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Feeding ecology of the Balkan Water frog (*Pelophylax kurtmuelleri*) in Greece with emphasis on habitat effect

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Abstract. The Balkan Water frog (*Pelophylax kurtmuelleri*) is the most widespread frog in Greece, occurring also in Albania, Montenegro and Serbia and recently introduced in Italy and Denmark. However, its biology remains surprisingly understudied. Here we assess for the first time the feeding ecology of the species. We focused on two populations from Attica (central Greece), one from a natural habitat (Erasinos River) and another from an artificial landscape (Diomidous Botanical Garden). We aimed to clarify the impact of the habitat on the feeding preferences of the species, and also to examine how food availability affects food composition and selectivity. For the purposes of the study a total of 75 individuals (41 from the Botanical Garden and 34 from Erasinos River) were captured and stomach content was removed following the flushing method. We measured body weight, snout-vent length and mouth width for all captured individuals and also width and length for each prey item. Prey availability was evaluated with the quadrat counts method. The diet of *P. kurtmuelleri* followed the same general feeding pattern of other ranid species in the broader area, with insects, spiders, isopods and gastropods dominating in stomach content. The habitat had a strong impact on the feeding preferences of the species: food composition, prey frequency, size and volume differed considerably between the two populations. Niche breadth was higher in the Botanical Garden, probably as a result of the lower invertebrate diversity and abundance.

Key words: *Pelophylax kurtmuelleri*, feeding habits, anthropogenic activities, terrestrial habitats, invertebrate diversity.

Introduction

The systematics of the frog genus *Rana* remained unclear for a long time. Dubois (1992) revised the status of the group and introduced a new subgenus (*Pelophylax*) that nowadays comprises 20 species (Frost 2015). Five *Pelophylax* species are present in Greece, among which *P. kurtmuelleri* (that was distinguished from *P. ridibundus* though this separation is not unanimously accepted, see Crochet and Dubois 2004). The Balkan Water frog (or Greek Marsh frog) occurs in Greece, Albania, Montenegro and south Serbia and has been introduced in Italy and Denmark (Frost 2015; Uzell et al. 2015). In Greece it is the most widespread frog, distributed in the largest part of the mainland (with the exception of the northeastern part) and numerous islands (Valakos et al. 2008). Surprisingly, there are only very few studies regarding this species and all of them dealt with phylogeographic and bioacoustic questions (Lymberakis et al. 2007; Vervust et al. 2013; Lukanov et al. 2014). Consequently, our knowledge on the basic biology of *P. kurtmuelleri* is extremely limited (Trochet et al. 2014).

Feeding ecology of amphibians and reptiles represents a classic scientific topic that attracted early attention (Cowles 1946; Savage 1967). Food quality and availability shape animal biology and play a pivotal role in the smooth function of organisms (Bennet & Dawson 1976). As such, trophic analyses still provide valuable data and remain a hot topic in herpetological studies (Rebouças et al. 2013; Brock et al. 2014; Sagonas et al. 2015). In amphibians, food acquisition and diet content revealed the important position of the group in food webs (Duellman & Trueb 1994). Amphibians comprise a large part of the biomass in riparian ecosystems and act as energy redirectors to higher trophic levels (Burton & Likens, 1975). Therefore, it is of great importance to detect their position in food webs and to understand how habitat overall energy flows to fuel riparian communities (Çiçek 2011).

In this study we focused on two *P. kurtmuelleri* populations, one from a natural ecosystem and one from an artificial landscape, in central Greece. Our aims were (1) to assess for the first time the feeding ecology of the species, (2) to compare the impact of the habitat on the feeding preferences of the Balkan Water frog, and (3) to examine how food availability affects food composition and se-

lectivity.

Materials and methods

Both sampling sites were located in Attica, in close proximity to Athens. Samplings took place from April to June 2009 for 3 days every month (between 12:00 to 20:00). The first site was Diomidous Botanical Garden at the southeastern part of Attica (38°00'35.60" N, 23°38'46.42" E, 148 m a.s.l.). Specimens were collected from 25 artificial permanent ponds that were scattered within the Garden. The ponds were made of stone and cement, varying in dimensions and depth, and their bottom was covered by mud. Two aquatic plants dominated the ponds, the common duckweed (*Lemna minor*) and water lilies (*Iris pseudacorus, Nymphaea spp*). The vegetation around the ponds was rather patchy, comprising mainly laurel (*Laurus nobilis*), pine trees (*Pinus halepensis*), oleander (*Nerium oleander*) and ivy (*Hedera helix*). The second site was Erasinos River, which runs through the wetland of Vravrona, at the west coast of Attica (37°55'34.61" N, 23°59'52.43" E, 3.5 m a.s.l.). Sampling took place at a swamp formed by the river and at the river itself, at a short distance from the ancient Sanctuary of Artemis. The vegetation in and around the river was dense, consisting mainly of plants such as spiny rush (*Juncus acutus*), common duckweed, ditch-grass (*Ruppia maritime*) and lesser bulrush (*Typha angustifolia*).

