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Amphibians in Eurasian otter Lutra lutra diet: osteological identification unveils hidden prey richness and male-biased predation on anurans.

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ABSTRACT

1. Amphibians form a major component of the diet of the otter *Lutra lutra* in several areas of its wide geographic range. Yet, amphibian remains are rarely identified to species level and therefore information on the diversity of this food resource is generally scarce.

2. The aims of this study were: 1) to assess the overall pattern and trends in the use of amphibians as a resource by otters at the range scale, and 2) to highlight current knowledge on the diversity of amphibians taken as prey by otters. Additionally, we carried out osteological identification of amphibian remains in otter spraints (faeces) from southern Italy, with the aim of demonstrating how this method may improve our knowledge on predator–prey relationships.

3. The frequency of occurrence of amphibians in 64 dietary studies averaged 12%. Predation of amphibians by otters increased with longitude and was the highest in the Alpine biogeographical region. Predation by otters was reported on 28 amphibian species (35% of European species). Peaks in their frequency of use were reported for all seasons, mostly in winter and spring. In southern Italy, we identified 355 individuals belonging to at least seven amphibian taxa (64% of available species; *Rana italica*, *Rana dalmatina*/*italica*, *Pelophylax* kl. *bergeri/hispanicus*, *Hyla intermedia*, *Bufo bufo*, *Bufotes balearicus*, and *Lissotriton italicus*), and pointed out male-biased predation within the Order Anura (frogs).

4. We conclude that the contribution of amphibians to the richness of the otter's prey community is far higher than commonly perceived, and that osteological analyses allow the detailed investigation of the feeding behaviour of this top predator of freshwater habitats.

INTRODUCTION

Predator–prey interactions play a major role in shaping community composition and dynamics at local scales, and can shape species richness at the global scale in combination with environmental gradients and interspecific competition (Sandom et al. 2013, Terborgh 2015).

Although terrestrial mammalian predators are, in general, highly specialised, only a minority of species are obligate specialists, i.e. rely on a single food or a handful of food resources; most usually integrate preferred food items with a number of locally and seasonally varying 'secondary resources' (Pineda-Munoz & Alroy 2014).

A positive effect of prey diversity on predator population stability was first hypothesised by MacArthur (1955), who envisaged that diversity increases the reliability of the resource pool on which each predator depends. Further mechanisms that enhance predator stability when several prey species are available have been revised by Petchey (2000) and, briefly, include increased total prey biomass, lowered temporal variation in food availability, and increased opportunities to combine complementary foods to achieve a balanced nutrient intake. Diversity of the prey community may also lower resource competition, as food partitioning increases with increasing prey diversity (Sanchez-Hernandez et al. 2017).

Assessing the nutritional requirements of a predator and its position along the specialist–generalist continuum is essential for reconstructing food webs and understanding the predator's function in the ecosystem (Remonti et al. 2016). Unfortunately, the investigation in detail of the diversity of species preyed on by elusive mammalian carnivores is often hindered by the difficulty of identifying prey remains to the species level through the analysis of the undigested remains found in either faeces or, to a lesser extent, stomachs (Britton et al. 2006). Low levels of prey identification can lead to unpredictable biases in the estimate of major food-niche and community parameters of mammalian carnivores (Greene & Jaksic´ 1983). The Eurasian otter *Lutra lutra* is a top predator of freshwater habitats (Chanin 2003, Prenda et al. 2006, Miranda et al. 2008, Ruiz-Olmo & Jimenez 2009, Clavero et al. 2010, Almeida et al. 2012). During the 20th century, this mustelid declined dramatically throughout Europe, mainly due to water pollution, hunting and poaching, increased road traffic, and habitat loss (Prigioni et al. 2007). In Italy, the otter is currently recovering (Balestrieri et al. 2016), but it is still classified as Endangered by the International Union for Conservation of Nature (IUCN; Panzacchi et al. 2010).

The semi-aquatic lifestyle of otters is metabolically costly compared to that of other mammals of similar size (Pfeiffer & Culik 1998, Kruuk 2006). Because of this, otters are ravening predators, but they have the most restricted trophic niche in the mustelid family (Jedrzejewska et al. 2001, McDonald 2002). The bulk of the diet of otters is formed by fish, which can constitute up to 95% of their prey (Ruiz-Olmo & Palazon 1997). Nonetheless, in most areas otters also rely on alternative prey, mainly amphibians, crabs, and crayfish (Mason & Macdonald 1986, Ruiz-Olmo & Palazon 1997, Jedrzejewska et al. 2001, Clavero et al. 2003, Prigioni et al. 2006, Remonti et al. 2008, 2009, Smiroldo et al. 2009), and, occasionally, birds (Mason & Macdonald 1986, de la Hey 2008). Habitat type and stability (Jedrzejewska et al. 2001, Smiroldo et al. 2009, Lanszki et al. 2016) and, ultimately, fish availability (Remonti et al. 2008) are the main factors determining the diversity of otter diet. Amphibian remains are frequently recorded in otter spraints (faeces; e.g. Weber 1990, Brzezinski et al. 1993, Parry et al. 2011), and in several localities amphibians form a major portion

of otter diet, particularly during late winter, when the availability of fish prey is reduced (Carss et al. 1990), and in spring, when anurans aggregate in high densities to spawn (Clavero et al. 2005). In Poland (Brzezinski et al. 1993, Jedrzejewska et al. 2001, Pagacz & Witczuk 2010) and Belarus (Sidorovich et al. 1998), frogs have been reported to outweigh fish in otter diet.

