheroid (Quiroga et al., 2009). We considered stomach contents as a random sample of food selected by an individual over an unspecified period of time prior to capture.

Data analysis

All identified prey were categorised into 17 broader categories ("prey categories"). They were assigned to categories defined by their ecomorphological characteristics to maximise clarity of biological interpretation and variation in diet composition (Vignoli et al., 2009).

To identify differences in diet composition between taxa and localities, we used a non-parametric similarity analysis (ANOSIM using Euclidean distance with N = 9,999 permutations) with pairwise comparisons based on a step-down sequential Bonferroni procedure. Variations in diet were compared between taxa and localities using a repeated measure permutational multivariate analysis of variance (PERMANOVA, with N = 9,999 permutations of the Euclidean distance matrix). SIMPER analysis was used to identify the specific prey group contributing the most to the observed differences and the possible existence of niche shifts. All tests were performed using the software programme PAST (Paleontological Statistics Software Programme, Ver. 4.13 Hammer et al., 2001).

For further analysis, an explanatory matrix was created in which each frog was assigned to a specific combination of locality and taxon (loc_tax): STR*Esc*, STR*Rid*, STR*Les*, DTD*Esc*, DTD*Rid*, DTD*Les*, JRG*Esc*, JRG*Rid* and JRG*Les*.

The evenness and richness of prey categories were estimated using the Simpson index (D') and Simpson dominance index (D), which are used in studies of niche differentiation as indices of specialised measures of niche width and can provide insights into details of trophic ecology (Krebs, 2014). All indices are given according to the notation in Hammer et al., (2001). The analysis was performed in the diversity module of PAST, with the options "unbiased" and "use ACE for S" selected. The ACE richness estimator corrects for the rare occurrence of food items and, in our case, the effects of prey sample size (see: Gotelli and Colwell 2011, Hammer et al., 2001). The standardised Levin's Index (B_A), as proposed by Hurlbert (1978), was used to calculate niche width values for each loc_tax combination using Ecological Methodology software, version 7.4 (Kenney and Krebs, 2003).

The available data were further analysed using multivariate statistical analysis. Following the suggestion of Leps and Smilauer (2003), we chose linear redundancy analysis (RDA), a multivariate direct gradient analysis (ter Braak and Prentice, 1988). It can be seen as a constrained form of PCA in which the ordination of the variables of interest is constrained by linear combinations of external explanatory variables. This allows us to assess how much of the variation in the structure of a multivariate data set (e.g., species composition, in our case diet composition) is explained (i.e., predicted) *a priori* by one or more independent external variables (Legendre and Legendre, 1998). We chose this procedure because we opted for a method that allows explicit prediction of food composition determination rather than exploratory post hoc interpretation. The resulting RDA ordination plot is presented as a triplot with the following symbology: 1) prey categories are shown as arrows (17 elements); 2) the three frog taxa and three sampling sites are shown as symbols (six in total); and 3) additionally, the centroids of the individual frogs sampled at each site are shown (nine in total). In this representation, the distance between symbols approximates the average dissimilarity of prey composition as measured by their Euclidean distances. These distances can be considered proportional to their trophic overlap. The scaling of loadings and scores (prey categories and frog taxa, localities, and individual frogs in our case) focused on standardised prey category scores and a scaling that preserves the relative distances between cases. The prey category arrows point in the direction of the steepest increase in prey category abundance in the samples. The length of a diet category arrow together with the angle with respect to an axis also indicates the relative contribution of that category to the axes shown in the triplot as well as the dominance and contribution of each diet category to the extracted gradients. The angle between the arrows indicates the approximate correlation between the dietary categories: The approximate correlation is positive when the angle is sharp and negative when the angle is more than 90 degrees. The projected orthogonal distance of the loc_tax symbols on the arrows of the dietary categories indicates the relative importance of this category in this sample. Ordination analysis was performed with CANOCO 5.15 (ter Braak and Smilauer, 2002). The significance of the two canonical axes was tested using a permutation test. Additional post-hoc tests (t-test with XLSTAT, ver. 3.1, 2021) were performed for the taxa and site assessment scores to confirm the trends observed by visual interpretation of the resulting ordinations. For all analyses, $P < 0.05$ was set as the significance level.

