Determinants of Eurasian otter (*Lutra lutra*) diet in a seasonally changing reservoir

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Abstract

Otter diet in reservoirs is known to experience seasonal changes. We selected a reservoir with a large population of exclusively wintering great cormorants and seasonal changes in stored water volume to test the relative influence of abiotic and biotic factors on otter foraging ecology. DNA metabarcoding of otter spraints revealed a dietary change from autumn to winter. Otters had a diet dominated by the exotic goldfish in autumn, but predated intensively on the native northern-straight-mouth nase in winter. This change was likely caused by predation of cormorants on goldfish and to fish biology. Secondly, macroscopic analysis of spraints revealed that otters shifted from a diet dominated by fish (in terms of biomass) to a diet dominated by red swamp crayfish during spring-summer, when the latter became overabundant. As revealed by modelling, this second shift was most likely influenced by the sudden increase in stored water volume in spring, but also by the cumulative effect of cormorant predation on fish during autumn-winter. Macroscopic analyses of otter spraints collected in a second reservoir with no cormorants revealed a lack of seasonality. Hence the combined influence of both biotic and abiotic factors explained otter diet seasonality in a lentic-water novel ecosystem.

Keywords: diet shifts, interspecific competition, foraging plasticity, DNA metabarcoding, changes in stored water volume.

Introduction

The Eurasian otter, *Lutra lutra* (Linnaeus, 1758), is a good example of how conservation measures have resulted in a substantial recovery of a formerly threatened species in Europe. After a dramatic decline in the 1960s and 1970s, mainly caused by water pollution in western and central Europe, many otter populations successfully rebounded to previous levels (Roos et al., 2015). Eurasian otters currently occur in all types of natural and human-made aquatic systems (Ayres & García, 2009; Weinberger et al., 2016) as a result of both conservation actions and their flexibility in habitat selection. Indeed, otters have increasingly colonised water reservoirs, particularly in southern Europe (Basto et al., 2011). Interestingly, reservoirs were initially seen as a threat to the persistence of otters in rivers worldwide (Palmeirim et al., 2014). However, Martínez-Abraín & Jiménez (2016) showed that otters are thriving in these novel ecosystems (Hobbs, 2018). Otters started using these new habitats in Spain by the 1980s and since then have fitted within them, ecologically (*sensu* Janzen, 1985), with great success (Delibes, 1990; Ruiz-Olmo & Delibes, 1998; López-Martín & Jiménez, 2009).

Several studies have highlighted a marked seasonality of the Eurasian otter diet in Iberian reservoirs. Otters are opportunistic predators that mainly consume fish while abundant, but shift to forage on the red swamp crayfish *Procambarus clarkii* (Girard, 1852) during periods when fish is not abundant (Pedroso & Santos-Reis, 2006; Sales-Luis et al., 2007; López-Martín & Jiménez, 2009; Basto et al., 2011; Pedroso et al., 2014). Both abiotic and biotic factors are known to affect fish abundance in reservoirs. For example, fluctuations in water level can affect fish recruitment differentially depending on nesting substrate, and inter-annual changes in temperature can influence inter-annual fish abundance through variations in the time of migration (de Lima et al., 2017; Kuczynski et al., 2017). Furthermore, the life-history of each fish species may

63 affect the abundance of fish prey for otters. In addition, large piscivorous vertebrates are
64 known to influence fish availability because of intense predation (Čech & Vejřík, 2011;
65 Klimaszyk & Rzymski, 2016). For instance, great cormorants *Phalacrocorax carbo*
66 (Linnaeus, 1758) are facultative specialists that are known to forage on a great variety
67 of fish prey but usually target those that are more common in each site (Orta et al.,
68 2018). Great cormorants have experienced a rapid population growth over the last four
69 decades because of the protection given to their breeding colonies in Central and
70 Northern Europe (Klimaszyk & Rzymski, 2016).

71 Here we set out to explore whether the intense predation exerted by wintering
72 great cormorants influenced the seasonality of otter foraging ecology in a reservoir. For
73 the present study, we selected a study site in Northwest Spain where otters can be
74 readily observed foraging during the day and in which there are large numbers of
75 wintering cormorants. We used DNA metabarcoding to determine if there is seasonal
76 variation in the diet of otters in a reservoir and if this seasonality is related with the
77 presence of another fish predator, the great cormorant, and/or abiotic factors that
78 influence the abundance of otter fish main prey.

Materials and methods

Study sites and prey species available

The Cecebre-Abegondo reservoir is located in Northwest Spain (central point coordinates: 43º16'56''N, 8º17'18''W) and is a core area of a Biosphere Reserve and a Special Conservation Area under the Nature 2000 network (Fig. 1). The reservoir was created in 1976 and covers 365 ha, holding a maximum of 22 million m^3 of water. It presents a marked artificial seasonality, with water levels kept low during fall-winter, to prevent flooding, and high during spring-summer, to supply tap water to approximately 400,000 people. The reservoir is located at ≈35 m above sea level, and it is quite shallow (6-15 m depth). Recreational boating is forbidden, but sport fishing is allowed from mid-March to the end of July. Temperatures are mild (14.4 ºC mean annual temperature) and rain is frequent throughout the year, although more common in winter (1037 mm mean annual precipitation). The reservoir has a narrow belt of riparian vegetation and oak forests (*Quercus robur* L.), where otters find shelter during their resting time.

Presence and diurnal activity of otters in this reservoir was firstly reported in 2004 (Mouriño et al., 2004). We observed otter foraging behaviour by direct observation with (x 10) binoculars and (x 20-60) terrestrial scopes. We determined that a minimum of seven resident otters made use of the reservoir during our study period (March 2015-February 2016), since two adult females with two cubs each, plus one adult male were observed simultaneously at least once. The red swamp crayfish (*Procambarus clarkii*) colonised the study area in the second half of the 1980s, becoming very abundant from the early 1990s onwards (Galán, 1997). There are only five fish species reported in the reservoir: two native species (brown trout *Salmo trutta*

103 Linnaeus, 1758 and northern straight-mouth nase *Pseudochondrostoma duriense*
104 (Coelho, 1985), hereafter referred to as 'nase') and three exotic species (goldfish
105 *Carassius auratus* (Linnaeus, 1758), complex, largemouth-bass *Micropterus salmoides*
106 (Lacepède, 1802), and mosquito fish *Gambusia holbrooki* (Girard, 1859)) (Augas de
107 Galicia, 2009). Anglers mostly target brown trout. Great cormorants first colonised the
108 study site in 1989, becoming a regular wintering species from 1993 onwards, hence
109 showing a similar time pattern of reservoir colonisation to that of red swamp crayfish
110 (X. Mouriño, pers. comm.).

111 The second study site, which was used as a control, was Eiras reservoir (central
112 point coordinates: 42º20'18''N, 8º25'51''W) ca. 100 km south of Cecebre (Fig. 1),
113 which has no wintering cormorants but where the exotic red-swamp crayfish has been
114 recorded. Eiras reservoir was created in 1977, has a maximum surface area of 149 ha
115 and supplies drinking water to a similar number of habitants than the Cercebre
116 reservoir. It also has a similar forced seasonal regime and its fish community includes
117 brown trout, largemouth-bass, European chub (*Squalius cephalus* (Linnaeus, 1758)),
118 and nase. To the best of our knowledge, goldfish are absent from this reservoir, which
119 may affect its comparability with Cecebre. Despite its small surface area it stores a
120 similar maximum amount of water (22 million m^3) due to its greater depth and steeper
121 banks. The height of the dam from the foundations to the spillway is 47 m. The areas
122 where the two tributaries (Oitavén and Barragán rivers) meet the lentic waters of the
123 reservoir (i.e. tributary outlets) have shallower banks and that is where we detected otter
124 activity. These areas (ecologically equivalent to Cecebre) were the areas sampled in
125 Eiras when searching for otter spraints. These two reservoirs are not stocked with fish
126 on a regular basis.