However, unlike fish remains, amphibian remains are rarely assigned to species level in dietary studies, and therefore information on the diversity of this important alternative resource is generally scarce (but see Clavero et al. 2005 and Parry et al. 2015). Although several previous reviews have analysed range-scale variability in otter diet, they were mainly focused on fish prey and the effectsof variation in its use or availability on the consumption of secondary food resources (Jedrzejewska et al. 2001, Clavero et al. 2003, Balestrieri et al. 2013, Krawczyk et al. 2016, Lanszki et al. 2016). Following on from Ruiz-Olmo's (1995) review on otter predation of reptiles, we reviewed the available literature with the aim of outlining the level of detail currently applied to the class Amphibia in otter dietary studies, and the overall pattern of use of this resource by otters throughout their European range.

Italy has the highest amphibian diversity in Europe (43 species, of which 15 are endemic; Sindaco et al. 2006, Sillero et al. 2014), and amphibians are the main alternative food resource for otters in freshwater ecosystems in Italy (Prigioni et al. 2006, Remonti et al. 2008, Smiroldo et al. 2009). The consumption of amphibians by otters is usually higher in Italy than the average for the Mediterranean region (Remonti et al. 2009). Thus, southern Italy may represent an area of choice for investigating amphibian prey diversity in otter diet. For these reasons, we applied a comparative osteological approach usually used for palaeontological and zooarchaeological studies, with the aim of identifying species and, whenever possible, gender and age of the amphibians preyed on by the Eurasian otters of the Sila Piccola Massif (Calabria region, southern Italy).

METHODS

Analysis of literature data

To assess the occurrence of amphibians in otter diet, data were collated from the available literature in May and June 2018, by searching the ISI Web of Science and Google Scholar online data bases (key words: 'otter', 'lutra', 'diet', 'food habits'). The lists of references from the downloaded articles were used to find further studies with the aim of reviewing as many studies of interest as possible. To standardise the comparison of results from different geographical areas, data were selected according to the following criteria: 1) studies covered at least one year and were based on the analysis of spraints only, so as to avoid differences in food type representation due to differential digestion (Balestrieri et al. 2011); 2) spraints were collected in freshwater systems; 3)

sample sizes were greater than 100 spraints, to distinguish moderate effect sizes (Trites & Joy 2005); and 4) diet composition was expressed as percent relative frequency (%RF = the number of occurrences of each prey item divided by the total number of occurrences of all prey items, times 100), or %RF could be derived from values or graphs. Results for several streams from the same area were pooled to avoid pseudoreplication (Hulbert 1984). When only seasonal data were reported, mean annual %RF was calculated from raw data when available.

Although %RF does not provide any information about the biomass or relative volume of each prey item, this index has the advantage of having been used frequently in inter-population dietary comparisons (e.g. Reynolds & Aebischer 1991, Zhou et al. 2011), and particularly in otter dietary studies (Balestrieri et al. 2013, Krawczyk et al. 2016, Lanszki et al. 2016). For otters, %RF values have been shown to be nearly as accurate as other indices (Jacobsen & Hansen 1996). When the geographical coordinates of the study area were not indicated, they were derived from ordnance survey maps; representative mean coordinates were used when samples were collected from a large area. Geodetic coordinates were projected to Eastings and Northings using a Transverse Mercator projection. Study sites were then grouped according to biogeographical regions. Spearman's correlations between geographical coordinates and arcsine transformed (Zar 1984) %RFs of amphibians were calculated. Seasonal variation in otter predation on amphibians was tested with the chi-squared test ($\chi 2$), using raw frequency data.

Case study: osteological identification of amphibians

Spraints were collected in a ca. 2040 km2 large area of southern Italy (the Sila Piccola Massif, Calabria region), as part of the long-term monitoring of a recently rediscovered otter population (see Gariano & Balestrieri 2018). Otters occurred on six rivers at a total of 12 sampling stations, and 357 spraints were collected, of which 91 in summer 2014 and 2015, and 266 in 2016–2017 (summer: 34; autumn: 30; winter: 85; spring: 117). Undigested remains were sorted according to standard methodologies (e.g. Smiroldo et al. 2009), and the bones of amphibians were identified using a comparative osteological approach. Taxonomic identification was based on available keys (e.g. Haller-Probst & Schleich 1994, Ratnikov & Litvinchuk 2007, 2009, for caudates; Bailon 1999, for anurans; Figs 1 and 2) and reference collections (see Appendix S1 for a brief description of the most significant diagnostic features). Bailon's diagnostic keys (1999) are based on anurans found in France, and do not allow discrimination between *Rana italica* and *Rana dalmatina*, both currently occurring on the Sila Piccola Massif. Consequently, most brown frog remains were attributed to *Rana dalmatina*/*italica*. Nonetheless, based on our observations on available comparative material, the frontoparietals of *Rana italica* (Fig. 2a–c) show a wide and deep groove on the posterolateral part of their dorsal surface which does not occur in *Rana dalmatina* (Fig. 2d–e). This

feature allowed us to distinguish the two species whenever we found well-preserved frontoparietals. When identification to species level from only osteological criteria was not possible (e.g. for *Lissotriton* spp. and *Pelophylax* spp.), a biogeographical rationale was applied, assigning the remains to the only species currently reported for the study area. Whenever possible, the age (juveniles vs. adults) and sex of each specimen were assessed. Age identification was mainly based on the size and degree of ossification of the bones. The sex of adult (large) Ranidae and Bufonidae was assessed on the basis of the sexually dimorphic mesial crest of the humerus (more robust in males than in females; Bailon 1999).

