# Diet composition of the Karpathos marsh frog (*Pelophylax cerigensis*): what does the most endangered frog in Europe eat?

P. Pafilis, G. Kapsalas, P. Lymberakis, D. Protopappas, K. Sotiropoulos

Pafilis, P., Kapsalas, G., Lymberakis, P., Protopappas, D., Sotiropoulos, K., 2019. Diet composition of the Karpathos marsh frog (*Pelophylax cerigensis*): what does the most endangered frog in Europe eat? *Animal Biodiversity and Conservation*, 42.1: 1–8.

**Abstract**

*Diet composition of the Karpathos marsh frog (*Pelophylax cerigensis*): what does the most endangered frog in Europe eat?* The Karpathos marsh frog (*Pelophylax cerigensis*) is considered the most endangered frog in Europe. Here we assess its feeding ecology and examine 76 individuals from the two known populations using the stomach flushing method. We also measured body weight, snout–vent length, mouth width and prey width and length. *Pelophylax cerigensis* follows the feeding pattern of green frogs of the adjacent areas, with Coleoptera, Araneae, Isopoda and Hymenoptera being the main prey groups. The two populations differed in body size but had similar values of prey abundance and frequency. It seems that *P. cerigensis* follows a strict feeding strategy. Further research on prey availability in its habitats will provide valuable insight.

Key words: Diet, Endangered species, Islands, Frogs, Mediterranean

**Resumen**

*Composición de la dieta de la rana de Kárpatos (*Pelophylax cerigensis*): ¿qué come la rana más amenazada de Europa?* La rana de Kárpatos (*Pelophylax cerigensis*) es considerada la rana más amenazada de Europa. Aquí evaluamos su ecología alimentaria y examinamos 76 individuos de las dos poblaciones conocidas usando el método del lavado de estómago. También medimos el peso corporal, la longitud desde el hocico hasta la cloaca y el ancho de la boca de las ranas y el ancho y largo de las presas. La dieta de *Pelophylax cerigensis*, compuesta principalmente por Coleoptera, Aranean, Isopoda e Hymenoptera, es similar a la de otras especies de ranas verdes de las zonas adyacentes. Las dos poblaciones difieren en el tamaño corporal, pero presentan valores similares de abundancia y frecuencia de presas. Parece que *P. cerigensis* sigue una estricta estrategia de alimentación. El estudio de la disponibilidad de presas en sus hábitats aportará información valiosa.

Palabras clave: Dieta, Especies en peligro de extinción, Islas, Ranas, Mediterráneo

*Received: 9 XII 17; Conditional acceptance: 12 II 18; Final acceptance: 12 III 18*

*Panayiotis Pafilis, Grigoris Kapsalas, Dept. of Biology, National and Kapodistrian Univ. of Athens, Panepistimioupolis, Ilissia, 15784 Greece.– Petros Lymberakis, Natural History Museum of Crete, Univ. of Crete, Irakleio, Greece.– Dinos Protopappas, Management Body of Karpathos–Saria, Olympos, 85700 Greece.– Konstantinos Sotiropoulos, Dept. of Biological Applications and Technology, Univ. of Ioannina, Bizani, 45500 Greece.*

Corresponding author: P. Pafilis. E–mail: ppafil@biol.uoa.gr

ISSN: 1578–665 X
eISSN: 2014–928 X

## Introduction

The extensive anthropogenic activity in the last century has changed natural ecosystems and put many species at stake (Wilson, 2002; Pimm et al., 2014). Successful protection and effective conservation of threatened species require good knowledge of their overall biology (Tracy et al., 2002; Wikelski and Cooke, 2006; Bertolero and Oro, 2009). Feeding ecology is one of the most important biological parameters as it shapes numerous aspects of animal life (Vervust et al., 2010; Brown et al., 2017; Olsen, 2017). Assessing the dietary regimes of endangered animals provides important insight into the identification of critical food resources and may contribute to integrated conservation plans for many animal taxa (Palazón et al., 2008; Pagani–Núñez et al., 2011; Butler et al., 2012).

The Karpathos marsh frog (*Pelophylax cerigensis*) (Beerli et al., 1994) is endemic to the island of Karpathos, south Aegean Sea, Greece (Valakos et al., 2008). Categorized as Critically Endangered by the IUCN (Beerli et al., 2009), it is considered the most endangered anuran amphibian in Europe because its range is restricted to y two small rivers (rivulets or brooks in reality) in the north part of the island (Temple and Cox, 2009). Such small, insular wetlands are nowadays considered the most endangered ecosystems in the Mediterranean Sea, representing isolated oases for birds, amphibians, aquatic reptiles and invertebrates (Cuttelod et al., 2008). The general biology of *P. cerigensis* is largely understudied since the first description of the species (Beerli et al., 1994). Feeding ecology is a classic scientific topic in frog studies as food quality and availability reveal the important position of the group in food webs (Duellman and Trueb, 1994). Frogs represent a considerable portion of the riparian biomass and serve as energy redirectors to higher trophic levels (Burton and Likens, 1975). Thus it is important to understand where they stand in food webs and to unravel how habitat global energy fuels riparian communities (Çiçek, 2011; Bogdan et al., 2013).