Frogs were captured by net or hand and were then immediately anesthetized with an MS-222 solution. Stomach content was removed following the stomach flushing method (Solé et al. 2005). For each individual we measured body weight (W) with a digital scale (i500 Backlit Display, My Weight, accurate to 0.1 g) and snout-vent length (SUL) and mouth width (MW) with a digital caliper (Silverline 380244, accurate to 0.01 mm). After collecting stomach contents all animals were released *in situ*. Stomach contents were then preserved in 70% alcohol and identified in the laboratory to order with the use of a stereomicroscope. For each prey item we measured width and length to the nearest 0.1 mm. Volume of prey items was calculated using the formula proposed by Duhnam (1983): $V= 4/3\pi(L/2)(W/2)^2$. For prey that was partially digested we used the mean values of the intact prey of the same order.

To assess prey availability we used the quadrat counts method, which produces a good estimate within a narrow time window (Díaz & Carrascal 1991; Pafilis et al. 2013). Taking care to cover as many types of microhabitats as possible, we tossed a 1 x 1 m wooden frame from a distance of 2 m. We then collected all invertebrates contained within the quadrates, stored them in 70% alcohol and then identified them to order in the lab following the *Fauna Europea* nomenclature. A supplementary sampling was also carried out on flying invertebrates using a butterfly net. The evaluation of prey availability was made on the same day frogs were captured.

We calculated the relative abundance (%A), the frequency of occurrence (%F) and the relative volume (%V) of each prey category. Food niche breadth was evaluated with the Simpson's (S) diversity index (Begon et al. 1986). Niche overlap estimation was estimated with Pianka's (1973) index. Animals with empty stomach were excluded from the analysis. The Mann-Whitney U-test was used to compare the diet composition between the two localities. We employed t-test to compare frog size and prey size in each locality. We calculated selectivity in feeding using Ivlev's (1961) index (E). Statistical analyses were performed according to Zar (2010). All tests were performed using STATISTICA ver. 7.0.

Results

A total of 75 individuals (41 from the Botanical Garden and 34 from Erasinos River) were caught. Frogs from the Botanical Garden were smaller (t-test, SUL: t= −4.80268, p=0.000010) and lighter (t-test, W: t= −2.51733, p=0.014299) than those from Erasinos River, while they also had smaller mouth width (t-test, MW: t = −1.00033, p=0.320858) (Table 1).

We found 381 prey items (221 from the Botanical Garden and 160 from Erasinos River). Eight individuals (one from the Botanical Garden and seven from Erasinos River) had empty stomachs and were excluded from further analyses. The number of mean prey items did not differ significantly between the two sites (t = −0.4837, df=65, p=0.63) and resulted in a median number of 5.69 (range 1–17) (Table 2). The highest number of prey items (17) was found in an individual from Erasinos River. Frogs at the Botanical Garden consumed significantly smaller prey items than the Erasinos River individuals (mean length, t = −6.11, p=0.00; mean width, t = −6.94, p=0.0001) (Table 2). The comparison of the mean prey maximum length, mean minimum length, mean maximum width and mean minimum width between the two populations corroborated the above results ($p<0.05$ for all comparisons).

Insecta was the most important class in both

Table 1. Values for snout–vent length and mouth width (in mm) and body mass (in g): means±standard deviation; range. Sample size in parenthesis.

Locality	Snout-Urostyle length Mean ± S.D.; Range	Mouth Width Mean ± S.D.; Range	Weight Mean ± S.D.; Range
Botanical Garden (41)	6.33±1.21; 3.4-8.6	2.22±0.36; 1.2-2.9	34.34±16.19; 26.6-71.62
Erasinos River (34)	7.56±0.76; 5.5-9.2	2.32±0.50; 1.4-3.3	43.07±9.58; 27.1-76.6

Table 2. The number of the frogs examined, the total number of prey items, feeding intensity, mean, minimum and maximum dimension of the consumed prey, the feeding diversity (Simpson's) and niche overlap estimation (Pianka's) indices.