Diagnostic bones were photographed using a Leica M205 microscope equipped with the Leica application suite V 4.10. This apparatus allows multiple pictures of a single specimen to be taken at different focus planes ('Z-stacks'). The different pictures are then merged by the image acquisition software to create a new, virtual picture showing the specimen entirely in focus. Chi-squared tests were used to test for seasonal variation in the raw frequencies of amphibians in otter diet; Kruskal–Wallis tests were used to test for seasonal variation in the mean number of prey individuals per spraint.

RESULTS

Amphibians in otter diet

Sixty-four studies, published as 48 articles between 1969 and 2017, met our criteria (Table 1). They were carried out in 20 European countries, from Sweden in the north to Greece in the south and from Portugal in the west to Lithuania in the east (Fig. 3). The %RF of amphibians in otter diet ranged between 0% in north-east Spain (Melero et al. 2008) to 43% in Poland (Pagacz & Witczuk 2010), averaging 12% ± 3.2 (mean %RF ± standard error; Fig. 4). In most studies, amphibians accounted for <15% of otter diet (Fig. 5). No latitudinal trend could be outlined, but predation of amphibians by otters increased with longitude (Spearman's rho = 0.47, $P < 0.001$), and from the Atlantic and Mediterranean biogeographical regions (mean %RF = 8.9% and 11%) to the Alpine region (mean %RF = 23%); the Pannonian, Continental, and Boreal regions showed intermediate values (%RF = 12–14%). Information on seasonal variation in otter diet was available for 34 studies. Otters preyed on amphibians mostly in winter (mean %RF = 53%) and/or spring (68%), but in some studies peaks in their frequency of use were reported for summer and autumn (mean %RF = 15% and 21%, respectively; $\chi 2 = 23.5$, 3 d.f., $P < 0.001$). Only a minority of studies (17%) classified amphibian prey at the species level: the number of species recorded ranged between 1 and 5 (mean = 3.0). Considering all available studies consulted for this review and our own results (see below), 28 amphibian species have been reported to be preyed on by otters (Table 2), comprising

eight Salamandridae (Order Urodela) and three Discoglossidae, two Pelodytidae, three Bufonidae, three Hylidae, and nine Ranidae (all Order Anura). Four of the 28 species (*Lissotriton boscai*, *Discoglossus galganoi*, *Pelodytes ibericus*, and *Rana iberica*) are endemic to the Iberian Peninsula, and three (*Lissotriton italicus*, *Hyla intermedia*, and *Rana italica*) are endemic to Italy (Sindaco et al. 2006, Speybroeck et al. 2016).

Amphibians preyed on by otters in southern Italy

Amphibian bones were found in 159 spraints (45%), allowing the identification of a minimum of 355 individuals (2.2 individuals/spraint, min-max: 1-21; Table 3) belonging to at least seven amphibian taxa (*Rana italica*, *Rana dalmatina*/*italica*, *Pelophylax* kl. *bergeri/hispanicus*, *Hyla intermedia*, *Bufo bufo*, *Bufotes balearicus*, and *Lissotriton italicus*). Most bone remains belonged to the Ranidae family (273 individuals, 77%), mainly either agile frogs or Italian stream frogs (*Rana dalmatina/ italica*, 168 individuals). Only three well-preserved frontoparietals were found, which belonged to two different individuals of *Rana italica*. Green frogs *Pelophylax* kl. *bergeri/hispanicus* were preyed on less frequently (24 individuals). Twenty-seven individuals belonged to Bufonidae (7.3%), of which 10 were assigned to European green toad *Bufotes balearicus* and four to common toad *Bufo bufo*. Hylidae were represented by the Italian tree frog *Hyla intermedia* (six individuals, 1.7%). The family Salamandridae included three Italian newts *Lissotriton italicus* (1%). Based on the size and degree of ossification of the remains, 330 of the amphibians represented in otter spraints were adults and 22 were juveniles. In total, sex determination was possible for 32% of the adults, 85 males and 19 females, corresponding to a male:female ratio of 4.5:1.

The frequency of amphibian remains in otter diet varied seasonally (Table 4), and was lowest in autumn, when frogs were recorded in otter spraints only once ($\chi 2 = 24.7$, 3 d.f., $P < 0.001$). In winter, spring and summer, the mean number of individual amphibians per spraint was statistically similar (1.7–2.0; Kruskal–Wallis test: $\chi 2 = 0.78$, 2 d.f., $P = 0.68$).