RESULTS

We captured 317 adult green frogs and obtained diet samples from 221 frogs. The breakdown of samples by location and frog taxa is given in Table 1. Empty stom-

Table 1. Breakdown of the total number of frogs caught by taxon and location (abbreviations are given in the text).

Locality/Taxon	*P. rid*	*P. les*	*P. esc*	Total
STR	46	11	129	186
DTD	11	9	53	73
JRG	6	9	43	58
Total	63	29	225	317

achs were present in 78 frogs (24.6%) and were excluded from further analysis. The taxon with the highest percentage of empty stomachs was *P. ridibundus* (30%), while this percentage was slightly lower in the other two taxa (18%). The site with the highest percentage of empty stomachs was STR (31%), while DTD and JRG had a lower percentage of empty stomachs (18% and 14%, respectively). Plant material (small leaves, seeds, lentils) was identified in 9 stomachs (3%) and was not included in further analysis as we considered it as unintended prey items. A further 9 stomachs (all *P.esc.*) contained unidentifiable prey items. In total, 1477 prey items were identified. The frogs' diet consisted mainly of insects (88%), while the remaining 12% consisted of other small invertebrates – spiders, crustaceans, molluscs (snails), and vertebrates. Insect larvae made up 20% of the total prey. In all three frog taxa, the most frequently observed prey groups were typical ground-dwelling terrestrial invertebrates, e.g., ants, ground beetles, weevils, caterpillars, and spiders (50%), followed by aerial groups (e.g., midges, flying ants and leafhoppers, 36%) and aquatic prey (6.5%).

The prey categories were as follows: Gastropoda (Gas.), Clitellata (Cli.), Arachnida (Ara.), Myriapoda (Myr.), Malacostraca (Mal.), Ephemeroptera (Eph.), Odonata (Odo.), Orthoptera (Ort.), Hemiptera (Hem.), Hymenoptera (Hym.), Coleoptera (Col.), Coleoptera larvae(Col_l), Lepidoptera (Lep.), Lepidoptera larvae(Lep_l), Diptera (Dip.), Diptera larvae(Dip_l) and Vertebrata (Ver.). The diet of all three frog taxa was dominated by insect orders Hymenoptera (28%), Coleoptera (18%), Lepidoptera (17%) and Diptera (11%). Other groups were represented with proportions below 10%. Rank-abundance curves of the 17 selected prey categories are shown in Fig. 2. Prey categories that were represented with a frequency of more than 10% in the whole sample were considered as staple diet prey - Hymenoptera, Coleoptera and Lepidoptera larvae. Those represented with a frequency of 5-10% were considered common prey - Diptera, Arachnida, Hemiptera. Rare and accessory prey groups were represented by the eleven remaining