127

128 *Diet variability between periods*

129 Based on prior literature as well as our own observations, we expected a
130 pronounced shift (fish-crayfish) on otter diet between the autumn-winter period and the
131 spring-summer period. Therefore, we analysed otter spraints macroscopically in order to
132 obtain relative indices of fish and crayfish consumption during autumn-winter and
133 spring-summer. For that purpose almost the entire length of the reservoir shore was
134 surveyed in search of otter spraints. This prevented pseudo-replication, that is,
135 collecting spraints belonging only to a few individuals. Most samples came from the
136 high density points identified in Fig. 1b. Adult females and their cubs use the same sites
137 repeatedly to mark their territories creating latrines. In such cases an individual sample
138 was arbitrarily defined as the amount of faeces that fitted approximately in half an 82 x
139 60 x 20 mm (50 cm^3) standard zip polythene bag.

140 Otter spraints (n = 230) were collected at two different times of the year. The
141 first collection (March-April 2015; n = 156) took place before the level of the reservoir
142 was artificially raised in spring, representing otter diet during the last part of the low
143 water period ("winter"). A second set (n = 74) was collected in September 2015 before
144 the water level was lowered, representing otter diet accumulated during the last part of
145 the high-water period ("summer"). It should be noted that these "winter"/"summer"
146 terms do not necessarily correspond with the chronology of seasons.

147 In order to study the differential consumption of fish and crayfish between
148 periods, we firstly observed the 230 spraints under the stereoscopic microscope to
149 quantify samples containing fish, crayfish or both. Crayfish-based spraints look red in
150 colour whereas fish-based spraints look greenish to grey in colour. Additionally, fish-
151 based spraints contain fish scales and bones whereas crayfish-based spraints have
152 remains of crayfish exoskeleton. Some spraints had mixed contents but fish and crayfish

153 remains were easy to differentiate. These 230 samples were used to develop a relative
154 index of fish and crayfish consumption by otters to compare between periods. Otter
155 physiology between periods (regarding spraint generation rate) was assumed to remain
156 constant, an assumption supported by the fact that the study otters were residents. Then
157 we converted the frequency of spraints containing crayfish and/or fish into a relative
158 index of biomass provided by each prey type (Supplementary data 1), as frequency data
159 does not necessarily reflect the actual relevance of prey items on the energy intake of
160 otters (Beja, 1996a; Kruuk, 2006).

161 At the Eiras reservoir, we collected 89 spraint samples for the summer period
162 (September 2016) and 51 for the winter period (February 2017). Samples were collected
163 at the tributary outlets. Macroscopic information was obtained following the same
164 procedures as for the Cecebre reservoir.

165 Considering that between-period dietary change was likely influenced by the
166 water temperature and/or artificial variability in water depth, we obtained monthly
167 values of both variables from the managers of the reservoir gates (EMALCSA,
168 www.emalcsa.es). Available data corresponds to 2014 (Figs. S1 and S2), a typical year
169 without severe droughts, extreme rainfall periods, or extreme cold or warm
170 temperatures. We also tested the possibility that the cumulative effect of foraging on
171 fish during the whole autumn-winter period could influence the ratio of fish/crayfish.
172 For this we used the cumulative average number of cormorants per month, starting in
173 September.

174

175 *Statistical analyses*

Proportional differences in diet composition (fish vs. crayfish, native vs. exotic) and biomass between periods (winter vs. summer) were explored by means of contingency tables together with the Chi-square statistic with Yate's correction. An independence test was also used to assess the ratio of exotic to native fish species consumed during early and late winter. Residuals of the Chi-square test were used to assess the positive and negative departure of observed frequencies in relation to expected frequencies. Seasonal diversity of otter autumn-winter fish diet (as obtained from DNA metabarcoding) was compared by means of the Shannon-Weaver index ($H' = -\Sigma(p_i * \ln p_i)$). Values of H' were compared by means of the equitability (J') calculated as $J' = H'/H_{max}$, where $H_{max} = \ln(S)$, that is the maximum value that H' can achieve in the study community (S = number of species).

The influence of the accumulated effect of great cormorants over winter and the influence of water temperature and stored water volume on otter dietary shift between periods was studied by means of Generalized Linear Models (Gaussian family of errors and identity link) in which the ratio of samples containing fish/samples containing crayfish was the dependent variable and cumulated number of cormorants, water temperature, and water volume were the independent variables. Biologically sound models were compared simultaneously by means of theoretical information criteria duly corrected for small sample size (AICc). The model with the lowest AICc was selected as it represents the most parsimonious model, the one explaining more deviance with the lowest structural complexity. Akaike weights were also used to study relative model probability. More specifically, the ratio of the Akaike weights of the models ranked first by means of AICc provides an idea of how likely one model is to be the best model in terms of Kullback-Leibler divergence or relative entropy. All the analyses were performed with the software and environment R (https://www.r-project.org/).

201

202 *Variability within the autumn-winter period*

203 Great cormorants arrive at the study area by the end of summer or the beginning
204 of autumn and leave in the spring. Great cormorants are solitary feeders during most of
205 their wintering season but they gather in groups of several hundreds in early autumn
206 (from mid-September to the end of October) probably because their main prey, goldfish,
207 is overabundant and still in shoals after their summer reproduction. Birds were counted
208 from vantage points with the aid of (x 10) binoculars and (x 20-60) terrestrial scopes.
209 Cormorants in rafts (i.e. dense flocks of birds on the water) were counted at Cecebre
210 during the autumn, because this is when they form large and dense fishing groups that
211 can have a greater impact on fish abundance. Eight rafts were detected in the autumn of
212 2016, and the number of birds in each raft was counted. For this, we took pictures with
213 a digital camera and the number of birds was counted on a computer screen by the same
214 observer. Additionally, we counted the cormorants at the major roosting site of Cecebre
215 reservoir during winter and spring, when rafts were no longer formed. The roosting site
216 is located on an island (Fig. 1b) and counts only referred to the area facing the
217 observation spot. These counts were performed with the aim of obtaining a relative
218 index of abundance over time. A long time series (n = 32 years including the complete
219 period from the first colonisation of the study site) of cormorant counts, was provided
220 by the environmental authorities (Consellería de Medio Ambiente, Xunta de Galicia).

221 We used DNA metabarcoding faecal analysis to determine the fish species
222 consumed by otters during the autumn-winter period, when the diet of otters is
223 dominated by fish. Morphological identification of fish in the diet of otter is not only
224 time consuming, but requires a high degree of expertise (Carss & Parkinson, 1996).
225 Genetic approaches based on DNA metabarcoding (Taberlet et al., 2012) overcome

these two difficulties and, in addition, may provide higher taxonomic precision and greater sensitivity (Nichols et al., 2016).

We collected 25 spraints with fish remains in October 2015 and another set of 25 spraints in February 2016, following the same sampling procedures described above (i.e. sampling was performed in two sessions in October and two sessions in February covering most of the reservoir shores; the zones sampled in October were also sampled in February). These 50 samples were exclusively used for genetic analyses, which were conducted at AllGenetics & Biology SL (www.allgenetics.eu). All DNA isolations were carried out using 200 mg of each spraint and the RealPure Spin Food-Stool kit (Real^{TM}, Valencia, Spain) following the manufacturer's protocol. All samples were resuspended in a final volume of 100 μL. A negative control that contained no sample was included to check for contamination. We assessed DNA content and quality using a NanoDrop 1000 spectrophotometer. The 50 DNA extractions were confirmed to be otter spraints using the diagnostic PCR methods developed by Schäffer et al. (2017) (*COI*, primers St_f and St_r) and Fernandes et al. (2008) (*cytb*, primers LlutraF1 and LlutraR1 as well as LlutraF2 and LlutraR2 if the former failed).

