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# Ecological role of the Eurasian otter, *Lutra lutra* (Mustelidae, Carnivora) as a seed dispersal species for riparian vegetation in Iberian fresh waters

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**Abstract.** Endozoochory is a mutualistic interaction between plants and animals. Such a relationship has rarely been examined in the Eurasian otter *Lutra lutra*. This study aimed to assess the use and electivity of fruit by this carnivore, along with the viability of ingested seeds. Otter spraints and fruit were collected from the River Bullaque (Guadiana River basin, central Spain) in June and September 2018-2019. A high occurrence of fruit (> 40%) was found in spraints during September. Otters ingested fruit in June from only one plant species: Iberian bushweed *Flueggea tinctoria* (Phyllanthaceae); whereas seeds ingested in September belonged to four plant species: apple mint *Mentha suaveolens* (Lamiaceae), common hawthorn *Crataegus monogyna*, dog rose *Rosa canina* and elmleaf blackberry *Rubus ulmifolius* (three Rosaceae species). According to the environmental availability, otters displayed avoidance for mint and rose, neutral selection for hawthorn and preference for blackberry. Germination was unsuccessful for mint seeds, whereas germination was the highest for blackberry (37%; 49% for blackbird *Turdus merula*, a well-known frugivorous species). Otters appear to display an ecological role as seed dispersers for riparian vegetation in Iberian fresh waters. These findings provide insights into this endozoochorous co-evolution between plants and Carnivora.

**Key words:** dietary traits, endozoochory, frugivory, germinability, spraint analysis

## Introduction

Endozoochory is a mutualistic relationship between plants and vertebrates, with the latter being mainly (frugivore) birds and mammals (Willson & Traveset 2000, Herrera & Pellmyr 2002). This seed dispersal

via ingestion has been observed in carnivores worldwide, belonging to various taxonomic families such as Ursidae, Procyonidae, Canidae and Mustelidae (Herrera 1989, Willson 1993, Koike et al. 2008). However, the endozoochorous plant-animal interaction is considered of scarce importance within

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this metazoan taxonomic group (i.e. order Carnivora), as these species are primarily predators. Regarding otter species in particular, these mustelids are mainly piscivorous top-predators within the aquatic food webs of inland waters, although fruit has also been found in spraints all around the world (e.g. Arcá & Prigioni 1987, Quadros & Monteiro-Filho 2000, Almeida et al. 2012a). Nevertheless, most research on dietary traits of otters does not include plant material as estimated ingested biomass is usually low for this food category (i.e. near 5% at the most; see Almeida 2008, Almeida et al. 2012a). Indeed, it is speculated whether fruit is found in the diet incidentally (e.g. when consuming a prey item laid out on the ground where the fruit is present) or indirectly (e.g. in the gut contents of prey that had recently consumed fruit) (Almeida et al. 2012a).

For the Eurasian otter *Lutra lutra* (Linnaeus, 1758) (hereafter “otter”), the observed high fruit occurrences in the spraints (over 30%; see Almeida 2008, Almeida et al. 2012a) strongly suggest that this species will intentionally select this food item as a dietary complement (e.g. see the same interpretation by Herrera 1989 for other carnivores in Spain).

Endozoochory implies that seeds are carried away from the parent plant to suitable sites where they can germinate under more favourable conditions of competence or herbivory (Willson & Traveset 2000, Herrera & Pellmyr 2002), facilitating the colonisation of new habitats (Pakeman 2001). Indeed, germination may be enhanced by softening hard seed coats during digestion (Baskin & Baskin 2014), and the deposition of seeds with faecal material may provide nutrients that promote seedling establishment (Traveset 1998). However, seed germination could also be inhibited due to the toxicity and hydrophobic nature of faeces of certain carrier taxa (Traveset 1998, Traveset et al. 2007). Thus, to determine whether a particular vertebrate species can contribute to plant dispersal, the level of seed transportation and the proportion of eventual viability should be quantified (Traveset et al. 2007, Baskin & Baskin 2014). To the best of our knowledge, information about the use of fruit by otters in the Iberian Peninsula is scarce (but see Almeida 2008 for one of few studies), and virtually no data exist on seed germination after defecation by this carnivore within this eco-region (but see an example for Neotropical otter *Lontra longicaudis* (Olfers, 1818) in Quadros & Monteiro-Filho 2000).