*Pelophylax cerigensis* remains an unknown animal in terms of ecology, and as such, no specific protection measures have been taken so far. Here we studied the two known populations of *P. cerigensis*. We aimed to (1) assess the diet of the species for the first time, (2) examine possible differences between the two populations, and (3) compare trophic niches and food composition to those of other frogs in the Balkans.

## Material and methods

We sampled both river sites at Argoni (35.6948°N, 27.1523°E) and Nati (35.7018°N, 27.1786°E) in the northern part of Karpathos Island. Frogs were collected during late spring (last week of May 2015, 2016 and 2017; average temperature and rainfall did not differ between the three years) from small ponds along riverbeds. Fieldwork was carried out in May, as this is the time of the year when the frogs are easier to observe and capture on Karpathos Island. During summer and early fall, the rivulets dry out and frogs resort to the nearby vegetation, being hard to catch. On the contrary, during late fall and winter, the rivulets turn to torrents, and frogs, once more, avoid them. The landscape in general is characterized by sparse and low vegetation in a rocky background with high erosion. The vegetation around the rivers consists of tall nerium shrubs (*Nerium oleander*), sparse pine trees (*Pinus brutia*) and low shrubs such as spiny rush (*Juncus articulatus*) and thyme (*Thymbra capitata*).

Seventy–six individuals (51 from Argoni and 25 from Nati) were captured by net or hand and were anesthetized with an MS–222 solution. To anaesthetize the frogs we followed the instructions of the Amphibian Research and Monitoring Initiative (U.S. Geological Survey) (ARMI SOP No. 104–Standard Operating Procedure) for safe anesthesia (Downes, 1995). We placed the animals in a plastic water bath (2 cm deep) containing a tricaine methane sulfonate solution (50 mg/L) for 15 min. After this period, we rinsed its skin with fresh water to avoid deeper levels of anesthesia. Frogs started to recover after 10 min. For each frog we took the basic morphometric measurements: body weight (W) with a digital scale (i500 Backlit Display, My Weight, accurate to 0.1 g) and snout–vent length (SVL) and mouth width (MW) with a digital caliper (Silverline 380244, accurate to 0.01 mm). To remove stomach content we used the stomach flushing method (Solé et al., 2005). Besides being simple and effective, this method provides high quality results without sacrificing animals as it can be applied in live individuals. It is the most widely used, non–invasive technique in frogs (e.g. Lamoureux et al., 2002; Çiçek, 2011; Rebouças et al., 2013; Bogdan et el., 2013; Plitsi et al., 2016) with significantly less impact than other methods (Bondi et al., 2015) and thus it can be used even in endangered species (Bower et al., 2014; Watson et al., 2017). Holding the animal with one hand, we gently opened the mouth with a spatula and then carefully introduced the infusion tube (made of supple silicon to avoid perforations of oesophagus) of a 20 ml syringe that contained water from the pond where the frogs were captured. We flushed the content of the syringe into the stomach forcing out the consumed prey items till no more stomach content appeared. The water with stomach content was stored in a plastic glass and then decanted into a sieve. Prey items were collected with forceps and preserved in 70% alcohol in small eppendorf tubes.

After measuring the frogs and collecting stomach contents, we waited for the captured individuals to recover. We kept the frogs into a plastic bin for 30 min to ensure that all of them were in good condition. None of the individuals died during this procedure and all of them were released in their habitat after fully recovering. The personnel of the Management Body of Karpathos that regularly patrolled the river did not encounter any dead individual during the days following the measurements.

Stomach contents were preserved in 70% alcohol and then transported to the lab (Dept. of Biology, National and Kapodistrian University of Athens) where

Table 1. Values for snout–vent length (SVL) and mouth width (MW) (in cm) and body weight (BW) (in g): means ± standard deviation; range (between brackets): N, sample size.

*Tabla 1. Valores para la longitud hocico–cloaca (SVL), el ancho de la boca (MW) (en cm) y el peso corporal (BW) (en g): media ± desviación estándar; rango (entre paréntesis); N, tamaño de muestra.*

| Site | N | SVL | MW | BW |
|---|---|---|---|---|
| Argoni | 51 | 4.29 ± 0.81 (2.5–5.6) | 1.68 ± 0.36 (0.9–2.3) | 10.39 ± 5.44 (1.7–22.3) |
| Nati | 25 | 3.76 ± 0.89 (2.3–5.4) | 1.42 ± 0.38 (0.8–2.2) | 7.43 ± 4.89 (1.5–18.2) |

they were identified to order with a stereomicroscope (Wild Heerburg M38). Prey item width (W) and length (L) were measured to the nearest 0.1 mm. Following Dunham (1983), prey volume (V) was calculated using the ellipsoid volumetric formula:

$$V = 4/3\ \pi\ (L/2) \cdot (W/2)^2$$

At this point, we should point out that although this is the typical method used in similar studies, the volume of prey groups such as Diplopoda or Formicidae calculated with this approach is rather unrealistic. However, for the sake of comparison with other studies on frog feeding ecology, we apply it here as well.

For each individual whose stomach contained prey items (contrary to empty stomachs), we calculated the minimum, mean and maximum prey item width, length and volume, while also counting the total number of prey items. For every identified prey category we calculated its relative abundance (%A), frequency of occurrence (%F) and relative volume (%V), per population as well as overall.