In order to prepare a DNA metabarcoding library, a pair of primers that met the following requirements was designed for the present study: (i) it should amplify a short fragment (ca. 200 bp), due to the degraded nature of DNA in spraints; (ii) it should work, at least, in the potential prey species revealed by Augas de Galicia (2009), i.e.: *C. auratus*, *S. trutta*, *P. duriense*, *M. salmoides*, and *G. holbrooki*; (iii) it should not amplify *L. lutra* DNA. Due to host DNA being expected to be in great proportion in the faecal samples, if the primers do not amplify host DNA, the sequencing depth needed to capture the sequences from all potential prey species is considerably reduced; and (iv)

250 the amplified region should be variable enough to allow for taxonomic identifications to
251 species level.
252 For primer design, complete mitochondrial genomes of *L. lutra* and all but one
253 (*P. duriense*) potential prey species were obtained from GenBank. *P. duriense* 12S
254 rRNA and 16S rRNA sequences were downloaded as well from the same source
255 (https://www.ncbi.nlm.nih.gov/genbank/). All the accession numbers are recorded in
256 Supplementary data 2. Sequences were aligned using Muscle (Edgar, 2004a, 2004b), as
257 implemented in Geneious 8.1.8 (www.geneious.com). The 12S rRNA and 16S rRNA
258 regions were subjected to a Primer3 search (Rozen & Skaletsky, 2000) as implemented
259 in Geneious 8.1.8 (http://www.geneious.com/). A pair of primers annealing to the 12S
260 rRNA region (Teleo-12S-F 5'-ACTGGGATTAGATACCCCACT-3' and Teleo-12S-R
261 5'-TCACAGGGTAAGCTGACGAC-3'; positions 1489 to 1707 of the mtDNA genome
262 (or 418-636 of the 12S rRNA gene) of *Oncorhynchus mykiss* (Walbaum, 1792)
263 (accession number L29771)) met the requirements mentioned above and were,
264 therefore, selected for biological validation. Primer Teleo-12S-F perfectly matched
265 sequences of both otter and fish. The only mismatch was at position 16 of the alignment
266 for two (accession numbers NC_001717 and L29771) out of the seven haplotypes of *O.*
267 *mykiss* analysed. However, amplification of DNA from otter or any other mustelid
268 present in the area is prevented by the fact that primers Teleo-12S-R perfectly matched
269 fish DNA and showed seven mismatches for otters (Supplementary Data 3).

270 In order to validate the selected primer pair, DNA was extracted from three *L.*
271 *lutra* tissue samples (using NZYTech's NZY Tissue gDNA isolation kit), and from one
272 tissue sample of each of the potential prey species using the High Pure PCR Template
273 Preparation Kit (Roche, Mannheim, Germany). PCRs were carried out in a final volume
274 of 25 μL, containing approximately 50 ng of template DNA, 0.5 μM of the primers

Teleo-12S-F and Teleo-12S-R, 12.5 μL of Phusion® High-Fidelity PCR Master Mix (Thermo Fisher Scientific, Carlsbad (CA), USA), and ultrapure water up to 25 μL. The reaction mixture was incubated as follows: an initial denaturation at 98 ºC for 30 s, followed by 35 cycles of 98 ºC for 10 s, 60 ºC for 20 s, 72 ºC for 20 s, and a final extension step at 72 ºC for 10 minutes. The PCR products were run in a 1% agarose gel stained with GreenSafe (NZYTech, Lisbon, Portugal), and visualised under UV light. This visual inspection confirmed the amplification on all the fish tissue samples, and the non-amplification on the *L. lutra* tissue samples. The fish PCR products (221 bp, but for *P. duriense* whose amplicon was 222 bp) were Sanger-sequenced in both directions using the PCR primers. Electropherograms were analysed with Geneious 8.1.8. Finally, a BLAST search (http://blast.ncbi.nlm.nih.gov/Blast.cgi) against the GenBank nr database confirmed that the primers had amplified the expected fish species in each sample (sequences and accession numbers are provided in Supplementary Data 4).

DNA metabarcoding libraries from each faecal sample were prepared with primers Teleo-12-S and Teleo-12-R in two different PCR steps. For the first one, the PCR conditions were identical to the PCR described above for the validation of the primers, with one exception: the primers had a 5' overhang, corresponding to the Illumina-specific sequencing primers. The index sequences (required for multiplexing different libraries in the same sequencing pool) were attached in a second PCR round with identical conditions but only 5 cycles. For a schematic overview of the library preparation process, please see Figure 1 in Vierna et al. (2017). The libraries were run on a 1% agarose gel stained with GreenSafe (NZYTech, Lisbon, Portugal), and imaged under UV light. Negative controls that contained no DNA were included to check for potential contamination during library preparation. Finally, all libraries were pooled in equimolar amounts into a single pool for sequencing in a fraction of an Illumina's 600-

300 cycle run (MiSeq Reagent Kit v3; PE300). All raw sequencing data from this study have
301 been deposited in the NCBI Sequence Read Archive (SRA) under BioProject accession
302 number PRJNA605969.
303 An initial assessment of the quality of the Illumina data was performed using the
304 software FASTQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/).
305 Paired-end assembly of the forward and reverse reads were performed with FLASH
306 (Magoc & Salzberg, 2011). The resulting sequences were processed using the
307 bioinformatic tool QIIME 1.9.1 (Caporaso et al., 2010). Sequences were filtered based
308 on read quality using a minimum quality score of 20. We checked for the presence of
309 chimeras *de novo* using the USEARCH algorithm (Edgar et al., 2011). The 12S rRNA
310 gene sequences were clustered into OTUs and the taxonomy was assigned using the
311 open-reference approach of QIIME. In this method, reads are clustered against a
312 reference database and those not hitting the reference sequence collection are
313 subsequently clustered *de novo*. We used an in-house reference database containing the
314 12S rRNA fragment for the eight potential fish prey species. Sequences not hitting the
315 reference database were compared against GenBank's nr database using BLASTN
316 (Altschul et al., 1990), as implemented in BLAST2.2.31+, under the megablast settings
317 and a percent identity of 97 %. Finally, based on the resulting OTU abundance table, we
318 removed OTUs that were observed less than twice in the whole dataset.
319 It has recently been observed in DNA metabarcoding studies that a low
320 percentage of the reads of a library can be assigned to another library. This
321 phenomenon, referred to as mistagging, is the result of the misassignment of the indices
322 during library preparation, sequencing, and/or demultiplexing steps (Esling et al., 2015;
323 Bartram et al., 2016). Mock communities (i.e. samples containing a known amount of
324 DNA from a defined set of taxa) are recommended to calibrate the filtering settings,

which are applied during the DNA metabarcoding pipeline to eliminate erroneous OTU assignments (Bokulich et al., 2013). Therefore, we created a mock community by pooling an equal mix of DNA from *C. auratus*, *P. duriense*, *M. salmoides*, *O. mykiss*, *S. trutta,* and *Salmo salar* Linnaeus, 1758. Two replicates of the mock community were analysed using the above-mentioned DNA metabarcoding protocol (Fig. S1, raw data available upon request). As a result, OTUs with a number of sequences lower than 0.5 % of the total number of sequences per sample were removed. Most of the reads of three samples from February-2016 (19, 21, and 23) did not get through the filtering of read quality and therefore no OTUs were assigned during the closed-reference selection step. These samples were not used for downstream analyses. The sequences that were clustered during the *de novo* picking process and subsequently compared to the NCBI database did not match any other fish species. The majority corresponded to bacteria and some green plants.

Results

Shift from fish to crayfish between periods

Stored water level changed seasonally, with higher levels being reached during the May-August period and lower levels during the September-April period. Changes between both periods amounted to a vertical difference of some 2.5 m (Fig. S1). Stored water temperature also changed seasonally with a period of higher temperatures from May to October, and a period of lower temperatures from November to April. Lowest water temperatures were reached in February (Fig. S2). The results of our modelling clearly showed that the main abiotic factor influencing the seasonal shift from fish to crayfish was the change in stored water volume rather than water temperature. The model containing only water volume as an explanatory variable was the most

parsimonious (had the smallest AICc) and the highest Akaike weight (0.52/0.38=1.4 times more likely to be the best model; Table 1). However, the second best model (model probability = 0.38; Table 1) also included the cumulative number of great cormorants.