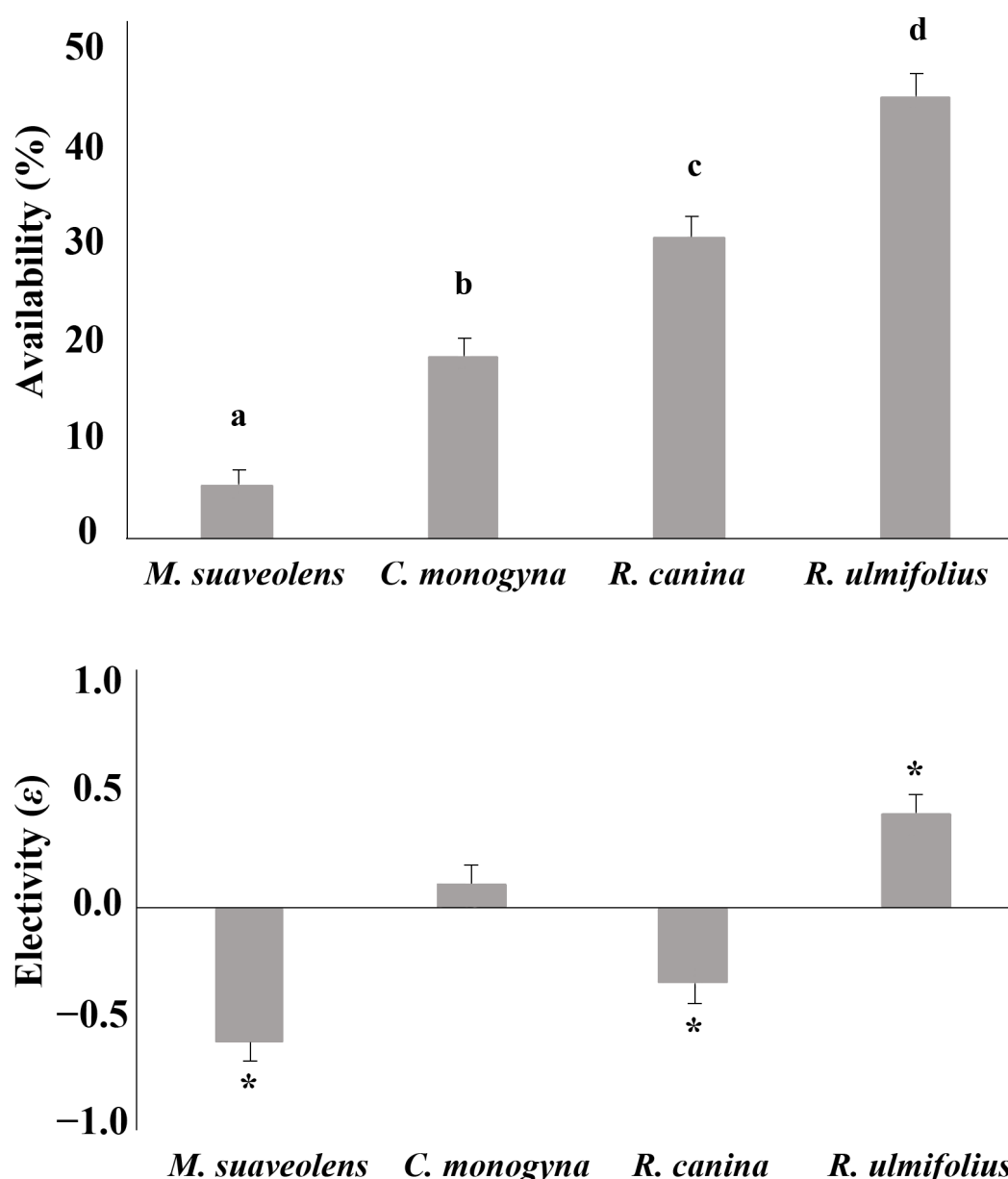
Consequently, to provide insights into this potential interaction (i.e. endozoochory) between otters and

riparian vegetation in the Iberian Peninsula, the present study aimed to analyse the feeding habits of this carnivore regarding the use of fruit in the River Bullaque (central Spain), as well as the viability of the ingested seeds. Specifically, this study assessed the overall diet composition of otters, the fruit electivity for particular plant species and the germinability of seeds extracted from spraints. Three hypotheses were stated: i) although the importance of ingested biomass will be low, the occurrence of fruit in the otter’s diet will be relatively high (Almeida 2008, Almeida et al. 2012a), which is of great interest for its contribution to the correct ecosystem functioning in terms of seed dispersal (Willson 1993, Koike et al. 2008); ii) the otter will use fruit according to its environmental availability, displaying an opportunistic foraging strategy (e.g. Almeida et al. 2012a, 2013a, Barrientos et al. 2014); and iii) the germination results will be different for each plant species, which will reveal particular adaptations to the variety of potential carrier species for seed dispersal (Traveset et al. 2001, Herrera & Pellmyr 2002).