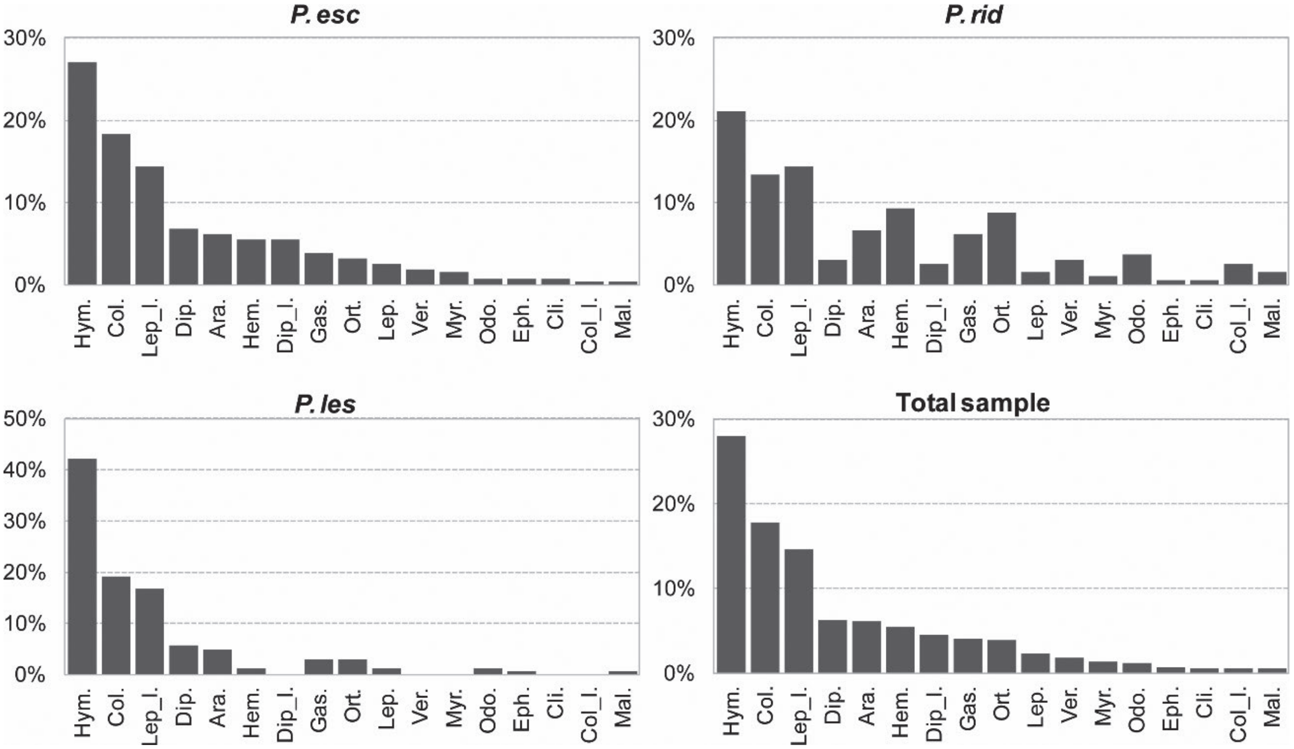

Fig. 2. Rank abundance curve for 17 selected prey categories (abbreviations are given in the text) for three taxa of green frogs. The order of the food categories for the individual species corresponds to their order in the total sample.

categories (less than 5%), e.g., snails, springtails, annelids and centipedes, and vertebrates (small fish, froglets, smaller voles). In *P. lessonae*, most prey items belonged to the dominant prey groups – Hymenoptera, Coleoptera and Lepidoptera larvae, while rare food items were present in small proportions. In contrast to *P. lessonae*, all 17 categories of prey were present in *P. ridibundus*. In addition to the predominant prey categories, *P. ridibundus* also consumed prey from other diet categories, e.g., Hemiptera, Gastropoda, Orthoptera, Odonata and Coleoptera larvae. The hybrid taxon also consumed all 17 prey categories, and as the largest number of frogs analysed were identified as *P. esculentus*, this influenced the pattern of the overall rank abundance curve. Rare prey categories were present in the diet of the hybrid taxon, but in lower proportions than in *P. ridibundus*.

The rank abundance curves for all three sites are shown in Fig. 3. The site STR was the only one where all 17 prey categories were present. Compared to the rank abundance curves of the total sample, there are some important differences: the most dominant prey categories were different, while some prey categories that are considered staple diet were hardly present (e.g. Lep_l. in the locality STR, < 2%). At the JRG and DTD sites, rare and accessory prey categories were represented by less than 2%. We note that a slightly larger proportion of Orthoptera and Lepidoptera were present in the DTD locality (11%) and Vertebrata in the STR locality (3%) in relation to the total sample.

Overall, individuals from the *P. esculentus* complex consumed 6.75 ± 0.58 (mean ± SE) prey items with an average length of 10.39 ± 0.23 mm and a volume of 830.86 ± 62.76 mm^3. The largest average prey length and volume were found in *P. ridibundus* (12.24 ± 0.65 mm and 1515.58 ± 257.1 mm^3, respectively) and the smallest in *P. lessonae* (9.07 ± 0.58 mm and 533.31 ± 91.62 mm^3, respectively). However, *P. ridibundus* consumed fewer individual prey items (4.97 ± 5.81), while *P. lessonae* consumed smaller prey items per stomach in greater numbers (7.43 ± 11.14). This is consistent with the body size pattern of these species. The breakdown of basic statistics and prey measurements in the total sample by taxon is shown in Table 2.