In terms of relative frequency, more samples contained crayfish than fish in both seasons (crayfish winter = 105; fish winter = 66; crayfish summer = 118; fish summer = 21). However, proportionally, crayfish was consumed more frequently in summer than in winter ($\chi^2 = 9.41$, d.f. = 1, $P = 0.002$; winter 39%; summer 85%), indicating diet seasonality. A modal-size goldfish captured by otters (using our field observations on approximate fish size and data on mean fish lengths from samples by Augas de Galicia (2009)) weighed 15.4 g after applying the equation by Andreu-Soler et al. (2006). Modal-size crayfish prey weighed 5.9 g, as from gastrolith analysis (Supplementary Data 1). Therefore, although crayfish was the most frequent otter prey in both periods, fish provided comparatively more biomass in winter (62%) than crayfish, whereas crayfish provided more biomass in summer (58%) compared to fish ($\chi^2 = 7.23$; d.f. = 1; $P = 0.007$).

By contrast, otters did not change their diet between periods in the absence of wintering cormorants. Proportional differences in spraints containing fish and crayfish consumed between periods were not found to be statistically significant at Eiras reservoir ($\chi^2 = 0.003945$, d.f. = 1, $P = 0.9499$) contrary to what was the case at Cecebre. Fish was consumed in a slightly higher proportion than crayfish in both periods at Eiras reservoir.

Shift from goldfish to nases and cormorant abundance

374 Numbers of cormorants in a single and dense raft ranged from 115 individuals
375 (by mid-September) to a maximum count of 567 individuals by the end of October
376 (median = 270, n = 8) (Fig. 3a). Relative numbers of cormorants in the roosting site at
377 Cecebre remained approximately constant during the winter (mean = 182.3 ± 28.69) and
378 declined rapidly and linearly during the spring ($r^2 = 0.93$) (Fig. 3b). The amount of
379 wintering cormorants recorded in January at Cecebre increased linearly during the
380 period 1987-2017 ($r^2 = 0.42$) (Fig. S4). January counts during the last few years are
381 around 200-300 individuals, a figure roughly coincident with our median estimate of
382 birds in rafts during the autumn.

383 All DNA extractions proved to be otter spraints and not from any other mammal.
384 Results from DNA metabarcoding revealed that the main fish prey during autumn-
385 winter were two species, the exotic goldfish (*C. auratus*) and the native nase (*P.*
386 *duriense*). Additionally, we found that otters foraged on largemouth-bass (*M.*
387 *salmoides*), brown trout (*S. trutta*), and even on a fish species that was not previously
388 recorded for the study reservoir, the rainbow trout (*O. mykiss*). We also detected the
389 presence of the small mosquito fish (*G. holbrooki*) in two of the spraints (Fig. 2).

390 The Shannon-Weaver index of diversity (H') (regarding autumn-winter fish
391 prey) was lower in October than in February (0.75 vs. 0.92). The equitability (J') of the
392 consumed fish prey community was 0.42 in October and 0.51 in February. The slightly
393 higher diversity in February was due to the fact that relative frequencies of DNA reads
394 were allocated more equitably among fish species. On the contrary, in October the
395 consumed fish prey community was dominated by *C. auratus*. In fact, the ratio of exotic
396 to native fish was shown to be different between both time periods ($\chi^2 = 240.31$, d.f. = 1,
397 $P < 0.05$). Residuals of the Chi-square test showed that the frequency of exotic fish was
398 higher than expected in October (5.19) but lower than expected in February (-5.90).

399 Conversely, frequencies of native fish were lower than expected in October (-8.85) and
400 higher than expected in February (10.07). Our results indicate that otters shifted from
401 foraging mainly on exotic goldfish in autumn to foraging on a wider variety of fish
402 species (mostly on native nases) in winter.

403

404 **Discussion**

405 *Shift from goldfish to nase*

406 DNA metabarcoding revealed that the most commonly consumed fish prey
407 during autumn was the exotic goldfish (*C. auratus*), which was the only truly lentic and
408 slow-moving species available at the study site. The native and endemic nase (*P.*
409 *duriense*) was consumed more often in winter. Predatory fish, with high muscle/fat
410 ratios and hence high reaction speed, were seldom consumed, as reported previously
411 (Blanco-Garrido et al., 2008). We postulate that the observed increase of otter predation
412 on nase from autumn to winter was caused by the scarcity of goldfish after cormorant
413 predation during the autumn. Great cormorants are known to eat large amounts of fish
414 during wintering. Their daily food intake is approximately half a kilogram of fish
415 (reviewed by Klimaszyk & Rzymski, 2016), whereas that of otters is approximately one
416 kilogram (Kruuk, 2006). Therefore, a median number of 270 cormorants during the
417 winter would be equivalent to the predation exerted on fish by 135 otters eating 1 kg of
418 fish per day in a reservoir of only 365 ha. However, variations in the composition of the
419 diet of otters could also be related with changes in the biology of prey species (reviewed
420 by Chanin, 2003). For example, goldfish might become scarce during winter as this is a
421 thermophilic species that reduces mobility, remains close to the bottom or even buries
422 itself in bottom mud during winter (Doadrio et al., 2011). In addition, nase might

become easier to catch in winter if individuals started gathering in groups before ascending to headwaters to reproduce in spring (Doadrio et al., 2011), although such gathering is expected to occur from March onwards (I. Doadrio, pers. comm.).

From a methodological point of view DNA metabarcoding has proven to be a successful methodology for identifying the species composition of a complex sample, even when DNA is degraded or in the presence of inhibitors (e.g. De Barba et al., 2014). Moreover, DNA metabarcoding is useful for identifying highly digested animal tissue in faeces or gut content, where morphological analyses might be challenging (e.g. Fernández-Álvarez et al., 2018). Recent literature emphasised that the advantages of molecular methods, namely a higher taxonomic resolution, definitely compensate for disadvantages such as the possibility of false negatives if prey DNA is not uniformly distributed throughout the faeces (Berry et al., 2015; Mumma et al., 2016; Oja et al., 2017). The fact that most of the readings of three samples from February showed low quality and could not be analysed (Fig. 3) might be due to this non-uniform distribution of prey DNA. Indeed, otter spraints consist of faeces and secretions from the anal scent glands (reviewed by Kean et al., 2011). It is possible that a part of the glandular secretion might have been taken for further DNA extraction in these three spraints. Alternatively, explanations for the lack of data in these three samples are that (i) they contained prey DNA from species with a substantially lower lipid content, so that mitochondrial DNA was more degraded through digestion or (ii) they were not taken from otter faeces, but from gut secretions usually referred to as “anal jelly” (reviewed by Thalinger et al., 2016). Although spraints and anal jelly are unmistakable, these three spraints might have been inadvertently contaminated with some anal jelly, as both of them are deposited in prominent locations for scent marking (Kean et al., 2011). At present we cannot rule out any of these hypotheses, but the finding of three false

448 negatives (a similar result to the one reported by Thalinger et al., 2016) highlights the
449 need for analysing more than one sample per spraint.

450

451 *Shift from fish to crayfish*

452 After the autumn-winter shift in the diet of otters (goldfish to nase), most likely
453 caused by the combined effect of competition with great cormorants and by prey
454 behaviour, we observed an expected second major shift. Otters moved from predating
455 mostly on fish in autumn-winter (in fixed hunting sites in the open water) to foraging
456 mainly on crayfish in spring-summer along the reservoir shores. Our results indicate
457 that this shift can be caused by the difficulties otters could be experiencing in reaching
458 the reservoir bottom in periods when stored water in the reservoir is artificially
459 increased. In addition, cormorant predation could decrease fish availability to otters.
460 The spring-summer massive emergence of crayfish from hibernation provides an
461 alternative prey. Evidence provided by otter diet at the control reservoir where
462 cormorants are absent (Eiras) supports the hypothesis that cormorant predation is an
463 important driver of the change in otter diet during this period. Although Eiras shows a
464 regime of changes in water level similar to that of Cecebre, otters do not show this
465 change in diet in Eiras. We believe this could be due to the fact that Eiras is a much
466 deeper reservoir, where changes in water level only influence the shallower areas.
467 However we cannot rule out other factors. Unfortunately, there are no reservoirs
468 supplying drinking water in the region that have a different water level regime that we
469 could use as control.