DISCUSSION

Eurasian otters have been recorded to prey on 28 amphibian species, corresponding to 35% of all species available throughout the European range of the otter (Speybroeck et al. 2016). These numbers, which are certainly underestimates, highlight the opportunistic feeding behaviour of the otter, which, although shaped by evolution as a fish-specialist predator, is able to shift to a large variety of semi-aquatic species when fish availability is low (Clavero et al. 2004, Remonti et al. 2008, Smiroldo et al. 2009). With eight species, Order Urodela consistently contributed to the diversity of otter diet. In the 1980s, Chanin (1985) suggested that newts are either not taken by

otters, or they are missed through standard spraint analysis. In the last decade, newt remains have been recovered in the stomachs of otter carcasses in both south-west England (*Triturus cristatus*; Britton et al. 2006) and Hungary (*Lissotriton vulgaris*; Lanszki et al. 2015), and in otter spraints in Wales (*Lissotriton helveticus*; Parry et al. 2015) and in the Czech-Moravian Highlands (Polednik et al. 2007). The negligible percentage of spraint-based studies reporting newts may be due to the difficulty in identifying amphibian remains (Parry et al. 2015). Further evidence has been obtained by direct observations of otters preying on *Triturus cristatus* in North Jutland (Bringsoe & Norgaard 2018) and on Iberian ribbed newts *Pleurodeles waltl* in southern Spain (Cogaˇlniceanu et al. 2010). In southern Spain, otters ate only the internal organs of ribbed newts, a behaviour that may lead to the underestimation of their contribution to the mustelid's diet (Cogaˇlniceanu et al. 2010). Many newts produce tetrodotoxin, a powerful neurotoxin and anti-predatory skin secretion, at various concentrations depending on the species (Hanifin 2010), which may explain why otters do not eat newts whole. Otherwise, otters may avoid the ingestion of the poison by skinning the newt, a behaviour that has been described in otters eating toads in Portugal (Beja 1996), Spain (Lizana & Perez Mellado 1990), Belarus (Sidorovich & Pikulik 1997), Finland (Sulkava 1996), and Wales (Slater 2002). As reported for toads (Lizana & Perez Mellado 1990), the large amount of time spent handling newts may explain otters' general preference for frogs, but recent observations (Cogaˇlniceanu et al. 2010) suggest that local availability may also play a major role. Previous reviews focused on the geographical variability in otter diet. Mason and Macdonald (1986) reported that in southern Europe otters generally eat more amphibians and reptiles than in the north, and Adrian and Delibes 1987) suggested that the frequency of occurrence of amphibians, reptiles, and insects in otter spraints decreases as latitude increases. In contrast, Jedrzejewska et al. (2001) did not find any latitudinal pattern in otter diet in Eurasia, while Clavero et al. (2003) did not include coastal otters in their analyses and found that otters inhabiting Mediterranean localities had more diverse diets than those in northern regions. Both Jedrzejewska et al. (2001) and Clavero et al. (2003) pointed out that habitat features (especially water availability) are important factors influencing the diet of otters. More recently, Balestrieri et al. (2013) found no relationship between otter dietary breadth and latitude, suggesting that habitat-related variations in fish assemblage richness and stability play a major role in shaping otter diet. Accordingly, otters prey on fish more frequently in standing waters surrounded by riparian vegetation than in flowing waters in open habitats (Krawczyk et al. 2016), and fish consumption decreases with stream elevation (Remonti et al. 2009). In agreement with previous studies (Jedrzejewska et al. 2001, Balestrieri et al. 2013), we could not highlight any latitudinal trend, but the frequency of amphibians in otter diet increased with longitude: the highest %RFs were recorded in the eastern Alpine region (Poland and Bulgaria)

and in Boreal regions (Finland, Belarus; see also Lanszki et al. 2016). As suggested by Kruuk (2006), this trend may result from the availability of large numbers of frogs and toads in those regions. Despite this general trend, we recorded a wide variation in amphibian contribution to otter diet (e.g. in southern Italy, %RF ranged between 7.2% and 34%), suggesting that it is shaped by the local availability of prey species, mainly fish (Remonti et al. 2008, Smiroldo et al. in press). While Anura may be most abundant in north-eastern Europe, the richness of amphibians communities is the highest in Mediterranean Europe (Sillero et al. 2014), as confirmed by the relatively large number of endemic species preyed on by otters in the Iberian and Italian peninsulas. Therefore, to unveil the actual diversity of otter prey in south-western Europe, a detailed analysis of bone remains should be undertaken. This task is time-consuming and, despite the availability of several useful keys, requires a certain degree of laboratory experience. For this reason, and because research has often been focused on fish prey, only a few of the selected studies reported sufficient information on the diversity of amphibian prey in otter diet. Osteological identification of amphibian remains allowed us to document otter predation on seven out of the 11 species recorded in our study area (64%; Sindaco et al. 2006). Three – Italian newt *Lissotriton italicus*, Italian tree frog *Hyla intermedia*, and Italian stream frog *Rana italica* – are endemic to the Italian peninsula and are listed as Least Concern in the Italian Red List of threatened species (Rondinini et al. 2013). The discrimination of *Rana italica* from *Rana dalmatina* was allowed by a newly described diagnostic feature, based on the morphology of frontoparietals,which, unfortunately, were seldom preserved in a condition good enough to allow species identification. The mean number of individual amphibians per spraint (2.2) was higher than that reported in the only previous study in which an attempt was made to assess numbers of otter prey (1.6; Clavero et al. 2005). As recorded throughout Europe, frogs formed the bulk of the amphibian fraction of the diet of the otter. According to published data, the frequency of otter predation on amphibians is usually the highest in late winter and spring, when amphibians aggregate in large numbers to spawn. However, in our study area, amphibian remains occurred in otter spraints throughout the year, with the exception of autumn, when otters mainly preyed on cyprinid fish (Smiroldo et al. in press). As frogs are often preyed on during spawning, it is hard to discriminate whether seasonal diet shifts depend on their availability or on shortages of fish (Kruuk 2006); nonetheless, in Mediterranean habitats, predation on amphibians is generally inversely related to fish biomass (Remonti et al. 2009, Novais et al. 2010, Krawczyk et al. 2016, but see Ayres & Garcia 2011 for an opposing opinion). Most brown frogs recovered in otter spraints in summer probably belonged to the widespread species *Rana italica*, which is more strictly associated with freshwater habitats throughout the year than *Rana dalmatina* (Romano et al. 2012), and which, in summer, is mainly crepuscular and nocturnal (Sindaco et al. 2006). Accordingly, in our study