ANOSIM identified a weak but significant difference in diet between localities (R = 0.1, P < 0.01), but not between taxa (R = -0.03, P = 0.72) (Fig. 4). Subsequent pairwise comparisons showed that each locality differed significantly from all others (Table S1). In addition, SIMPER analysis showed that the overall dissimilarity between localities was 85.74% and 3 of the total 17 prey categories (Coleoptera, Hymenoptera and Lepidoptera larvae) yielded more than 50.65% of cumulative dissimilarity (Table S2). PERMANOVA analysis supported these results, finding a significant effect of locality (F = 58.44, P < 0.01) but not between taxa (F = 0.68, P = 0.65) and the interaction between these two factors (F = 0.94, P = 0.45).

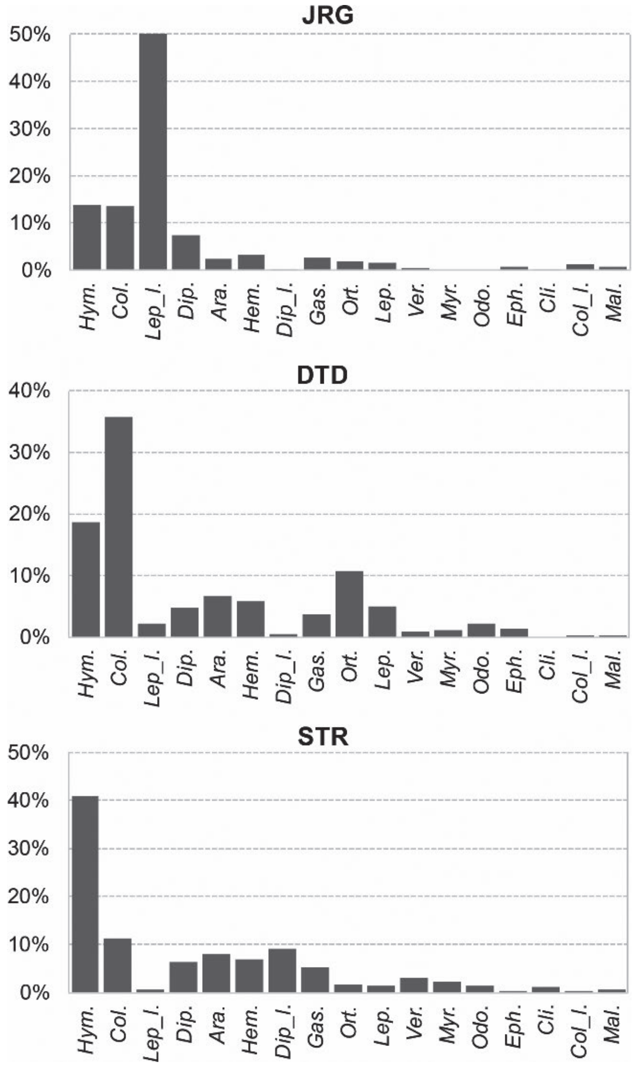

Fig. 3. Rank abundance curve for three localities (abbreviations are given in the text).

The estimates of prey diversity and niche width for the nine loc_taxa groups are shown in Table 3. Among the sites, the highest number of prey categories is documented at site STR (S = 17), followed by DTD (S = 16), while the site with the lowest number of prey categories is JRG (S = 15). In general, the lowest diversity of frog prey was found in frogs at site JRG, which was accompanied by correspondingly higher values of dominance.