470 Otters are known to switch to suboptimal prey during periods of low fish
471 availability (Kruuk, 2006: Lanszki et al., 2016; Bouroş & Murariu, 2017; Britton et al.

472 2017). Summer was also the period when native fish resources were lowest in Portugal
473 (Beja, 1996b), which made the presence of exotic crayfish especially useful as an
474 alternative food source for otters (Kruuk, 2006). The role of crayfish (native or exotic)
475 as an alternative food for otters after fish become scarce has also been reported for
476 Mediterranean rivers (Ruiz-Olmo & Palazón, 1997; Jiménez, 2005). In spite of fish
477 being the main prey in autumn-winter (low water period) at both reservoirs, otters also
478 managed to capture a substantial proportion of crayfish during that time, when crayfish
479 burrow into the bottom and go dormant. Otters seem to be skilled in locating their
480 burrows, which is congruent with the findings of Lyach & Čech (2017) in more severe
481 winter conditions. Scent, rather than vibrissae, might play a role in detecting dormant
482 crayfish (reviewed by Noordhuis, 2002).

483 We have shown that otters first preyed upon goldfish, the most abundant fish
484 resource during autumn, but then increased their dietary diversity in winter and preyed
485 more on nase. This was likely influenced by both the intense predation by great
486 cormorants on the same resource and by changes in fish biology during winter. Then the
487 sudden change in water depth in spring, coupled with the cumulative effect of
488 cormorant predation on fish, coincided with a major shift from fish to red swamp
489 crayfish in spring-summer. Hence, the combined effect of biotic and abiotic changes
490 throughout the year can explain the observed shifts in otter foraging ecology. We cannot
491 rule out variations of the seasonality pattern among years, as our study was carried out
492 during one year.

493 Although cormorants seemingly are severe competitors of otters during the
494 autumn-winter period, otters are known to have a high foraging and dietary plasticity in
495 unstable environments (Clavero et al., 2003; Jiménez, 2005; Remonti et al., 2008;
496 Krawczyk et al., 2016). This high plasticity explains how otters can make use of a

lentic-water novel ecosystem, full of exotic species, all year round. Cormorants could also have some beneficial effect on otters: fish move to shallower areas and shelters (Eckman & Imbrock, 1996) to avoid predation by cormorants, but this makes them easier to be captured by otters. However, this ambitious hypothesis needs to be further investigated.

Acknowledgments

Fish samples to be used as positive controls for molecular analysis were kindly provided by Carlos Antunes, except for *O. mykiss* and Atlantic salmon *S. salar* that were purchased at a local fish market. David Stanton generously provided us with four otter tissue samples used during DNA metabarcoding optimisation. EMALCSA kindly provided data on water level variation and Xunta de Galicia (Consellería de Medio Ambiente e Ordenación do Territorio) allowed us to have a copy of an unpublished report with detailed information about the structure of the fish community at the study reservoir. The long-term time series on great cormorant counts at the study reservoir was supplied by Xunta de Galicia (Dirección de Patrimonio Natural). Pablo Sierra helped us during field work at Eiras reservoir. We are thankful to Ignacio Doadrio, Juan Jiménez, Daniel Oro, three editors, Martin Čech, and two anonymous referees for their valuable comments and suggestions. This work received funding from Xunta de Galicia (grants GRC2014/050 and ED431C 2018/57) and Universidade da Coruña. A Martínez-Abraín was supported by an *Isidro Parga Pondal* research contract by Xunta de Galicia during the period 2011-2016.

References

521 Altschul, S. F., W. Gish, W. Miller, E. W. Myers & D. J. Lipman, 1990. Basic local
522 alignment search tool. Journal of Molecular Biology 215: 403-410.

523 Andreu-Soler, A., F. J. Oliva-Paterna & M. Torralva, 2006. A review of length–weight
524 relationships of fish from the Segura River basin (SE Iberian Peninsula). Journal
525 of Applied Ichthyology 22: 295-296.

526 Augas de Galicia, 2009. Asistencia técnica para la toma de datos del indicador biológico
527 peces en las redes de monitorización de aguas superficiales en el ámbito de la
528 demarcación hidrográfica Galicia-costa (Expte. OH.688.244.SV). Consellería de
529 Medio Ambiente, Territorio e Infraestructuras. Xunta de Galicia, Spain.

530 Ayres. C. & P. García, 2009. Abandoned clay mines: an opportunity for Eurasian otters
531 in NW Spain. IUCN Otter Specialist Group Bulletin 26: 67-72.

532 Bartram, J., E. Mountjoy, T. Brooks, J. Hancock, H. Williamson, G. Wright, J. Moppett,
533 N. Goulden & M. Hubank, 2016. Accurate sample assignment in a multiplexed,
534 ultrasensitive, high-throughput sequencing assay for minimal residual disease.
535 Journal of Molecular Diagnostics 18: 494-506.

536 Basto, M. P., N. M. Pedroso, A. Mira & M. Santos-Reis, 2011. Use of small and
537 medium-sized water dams by otters in a Mediterranean ecosystem. Animal
538 Biology 61: 75-94.

539 Beja, P. R., 1996a. An analysis of otter *Lutra lutra* predation on introduced American
540 crayfish Procambarus clarkii in Iberian streams. Journal of Applied Ecology 33:
541 1156-1170.

542 Beja, P. R., 1996b. Temporal and spatial patterns of rest-site use by four female otters
543 *Lutra lutra* along the southwest coast of Portugal. Journal of Zoology 239: 741-
544 753.

545 Berry, O., C. Bulman, M. Bunce, M. Coghlan, D.C. Murray & R. D. Ward, 2015.
546 Comparison of morphological and DNA metabarcoding analyses of diets in
547 exploited marine fishes. Marine Ecology Progress Series 540: 167-181.

548 Blanco-Garrido, F., J. Prenda & M. Narvaez, 2008. Eurasian otter (*Lutra lutra*) diet and
549 prey selection in Mediterranean streams invaded by centrarchid fishes.
550 Biological Invasions 10: 641-648.

551 Bokulich, N. A., S. Subramanian, J. J. Faith, D. Gevers, J. I. Gordon, R. Knight, D. A.
552 Mills & J. G. Caporaso, 2013. Quality-filtering vastly improves diversity
553 estimates from Illumina amplicon sequencing. Nature Methods 10: 57-59.

554 Bouroş, G. & D. Murariu, 2017. Comparative diet analysis of the Eurasian otter (*Lutra*
555 *lutra*) in different habitats: Putna-Vrancea Natural Park and Lower Siret Valley,
556 south-eastern Romania. North-Western Journal of Zoology 13: 311-319.

557 Britton, J. R., M. Berry, S. Sewell, C. Lees & P. Reading, 2017. Importance of small
558 fishes and invasive crayfish in otter *Lutra lutra* diet in an English chalk stream.
559 Knowledge and Management of Aquatic Ecosystems 418: 13.

560 Caporaso, J. G., J. Kuczynski, J. Stombaugh, K. Bittinger, F. D. Bushman, E. K.
561 Costello, N. Fierer, A. Gonzalez Peña, J. K. Goodrich, J. I. Gordon, G. A.
562 Huttley, S. T. Kelley, D. Knights, J. E. Koenig, R. E. Ley, C. A. Lozupone, D.
563 McDonald, B. D. Muegge, M. Pirrung, J. Reeder, J. R. Sevinsky, P. J.
564 Turnbaugh, W. A. Walters, J. Widmann, T. Yatsunenko, J. Zaneveld & R.
565 Knight, 2010. QIIME allows analysis of high-throughput community sequencing
566 data. Nature Methods 7: 335-336.

567 Carss, D. N. & S. G. Parkinson, 1996. Errors associated with otter *Lutra lutra* faecal
568 analysis I. Assessing general diet from spraints. Journal of Zoology 238: 301-
569 317.