area, *Rana italica* has been reported to be far more widespread than *Rana dalmatina* (the two species were found at 41% and 8.0% of 36 sampling stations, respectively; Montillo 2017). The analysis of bone remains allowed us to documentmale-biased predation on anurans. Sex-biased mortality induced by predation is a general phenomenon, and males are often the sex paying the heavier toll (Christe et al. 2006). Otters, as predators, are not an exception to this general rule: male Atlantic salmon *Salmo salar* are more likely to be killed by otters than females (Carss et al. 1990). As with fish, male-biased predation on anurans is likely to result from the breeding behaviour of the prey. First, the number of male anurans at breeding sites greatly exceeds that of females (Merrell 1968, Calef 1973), as females leave the pond soon after laying their eggs (Hartel et al. 2007), while males stay close to breeding sites for longer in order to mate with more females. Moreover, during the breeding season advertisement calls are mainly produced by males, exposing them to a greater risk of being detected by predators. Although we do not know to what extent mustelids rely on auditory cues to find their prey, European polecats *Mustela putorius* take large numbers of male agile frogs during their spawning season (Lode 1996, Lode et al. 2004). Otters preyed on juvenile amphibians only in spring and summer, when their availability is highest, but in very low numbers, suggesting that otters prefer the more profitable adult individuals.

CONCLUSIONS

Our results show that, although Eurasian otters prey mainly on fish, amphibians represent an important secondary food resource. The contribution of amphibians to the richness of the otter's prey community is higher than commonly perceived, and osteological analyses allowed us to investigate to a deeper extent the feeding behaviour of this top predator of freshwater habitats. Throughout its wide range, the otter can rely on amphibians wherever fish availability is insufficient, and thus this resource may play a major role in the ongoing recovery of otter populations, especially in parts of the otter's range where freshwater habitats have been deeply altered by human activities (Smiroldo et al. in press). The dramatic decline affecting amphibian populations at the global scale (Wake & Vredenburg 2008, Blaustein et al. 2011) might threaten the viability of otters living in freshwater systems that have been depleted of their fish communities. In turn, expanding otter populations may affect the survival of small populations of endemic amphibian species, as otters are able to kill several individual amphibians in a very short period of time (Cogălniceanu et al. 2010, Parry et al. 2015). Further studies are needed to understand the potential role played by otters in amphibian conservation, and a detailed knowledge of predator–prey interactions is a first step in that direction.

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SUPPORTING INFORMATION

Additional supporting information may be found in the online version of this article at the publisher's web-site.

Appendix S1. Methods used in the identification of bone remains in the faeces of Eurasian otter *Lutra lutra*.