We assessed food niche breadth using Levin's standardized index B*i* (Levins, 1968), where $p_i$ is the relative abundance of every prey category in each population:

$$Bi = 1/\ \Sigma(p_i)^2$$

Niche overlap (O) was evaluated with Pianka's (1973) index, where $p_i$ is the relative proportion of prey category *i* in each of two populations A and B:

$$O = \frac{\Sigma_i^n\ p_{iA} \cdot p_{iB}}{\Sigma_i^n\ p_{iA}{}^2 \cdot \Sigma_i^n\ p_{iB}{}^2}$$

We used *t*–tests to compare frog and prey morphometric variables between the two populations, unless the assumptions of normality (Shapiro–Wilk test, $p < 0.05$) and homogeneity of variances (Levene's test, $p < 0.05$) were violated, in which case the non–parametric Mann–Whitney test was used instead. A Mann–Whitney test was used to compare the number of prey items between the two populations. The relationship between frog body size and prey size was assessed with Pearson's product–moment correlation. In order to examine the similarity of diet composition between the two populations, $\chi^2$–tests of independence were performed on absolute and relative abundance (N, %A) and frequency of occurrence (F, %F) of the five most common overall prey categories respectively. All statistical analyses were performed according to Zar (2010) using R 3.4.2 (R Core Team, 2017).

## Results

The Argoni population consisted of larger (SVL: *t*–test, $t = 2.574$, df = 74, $p = 0.0121$) and heavier (W: *t*–test, $t = 2.301$, df = 74, $p = 0.0242$) individuals than those of Nati. Also, frogs from the latter study had smaller mouth widths (MW: *t*–test, $t = -2.943$, df = 74, $p = 0.0043$) (table 1).

Stomach analyses yielded 296 prey items (199 from Argoni and 97 from Nati). Fifteen frogs, nine from Argoni and six from Nati, had empty stomachs. The two populations did not differ in the number of prey items consumed (Mann–Whitney U = 640, $p = 0.98$). A frog from the Argoni River had the highest number of prey items in a single individual (11).

The main prey category was insects, with an overall value between the two populations reaching 64.65 %. Coleoptera and Araneae topped the list of the most abundant and the most frequently consumed prey taxa (table 2). Finally, Coleoptera occupied the largest global relative volume in frog stomachs (35 %).

Eleven prey categories were present in both sites, while six taxa were found only at Argoni and three only at Nati. These unique prey groups, however, were of minor importance as they were represented by low relative abundance and frequency in the stomachs examined (table 2).

Argoni frogs ate larger prey items than the Nati population (mean length: Mann–Whitney U = 703.5, $p < 0.0001$; mean width: Mann–Whitney U = 696, $p < 0.0001$) (table 3). When we additionally compared the mean minimum and mean maximum prey item lengths and widths, we found that the two populations differed considerably in all prey size variables (Mann–Whitney U, $p < 0.05$ for all comparisons). As a consequence, prey volume was also higher in the

Table 2. The relative abundance (A%), frequency of occurrence (F%) and relative volume (V%) of prey consumed by the two *P. cerigensis* populations.

*Tabla 2. Abundancia relativa (A%), frecuencia de presencia (F%) y volumen relativo (V%) de las presas consumidas por las dos poblaciones de* P. cerigensis.

| | Argoni River | | | Nati River | | | Overall | | |
|---|---|---|---|---|---|---|---|---|---|
| Prey type | %A | %F | %V | %A | %F | %V | %A | %F | %V |
| Araneae | 14.07 | 50.00 | 2.51 | 13.40 | 57.89 | 5.65 | 13.85 | 52.46 | 2.81 |
| Coleoptera | 29.15 | 69.05 | 38.19 | 13.40 | 52.63 | 5.09 | 23.99 | 63.93 | 35.00 |
| Coleoptera larvae | 1.01 | 2.38 | 0.08 | 1.03 | 5.26 | 1.19 | 1.01 | 3.28 | 0.19 |
| Dermaptera | 2.51 | 11.90 | 1.23 | 3.09 | 15.79 | 8.38 | 2.70 | 13.11 | 1.92 |
| Dictyoptera | 1.01 | 4.76 | 0.37 | 1.03 | 5.26 | 1.64 | 1.01 | 4.92 | 0.49 |
| Diplopoda | 2.51 | 11.90 | 0.47 | 3.09 | 15.79 | 2.78 | 2.70 | 13.11 | 0.69 |
| Diplura | 0.00 | 0.00 | 0.00 | 1.03 | 5.26 | 0.00 | 0.34 | 1.64 | 0.00 |
| Diptera | 10.05 | 42.86 | 10.32 | 8.25 | 31.58 | 3.49 | 9.46 | 39.34 | 9.66 |
| Ephemeroptera | 0.50 | 2.38 | 0.00 | 0.00 | 0.00 | 0.00 | 0.34 | 1.64 | 0.00 |
| Gasteropoda | 5.03 | 21.43 | 2.94 | 7.22 | 31.58 | 16.60 | 5.74 | 24.59 | 4.25 |
| Hemiptera | 9.05 | 28.57 | 1.46 | 15.46 | 42.11 | 8.41 | 11.15 | 32.79 | 2.13 |
| Hymenoptera | 7.04 | 21.43 | 2.84 | 18.56 | 52.63 | 11.90 | 10.81 | 31.15 | 3.72 |
| Isopoda | 12.06 | 47.62 | 11.59 | 12.37 | 42.11 | 33.92 | 12.16 | 45.90 | 13.74 |
| Lepidoptera | 1.51 | 7.14 | 17.54 | 0.00 | 0.00 | 0.00 | 1.01 | 4.92 | 15.85 |
| Lepidoptera larvae | 1.01 | 4.76 | 0.27 | 0.00 | 0.00 | 0.00 | 0.68 | 3.28 | 0.24 |
| Odonata | 1.51 | 7.14 | 1.27 | 0.00 | 0.00 | 0.00 | 1.01 | 4.92 | 1.15 |
| Odonata larvae | 1.51 | 7.14 | 1.06 | 0.00 | 0.00 | 0.00 | 1.01 | 4.92 | 0.96 |
| Orthoptera | 0.00 | 0.00 | 0.00 | 1.03 | 5.26 | 0.96 | 0.34 | 1.64 | 0.09 |
| Trichoptera | 0.00 | 0.00 | 0.00 | 1.03 | 5.26 | 0.00 | 0.34 | 1.64 | 0.00 |
| Lizards | 0.50 | 2.38 | 7.86 | 0.00 | 0.00 | 0.00 | 0.34 | 1.64 | 7.10 |