570 Čech, M. & L. Vejřík, 2011. Winter diet of great cormorant (*Phalacrocorax carbo*) on
571 the river Vltava: estimate of size and species composition and potential for fish
572 stock losses. Folia Zoologica 60: 129-142.

573 Chanin, P., 2003. Ecology of the European Otter. Conserving Natura 2000 Rivers
574 Ecology Series No. 10. English Nature, Peterborough.

575 Clavero, M., J. Prenda & M. Delibes, 2003. Trophic diversity of the otter (*Lutra lutra*
576 L.) in temperate and Mediterranean freshwater habitats. Journal of Biogeography
577 20: 761-769.

578 De Barba, M., C. Miquel, F. Boyer, C. Mercier, D. Rioux, E. Coissac & P. Taberlet,
579 2014. DNA metabarcoding multiplexing and validation of data accuracy for diet
580 assessment: application to omnivorous diet. Molecular Ecology Resources 14:
581 306-323.

582 De Lima, F. T., D. A. Reynalte-Tataje & E. Zaniboni-Filho, 2017. Effects of reservoirs
583 water level variations on fish recruitment. Neotropical Ichthyology 15: e160084.

584 Delibes, M. (ed.), 1990. La nutria (*L. lutra*) en España. ICONA Serie Técnica, Madrid.

585 Doadrio, I., S. Perea, P. Garzón-Heydt & J. L. González, 2011. Ictiofauna continental
586 española. Bases para su seguimiento. DG Medio Natural y Política Forestal.
587 MARM, Madrid.

588 Eckman, R. & F. Imbrock, 1996. Distribution and diel vertical migration of Eurasian
589 perch (*Perca fluviatilis*) during winter. Annales Zoologici Fennici 33: 679-686.

590 Edgar, R. C., 2004a. MUSCLE: multiple sequence alignment with high accuracy and
591 high throughput. Nucleic Acids Research 32: 1792-1797.

592 Edgar, R. C., 2004b. MUSCLE: a multiple sequence alignment method with reduced
593 time and space complexity. BMC Bioinformatics 5: 113.

594 Edgar, R. C., B. J. Haas, J. C. Clemente, C. Quince & R. Knight, 2011. UCHIME
595 improves sensitivity and speed of chimera detection. Bioinformatics 27: 2194-
596 2200.

597 Esling, P., F. Lejzerowicz & J. Pawlowski, 2015. Accurate multiplexing and filtering
598 for high-throughput amplicon-sequencing. Nucleic Acids Research 43: 2513-
599 2524.

600 Fernandes, C. A., C. Ginja, I. Pereira, R. Tenreiro, M. W. Bruford & M. Santos-Reis,
601 2008. Species-specific mitochondrial DNA markers for identification of non-
602 invasive samples from sympatric carnivores in the Iberian Peninsula.
603 Conservation Genetics 9: 681-690.

604 Fernández-Álvarez, F. Á., A. Machordom, R. García-Jiménez, C. A. Salinas-Zavala &
605 R. Villanueva, 2018. Predatory flying squids are detritivores during their early
606 planktonic life. Scientific Reports 8: 3440.

607 Galán, P., 1997. Declive de poblaciones de anfibios en dos embalses de La Coruña
608 (Noroeste de España) por introducción de especies exóticas. Boletín de la
609 Asociación Herpetológica Española 8: 38-40.

610 Hobbs, R., 2018. Novel ecosystems: can't we just pretend they're not there? In Kareiva,
611 P., M. Marvier & B. Silliman (eds), Effective conservation science: data not
612 dogma. Oxford University Press, Oxford: 45-50.

613 Janzen, D. H., 1985. On Ecological Fitting. Oikos 45: 308-310.

614 Jiménez, J., 2005. Ecology of the otter in tributaries of the river Ebro subjected to strong
615 fluctuations of resources. PhD Dissertation. University of Valencia, Spain.

616 Kean, E. F., C. T. Müller & E. A. Chadwick, 2011. Otter Scent Signals Age, Sex, and
617 Reproductive Status. Chemical Senses 36: 555-564.

618 Klimaszyk, P. & P. Rzymski, 2016. The complexity of ecological impacts induced by
619 great cormorants. Hydrobiologia 771: 13-30.

620 Krawczyk, A. J., M. Bogdziewicz, K. Majkowska & A. Glazaczow, 2016. Diet
621 composition of the Eurasian otter *Lutra lutra* in different freshwater habitats of
622 temperate Europe: a review and meta-analysis. Mammal Review 46: 106-113.

623 Kruuk, H., 2006. Otters: ecology, behavior and conservation. Oxford University Press,
624 Oxford.

625 Kruuk, H. & A. Moorhouse, 1990. Seasonal and spatial differences in food selection by
626 otters (*Lutra lutra*) in Shetland. Journal of Zoology 221: 621-637.

627 Kuczynski, L., M. Chevalier, P. Laffaille, M. Legrand & G. Grenouillet, 2017. Indirect
628 effect of temperature on fish population abundances through phenological
629 changes. PLoS ONE 12: e0175735.

630 Lanszki, J., I. Lehoczky, A. Kotze & M. J. Somers, 2016. Diet of otters (*Lutra lutra*) in
631 various habitat types in the Pannonian biogeographical region compared to other
632 regions of Europe. PeerJ 4: e2266.

633 Lyach, R. & M. Čech, 2017. Do otters target the same fish species and sizes as anglers?
634 A case study from a lowland trout stream (Czech Republic). Aquatic Living
635 Resources 30: 11.

López-Martín, J. M. & J. Jiménez (eds.), 2009. La nutria en España, veinte años de seguimiento de un mamífero amenazado. SECEM, Málaga, Spain.

Magoc, T. & S. L. Salzberg, 2011. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics 27: 2957-2963.

Martínez-Abraín, A. & J. Jiménez, 2016. Anthropogenic areas as incidental substitutes for original habitat. Conservation Biology 30: 593-598.

Mouriño, J., X. L. Otero, R. Salvadores, P. Alonso, F. Sierra-Abraín & F. Arcos, 2004. Los espacios naturales de Galicia. Edicións Nigra Trea S.L., Vigo, Spain.

Mumma, M. A., J. R. Adams, C. Zieminski, T. K. Fuller, S. P. Mahoney & L. P. Waits, 2016. A comparison of morphological and molecular diet analyses of predator scats. Journal of Mammalogy 97: 112-120.

Nichols, R. V., M. Åkesson & P. Kjellander, 2016. Diet Assessment Based on Rumen Contents: A Comparison between DNA Metabarcoding and Macroscopy. PLoS ONE 11: e0157977.

Noordhuis, R., 2002. The River Otter (*Lontra canadensis*) in Clarcke County (Georgia, USA) - Survey, Food Habits and Environmental Factors. IUCN Otter Specialist Group Bulletin 19: 75-86

Oja, R., E. Soe, H. Valdmann & U. Saarma, 2017. Non-invasive genetics outperforms morphological methods in faecal dietary analysis, revealing wild boar as a considerable conservation concern for ground-nesting birds. PLoS ONE 12: e0179463.

