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Local habitat preferences of a semi-aquatic mammal, the Pyrenean desman *Galemys pyrenaicus*

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Abstract: To date, the large-scale distribution of the endangered Pyrenean desman, *Galemys pyrenaicus*, has been related to various abiotic factors (e.g. riverbed and riverbank characteristics, hydrology, topography, climate). However, none could fully explain the recent observed range contraction of the species, suggesting the influence of other drivers. In this study, the potential effect of some unexplored variables on the Pyrenean desman presence was investigated at the local scale (i.e. stream reaches) in the French Pyrenees. They described not only the riverbed, riverbanks, water chemistry, topography and pedology but also the presence of potential interacting species. Generalised linear models were implemented to select the best drivers of the Pyrenean desman presence. Our results stressed the relevance of considering human impacts at the riverbed scale, river narrowing and water chemistry to explain the local distribution of the Pyrenean desman. The presence of two potential competitors, the Eurasian water shrew *Neomys fodiens* and the dipper *Cinclus cinclus*, was also highly correlated to the species presence in stream reaches. This suggests that all three species may use the same resources within streams, which could be a potential source of competitive interactions. This study not only highlights the importance of maintaining the riverbed integrity for the Pyrenean desman but also stresses the need to assess the extent to which biotic interactions may affect its distribution in order to understand its current decline.

Keywords: French Pyrenees; habitat use; human impact; stream habitat.

Introduction

For semi-aquatic species, the number of abiotic and biotic potential drivers of communities' structure and species distribution is duplicated as they are closely linked to both aquatic and terrestrial environments (e.g. Greenwood et al. 2002, Mate et al. 2013). In aquatic environments, these organisms need to be adapted to inherent characteristics of water bodies such as geo-morphology, hydrology, water quality or riverbed fine scale features. For instance, river narrowing and stream slope increase hydraulic harshness of rivers by allowing fast responses to rainfall and sudden high flow peaks (Camana et al. 2016). Arvicolid (Mate et al. 2013) and river birds (Royan et al. 2014) have been reported to suffer from drowning and to be impacted by the reduced availability of suitable microhabitats for food and shelters at high flow levels. Water quality is another driver of aquatic and semi-aquatic species distribution and abundance (e.g. Kibichii et al. 2015, Mieza-Paez et al. 2016) with potential direct effects on species physiology (e.g. increased vulnerability to parasites, reduced fertility: Benejam et al. 2010, Peterson and Schulte 2016) and/or indirect effects through their environment (e.g. anoxia, eutrophication: Azevedo et al. 2015) or their prey (Bedford 2009).

Terrestrial environments are used by semi-aquatic species to satisfy their needs in terms of shelters and food resources. For instance, the presence of the Eurasian water shrew *Neomys fodiens* (Pennant, 1771) or the Southern water vole *Arvicola sapidus* (Miller, 1908) is favoured in rivers where banks provide existing cavities or the opportunity for digging burrows, respectively (Mate et al. 2013, Keckel et al. 2014). A dense riparian vegetation also provides suitable shelters and refuges to hide from predators. In addition, riparian vegetation may be a direct (i.e. for herbivores) or an indirect (i.e. by hosting terrestrial prey) source of food, which benefits several semi-aquatic mammals (Greenwood et al. 2002, Eubanks et al. 2011, Hysaj et al. 2013, Mate et al. 2013, Speldewinde et al. 2013).

In addition to abiotic factors, biotic interactions are known to be important in explaining species presence in a habitat at different spatial scales (Boulangeat et al. 2012, Kissling et al. 2012, Wisz et al. 2013). It is acknowledged that

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food availability strongly influences the presence and persistence of wildlife populations (Manly et al. 2002) and that competition between species plays a key role in shaping communities (Alexandrou et al. 2011), with consequences on the spatial and temporal use of resources (e.g. niche partitioning: Crow et al. 2010, Cozzi et al. 2012, Sánchez-Hernández et al. 2016). Predator-prey interactions are also important for the conservation of vulnerable species as they may affect the recovery of populations (Mate et al. 2013).