Argoni population (mean volume: Mann–Whitney U = 709, $p < 0.0001$). Overall, there was a positive correlation between frog SVL and mean prey length [r = 0.603, $t(59) = 5.81$, $p < 0.0001$], as well as frog SVL and mean prey width [r = 0.463, $t(59) = 4.02$, $p = 0.0002$].

Levin's index was lower at Argoni than Nati (B = 6.787 and B = 8.063 respectively) (table 2). According to Pianka's index, the two populations share a high diet similarity (O = 0.840) (table 3).

The most abundant prey taxa and the most frequently eaten prey items were similar in both populations (table 2). The two populations differed significantly when absolute abundance of the five most common prey groups was tested ($\chi^2 = 17.004$, df = 4, $p = 0.0019$). However, relative abundance differences were not statistically significant ($\chi^2 = 0.13$, df = 4, $p = 0.998$). No statistical differences were found between the two populations, either in absolute ($\chi^2 = 1.91$, df = 4, $p = 0.753$) or in relative ($\chi^2 = 0.07$, df = 4, $p = 0.999$) frequencies of occurrence.

Finally, a notable deviation was observed in the relative volume between the two populations: while Coleoptera were the dominant group in frog stomachs at the Argoni River (38.19 %), they reached only a small percentage (5.09 %) at Nati, where Isopoda was the first pray taxon in volume (33.92 %) (table 2).

## Discussion

Karpathos is one of the oldest islands in the eastern Mediterranean (around 8 my). This long isolation is reflected in the two endemic amphibians occurring on the island, the Karpathos Lycian salamander (*Lyciasalamandra helverseni*) and *P. cerigensis*. However, although *L. helverseni* maintains dense populations, *P. cerigensis* is known from only two sparse populations in the north of Karpathos (Lymberakis et al., 2018). Climate change strongly affects the east Mediterranean, with increasingly fewer rainfalls (Giorgi and Lionello, 2008), further exacerbating the general

Table 3. The number of examined individuals, the total number of prey items, mean dimensions of the consumed prey, and the feeding diversity (Levin's, B) and niche overlap estimation (Pianka) indices.

*Tabla 3. Número de individuos examinados, número total de presas, dimensiones medias de las presas consumidas, e índices de diversidad de la dieta (Levin, B) y de superposición de nicho (Pianka).*

| | Argoni | Nati |
|---|---|---|
| No of individuals | 51 | 25 |
| Empty stomachs (%) | 17.6 | 24 |
| Total number of prey | 199 | 97 |
| Average no of prey/range | 3.90/0–11 | 3.88/0–10 |
| Mean prey length/range | 9.43/2.62–32.00 | 5.56/1.30–25.00 |
| Mean prey width/range | 3.60/0.59–20.20 | 2.13/0.20–5.50 |
| Simpson's diversity index | 6.787 | 8.063 |
| Pianka's index | 0.840 | |

water scarcity on Karpathos. Thus, and also due to its restricted range, *P. cerigensis* faces severe risks in the immediate future. Here we present the first data on its diet. These findings should be taken into account to effectively protect the species. We should point out, however, that the most important protection task undertaken on the island should be to conserve the Karpathos environment as a whole.

The Karpathos marsh frog adopted a rather simple diet, comprising fewer prey taxa than other ranid frogs in the area (Cogălniceanu et al., 2000; Mollov et al., 2006; Bisa et al., 2007; Bogdan et al., 2013). The dominant prey groups were insects, spiders and isopods. Frogs from the two examined populations prey on the same invertebrate taxa (11 common groups), with limited differentiation between them (table 2).