	Botanical Garden	Erasinos River
No of individuals	41	34
Empty stomachs %	2.4	20.59
Total number of prey items	221	160
Average no of prey / Range	5.53 / 2-14	5.92 / 1-17
Mean prey length / Range	6.56 / 1.00-30.00	10.14 / 2.00-38.50
Min. prey length / Range	3.46 / 1-6.5	4.70 / 2-10.1
Max. prey length / Range	10.67 / 3.3-30	18.19 / 4.4-38.5
Mean prey width / Range	2.00 / 0.20-5.50	3.84 / 1.20-20.20
Min. prey width / Range	0.97 / 0.2-3.2	2.16 / 1.20-10
Max. prey width / Range	3.13 / 0.6-5.5	6.44 / 2-20.2
Simpson's diversity index	9.494	5.992
Pianka's index	0.669	

locations comprising 61.16% of the diet, mostly belonging to terrestrial and flying orders (main groups: Coleoptera, Diptera and Hymenoptera). The most abundant prey orders in the stomach content were Coleoptera, Isopoda, Araneae, Hymenoptera and Diptera (Table 3). Coleoptera were the most frequently consumed prey, followed by Araneae, Diptera, Isopoda and Hymenoptera (Table 3). Taking into account the most important prey groups together with their relative volume, Coleoptera dominated in the diet, along with Isopoda and Diptera (Table 3). The limited presence of aquatic prey-items (such as Osteichthyes, Amphipoda, and tadpoles) suggests that feeding in the water occurs to a lesser extent.

The taxonomical composition of the diet differed considerably between the two localities (Mann-Whitney U-test, $U = 8.34$, $p < 0.05$). Fourteen prey categories were common in both populations, while nine taxa were found only at the Botanical Garden and seven exclusively at Erasinos River. However, these prey groups (unique to each population) were of minor importance in terms of relative abundance, frequency and volume (Table 3). The 14 more common prey taxa were much more abundant, frequent and occupied more stomach volume in both biotopes (Table 3).

We detected statistically significant differences in the abundance of the five more common groups between the two biotopes (Mann-Whitney U-test, $U = 6.25$, $p < 0.05$). The more abundant prey groups at the Botanical Garden frog stomachs were Hymenoptera, Isopoda, Hemiptera, Diptera and Araneae, whereas at Erasinos River – Coleoptera, Araneae, Isopoda and Hymenoptera (Table 3). Differences also occurred regarding the frequency of occurrence of the five most important prey groups (Mann- Whitney U-test, $U = 7.41$, $p < 0.05$). The more frequently consumed prey taxa at the Botanical Garden were Diptera, Hemiptera, Araneae, Coleoptera and Hymenoptera while at Erasinos River Coleoptera, Araneae, Isopoda, Diptera and Hymenoptera (Table 3). Isopoda and Gastropoda were the largest prey items (highest volume) found in the stomach of the Botanical Garden frogs, whereas Coleoptera, Isopoda and Diptera were the most important prey categories in volume contribution at Erasinos River (Table 3). In the case of the Botanical Garden three stomachs contained one tadpole each, indicating cannibalistic tendencies. There were no similar findings at Erasinos River.

The Simpson's index was higher in the Botanical Garden compared to Erasinos River (S=9.949 and S=5.992 respectively) (Table 2). The Pianka's index denoted a moderate diet similarity between the two populations (0.669) (Table 2).

Ivlev's index of selectivity for the five most common groups (in stomach content and the environment) indicated a preference for Araneae and a strong avoidance for Gastropoda at both sites (Table 4). Frogs at the Botanical Garden did not demonstrate any further particular preference, whereas frogs at the Erasinos River showed a preference for Coleoptera and avoidance of Hymenoptera (Table 4).

Discussion

The food preferences of *P. kurtmuelleri* conform to the general feeding patterns of other ranid species on the Balkans (Cogălniceanu et al. 2000; Çiçek & Mermer 2006; Bisa et al. 2007; Sas et al. 2007; Cicort-Lucaciu et al. 2013). Insects dominate in stomach content, whereas spiders, isopods and gastropods comprise an important part of the diet. The two populations differed in all partial prey parameters examined (composition, frequency, size and volume), indicating a clear effect of habitat on diet. Furthermore, the two populations

Table 3. The percentage abundance (A%), the frequency of occurrence (F%) and the relative volume (V%) of the consumed prey items in the stomachs of *P. kurtmuelleri* from the two populations.