In terms of trophic niche width, the widest Levins-standardised niche width for all sites was found in *P. ridibundus* (B_A = 0.489), while the narrowest niche width

Table 2. Basic statistics of green frogs and their stomach contents by taxon

	P. rid ($n = 63$)	*P. les* ($n = 29$)	*P. esc* ($n = 225$)
Average body size of frogs (mm)	75.8 ± 1.32	69.06 ± 1.48	72.62 ± 0.71
Average number of consumed prey items per stomach	4.97 ± 5.81	7.43 ± 11.14	6.95 ± 8.70
Average length of the consumed prey items (mm)	12.24 ± 0.65	9.07 ± 0.58	10.26 ± 0.26
Average volume of the consumed prey items (mm^3)	1515.58 ± 257.1	533.31 ± 91.62	757.27 ± 68.1

Table 3. Summary of frogs' diet diversity indices and niche breadth

Loc_tax	STR*Rid*	DTD*Rid*	JRG*Rid*	STR*Les*	DTD*Les*	JRG*Les*	STR*Esc*	DTD*Esc*	JRG*Esc*
Number of prey categories (S)	16	11	9	7	11	8	17	14	15
Prey abundance in sample (n)	99	43	52	73	40	48	537	277	308
Simpson Index (D')	0.86	0.83	0.69	0.40	0.76	0.64	0.81	0.80	0.71
Simpson's dominance index (D)	0.15	0.17	0.31	0.60	0.24	0.36	0.19	0.20	0.29
Levins' standardized measure of trophic niche breadth (B_A)	0.3	0.37	0.14	0.04	0.2	0.11	0.28	0.25	0.15

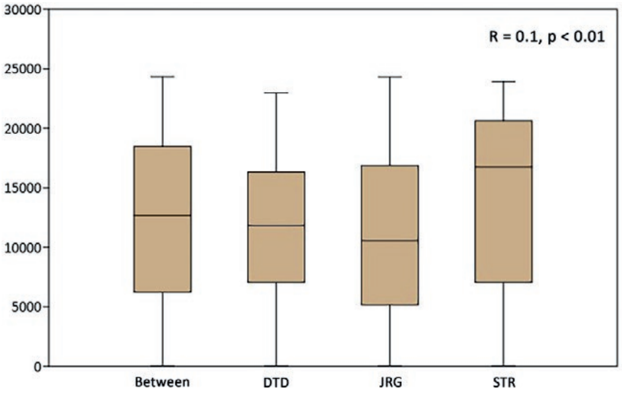

Fig. 4. Box whisker plot of the ANOSIM analysis comparing the diet of frogs at three different localities. Boxes indicates values from 25th (bottom) to 75th (top) percentile; horizontal black line indicates the median.

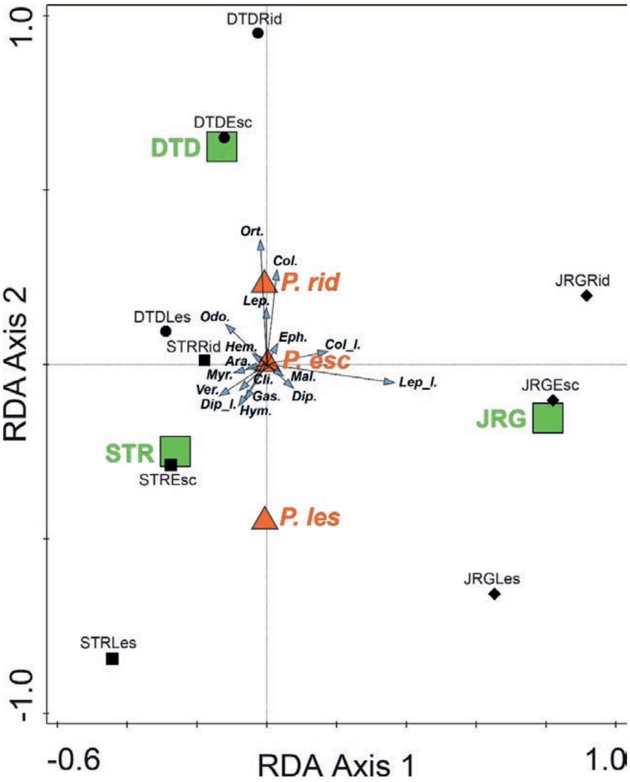

Fig. 5. Redundancy analysis (RDA) triplot of standardised taxon/locality scores derived from correlation matrices of 17 prey categories analysed (abbreviations as in text). The scaling preserves the distances between the loc_tax combinations, the angles between the vectors indicate the correlation, the length of the arrows the dominance.