The Pyrenean desman *Galemys pyrenaicus* (É. Geoffroy Saint-Hilaire, 1811) is a semi-aquatic mammal endemic to the Iberian Peninsula and the Pyrenean Mountains. The species is listed as Vulnerable by the International Union for Conservation of Nature (IUCN) (Fernandes et al. 2008) and is legally protected in the four countries where it is present. Recent local, national and European initiatives (e.g. in France: Némot et al. 2011, Life+ Desman 2013) have given priority to complement the knowledge about its ecology and habitat requirements. Several environmental factors acting both at fine (i.e. riverbed, riverbanks: Biffi et al. 2016) and large (i.e. hydrology, climate, connectivity, topography: Charbonnel et al. 2015, 2016) scales have been recently identified as major drivers shaping the Pyrenean desman distribution in the French Pyrenees. However, none could fully explain the substantial contraction of the species range observed over the past decades (Fernandes et al. 2008, Gisbert and García-Perea 2014, Charbonnel et al. 2016) and its disappearance in several watersheds of the French Pyrenees, suggesting the influence of other and still unknown drivers. Despite this recent enhanced knowledge on the Pyrenean desman ecological preferences, important gaps remain about the potential effect of the water physico-chemistry (i.e. indirect effects on invertebrate prey suggested by Santamarina 1992 and Bertrand 1994), human pressures on the riverbed (e.g. trampling or water abstraction: Lake 2003, Evans et al. 2006, Benejam et al. 2010, Escarpinati et al. 2014) and soil pedology (e.g. indirect effects on invertebrate communities: Kennedy and Fitzmaurice 1971) on the local distribution of the species. Moreover, co-occurrence patterns between the Pyrenean desman and some potential competitors for food resources and habitats (e.g. the dipper *Cinclus cinclus* (Linnaeus, 1758) and the Eurasian water shrew: Santamarina 1993, Castién and Gosálbez 1999, Biffi et al. 2017b) have been overlooked.

In this context, the aim of this study was to investigate some abiotic and biotic factors that may explain the local distribution of the Pyrenean desman in the French Pyrenees. More specifically, we tested the relative contribution of different categories of variables including riverbank and riverbed descriptors, water quality, topography, pedology, human pressures in the riverbed and the presence

of two potential competitors. These variables were chosen either because their influence on the Pyrenean desman has never been tested as far as we are aware, or for comparison purposes because their effect has already been reported (Biffi et al. 2016, Charbonnel et al. 2016).

Materials and methods

Study area

The study was conducted in the Ariège department, a French administrative land in the Pyrenees Mountains (Figure 1) where the Pyrenean desman has a high occurrence (Charbonnel et al. 2015, Biffi et al. 2016). The study area is 2885 km² with a low human density [mean $\pm$ standard deviation (SD): 35 ± 71 inhabitants per km², minimum: 1, maximum: 500; GEOFLA© DB 2016]. It is a mountainous zone (elevation range: 357–3109 m) with a continental climate. The mean annual temperature is $8.7 \pm 2.5^\circ\text{C}$ and the mean annual rainfall is 1209.4 ± 173.0 mm. The land is mainly occupied by natural zones with forests (45.1%), herbaceous or shrubby vegetation (23.5%) and agriculture (13.6%; Corine Land Cover© DB 2012).

Pyrenean desman, Eurasian water shrew and dipper occurrence records

Given the cryptic behaviour of the Pyrenean desman, faeces were searched twice between June and September 2015 in 65 sites spread over the entire study area (Figure 1). One site was a 250-m-long river transect where each emergent item of the riverbed (i.e. rock, tree root or branch) was meticulously inspected by skilled observers. This length was chosen as a compromise between high detection rate and reasonable sampling time. It was also in accordance with Aymerich and Gosálbez (2014) who found a high probability of detecting the species when conducting sampling on 200-m-long sections. Sampling was conducted under good and similar environmental conditions for both surveys, excluding periods during or just after (i.e. more than 4 days) fluctuating waters (e.g. rainfall, dam release), and by the same observers, to avoid biasing the detection of Pyrenean desman faeces (Charbonnel et al. 2014). The particularly rainy 2015 summer guaranteed the regular washing of old faeces and the presence of freshly deposited faeces in all sites at the time of sampling.