A clear difference arose from the body size measurements: the frogs from Argoni were larger and heavier, and had a larger mouth width than their Nati peers (table 1). As a consequence, Argoni frogs ate larger food items despite the fact that the number of prey items per stomach was similar (Cogălniceanu et al., 2000) (table 3). Intrapopulation analysis revealed that body size also affected diet: larger frogs consumed larger prey in both populations. Argoni and Nati share the same ecological and abiotic parameters (vegetation, substrate, slope, exposure to winds and sunlight), and hence it is difficult to identify the reason underlying discrepancies in their diets. The strong relation between body and prey size may account for the differences in the taxonomy (limited though, as mentioned above) and size of the prey consumed.

Diet compositions were quite similar between the two populations, suggesting a high dietary/niche overlap (Pianka's index, O = 0.840). The reason for such a high dietary overlap should be sought in habitat similarity: the two focal populations share the same vegetation type and main abiotic characteristics. The values of Levin's index (Bi) fall within the range of other Balkan frogs (Covaciu–Marcov et al., 2010; Çiçek, 2011; Cicort–Lucaciu et al., 2011; Bogdan et al., 2012), indicating a rather broad feeding niche for the species.

The two populations did not differ in the frequency of consumed prey. Argoni frogs primarily consumed Coleoptera (76.19 %), whereas Araneae was the most frequent prey taxon in Nati (table 2). Our findings confirm previous research reporting that Coleoptera and Araneae are a typical food resource for *Pelophylax* frogs (Sas et al., 2007; Balint et al., 2010; Mollov et al., 2010; Bogdan et al., 2012; Plitsi et al., 2016). Frequency, taken together with relative abundance in stomach content, provides a reliable evaluation of feeding homogeneity (Cogălniceanu et al., 2000). In both populations, frequency and relative abundance received high values (table 2).

It is worth highlighting two particularities in the diet of *P. cerigensis*: the small percentage of aquatic taxa and the finding of a lizard tail in a single stomach from a frog in Nati. The limited consumption of aquatic prey is not a surprising feature among ranid frogs that typically seek their food in the banks of their habitats (Çiçek and Mermer, 2007; Mollov, 2008; Covaciu–Marcov et al., 2010; Çiçek, 2011). However, the particularly low portion in the *P. cerigensis* stomach content should be attributed to the physical water scarcity on the island. From April to October the rivers practically survive by small, shallow pools that cannot support a typical aquatic invertebrate fauna. Thus, frogs largely consume terrestrial and flying prey, and may include unusual food, such as the observed lizard tail. The latter belongs to the European copper skink (*Ablepharus kitaibelii*), a small lizard of the Scincidae family. Though the consumption of vertebrate prey is not uncommon within the genus *Pelophylax* (Ruchin and Ryzhov, 2002; Covaciu–Marcov et al., 2005; Çiçek and Mermer, 2006), this is only the second time an incident of saurophagy is reported (Nicolaou et al., 2014). Mediterranean insular herpetofaunas are known for extreme feeding behaviors in response to low food resources (Castilla et al., 2009; Brock et al., 2014; Cooper et al., 2015). Our finding may echo such behaviors but it is probably of an accidental nature. We stress here that our results refer only to spring. Frog diet is known to change through the seasons, depending on food availability and microhabitat use (Das, 1996; Covaciu–Marcov et al., 2005). To assess the annual diet of the species, similar work should be carried out even during the unfavorable periods of the year that were mentioned earlier.

The dramatic changes in the Miocene eastern Mediterranean and their impact on *Pelophylax* phylogenetic history have been well studied (Lymberakis et al., 2007; Akin et al., 2010; Plötner et al., 2010; Poulakakis et al., 2015). Though the feeding ecology of the mainland *Pelophylax* frogs is also well studied (Çiçek and Mermer, 2006, 2007; Sas et al., 2007; Mollov, 2008; Sas et al., 2009; Mollov et al., 2010; Bogdan et al., 2012; Plitsi et al., 2016), in striking contrast there is an intense lack of general biology studies in the case of insular species and populations. To the best of our knowledge, this is the first systematic work on the diet of an island frog in the eastern Mediterranean. The significance of the dietary profile of species that requires protection has been highlighted in a comparative frame (Pope and Matthews, 2002; Fisher and Owens, 2004; Wiens et al., 2010). Our results stress the importance of beetles and spiders as primary food sources in the focal habitats. Measurements that will attract these two prey taxa (e.g. specific plants, suitable microhabitats) will ensure the smooth energy flow to the frogs. Fresh water is a rare commodity on Mediterranean islands, directly affecting frogs' distribution and future survival (Vervust et al., 2013). The conservation of these unique populations is a demanding task of the highest priority.

## Acknowledgements

This work was fully supported by a grant from the Mohamed bin Zayed Species Conservation Fund (Project Number: 14259483). Sampling took place according to the Hellenic National Law (Presidential Decree 67/81) and under a special permit (code: 6ΨΤ04653Π8–ΥΟ3) issued by the Ministry of the Environment.

## References

Akın, Ç., Bilgin, C. C., Beerli, P., Westaway, R., Ohst, T., Litvinchuk, S. N., Uzzell, T., Bilgin, M., Hotz, H., Guex, G.–D., Plötner, J., 2010. Phylogeographic patterns of genetic diversity in eastern Mediterranean water frogs have been determined by geological processes and climate change in the Late Cenozoic. *Journal of Biogeography,* 37: 2111–2124.