## Material and Methods

### Study area

Field surveys were carried out in the River Bullaque (94 km length, 550–620 m a.s.l.), a small tributary of the River Guadiana (one of the main Iberian basins, central Spain). Climatic conditions, geo-morphology, limnological features and biological communities of this study area have been previously well-described in Almeida (2008), Almeida et al. (2012b, 2013b) and Bueno-Enciso et al. (2014) (see geographic maps herein these references). The middle and lower reaches of the River Bullaque (60 km to the confluence with the River Guadiana) were specifically selected due to the high density of otter spraints (estimated population of 25 individuals by faeces genotyping, see Almeida et al. 2012b) and previous knowledge on particular marking sites, i.e. “latrines” (Almeida 2008, Almeida et al. 2012b). Moreover, the two surveyed river reaches were selected because the flow regime is regulated by a dam forming the Torre Abraham Reservoir (see Fig. 1 in Bueno-Enciso et al. 2014). Therefore, this river section provides stability in food resources and refuge for otters throughout the year, unlike the higher reach of the River Bullaque before the dam, where the fluvial system changes to a series of disconnected pools during the summer drought (i.e. typical of Mediterranean rivers under natural flow regimes, Gasith & Resh 1999). Thus, results about the use of fruit by otters as a genuine addition to the diet will be more conclusive, as this carnivore



**Fig. 1.** Percentage of fruit availability (environmental biomass, above) and fruit electivity index ( $\epsilon$ , below) of the Eurasian otter *Lutra lutra* are presented for each plant species (only in September). Results are means  $\pm$  SE. The letters above bars indicate significant differences in fruit availability between plant species (Tukey HSD tests). Significant differences from zero for  $\epsilon$  are indicated by asterisks (t-tests). Significant differences in fruit availability and electivity were denoted when  $P <$  critical  $P$  value from Bonferroni corrections.

would not be forced to consume plant material due to a lack of prey supply within the selected river section.

### Field sampling

The collection of otter spraints was adapted from Almeida et al. (2012b) and Bueno-Enciso et al. (2014). Briefly, sampling sites ( $n = 15$ , 400-500 m river long) were separated by  $> 3$  km and evenly distributed along the middle and lower reaches of the River Bullaque to include a representative data-set of the existing environmental variability in the study area. According to previous surveys (Almeida 2008, pers. observ. in 2017), seeds were found in otter spraints

during the following months: May, June, August, September and October. For the present study, otter spraints were collected for two weeks in mid-June and mid-September 2018-2019. These sampling months were selected because they are the typical fruit-set periods according to the phenology of plant species used by otters within this study area (Almeida 2008), with the highest occurrence of seeds being found in spraints. Additionally, this was a two-year study, which allowed us to acquire a more representative data set than a single-year study, as per Fausch et al. (2002). These authors recommended a minimum of two years of sampling in fluvial habitats to encompass

all the variability in biological communities from such dynamic ecosystems. Furthermore, 2018 and 2019 are considered to have been hydrologically “average” in the study area, according to public data recorded from rainfalls and temperatures (Ministry of Environment Spain 2022). As a result, the effects of particularly dry or wet years on food supply for otters are avoided within the surveyed period, which allows the data to be considered more representative of the dietary traits of this carnivore in the study area. Consequently, these sampling conditions increase the potential generality of the present findings and conclusions.

Only fresh spraints ( $n = 200$ , 50 spraints  $\times$  two months  $\times$  two years, 3–4 spraints per site) were collected to ensure the least time since defecation; thus, a low alteration of food remains for subsequent estimations of ingested biomasses. Moreover, fresh spraints indicate a current use of the food sources in the study area (Almeida et al. 2012b). Adapted from Quadros & Monteiro-Filho (2000), Hampe & Arroyo (2002) and Siemens et al. (2020), three river transects (50 m) were surveyed at each sampling site to estimate the available fruit biomass of target plant species, i.e. particular seed types found in spraints. These transects were located at the beginning, middle and end of each sampling site and extended 10 m from the water's edge on both banks. This distance (i.e. 10 m) was used because no spraint was found beyond this limit in previous surveys (Almeida et al. 2012b). At each river transect, plant individuals were counted per species, and the “fruit load” (in g) from 10 random individuals (whenever possible) of different sizes was recorded to estimate the total available fruit biomass of that particular transect. Specifically, for generating the values of fruit load per selected plant individual, 20 ripe fruit were randomly collected and weighed (portable electronic balance,  $\pm 0.01$  g) to calculate the mean fruit mass, which was multiplied by the total number of fruit on that individual plant. The fruit was considered ripe if it detached easily from the receptacle but was not shrivelled or mouldy. Specifically for herbaceous species (i.e. *Mentha*, see below), a whole sprig (infructescence – 5 cm) was considered to be ingested by otters, according to the high number of seeds found per single spraints. Thus, the number and mass of sprigs were used to calculate the available “fruit load” (see below in Data analyses). For shrub/tree species, fruit biomass was only estimated from the parts of the plant accessible to the otter (i.e. up to 1 m height). Field surveys were carried out by a research team of six people: two persons for the spraint collection (D. Latorre and D. Almeida) and four persons (R. Merino-Aguirre,