All putative faeces of the Pyrenean desman as well as those of the Eurasian water shrew were collected based

Figure 1: Location of the study area and sampling sites (black dots) in the French Pyrenees.

on their colour, size, smell and position. The search for faeces is an effective protocol for detecting the presence of the Pyrenean desman (Charbonnel et al. 2014) and the Eurasian water shrew which deposits its faeces on similar places (i.e. emerging items near or within the riverbed, Aymerich and Gosálbez 2004). For each faeces, the observer qualitatively assessed the degree of certainty about species identity. Among the faeces collected in each site, a maximum number of seven faeces per mammal species was then selected for further molecular analyses to confirm the presence of species in each site. This number depended on the total number of faeces collected in the site, the degree of certainty in the identification on the field (i.e. more faeces were analysed when it was uncertain) and the freshness and size of the collected faeces (i.e. fresh and large faeces were given priority as molecular analyses are known to perform better than with old and small faeces, McInnes et al. 2017). In addition, the presence of the dipper was recorded during sampling of mammal faeces based on direct observations of the species or indirect presence signs (i.e. pellets).

DNA was extracted from faeces ($n=464$) using the Stool Mini Kit (Qiagen Inc., Hilden, Germany) and following the instructions from the manufacturer. Polymerase chain reaction (PCR) amplification was duplicated for

each sample on a portion of the mitochondrial cytochrome oxidase I gene (*COI*, for details, see Gillet et al. 2015). The procedure included negative DNA extraction and PCR controls. PCR products were purified and purified amplicons were quantified. The quantification step allowed to pool final products at equimolarity before being sent to the GIGA Genomics platform (University of Liège, Belgium) for sequencing. Raw sequences were sorted and filtered using a script mixing FASTX Toolkit (http://hannonlab.cshl.edu/fastx_toolkit; 23-09-16) and USEARCH (Edgar 2010) functions (see André et al. 2017 for details on bioinformatics). Sequences originating likely from extraction or PCR contaminants were excluded from further analyses. The remaining sequences were compared with the reference online BOLD database for *COI* (Ratnasingham and Hebert 2007). Sequences that had a unique best-hit were considered to be positive matches with an identity score higher or equal to 99% for the identification of the species producing the faeces.

Abiotic and biotic variables

A set of 18 abiotic variables (Table 1) was selected to describe local conditions at the reach scale. They can be

Table 1: Abiotic and biotic factors expected to influence the abundance of Pyrenean desman faeces in streams.

Category	Code	Description
Riverbed	DEPOS	Proportion (%) of the heterogeneity of emerging items (e.g. rocks, trees, roots or branches) and cavities in the riverbed and riverbanks representing an estimate of the availability of habitats for the semi-aquatic mammals studied and of deposit areas for faeces. Low proportions indicate a riverbed with homogeneous substrate and few or no cavities, whereas high proportions indicate a riverbed with a high diversity of emerging items and cavities
	CURRENT	Index of current velocity derived from a principal component analysis (PCA) conducted to summarise the proportion of six stream facies categories defined according to stream current velocity and depth: sheet, run, riffle, pool with no current, fast-flowing pool, falls. This index is the first axis of the PCA summarising 35.9% of the total variability. Small values indicate fast flowing waters
	GRAINS	Index of riverbed substrate size derived from a PCA conducted to summarise the proportion of seven grain-size categories: silt/mud (<0.05 mm), sand (0.05–2 mm), gravel (2 mm–2 cm), pebbles (2–7 cm), cobbles (7–25 cm), boulders (25 cm–1 m), larger boulders (>1 m). This index is the first axis of the PCA summarising 36.5% of the total variability. Small values correspond to the coarsest grain sizes
	WIDTH	Mean width of the riverbed (m)
	HUMIMP	Proportion (%) of the stream reach where a human impact was observed (e.g. presence of fishermen, cattle, bathers, wastewater discharges, water abstraction)
Riverbank	ROOTS	Proportion (%) of banks with roots
	DIRT	Proportion (%) of banks with bare soil or herbaceous vegetation
	HVEG	Index of vegetation height on the riverbanks derived from a PCA conducted to summarise the proportion of the riverbanks covered by bare soil, short grasses, tall grasses and shrubby vegetation. This index is the first axis of the PCA summarising 43.3% of the variability. Small values correspond to tall vegetation
	TREE	Proportion (%) of the riverbanks covered by trees
Water chemistry	DOC	Concentration of dissolved organic carbon (mg/l) in water
	NITRAT	Concentration of nitrate ions (N-NO_3^- mg/l) in water
	AMMON	Concentration of ammonium ions (N-NH_4^+ mg/l) in water
	PHOSPH	Concentration of phosphate ions (P-PO_4^{3-} mg/l) in water
Topography	CONDUC	Water conductivity ($\mu\text{S/cm}$)
	SLOPE	Mean slope of the river section (%)
	NARROW	Ratio (%) of the width of the bottom of the valley divided by the width of the riverbed. Small values correspond to enclosed streams flowing in a V-shaped valley (e.g. gorges)
	STRAH	Stream order as defined by the “top down” system of Strahler (1957) indicating the level of branching in a river network. Headwater streams at the source are ranked one. When two rivers of the same order merge, the resulting stream takes one order higher. The higher order number is kept when two rivers with different order merge
Pedology	PEDO	Category of soil type in the floodplain: calcareous vs. not calcareous
Biotic interactions	NEOMYS	Occurrence of the Eurasian water shrew (1: detection vs. 0: no detection) validated from molecular analyses of faeces collected
	CINCLUS	Occurrence of the dipper (1: detection vs. 0: no detection) from direct observations or detection of pellets