(B_A = 0.186) was found in the second parental species *P. lessonae* at all three sites. The trophic niche width values for the hybrid taxon *P. esculentus* were between the values of the parental species (B_A = 0.365). The observed differences in the standardised Levin's measure of niche width between loc_tax were not statistically significant.

The RDA triplot is shown in Fig. 5. The first RDA axis explains 4.16% of the variance in Euclidean distance, and the proportion increases to 5.38% when the second axis is added. About 50% of the unconstrained ordination is explained by the constrained axes, while the first two axes of the unconstrained ordination were able to extract 17% of the variance in individual frog diet composition.

As observed, frog taxa and sites are arranged such that the first RDA axis separates the JRG site from DTD and STR (two-tailed t-test of ordination values: $t_{219} = 73.25$, $P < 0.01$). The second RDA axis separates two parental taxa, with *P. ridibundus* being positive and *P. lessonae* being negative (two-tailed t-test of ordination scores: $t_{57} = -6.31$, $P < 0.01$). The hybrid taxon lies approximately in the middle of the ordination. Moreover, the second axis separates the DTD locality from the other two localities (two-tailed t-test of ordination values: $t_{219} = 26.37$, $P < 0.01$). The RDA with the two extracted gradients was statistically significant ($F = 3.4$, $P < 0.01$).

DISCUSSION

The frogs of the *P. esculentus* complex were found to feed on different invertebrates, especially ground-dwelling arthropods and aerial insect groups. Aquatic prey made up only a small proportion of the total prey, suggesting that the green frogs feed mainly on land or wait for terrestrial prey to come close to the water ("sit and wait" strategy). According to our data, Hymenoptera, Coleoptera, and Lepidoptera predominate in the diet of all three frog taxa of the complex. A comparison of our results with the only published data on the diet of the green frog in a REL system from Serbia, reported by Paunović et al., (2010), showed a similar dietary spectrum, albeit with differences in the proportions of prey. In their study, the dominant prey groups were Coleoptera, Hymenoptera, and Gastropoda (each with a frequency of > 30%). In our study Gastropoda were more abundant in the diet only in the STR locality (5%), a marshy locality with similar characteristics to the locality in Paunović et al., (2010). Insect larvae were less present in Paunović et al., (2010) (11%) than in our study (20%). In both studies, no vertebrate prey was observed in *P. lessonae*, while in the other two species the proportion of vertebrate prey was low (1 – 3%). Our results also agree with those of Karaica et al., (2016) from REL population systems in the north-western part of Croatia, where the predominant prey groups were Coleoptera, Diptera, and Hymenoptera. In both studies, a higher proportion of aquatic prey was observed in *P. ridibundus* than in the other two taxa. In contrast to our results, no vertebrate prey was observed, and insect larvae were present in a much lower proportion (1%). The diet composition of the frogs of the *P. esculentus* complex from Serbia was similar to that observed in populations of *P. ridibundus* in Turkey (ÇiÇek and Mermer, 2006; ÇiÇek and Mermer, 2007), Bulgaria (Mollov, 2006; Mollov et al., 2010) and Russia (Ruchin and Ryzhov, 2002), and to that in a mixed LE population system in Romania (Sas et al., 2007).