A. Cruz, E. Lantero and A.M. Arroyo) at both banks for the estimation of fruit biomass. Geographic coordinates were recorded using a hand-held GPS (GARMIN model 72, USA,  $\pm 3$  m WAAS system) for the location of spraints and transects. Additionally, in the same study area during September, fresh faeces ( $n = 200$ ) were collected from the common blackbird *Turdus merula* (L., 1758), a well-known frugivorous species on elmleaf blackberry *Rubus ulmifolius* (Schott, 1818) (see Results below).

All field procedures complied with plant/animal use and care regulations of Europe and Spain. In addition, spraints and fruit were collected by trained research personnel. Thus, no adverse effects were caused to wildlife (both flora and fauna) in the study habitats.

### Laboratory procedures

Spraints were examined on the collection day as per Almeida et al. (2012a, b). Briefly, they were partly dried on blotting paper (no oven) under dark conditions (1 h) to avoid damaging seeds. After that, spraints were examined under a dissecting microscope (40 $\times$ ) and seeds were extracted with fine-point tweezers. Next, spraints were soaked in soapy water for 24 h and dried in the oven at 60 °C for 1 h. Food categories (plants and animals) were identified to the lowest possible taxonomic level using dedicated reference collections for the study area and the published literature (e.g. Conroy et al. 1993, Miranda & Escala 2002, Tachet et al. 2003). For crayfish and fish, diagnostic structures (e.g. appendages, carapaces, scales, pharyngeal arches) were used for identification and measured with a digital calliper ( $\pm 0.01$  mm). The remaining prey categories (i.e. other invertebrate and vertebrate taxa) were identified using diagnostic structures such as exoskeletons, feathers, bones, scales (reptile skin, bird legs) and fur. All spraints containing seeds were used for the germination experiment. All seeds present were extracted and identified to ensure good representation. On the same day of extraction, seeds were individually planted in small pots (5 cm diameter). Since the main goal was to assess seed viability for dispersion, planting every seed individually avoided any effects of competition, e.g. allelopathic interactions (Muller 1969). Indeed, isolated or very few seeds are commonly found in a single spraint (D. Almeida, pers. observ.). Specifically for *Mentha*, the observed range was 5–27 seeds per spraint. For this plant species, seeds were planted: 1) individually; 2) in groups of five; 3) in groups of ten (see Results below). Germination was monitored for two years in an experimental garden to overcome any effect of seed dormancy (see a similar procedure



in Cáceres & Monteiro-Filho 2007), which is typical for many Rosaceae species (even double dormancy, see Wada & Reed 2011). To more naturally reproduce the environmental conditions on planted seeds, the following study design was applied: 1) each pot was partly filled with soil previously taken near where each spraint was found and also nearby specimens of the target plant species; 2) fresh spraint matter (2 g) was added into the pots around the seed to discard any potential inhibitory effect of decomposition by otter faeces on germination (Traveset et al. 2007); and 3) day-light period, temperature, humidity and rainfall were simulated according to data from nearby meteorological stations to the study area (Ministry of Environment Spain 2022). For comparative purposes, blackberry seeds from blackbird faeces were processed and planted under the same conditions as for otters. For more detailed information on seed plantation, this methodology was adapted from Traveset et al. (2001), as these authors carried out studies on seed germination of similar plant taxa after defecation of frugivore birds (e.g. blackbird).

### Data analyses

Regression equations were used between the size of hard structures (e.g. crayfish appendages and fish bones) and the body weights to estimate the ingested biomasses of consumed crayfish and fishes (see Conroy et al. 1993 and Miranda & Escala 2002 for specific formulae). Biomasses were allocated to the remaining prey categories according to data from collections in the study area (adapted from Bueno-Enciso et al. 2014). Data on weights from plant samples were used for estimations of ingested fruit biomass. Dietary traits were analysed by calculating the percentages of occurrence and ingested biomass for each food category (e.g. Almeida et al. 2012a, b, 2013a). Examination of these percentages indicates whether a given food item is commonly eaten within the otter population and whether this food category is energetically important to that particular population. To compare the relationship between dietary use and environmental availability (i.e. fruit selection), Vanderploeg & Scavia (1979) normalised electivity index ( $\epsilon$ ) was used (see Almeida et al. 2012a and Bueno-Enciso et al. 2014 for other examples with a detailed formula of this index). Electivity was calculated per spraint with seed presence in September ( $n = 42$ ), as only one plant species was found in spraints from June (see Results below). Values can range from  $-1$  (avoidance) to  $1$  (preference), with zero implying neutral selection. Amongst many electivity indices,  $\epsilon$  was chosen because Lechowicz (1982) showed how this particular calculation best represented the