Balint, N., Indrei, C., Ianc, R., Ursuţ, A., 2010. On the diet of the *Pelophylax ridibundus* (Anura, Ranidae) in Ţicleni, Romania. *South Western Journal of Horticulture, Biology and Environment,* 1: 57–66.

Beerli, P., Hotz, H., Tunner, H. G., Heppich, S., Uzzell, T., 1994. Two new water frog species form the Aegean Islands Crete and Karpathos (Amphibia, Salientia, Ranidae). *Notulae Naturae, Academy of Natural Sciences of Philadelphia*, 470: 1–9.

Beerli, P., Uzzell, T., Lymberakis, P., 2009. *Pelophylax cerigensis*. In: *The IUCN Red List of Threatened Species 2009*: e.T58567A11787309, http://dx.doi.org/10.2305/IUCN.UK.2009.RLTS.T58567A11787309.en [Accessed on 12 October 2018].

Bertolero, A., Oro, D., 2009. Conservation diagnosis of reintroducing Mediterranean pond turtles: what is wrong? *Animal Conservation,* 12: 581–591.

Bisa, R., Sfenthourakis, S., Fraguedakis–Tsolis, S., Chondropoulos, B., 2007. Population density and food analysis of *Bombina variegata* and *Rana graeca* in mountainous riverine ecosystems of northern Pindos (Greece). *Journal of Biological Research–Thessaloniki*, 8: 129–137.

Bogdan, H. V., Covaciu–Marcov, S.–D., Cupsa, D., Cicort–Lucaciu, A.–S., Sas, I., 2012. Food composition of a *Pelophylax ridibundus* (Amphibia) population from a thermal habitat in Banat region (southwestern Romania). *Acta Zoologica Bulgarica*, 64: 253–262.

Bogdan, H. V., Covaciu–Marcov, S.–D., Gaceu, O., Cicort–Lucaciu, A.–S., Ferenţi, S., Sas–Kovács, I., 2013. How do we share food? Feeding of four amphibian species from an aquatic habitat in south–western Romania. *Animal Biodiversity and Conservation*, 36: 89–99.

Bondi, C. A., Green, S., Beier, C. M., 2015. Evaluation of Gastric Lavage Method for Diet Analysis of the Eastern Red–backed Salamander (*Plethodon cinereus*). *Herpetological Review*, 46: 196–199.

Bower, D. S., Pickett, E. J., Garnham, J. I., Deboo, M. L., McCurry, M. R., Mengerink, R. M., Mahony, M. J., Clulow, J., 2014. Diet of a threatened pond frog differs over a small spatial scale. *Endangered Species Research*, 23: 93–98.

Brock, K. M., Donihue, C. M., Pafilis, P., 2014. Novel records of frugivory and ovophagy in *Podarcis* (Lacertidae) lizards from East Mediterranean Islands. *North–Western Journal of Zoology*, 10: 223–225.

Brown, G. P., Madsen, T. R. L., Shine, R., 2017. Resource availability and sexual size dimorphism: differential effects of prey abundance on the growth rates of tropical snakes. *Functional Ecology*, 31: 1592–1599.

Burton, T. M., Likens, G. E., 1975. Energy flow and nutrient cycling in salamander populations in the Hubbard Brook Experimental Forest, New Hampshire. *Ecology*, 56: 1068–1080.

Butler, D. A., Palmer, W. E., Cook, M. P., 2012. The invertebrate diet of northern bobwhite chicks in Georgia, United States. *Animal Biodiversity and Conservation*, 35: 415–418.

Castilla, A. M., Herrel, A., Gosá, A., 2009. Marine prey in the diet of *Podarcis atrata* from the Columbretes Islands. *Munibe Ciencias Naturales*, 57: 295–298.

Çiçek, K., 2011. Food composition of Uludağ frog, *Rana macrocnemis* Boulenger, 1885 in Uludağ (Bursa, Turkey). *Acta Herpetologica*, 6: 87–99.

Çiçek, K., Mermer, A., 2006. Feeding biology of the Marsh Frog, *Rana ridibunda* Pallas 1771, (Anura, Ranidae) in Turkey's Lake District. *North–Western Journal of Zoology*, 2: 57–72.

– 2007. Food composition of the Marsh Frog, *Rana ridibunda* Pallas, 1771, in Thrace. *Turkish Journal of Zoology*, 31: 83–90.

Cicort–Lucaciu, A.–Ş., Cupşa, D., Ilieş, D., Ilieş, A., Baiaş, Ş., Sas, I., 2011. Feeding of two amphibian species (*Bombina variegata* and *Pelophylax ridibundus*) from artificial habitats from Pădurea Craiului Mountains (Romania). *North–Western Journal of Zoology*, 7: 297–303.

Cogălniceanu, D., Palmer, M. W., Ciubuc, C., 2000. Feeding in anuran communities on islands in the Danube floodplain. *Amphibia–Reptilia*, 22: 1–19.

Cooper, W. E., Dimopoulos, I., Pafilis, P., 2015. Sex, age and population density affect aggressive behaviors in island lizards promoting cannibalism. *Ethology*, 121: 260–269.