	Botanical Garden			Erasinos River			Overall		
Prey Type	%A	%F	%V	%A	%F	%V	%A	%F	%V
Amphipoda	0.00	0.00	0.00	1.25	11.11	0.33	0.52	4.48	0.25
Arachnida	10.41	40.00	3.13	15.63	70.00	4.84	12.6	52.24	4.46
Acari	0.00	0.00	0.00	0.63	3.70	0.00	0.26	1.49	0.00
Araneae	10.41	40.00	3.13	15.00	66.67	4.84	12.34	50.75	4.46
Myriapoda	9.50	32.5	4.56	2.50	3.70	0.67	6.56	20.9	1.54
Chilopoda	2.71	12.50	0.24	2.50	3.70	0.67	2.62	8.96	0.58
Diplopoda	6.79	20.00	4.32	0.00	0.00	0.00	3.94	11.94	0.96
Coleoptera	8.60	37.50	1.81	33.13	74.07	49.67	18.90	52.24	39.07
Coleoptera larvae	0.45	2.50	0.00	1.88	7.41	0.26	1.05	4.48	0.20
Dermaptera	1.81	10.00	2.90	2.50	7.41	2.13	2.10	8.96	2.30
Dictyoptera	0.00	0.00	0.00	0.63	3.70	0.21	0.26	1.49	0.17
Diptera	11.76	47.50	6.08	10.00	40.74	10.71	11.02	44.78	9.69
Diptera larvae	0.00	0.00	0.00	0.63	3.70	0.01	0.26	1.49	0.01
Hemiptera	13.12	40.00	8.88	0.63	3.70	0.04	7.87	25.37	2.00
Hymenoptera	15.38	37.50	2.99	6.25	29.63	4.25	11.55	34.33	3.97
Hymenoptera larvae	0.00	0.00	0.00	0.63	3.70	0.06	0.26	1.49	0.05
Lepidoptera	0.45	2.50	0.00	1.25	7.41	0.91	0.79	4.48	0.71
Lepidoptera larvae	4.07	17.50	6.83	0.63	3.70	0.14	2.62	11.94	1.62
Odonata	0.90	5.00	0.00	2.50	14.81	4.23	1.57	8.96	3.29
Odonata larvae	0.00	0.00	0.00	1.88	7.41	1.29	0.79	2.99	1.00
Plecoptera	0.45	2.50	0.00	0.63	3.70	0.44	0.52	2.99	0.34
Ephemeroptera	0.90	5.00	0.76	0.00	0.00	0.00	0.52	2.99	0.17
Mecoptera	0.45	2.50	0.00	0.00	0.00	0.00	0.26	1.49	0.00
Orthoptera larvae	0.45	2.50	0.00	0.00	0.00	0.00	0.26	1.49	0.00
Psocoptera	0.45	2.50	0.00	0.00	0.00	0.00	0.26	1.49	0.00
Trichoptera	0.45	2.50	0.00	0.00	0.00	0.00	0.26	1.49	0.00
Diplura	0.45	2.50	0.00	0.00	0.00	0.00	0.26	1.49	0.00
Isopoda	13.12	32.50	39.94	12.50	29.63	14.44	12.86	31.34	2.90
Gastropoda	4.98	17.50	12.61	4.38	14.81	1.50	4.72	16.42	3.96
Annelida	0.45	2.50	3.74	0.00	0.00	0.00	0.26	1.49	0.82
Tadpoles *P. kurtmuelleri*	1.36	5.00	5.77	0.00	0.00	0.00	0.79	2.99	1.28
Actinopterygii	0.00	0.00	0.00	0.63	3.70	3.88	0.26	1.49	3.02

Table 4. Ivlev's selectivity index (Ei) for the five main prey taxa of *P. kurtmuelleri* in the two populations. Prey abundance (PA%) in the environment in percentage, as measured from quadrat counts method. Preferred prey taxa (Ei > 0.5).

	Botanical Garden		Erasinos River	
Prey type	PA%	Ei	PA%	Ei
Coleoptera	12.63	0.12	15.94	0.47
Gastropoda	32.03	-0.55	35.25	-0.72
Hymenoptera	22.33	0.13	25.7	-0.51
Isopoda	23.31	0.03	14.15	0.08
Araneae	7.77	0.43	3.58	0.69

showed a different degree of selectivity in regard to the available food resources in each habitat.

The frogs from the Botanical Garden were smaller and lighter than their Erasinos River kin, whereas they also had smaller mouth width (Table 1). The latter finding was reflected in the smaller prey size they consumed compared to the Erasinos River population (Table 2). Also, the intensity of feeding (number of prey individuals/stomach, Cogălniceanu et al. 2000) was higher in the case of the Erasinos River population.