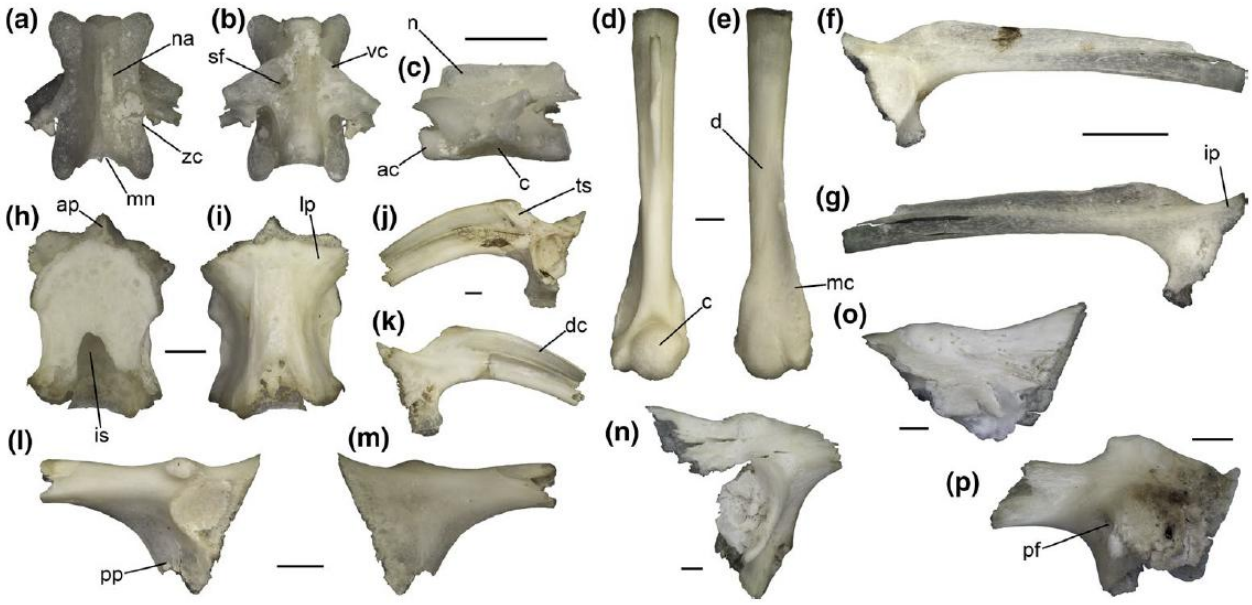

Fig. 1. Bone fragments, used in the identification of amphibian prey, from the faeces of the Eurasian otter *Lutra lutra*. Trunk vertebra of *Lissotriton italicus* in dorsal (a), ventral (b), and left lateral (c) views; left humerus of *Rana dalmatina/italica* in ventral (d) and dorsal (e) views; right ilium of a juvenile *Rana dalmatina/italica* in lateral (f) and medial (g) views; sphenetmoid of *Pelophylax* sp. in dorsal (h) and ventral (i) views; left ilium of *Pelophylax* sp. in lateral (j) and medial (k) views; left ilium of *Hyla intermedia* in lateral (l) and medial (m) views; right ilium of *Bufo bufo* in lateral view (n); left ilium of *Bufo bufo* in lateral view (o); left ilium of *Bufotes* sp. in lateral view (p). Abbreviations: ac, anterior condyle; ap, anterior process; c, centrum; d, diaphysis; dc, dorsal crest; ip, ischiadic process; is, incisura semielliptica; lp, lateral process; mc, medial crest; mn, median notch; n, neurapophysis; na, neural arch; pf, preacetabular fossa; pp, pubic process; sf, subcentral foramina; ts, tuber superior; vc, ventral crest; zc, zygapophyseal crest. Scale bars equal 1 mm.

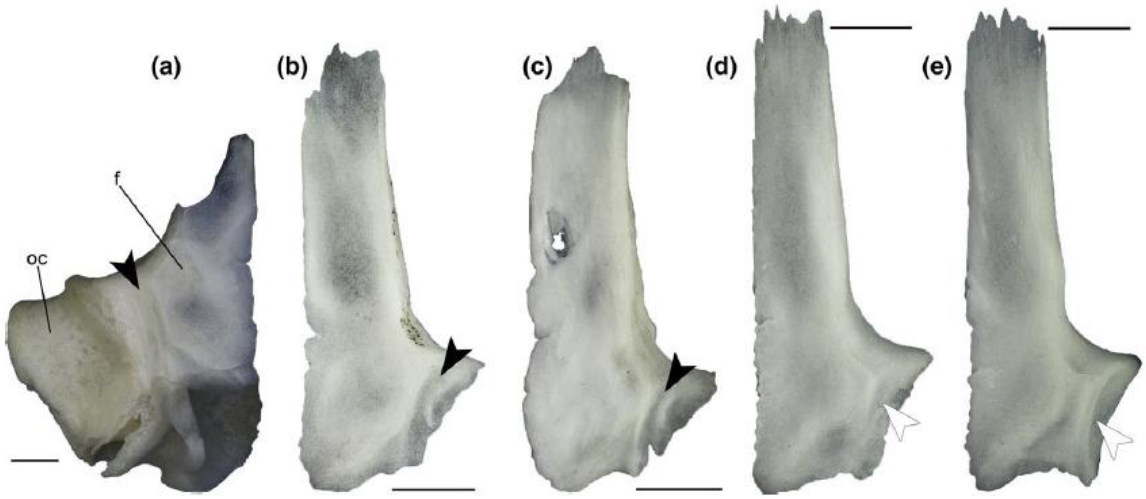

Fig. 2. Bone fragments, used in distinguishing the brown frogs *Rana italica* and *Rana dalmatina*, from the faeces of the Eurasian otter *Lutra lutra*. Fragmentary left frontoparietal of *Rana italica* fused with the otic complex (a) in dorsal view; dorsal view of right frontoparietals of *Rana italica* (b, c), and *Rana dalmatina* (d, e). Abbreviations: f, frontoparietal; oc, otooccipital complex. Scale

bars equal 1 mm. Black arrows mark the groove on the frontoparietals of *Rana italica*, whereas white arrows mark its absence in *Rana dalmatina*.