Covaciu–Marcov, S.–D., Cupşa, D., Ferenţi, S., David, A., Dimancea, N., 2010. Human influence or natural differentiation in food composition of four Amphibian species from Histria Fortress, Romania? *Acta Zoologica Bulgarica*, 62: 307–313.

Covaciu–Marcov, S.–D., Sas, I., Cupşa, D., Bogdan, H., Lukács, J., 2005. The seasonal variation of the food of a non–hibernated *Rana ridibunda* Pallas 1771 population from the thermal lake from 1 Mai Spa, Romania. *Analele Universitătii din Oradea, Fascicula Biologie*, 12: 77–85.

Cuttelod, A., García, N., Abdul Malak, D., Temple, H., Katariya, V., 2008. The Mediterranean: a biodiversity hotspot under threat. In: *The 2008 Review of The IUCN Red List of Threatened Species* (J.–C. Vié, C. Hilton–Taylor, S. N. Stuart, Eds.). IUCN Gland, Switzerland.

Das, I., 1996. Folivory and seasonal changes in diet in *Rana hexadactyla* (Anura: Ranidae). *Journal of Zoology*, 238: 785–794.

Downes, H., 1995. Tricaine Anesthesia in Amphibia: A Review. *Bulletin of the Association of Reptilian and Amphibian Veterinarians,* 5: 11–16.

Duellman, W. E., Trueb, L., 1994. *Biology of Amphibians*. Johns Hopkins University Press, Baltimore and London.

Dunham, A. E., 1983. Realized niche overlap, resource abundance, and intensity of interspecific competition. In: *Lizard ecology: studies of a model organism*: 261–280 (R. B. Huey, E. R. Pianka, T. W. Schoener, Eds.). Harvard University Press, Cambridge.

Fisher, D. O, Owens, I. P. F., 2007. The comparative method in conservation biology. *Trends in Ecology and Evolution*, 19: 391–398.

Giorgi, F., Lionello, P., 2008. Climate change projections for the Mediterranean region. *Global and Planetary Change*, 63: 90–104.

Lamoureux, V., Maerz, C. M., Madison, D. M., 2002. Premigratory autumn foraging forays in the Green frog, *Rana clamitans*. *Journal of Herpetology*, 36: 245–254.

Levins, R., 1968. *Evolution in changing environments*. Princeton University Press. Princeton, New Jersey.

Lymberakis, P., Pafilis, P., Poulakakis, N., Sotiropoulos, K., Valakos, E. D., 2018. The Amphibians and Reptiles of the Aegean Sea. In: *Biogeography and Biodiversity of the Aegean. In honour of Prof. Moysis Mylonas*: 169–189 (S. Sfenthourakis, P. Pafilis, A. Parmakelis, N. Poulakakis, K. A.Triantis, Eds). Broken Hill Publishers Ltd, Nicosia, Cyprus.

Lymberakis, P., Poulakakis, N., Manthalou, G., Tsigenopoulos, C. S., Magoulas, A., Mylonas, M., 2007. Mitochondrial phylogeography of *Rana* (*Pelophylax*) populations in the Eastern Mediterranean region. *Molecular Phylogenetics and Evolution*, 44: 115–125.

Mollov, I. A., 2008. Sex based differences in the trophic niche of *Pelophylax ridibundus* (Pallas, 1771) (Amphibia: Anura) from Bulgaria. *Acta Zoologica Bulgarica*, 60: 277–284.

Mollov, I. A., Boyadzhiev, P. S., Donev, A. D., 2006. A synopsis on the studies of the trophic spectrum of the Amphibians in Bulgaria. *Scientific Studies of the University of Plovdiv–Biology*, *Animalia*, 42: 115–131.

– 2010. Trophic role of the marsh frog *Pelophylax ridibundus* (Pallas, 1771) (Amphibia, Anura) in the aquatic ecosystems. *Bulgarian Journal of Agricultural Science*, 16: 298–306.

Nicolaou, H., Zogaris, S., Pafilis, P., 2014. Frog vs. lizard: An unusual feeding behaviour in the Levantine Marsh Frog, *Pelophylax bedriagae* from Cyprus. *North–Western Journal of Zoology*, 10: 221–222.

Olsen, A. M., 2017. Feeding ecology is the primary driver of beak shape diversification in waterfowl. *Functional Ecology*, 31: 1985–1995.

Pagani–Núñez, E., Ruiz, Í., Quesada, J., Negro, J. J., Senar, J. C., 2011. The diet of Great Tit *Parus major* nestlings in a Mediterranean Iberian forest: the important role of spiders. *Animal Biodiversity and Conservation*, 34: 355–361.

Palazón, S., Ruiz–Olmo, J., Gosálbez, J., 2008. Autumn–winter diet of three carnivores, European mink (*Mustela lutreola*), Eurasian otter (*Lutra lutra*) and small–spotted genet (*Genetta genetta*), in northern Spain. *Animal Biodiversity and Conservation*, 31: 37–43.

Pianka, E. R., 1973. The structure of lizard communities. *Annual Review of Ecology and Systematics*, 4: 53–74.

Pimm, S. L., Jenkins, C. N., Abell, R., Brooks, T. M., Gittleman, J. L., Joppa, L. N., Raven, P. H., Roberts, C. M., Sexton, J. O., 2014. The biodiversity of species and their rates of extinction, distribution, and protection. *Science*, 344: 1246752.