properties of true selection. Student's  $t$ -tests were used to assess whether the mean fruit electivity index differed significantly from zero per plant species (Almeida et al. 2012a, Bueno-Enciso et al. 2014). For the formula of this electivity index, the proportion of "fruit use" was calculated as a percentage of the ingested biomass per spraint. Given that otters can display high ambulation rates across their territories (see Almeida et al. 2013a), the proportion of "fruit availability" was calculated from the environmental biomass within 5 km upstream and downstream of every particular fresh spraint (i.e. 10 km river section), and thus fruit electivity was more accurately assessed per spraint. Specifically, the mean fruit load from the 10 assessed specimens was multiplied by the number of total plant individuals in the surveyed transect. A summation from the three transects per site was calculated, and finally, percentages of fruit availability were computed among the target plant species per site. The mean percentages from sampling sites were compared by one-way ANOVA, followed by a post hoc Tukey-Kramer honestly significant difference (HSD) test to detect the particular significant differences in fruit availability among plant species. Germinability (or final proportion of germination, i.e. proportion of seeds that germinate in a period long enough to obtain total germination) was calculated per plant taxon as a percentage, according to the following formula (e.g. Quadros & Monteiro-Filho 2000, Traveset et al. 2001):  $[\text{number of germinated seeds} \div \text{total number of planted seeds}] \times 100$ . Pairwise chi-square tests ( $\chi^2$ ) were used to compare germinability between plant species with Yates correction. Spearman's rank correlation was used to test for a significant relationship between  $\epsilon$  index and germinability.

A Generalised Linear Mixed Model (GLMM) was fitted for each response variable (i.e. ingested biomass, fruit availability, electivity), with "year" as the random factor to test for temporal dependences (see a comprehensive review of this statistical technique in Johnson et al. 2015). No significant difference was detected between 2018 and 2019 (Table 1, all  $P$  values  $> 0.05$ ). Spearman's rank correlations were used to test for spatial dependence in response variables along the study river section ( $n = 15$  sampling sites). No significant relationship was found between the response variables and sites (Table 1, all  $P$  values  $> 0.05$ ). This result is probably because of the low slope and limnological homogeneity of the surveyed reaches in the River Bullaque (e.g. see similar habitat conditions for Almeida et al. 2013b). Given that no significant temporal (year) or spatial (site gradient)

**Table 1.** Effects of the factors “year” and “site” on response variables (i.e. ingested biomass, fruit availability, electivity). *F*-ratios, degrees of freedom (df), Spearman’s rank correlation coefficients ( $\rho$ ) and significance levels (*P*) are presented per month/plant species (see Data analyses for details).

	<i>F</i>	df	<i>P</i>	$\rho$	<i>P</i>
Ingested biomass					
June	0.118	1, 28	0.733	0.204	0.466
September	0.905	1, 28	0.350	0.391	0.149
Availability					
<i>Mentha suaveolens</i>	0.115	1, 28	0.737	−0.210	0.452
<i>Crataegus monogyna</i>	0.001	1, 28	0.983	−0.077	0.785
<i>Rosa canina</i>	0.380	1, 28	0.543	−0.097	0.732
<i>Rubus ulmifolius</i>	0.413	1, 28	0.526	0.310	0.260
Electivity					
<i>Mentha suaveolens</i>	0.290	1, 40	0.593	−0.001	0.997
<i>Crataegus monogyna</i>	2.022	1, 40	0.163	0.209	0.184
<i>Rosa canina</i>	1.227	1, 40	0.275	−0.088	0.577
<i>Rubus ulmifolius</i>	0.453	1, 40	0.505	−0.133	0.402

effects were observed on response variables (Table 1, all *P* values > 0.05), data independence was established and values for every study variable were pooled. In particular, dietary data were pooled from 30 sites in June 2018–2019 and 30 sites in September 2018–2019, whereas availability (30 sites) and electivity (42 spraints) data were pooled only in September 2018–2019 per plant species (Table 1). This analytical approach (i.e. pooled data) considerably improved the clarity of results and facilitated biological interpretation. For statistical analyses, percentage data were logit-transformed, as Warton & Hui (2011) recommended this method to analyse proportions in ecology research as an alternative to arcsine square-root transformation. Assumptions of normality in data distributions and homogeneity of variances were verified using Shapiro-Wilk and Levene tests, respectively. Statistical analyses were performed with R v. 3.6.3 (R Core Team 2020). The significance level was set at  $\alpha = 0.05$ . Sequential Bonferroni corrections were performed for every set of multiple tests.