Orta, J., E. F. J. Garcia, F. Jutglar, G. M. Kirwan & P. Boesman, 2018. Great Cormorant (*Phalacrocorax carbo*). In del Hoyo, J., A. Elliott, J. Sargatal, D. A. Christie &

659 W. de Juana (eds), Handbook of the Birds of the World Alive. Lynx Edicions,
660 Barcelona.
661 Palmeirim, A.F., C. A. Peres & F. C. W. Rosas, 2014. Giant otter population responses
662 to habitat expansion and degradation by a mega hydroelectric dam. Biological
663 Conservation 174: 30-38.
664 Pedroso, N. M. & M. Santos-Reis, 2006. Summer diet of Eurasian otters in large dams
665 of South Portugal. Hystrix, the Italian Journal of Mammalogy 17: 117-128.
666 Pedroso, N. M., T. A. Marques & M. Santos-Reis, 2014. The response of otters to
667 environmental changes imposed by the construction of large dams. Aquatic
668 Conservation: Marine and Freshwater Ecosystems 24: 66-80.
669 Remonti, L., C. Prigioni, A. Balestrieri & G. Priore, 2008. Trophic flexibility of the
670 otter (*Lutra lutra*) in southern Italy. Mammalian Biology 73: 293-302.
671 Roos, A., A. Loy, P. de Silva, P. Hajkova & B. Zemanová, 2015. *Lutra lutra*. The
672 IUCN Red List of Threatened Species 2015: e.T12419A21935287.
673 Rozen, S. & H. J. Skaletsky, 2000. Primer3 on the WWW for general users and for
674 biologist programmers. In Krawetz, S. & S. Misener (eds), Bioinformatics
675 Methods and Protocols: Methods in Molecular Biology. Humana Press, Totowa,
676 NJ: 365-386. Source code available at http://sourceforge.net/projects/primer3/
677 Ruiz-Olmo, J. & M. Delibes (eds), 1998. La nutria en España ante el horizonte del año
678 2.000. SECEM, Málaga, Spain.
679 Ruiz-Olmo, J. & S. Palazón, 1997. The diet of the otter (*Lutra lutra*) in Mediterranean
680 freshwater habitats. Journal of Wildlife Research 2: 171-181.

681 Sales-Luis, T., N. M. Pedroso & M. Santos-Reis, 2007. Prey availability and diet of the
682 Eurasian otter (*Lutra lutra*) on a large dam and associated tributaries. Canadian
683 Journal of Zoology 85: 1125-1135.

684 Schäffer, S., F. E. Zachos & S. Koblmüller, 2017. Opening the treasure chest: A DNA-
685 barcoding primer set for most higher taxa of Central European birds and
686 mammals from museum collections. PLoS ONE 12: e0174449.

687 Taberlet, P., E. Coissac, R. Pompanon, C. Brochmann & E. Willerslev, 2012. Towards
688 next-generation biodiversity assessment using DNA metabarcoding. Molecular
689 Ecology 21: 2045-2050.

690 Thalinger, B., J. Oehm, H. Mayr, A. Obwexer, C. Zeisler & M. Traugott, 2016.
691 Molecular prey identification in Central European piscivores. Molecular
692 Ecology Resources 16: 123-137.

693 Vierna, J., J. Doña, A. Vizcaíno, D. Serrano & R. Jovani, 2017. PCR cycles above
694 routine numbers do not compromise high-throughput DNA barcoding results.
695 Genome 60: 868-873.

696 Weinberger, I. C., S. Muff, A. de Jongh, A. Kranz & F. Bontadina, 2016. Flexible
697 habitat selection paves the way for a recovery of otter populations in the
698 European Alps. Biological Conservation 199: 88-95.
699

700 Table 1. Models explaining the shift from fish to crayfish in otter diet at the study site
701 (Ratio) as a function of seasonal changes in stored water volume (vol), water
702 temperature (temp) and cumulated number of great cormorants (cormo). K = number of
703 estimable parameters; AICc = Akaike's Information Criterion corrected by small
704 sample size; Δ AICc = Difference between the AICc value of each model in relation to
705 the AICc value of the model with the lowest AICc; w_i = Akaike's weight; LL = Log-
706 likelihood. The best models of the set are highlighted in bold.
707

Model	K	AICc	Δ AICc	w_i	LL
Ratio~vol	**3**	**-12.16**	**0.00**	**0.52**	**10.58**
Ratio~vol+cormo	**4**	**-11.52**	**0.63**	**0.38**	**12.62**
Ratio~vol+temp	4	-8.36	3.80	0.08	11.03
Ratio~vol*temp	5	-5.60	6.55	0.02	12.80
Ratio~temp	3	-2.64	9.52	0.00	5.82
Ratio~temp+cormo	4	-1.41	13.57	0.00	6.15
Ratio~cormo	3	-5.57	17.73	0.00	1.71

708
709
710

711 Figure legends

712

713 Fig. 1. Location of the Cecebre-Abegondo and Eiras reservoirs (a) and locations and
714 density of otter spraints at the study reservoir (b).

715

716

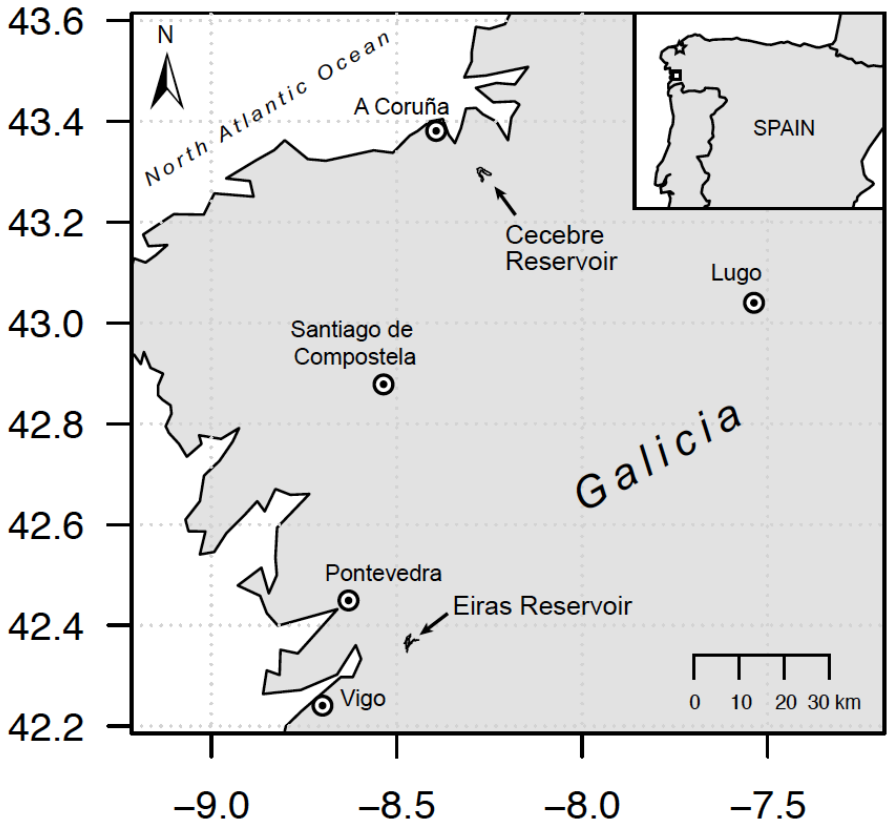
717 Fig. 2. Percent occurrence of all fish species found in 47 out of the 50 otter spraints
718 collected at Cecebre reservoir in autumn (October) and winter (February) as determined
719 by DNA metabarcoding. Most of the reads of three samples from February (Feb19,
720 Feb21, Feb23) showed low quality and could not be analysed.

721

722 Fig. 3. Counts of cormorants in rafts (i.e. dense groups of foraging birds in the water)
723 during the 2016 autumn at Cecebre reservoir. Counts are absolute numbers of birds in a
724 raft (a). Counts of cormorants at the only roosting site in the study area (i.e. reservoir
725 island) performed around half an hour before sunset from winter to spring. No
726 cormorants were present during the summer. Counts in this case are a relative index of
727 abundance as birds were counted only in half the roost (b).