Table 1. Amphibians in the diet of the Eurasian otter *Lutra lutra* in the 64 studies included in this review. Location (country), bio-regions, study period, latitude and longitude, number of otter spraints (faeces) that were analysed (*N*), classification level used for prey fragments, and percent relative frequency (%RF) of amphibians in otter diet for each of the 64 studies in Europe. Biogeographical regions: 6-BOR = Boreal, 9-MED = Mediterranean, 4-ATL = Atlantic, 7-CON = Continental, 1-ALP = Alpine, 11-PAN = Pannonian. Locations of study codes are shown in Fig. 3, and codes are used in Table 2

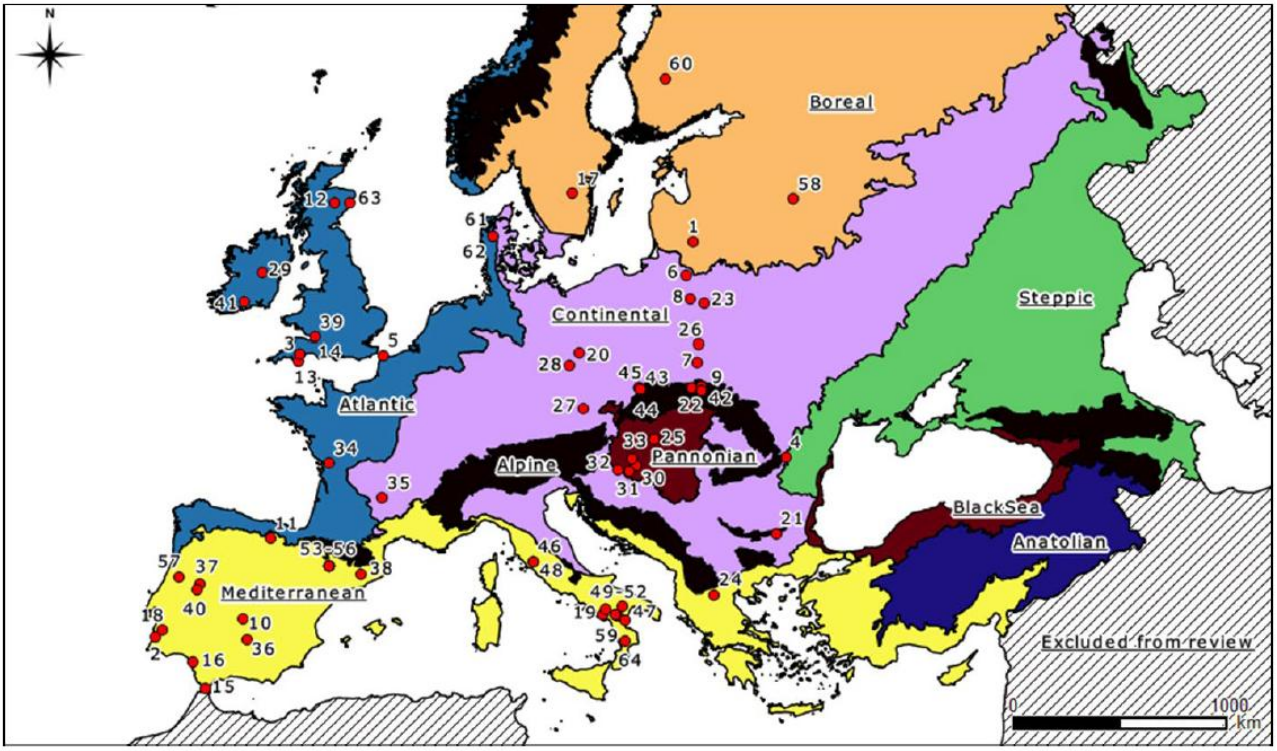

Fig. 3. Distribution of the 64 reviewed European studies on the diet of the Eurasian otter *Lutra lutra* (numbers correspond to those in Table 1). Biogeographical regions are shown.