According to studies on the diet of green frogs, arthropods accounted for 90.1-97.3% of the total prey in different population systems (Cogălniceanu et al., 2000; Ruchin and Ryzhov, 2002; ÇiÇek and Mermer, 2007; Rakojević et al., 2022), which is consistent with the results of our study (92.4%). Most previous studies show that green frogs feed predominantly on terrestrial prey (e.g. ÇiÇek and Mermer, 2007; Karaica et al., 2016; Rakojević et al., 2022), which is consistent with our results (93.46%), with the largest proportion of aquatic prey found in *P. ridibundus* (15.46%) and the smallest in *P. lessonae* (1.90%). However, according to Ruchin and Ryzhov (2002), who based their results on a single taxon population (*P. ridibundus*), the frogs consumed aquatic rather than terrestrial prey. This phenomenon is generally not confirmed in other studies. Adult forms of invertebrates dominated the diet of green frogs in our sample (79.83%), which is consistent with previous studies (e.g. ÇiÇek and Mermer, 2007; Paunović et al., 2010). This suggests that members of the *P. esculentus* complex generally seize more active prey, as adult invertebrates are generally more active than their larvae. All three taxa had an almost equal proportion of larvae in their diet (about 20%), although interestingly a high dominance of caterpillars (mainly moth larvae of the family Geometridae) was recorded in the JRG locality (as much as 51.57% of the total prey, while in other localities this percentage was much lower 10.44% in STR and only 3.9% in DTD). The abundance of caterpillars in the diet of green frogs is probably not the result of their preference, but the result of the abundance of caterpillars at the particular site, especially in samples collected in late summer/early autumn. Although this result is not the subject of this article, it could indicate a possible phenological effect specific to that site. However, this hypothesis needs to be further confirmed.

Vertebrates were "on the menu" of *P. esculentus* and *P. ridibundus* at all three sites, with the largest proportion found at STR. The most numerous vertebrate prey were newly-metamorphosed frogs from the *P. esculentus* complex (besides small fish and voles). The STR site with the highest percentage of cannibalism (3%) was also the site with the highest percentage of empty stomachs. Intraspecific predation has been previously documented in green frogs as a mechanism that enhances the survival of the individual under certain conditions such as drought, lower food availability, etc. (Crump, 1992; Çiçek and Mermer, 2007). Since in our study vertebrate prey was mainly present in autumn, the possibility of a phenological effect of prey preference cannot be completely excluded. However, we consider it more likely that an explanation for the observed phenomena is related to a relative prey deficit associated with season and/or location.

The diet of all three green frog taxa is dominated by the same prey categories, which means that there is no difference in the staple diet between the taxa. However, in the diet of the parental species there is a difference in terms of rare and accessory (marginal) prey, especially those that are present in the diet of *P. ridibundus*, while they are almost absent in the diet of *P. lessonae*. In the diet of the hybrid taxon, most of these prey categories were present, but in smaller or negligible proportions. These marginal prey categories are mostly represented by larger prey such as Odonata, Gastropoda and Orthoptera, and various species of vertebrates. This could be due to the fact that *P. ridibundus* frogs are larger and can therefore consume larger prey, whereas *P. lessonae* frogs fulfill their nutritional requirements with smaller prey, but in larger numbers (Löw and Török, 1998). This could also indicate a tendency of smaller green frog taxa to concentrate on fewer prey categories when foraging and would explain the dominance of only three prey categories in the diet of *P. lessonae*. Each of the dominant prey categories was most common in different locations. These differences in the dominance of prey at all three sites can be explained by differences in the type and configuration of the habitats. As the STR and JRG sites are floodplains under the strong influence of the surrounding rivers (Danube and Nera), the presence of hydrophilic and aerial categories was to be expected. The orchards surrounding the JRG site may also have had an influence on the high dominance of moth larvae. At the DTD site, a deforested artificial embankment planted with clover influenced the dominance of epigeobiont species.