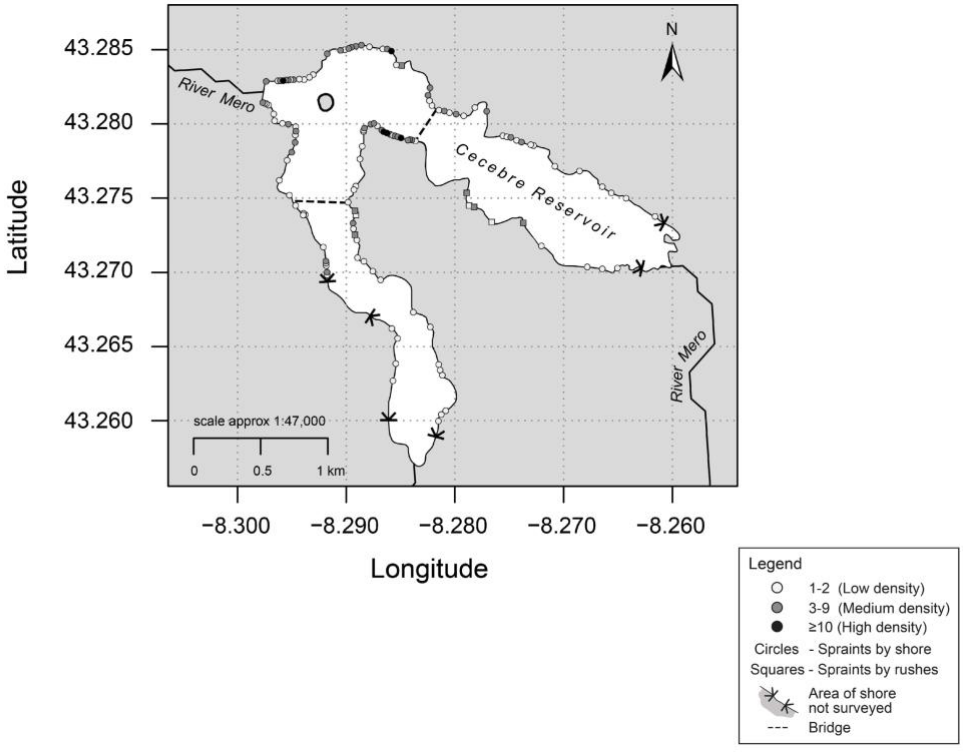
728
729

730 Fig. 1
731 a)



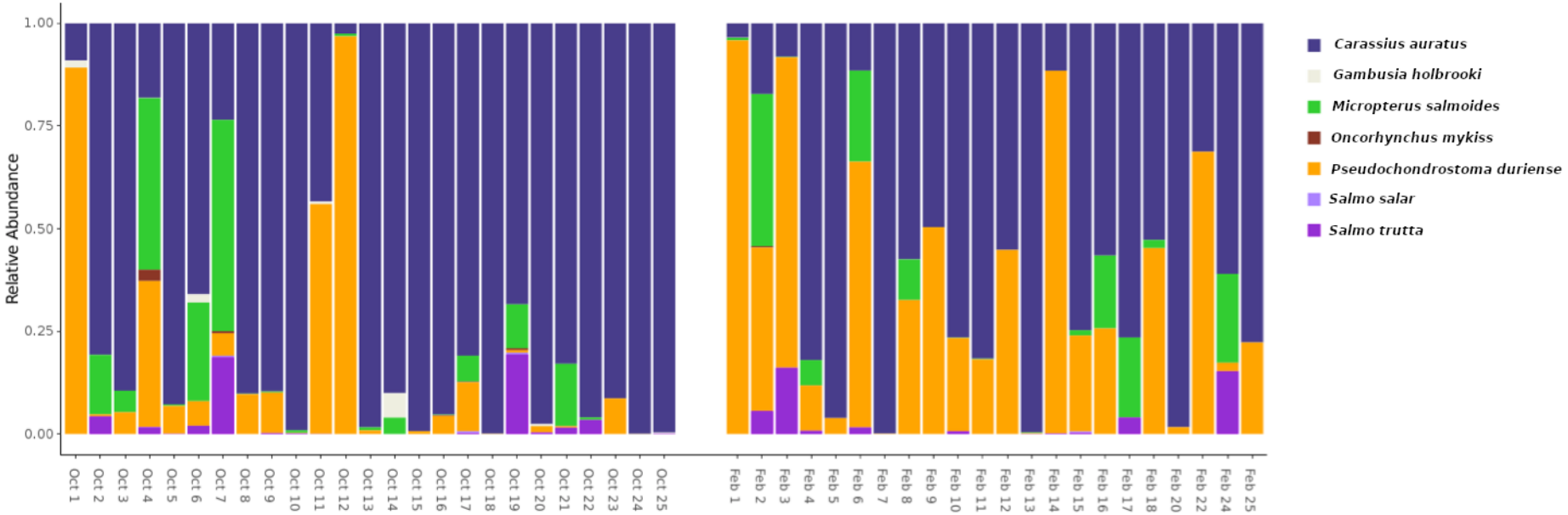
732

733 b)



734

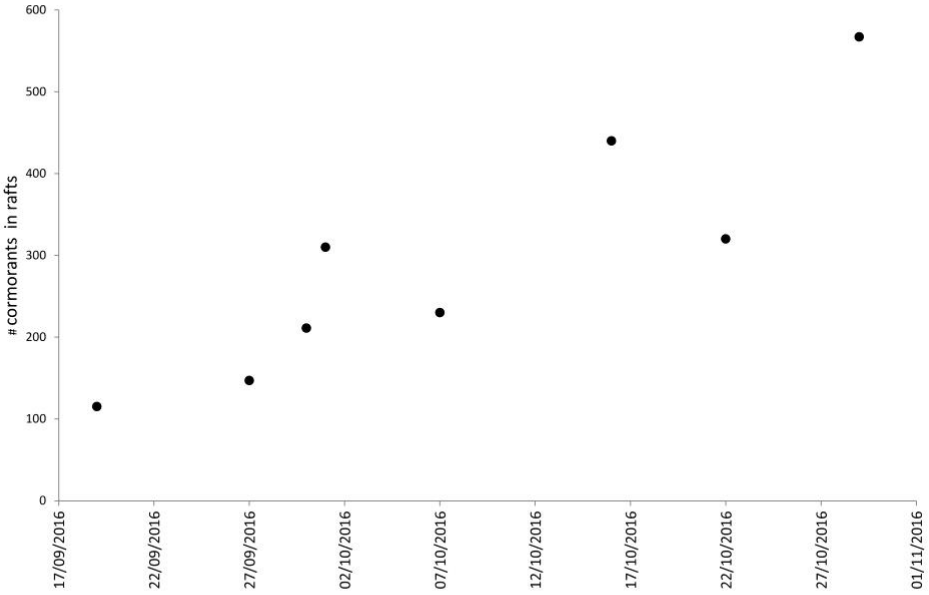
735 Fig. 2.



736

737

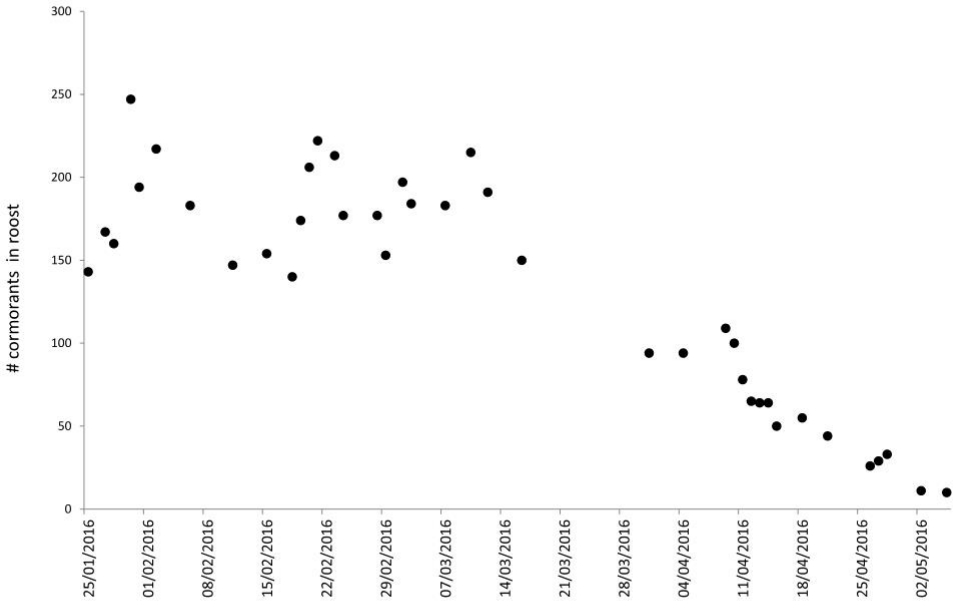
738 Fig. 3a



739

740

741 Fig. 3b



742