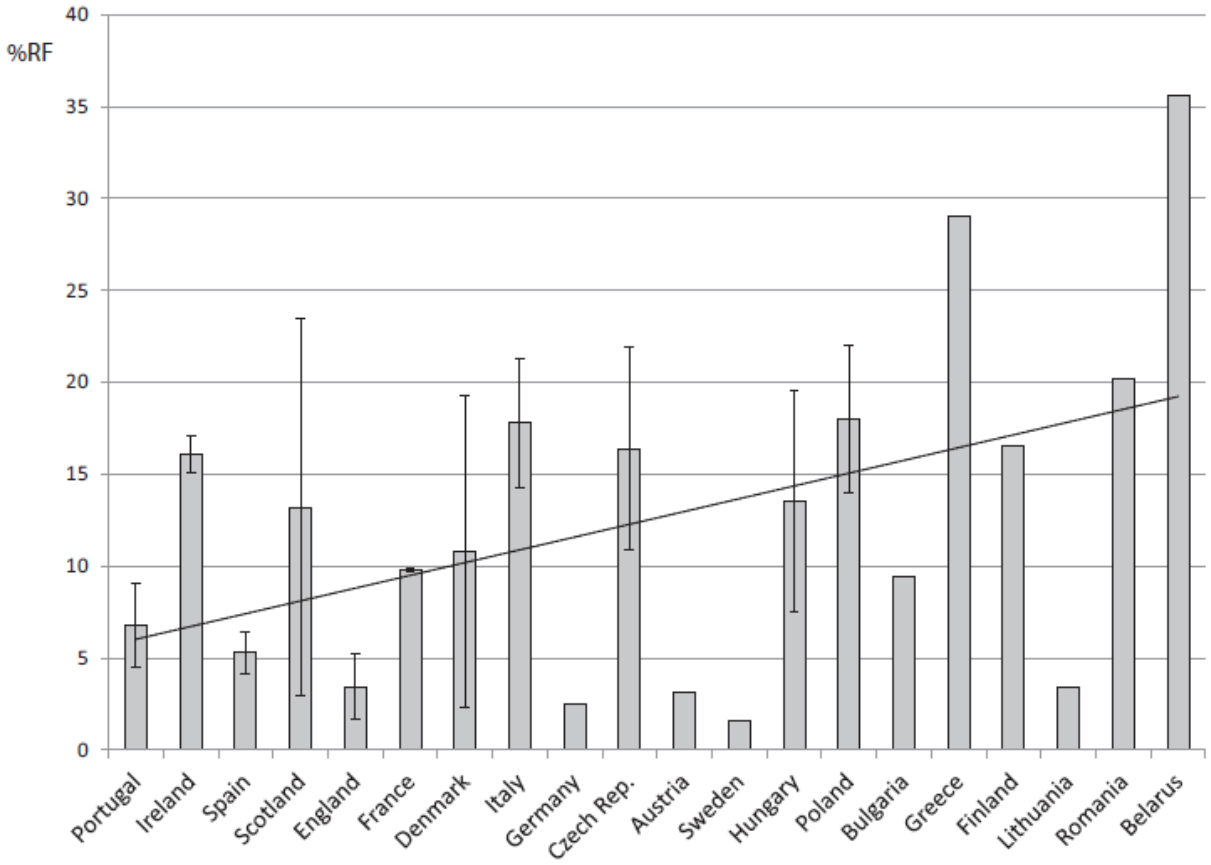

Fig. 4. Country-level variation in the mean ± SE percent relative frequency of occurrence (%RF) of amphibians in the diet of the Eurasian otter *Lutra lutra*. Countries are listed from west to east, based on the longitude of their capital cities. The longitudinal trend in predation of amphibians by otters is shown by the dotted line.

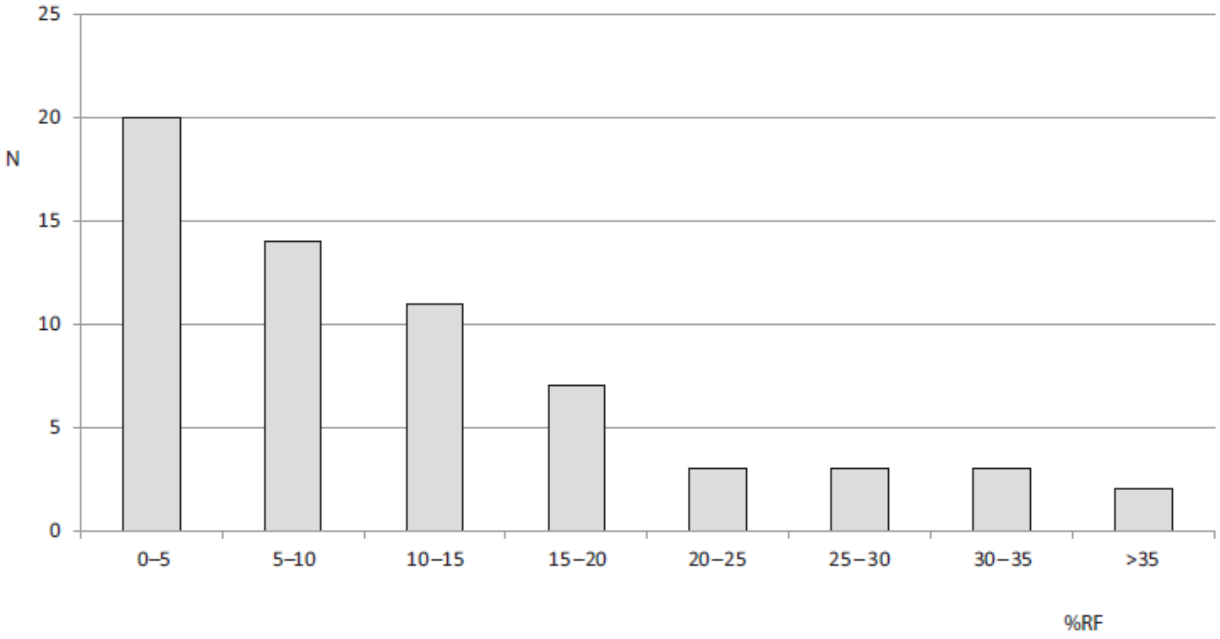

Fig. 5. Histogram of percent relative frequency of occurrence (%RF) of amphibians in the diet of the Eurasian otter *Lutra lutra* as reported in 64 studies published between 1969 and 2017 (N = number of studies).

Table 2. Amphibian species preyed on by Eurasian otters *Lutra lutra* as assessed by reviewing the available literature, including data collected for this Study

Table 3. Numbers of adult, juvenile, female, and male amphibians preyed on by Eurasian otters *Lutra lutra* in the rivers of the Sila Piccola Massif, Italy. Abbreviations: a.s.l.: above sea level; *N*tot: total number of spraints (faeces); *N*amp: number of spraints containing amphibian remains

Table 4. Seasonal distribution of the numbers of adult, juvenile, female, and male amphibians found in Eurasian otter *Lutra lutra* spraints (faeces) from the Sila Piccola Massif, Italy