## Results

Crayfish and fishes were the primary food categories for otters in the study area, both in terms of occurrence and ingested biomass. Specifically, invasive red-swamp crayfish *Procambarus clarkii* (Girard, 1852) and the endemic Iberian minnow *Squalius alburnoides* (Steindachner, 1866) were the two species with higher values of occurrence (> 80 and > 70%, respectively) and ingested biomass (45 and 20%, respectively) (Table 2). Other invasive fishes were

also important, such as largemouth bass *Micropterus salmoides* (Lacépède, 1802) and pumpkinseed sunfish *Lepomis gibbosus* (Linnaeus, 1758). Other vertebrates (i.e. tetrapods) were also frequent in the diet (25% of occurrence) during late spring (mid-June), such as frogs, terrapins, lizards, aquatic snakes, coot, moorhen and aquatic rodents. Regarding plant material, otters consumed fruit from five plant species belonging to three taxonomic Families: Iberian bushweed *Flueggea tinctoria* (Webster, 1984) (Phyllanthaceae), apple mint *Mentha suaveolens* (Ehrh, 1792) (Lamiaceae), common hawthorn *Crataegus monogyna* (Jacq, 1775), dog rose *Rosa canina* (Linnaeus, 1753) and elmleaf blackberry (three Rosaceae species). Fruit from bushweed was only found in June, whereas the remaining plant species were only found in September (Table 2). Fruit was of low importance in June (< 1% of biomass). However, in September, this plant trophic resource was consumed more often (> 5% of biomass) than other prey of minor importance for otters (i.e. invertebrates such as snails, spiders, beetles and benthic insects; < 2%). Indeed, the occurrence increased (> 40%), with blackberry exceeding 30% (Table 2).

Fruit availability was significantly different among plant species ( $F_{3,56} = 100.97$ ,  $P < 0.001$ ) (Fig. 1 above). In particular, blackberry was the most abundant species in the environment (near 50% among fruit in the study), whereas estimated biomass for mint showed the lowest value (5%). Availability of fruit biomass was 30% and 20% for rose and hawthorn, respectively (Fig. 1 above). The  $\epsilon$  index was significantly lower than zero for mint ( $t = -7.78$ ,  $P < 0.001$ ) and rose ( $t = -3.67$ ,

**Table 2.** Diet composition of the Eurasian otter *Lutra lutra* in the River Bullaque. Percentages of occurrences (Oc., %) and estimated ingested biomasses (Biom., %) are presented per month.

Month	June		September	
Food category	Oc.	Biom.	Oc.	Biom.
Fruit	15	0.8	42	5.4
<i>Flueggea tinctoria</i>	15	0.8	-	-
<i>Mentha suaveolens</i>	-	-	7	0.1
<i>Crataegus monogyna</i>	-	-	25	1.1
<i>Rosa canina</i>	-	-	22	1.1
<i>Rubus ulmifolius</i>	-	-	35	3.1
Invertebrates	90	43.3	93	48.2
Terrestrial taxa <sup>a</sup>	16	0.9	6	0.6
<i>Procambarus clarkii</i>	82	41.4	85	46.4
Other aquatic taxa <sup>b</sup>	11	1.0	17	1.2
Fishes	89	46.8	85	43.1
<i>Iberochondrostoma lemmingii</i>	33	5.3	28	5.0
<i>Luciobarbus microcephalus</i>	13	2.5	10	1.9
<i>Squalius alburnoides</i>	76	19.1	72	21.6
<i>Squalius pyrenaicus</i>	24	2.4	10	1.9
<i>Cobitis paludica</i>	25	2.1	24	2.3
<i>Gambusia holbrooki</i>	15	0.3	-	-
<i>Lepomis gibbosus</i>	27	5.7	22	4.8
<i>Micropterus salmoides</i>	23	9.4	15	5.6
Other vertebrates	26	9.1	6	3.3
Anura ( <i>Pelophylax perezi</i> )	7	1.2	3	1.4
Geoemydidae ( <i>Mauremys leprosa</i> )	1	0.2	-	-
Lacertidae ( <i>Podarcis hispanicus</i> )	2	0.3	-	-
Colubridae ( <i>Natrix</i> sp.)	11	3.2	2	1.1
Birds (Rallidae)	9	3.1	-	-
Mammalia ( <i>Arvicola sapidus</i> )	3	1.1	1	0.8