Plitsi, P., Koumaki, M., Bei, V., Pafilis, P., Polymeni, R. M., 2016. Feeding ecology of the Balkan Water frog (P*elophylax kurtmuelleri*) in Greece with emphasis on habitat effect. *North–Western Journal of Zoology*, 12: 292–298.

Plötner, J., Uzzell, T., Beerli, P., Akın, Ç., Bilgin, C. C., Haefeli, C., Ohst, T., Köhler, F., Schreiber, R., Guex, G.–D., Litvinchuk, S. N., Westaway, R., Reyer, H.–U., Pruvost, N., Hotz, H., 2010. Genetic divergence and evolution of reproductive isolation in eastern Mediterranean water frogs. In: *Evolution in Action*: 373–403 (M. Glaubrecht, Ed.). Springer, Berlin, Heidelberg.

Pope, K. L., Matthews, K. R., 2002. Influence of anuran prey on the condition and distribution of *Rana muscosa* in the Sierra Nevada. *Herpetologica*, 58: 354–363.

Poulakakis, N., Kapli, P., Lymberakis, P., Trichas, A., Vardinoyiannis, K., Sfenthourakis, S., Mylonas, M., 2015. A review of phylogeographic analyses of animal taxa from the Aegean and surrounding regions. *Journal of Zoological Systematics and Evolutionary Research*, 53: 18–32.

R Core Team, 2017. *R: A language and environment for statistical computing*. R Foundation for Statistical Computing, Vienna. (https://www.R–project.org/).

Rebouças, R., Castro, I. M., Solé, M., 2013. Diet of *Haddadus binotatus* (Spix, 1824) (Anura: Craugastoridae) in Brazilian Atlantic Rainforest, Bahia state. *North–Western Journal of Zoology*, 9: 293–299

Ruchin, A. B., Ryzhov, M. K., 2002. On diet of the Marsh Frog (*Rana ridibunda*) in the Sura and Moksha Watershed, Mordovia. *Advances in Amphibian Research in the Former Soviet Union*, 7: 197–205.

Sas, I., Covaciu–Marcov, S. D., Strugariu, A., David, A., Ilea, C., 2009. Food habit of *Rana* (*Pelophylax*) kl. *esculenta* females in a new recorded E–system population from a forested habitat in North–Western Romania. *Turkish Journal of Zoology,* 33: 1–5.

Sas, I., Kovács, E.–H., Covaciu–Marcov, S.–D., Strugariu, A., Covaci, R., Ferenţi, S., 2007. Food habits of a Pool frog *Pelophylax lessonae*–Edible frog *Pelophylax* kl. *esculentus* population from North–Western Romania. *Biota*, 8: 71–78.

Solé, M., Beckmann, O., Pelz, B., Kwet, A., Engels, W., 2005. Stomach–flushing for diet analysis in anurans: an improved protocol evaluated in a case study in Araucaria forests, southern Brazil. *Studies on Neotropical Fauna and Environment*, 40: 23–28.

Temple, H. J., Cox, N. A., 2009. *European Red List of Amphibians*. Office for Official Publications of the European Communities, Luxembourg.

Tracy, C. R., Nussear, K. E., Esque, T. C., Dean–Bradley, K., Tracy, C. R., DeFalco, L. A., Castle, K. T., Zimmerman, L. C., Espinoza, R. E., Barber, A. M., 2002. The importance of physiological ecology in conservation biology. *Integrative and Comparative Biology*, 46: 1191–1205.

Valakos, E. D., Pafilis, P., Sotiropoulos, K., Lymberakis, P., Maragou, P., Foufopoulos, J., 2008. *The amphibians and reptiles of Greece*. Edition Chimaira, Frankfurt am Main.

Vervust, B., Brecko, J., Pafilis, P., 2013. Preliminary data on the differentiation of Milos Island water frogs. *Journal of Biological Research–Thessaloniki*, 20: 290–295.

Vervust, B., Pafilis, P., Valakos, E. D., Van Damme, R., 2010. Anatomical and physiological changes associated with a recent dietary shift in the lizard *Podarcis sicula*. *Physiological and Biochemical Zoology*, 83: 632–642.

Watson, A. S., Fitzgerald, A. L., Damián Baldeón, O. J., 2017. Diet composition and prey selection of *Telmatobius macrostomus*, the Junín giant frog. *Endangered Species Research,* 32: 117–121.

Wiens, J. J., Ackerly, D. D., Allen, A. P., Anacker, B. L., Buckley, L. B., Cornell, H. V., Damschen, E. I., Davies, T. J., Grytnes, J. A., Harrison, S. P., Hawkins, B. A., Holt, R. D., McCain, C. M., Stephens, P. R., 2010. Niche conservatism as an emerging principle in ecology and conservation biology. *Ecology Letters*, 13: 1310–1324.

Wikelski, M., Cooke, S. J., 2006. Conservation physiology. *Trends in Ecology and Evolution*, 21: 38–46.

Wilson, E. O., 2002. *The Future of Life*. Knopf Doubleday Publishing Group, NY, United States.

Zar, J. H., 2010. *Biostatistical Analysis*. Prentice Hall, New Jersey.