The RDA showed a high variance in the diet of the individual frogs. This is not an unexpected result, as frogs are known to be opportunistic feeders, from which we conclude that there should be a large heterogeneity in diet between individual frogs. The RDA revealed a differentiation between the localities according to the dominance of certain prey categories. In the JRG locality, for example, Lepidoptera larvae had the largest contribution, in the DTD Coleoptera and Orthoptera stood out, while in the STR locality only Vertebrata and Hymenoptera had relevant loadings. A very high dominance of a single prey category (e.g. Lepidoptera larvae in JRG) indicates a lower diversity at this site, which is confirmed by the values of the diversity indices. At STR, on the other hand, the arrows of the prey categories were about the same length, which, together with the values of the diversity indices, indicates higher prey diversity. The "swarm" of arrows pointing to a sampling site is an indicator of the diversity of the food composition of that sample and consequently may indicate higher trophic production/availability at that site. According to the results of the multivariate analyses, the frog taxa are well separated both in ordination and at specific sites that differ in diet. For each locality, the position of each frog score corresponds to the position of the respective taxon on the second axis. The position of the centroids of the hybrid taxon was always close to the locality score, whereas the centroids of the parental species were always above (*P. ridibundus*) or below (*P. lessonae*) the locality score, reflecting their relative position on the secondary gradient. We note that the ordination of taxa on the secondary gradient corresponds to the ordination of the localities on the same gradient (P. ridibundus and the DTD locality have higher positive scores on the second axis, P. lessonae and the STR and JRG have negative scores on this axis). Furthermore, *P. lessonae* had the lowest values for prey diversity (as well as the highest dominance of certain prey categories) and Levins' standardised niche breadth. We conclude that *P. lessonae* has the narrowest trophic niche among the three taxa, which contradicts previous studies in mixed population systems where the hybrid taxon had the narrowest niche (Paunović et al., 2010; Karaica et al., 2016). Trophic diversity for the other two taxa was approximately the same at all three sites, with the lowest values at the JRG site and the highest at the STR site. The positions of their centroids on the RDA plot support this result. The direct gradient analysis presented in this study provides further insight into these patterns. Since the gradient extraction in RDA is hierarchical, we can interpret the two gradients as follows: 1) the first dominant gradient, predicting site position, is an environmental gradient related to habitat quality, defining the differential trophic availability of potential food components specific to a site through differences in secondary production; 2) the second, subdominant gradient predicting species position is a taxon-specific gradient separating parental species from hybrids. It probably follows the characteristics of their life form and differences, especially between parental species – different details of foraging strategy, size-related prey acquisition, etc.

The success of the "sit-and-wait" predation strategy depends, among other conditions (high mobility of prey and low energy requirements of the predator), on a fairly high prey density (Moore and Biewener, 2015). A high proportion of certain taxa in the diet could therefore also be due to the fact that green frogs, as "sit-and-wait" predators, compensate for the rarity or low specific mass of the prey taken by the number of individuals taken (Löw and Török, 1998). This would also suggest that prey items are acquired in proportion to their dominance in the immediate environment. We hypothesise that differences in the prey composition of all three taxa in the study area are influenced by differences in the local availability of prey groups, possibly resulting from differences in habitats and their secondary production. A review of the diet composition of the *P. esculentus* complex across its range revealed that some dominant taxonomic prey groups were consistent, but other prey categories varied in abundance. This suggests that the diet of frogs of the *P. esculentus* complex is determined by prey availability and habitat characteristics rather than active selection. Consequently, niche shifts among localities and variations in prey composition are to be expected, as green frogs from the *P. esculentus* complex can be characterised as non-selective predators that show a generalist feeding habit and consume prey according to their body size and other morphological features such as the mouth gap (Mollov, 2008).

In summary, the diet of all three taxa of the *P. esculentus* complex is diverse but regionally specific. There is no difference in the staple diet between the taxa of the complex, but in the less frequent and rare prey categories, especially in the parental species *P. ridibundus* and *P. lessonae*. Understanding feeding habits and trophic ecology is of particular importance for syntopic and marginal populations of the complex and for understanding the details of co-occurrence in human-modified landscapes. This is particularly important for *P. lessonae*, one of the parental species, as the southern Banat represents its southern geographical boundary. Although *P. lessonae* is classified as a Least Concern Species (LC) by the International Union for Conservation of Nature and populations are declining worldwide (Kuzmin et al., 2009), it can be considered threatened at the regional level as it is exposed to constant and increasing environmental threats throughout its range, which increase the risk of local extinction (Sjögren-Gulve, 1994; Vukov et al., 2015).

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SUPPLEMENTARY MATERIAL

Supplementary material associated with this article can be found at <http://www-9.unipv.it/webshi/appendix/index.html> manuscript number 13529

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