<sup>a</sup>Gastropoda (Pulmonata), Araneae, Orthoptera, Coleoptera<sup>b</sup>Anisoptera nymphs, Coleoptera adults (Dytiscidae)

$P < 0.001$ ), whereas this pattern was significantly inverse ( $t = 4.43$ ,  $P < 0.001$ ) for blackberry (Fig. 1 below). A positive value was also observed for fruit from hawthorn (Fig. 1 below), although this deviation from zero was non-significant ( $t = 1.08$ ,  $P = 0.286$ ).

The highest germinability was found for blackberry (37% from 107 planted seeds), followed by hawthorn (24% from 29 seeds), rose (18% from 33 seeds) and bushweed (9% from 22 seeds). No germinated seeds were observed for mint from 103 planted seeds (33 pots  $\times$  one seed + six pots  $\times$  five seeds + four pots  $\times$  10 seeds). Germinability was 49% for blackberry (110 planted seeds) from blackbird faeces. Pairwise comparisons (with Yates corrections) highlighted

significant differences between germinabilities for “mint- rose” ( $\chi^2 = 9.77$ ,  $P = 0.0018$ ), “mint-hawthorn” ( $\chi^2 = 13.70$ ,  $P = 0.0002$ ) and “mint-blackberry” ( $\chi^2 = 28.82$ ,  $P < 0.0001$ ), after Bonferroni corrections. Among plants with successful germination, the pair “bushweed-blackberry” was marginally non-significant ( $\chi^2 = 3.01$ ,  $P = 0.0825$ ). A highly significant and positive relationship was observed between the  $\varepsilon$  index and germinability ( $\rho = 1$ ,  $P < 0.001$ ).

## Discussion

As with previous studies on otter diet in Mediterranean areas (e.g. Arcá & Prigioni 1987, Almeida et al. 2012a), an elevated piscivory was also





observed in the present study. However, invasive red-swamp crayfish was the most important prey species for otters, a trophic resource surely more profitable in terms of cost-benefit (Schoener 1971, Almeida et al. 2012b). Indeed, otters have profoundly changed their diet in the study area within a few decades towards invasive species, crayfish and fishes (Barrientos et al. 2014), with similar findings also being shown in the present paper. Regarding the intake of fruit, this food category has been found in several studies on otter diet (e.g. Arcá & Prigioni 1987, Georgiev 2006, Smiroldo et al. 2009, Almeida 2008). However, this trophic resource has usually been considered of low importance, almost an “accidental event” within the otter foraging activity (Almeida et al. 2012a). Riparian vegetation is a critical element of freshwater habitats where otters dwell. Thus, Prenda & Granado-Lorencio (1996) stated the importance of plant community structure and taxonomic composition (including species of rose, blackberry and hawthorn) for otter sprainting activity. Nevertheless, such associations were only explained as mere physical components of these aquatic environments (i.e. refuge for otters or their prey), with no mention of the potential trophic exploitation of plant resources. In this respect, frugivory in the taxonomic order Carnivora (including mustelid species) has already been well-described in the scientific literature (e.g. Herrera 1989), with pivotal implications for the ecosystem functioning (i.e. seed dispersal) (Willson 1993, Koike et al. 2008). In the present study and according to the first hypothesis, the relatively high occurrence of fruit in the diet merits special consideration for ecological processes, such as the distribution and structure of riparian vegetation. Indeed, this elevated use of fruit throughout particular months is in accordance with the species-specific phenology (i.e. main fruition periods). Also, as expected, the ingested biomass was low. However, a few grams of fleshy fruit may provide an individual with nutritious fibre, vitamins, proteins, carbohydrates and minerals (see Table 3 in Herrera 1989 for biochemical data), which are essentials for normal bodily development in Carnivora, including Mustelidae (Balestrieri et al. 2019).