Both populations fed mainly on terrestrial and flying orders while aquatic prey was underrepresented (Table 3). This scheme is rather common among ranids that principally capture their prey in the land surrounding the water-bodies where they live (Çiçek & Mermer 2007; Mollov 2008; Covaciu-Marcov et al. 2010). The limited presence of aquatic groups in the stomach of *P. kurtmuelleri* suggests hat this species forages only sporadically in the water, like other ranid frogs do (Sas et al. 2009; Çiçek 2011). Apart from this similarity though, the two populations showed considerable differences. In the Botanical Garden the food content consisted of Diptera (47.50%), Hemiptera (40%), Araneae (40.00%), Hymenoptera (37.50%)

and Coleoptera (37.50%), while the frogs from Erasinos River ate largely Coleoptera (74.07%), Araneae (66.67%) and Diptera (40.74%) (Table 3). *Pelophylax* frogs demonstrate a strong preference to Araneae and Coleoptera (Sas et al. 2007; Balint et al. 2008, 2010; Mollov et al. 2010; Bogdan et al. 2012). The somewhat lower percentage of these two prey taxa in the Botanical Garden population could reflect a more restricted habitat abundance, as in the case of Coleoptera (Table 4) or different dietary preferences. Besides the difference between the predominant prey groups, diet composition also differed between the two populations with nine and seven categories being present solely at the Botanical Garden and Erasinos River frogs respectively (Table 3).

Consideration of both abundance and frequency for the same sample offers an informative estimate of the homogeneity of feeding (Cogălniceanu et al. 2000). In Erasinos River these two metrics reached equally high values for prey taxa, indicating that this population exploits its habitat sources in an effective way. On the contrary, the Botanical Garden frogs deviated from this pattern: the prey taxa with the higher numerical abundance values (Hymenoptera, Isopoda and Hemiptera) did not receive the higher frequencies (Table 3). This means that only some individuals fed on these groups, while the majority ate other prey taxa that may be not available in high numbers, such as Diptera and Coleoptera. This could be attributed to the artificial character of the Botanical Garden and the limited diversity of the invertebrate fauna in this location.

Among the stomach findings we detected tadpoles that were consumed by three individuals in the Botanical Garden. The consumption of vertebrate prey has been repeatedly reported from *Pelophylax* frogs (Ruchin & Ryzhov 2002; Covaciu-Marcov et al. 2005; Nicolaou et al. 2014). Furthermore, cannibalism, particularly in the form of infanticide, is not uncommon in this genus (Mollov et al. 2006, 2010; Paunovic et al. 2010). Anurans may shift to cannibalism when food availability comes in short supplies or when space is limited (Petranka & Kennedy 1999; Jordan et al. 2004; Kovacs & Sas 2009). The lower resource availability in combination with the small size of the ponds (and the concomitant lack of space) in the Botanical Garden should account for this particular behavior. In the natural habitat of Erasinos River, where food was in general more abundant, frogs did not eat conspecifics.

The Simpson's index (S) revealed a broad feeding niche for both *P. kurtmuelleri* populations and falls within the range of other Balkan frogs (Covaciu-Marcov et al. 2010; Çiçek 2011; Cicort-Lucaciu et al. 2011; Bogdan et al. 2012). The higher value for the Botanical Garden frogs (Table 2) most probably reflects the pressure that the abovementioned limited food resources exert to this population. In other words, the Botanical Garden frogs have to broaden their trophic spectrum and thus achieve a higher food niche breadth.

According to our results, habitat had a clear effect on *P. kurtmuelleri* diet. The type and quality of habitat are known to influence the feeding preferences in frogs (Ferenti & Covaciu-Marcov 2009; Ferenti et al. 2010). Moreover, anthropogenic activities have a direct impact on food preferences and populations living in artificial habitats have adopted different feeding behaviors (Cicort-Lucaciu et al. 2011). The environment at the Botanical Garden has been shaped by humans and represents an assortment of plant species in uneven communities. That fact had an impact on stomach content. For instance, frogs deriving from ponds that were closer to flower gardens consumed more flying insects. Anthropogenic habitats differ considerably from the natural ones and affect amphibian populations (Vallan 2002; Hamer & McDonnell 2008; Covaciu-Marcov et al. 2011). Further research that will focus on such habitats will elucidate the extent and the nature of similar interferences.

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