As mentioned before, Barrientos et al. (2014) observed significant changes in otter diet throughout a few decades in the River Bullaque, which are related to a clear opportunistic feeding strategy. Similarly, Almeida et al. (2012a, 2013a) found an opportunistic seasonal use of ponds as foraging territories in eastern England, a very different habitat type with respect to fluvial waters. In the present study, fruit was also used seasonally as a trophic resource according

to contrasting plant phenology (i.e. fruition in late spring or late summer). However, partial support was found for the second hypothesis on the consumption of fruit relative to its environmental availability, as neutral selection (i.e. electivity – 0) was only observed for hawthorn. Thus, otters displayed avoidance for rose and (mainly) for mint, whereas preference was observed for blackberry, even when this plant species was the most available in the environment (i.e. displaying significant ingestion of blackberry fruit). These findings highlight the wide trophic plasticity of otters in Iberian fresh waters (Barrientos et al. 2014), including the elective intake of plant material. More specifically, otters may avoid a high level of mint consumption because this herbaceous species does not provide a suitable “fleshy” fruit material, and rosehip may pose a moderate degree of digestive toxicity from the high levels of tannins (Ayati et al. 2018, Barrett et al. 2018). Particularly for blackberry, otters displayed the greatest positive selection, in terms of fruit ingested biomass, amongst the target plant species. This finding, along with the highest germination success after otter defecation, is remarkable considering the observed data for the blackbird. This passerine species is a critical seed dispersal agent for many species of riparian vegetation in the Iberian Peninsula, especially for blackberry, due to a mutualistic co-evolution (Herrera 1984). As an example, Traveset et al. (2001) found a 60% germinability for blackberry seeds after extraction from blackbird faeces. This value was higher than the present study (50%), possibly because of slight differences in experimental protocols or intrinsic features of blackberry-blackbird interactions in this freshwater system. Blackberry seeds collected from otter spraints showed < 15% of lower germinability in comparison with blackbird faeces from the River Bullaque (37% *vs.* 49%, respectively). Thus, the otter may be a complementary species for a dual “bird-mammal” seed dispersal system, as Herrera (1989) suggested for Iberian passerines and carnivores. Furthermore, electivity values and percentages of germinability for the study plant species appeared to be positively correlated. Such result amongst plant taxa is in line with the third hypothesis, which may reveal a gradient of co-evolutionary history between contrasting “carrier” vertebrate species and “donor” plants (Willson 1993, Herrera & Pellmyr 2002).

In terms of specific mechanisms for such seed dispersal, otters can ingest significant quantities of fruit due to their large body sizes and fertilise them with faeces in particular sites cleared of dense vegetation (i.e. latrines), reducing plant competition

for seed settlement. Additionally, otters generally have broad (e.g. > 10 km<sup>2</sup>) home ranges, and they can display high ambulation rates, transporting seeds many kilometres from the parental plants along water courses (Almeida et al. 2012a, b) or across the landscape among ponds (Almeida et al. 2013a). Despite otters being top predators, this carnivore showed a high number of seeds in the spraints, and positive germinabilities were observed for most plant taxa (except for mint). According to these overall results, otters potentially act as a dispersal agent for seeds in the study area, an ecological role that has also been assigned to Neotropical otters in a Brazilian Atlantic forest (Quadros & Monteiro-Filho 2000). As already mentioned, germination was unsuccessful for mint seeds. A potential explanation of this observation concerns the type of “consumer guts”, as successful germination has been observed for this mint species from the dung of wild herbivores in the Iberian Peninsula (Malo & Suárez 1995). In otters, this “carnivore” digestive environment (e.g. pH, enzymes) may degrade the integrity of the seed coat leading to irreversible damage to the seeds. However, otters may still be able to spread undamaged mint seeds while they feed on sprigs, with nutlets being eventually attached to the wet fur by the mucilage (a myxocarpic process, see Ferreira et al. 2020). Opposite to the situation with mint seeds, such chemical scarification through the otter digestive tract may be suitable for germination of the taxonomic family Rosaceae (over 15% for rose at the minimum), whose seeds are more resistant (see examples in Wada & Reed 2011).

As the main conclusions of the present study, otters appear to i) use a variety of fruit as complementary food items; and ii) display an ecological role as seed

dispersal species for riparian vegetation in Iberian fresh waters. However, more research will be needed to quantify the specific contribution of fruit to otter nutritional status in terms of biochemical levels and metabolism. Also, as a complement for experimental data, germination patterns should be assessed in the wild to reveal potential inhibitory, enhancement or neutral effects of spraints on seedling establishment among plant taxa (e.g. see different traits in Traveset et al. 2001). This finding could provide evolutionary insights into the interactions between plants and carnivores, which have not traditionally been considered relevant for an endozoochorous ecosystem function (Herrera & Pellmyr 2002).

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### Author Contributions

*D. Latorre and D. Almeida – conceptualisation, methodology, investigation and writing original draft; R. Merino-Aguirre, A. Cruz, E. Lantero and A.M. Arroyo – investigation and formal analysis; D.H. Fletcher – formal analysis, review and editing.*

### Data Availability Statement

*The data supporting this study’s findings are available in the FigShare Digital Repository: <https://doi.org/10.6084/m9.figshare.20364714.v1>.*

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