grouped into six categories of variables: biotic, riverbed, riverbank, water chemistry, topography and pedology.

Riverbed and riverbank variables were described in the field and computed for each 250-m-stream reach (see Biffi et al. 2016 for more details). Riverbed variables give information on emergent items (DEPOS), flow conditions (CURRENT), riverbed substrate size (GRAINS), mean width of the riverbed (WIDTH) and presence of human impacts (HUMIMP) in the riverbed. Riverbank variables

describe the bank composition (i.e. with roots: ROOTS; bare or grassy soil: DIRT) and the riparian vegetation (i.e. index of vegetation height: HVEG; presence of trees: TREE).

The chemistry of stream water was assessed through the sampling of 1 l of water collected for each site which was immediately filtered (mesh size: 0.45 μm). Filtered water samples were frozen and analysed using the chemical analytical platform of the EcoLab Laboratory

(Toulouse, France). The dissolved organic carbon (DOC), nitrates (NITRAT), ammonium (AMMON) and phosphate (PHOSPH) concentrations were measured. The water conductivity (CONDUC) was measured *in situ* in shallow and non-turbulent fast waters (EUTECH Instruments Série 600 device, Fisher Scientific France, Illkirch, France).

The Strahler stream order (STRAH, Strahler 1957), indicating the location of the sampled site along the upstream-downstream gradient, was derived from the stream network of the study area (CARTHAGE© DB, French database on thematic mapping of the Water Agencies, 2011 version <http://www.sandre.eaufrance.fr>). It starts at one for headwater tributaries and increases by one when two streams of the same order merge. The narrowness of the river valley (NARROW) and the site slope (SLOPE) were obtained from Valette et al. (2012) and Charbonnel et al. (2016), respectively.

The pedology in the surrounding floodplain soil of each site was obtained from a map of soil landscape units (Guiresse et al. 2014, GIS Sol, <https://www.gissol.fr/>) which are unique in terms of lithology, geomorphology, land use and pedological features. One single variable related to pedology (PEDO) was retained, namely the presence of limestone in the first soil strata, as it is known to affect aquatic species (e.g. Kennedy and Fitzmaurice 1971).

In addition, the occurrence of the Eurasian water shrew (NEOMYS) and the dipper (CINCLUS) was recorded in the sampled sites. The presence of the Mediterranean water shrew, *Neomys anomalus* (Cabrera, 1907), was detected in nine sites but was not considered for further analyses regarding its marginal presence and its higher use of terrestrial habitats and terrestrial preys compared to the Pyrenean desman and the Eurasian water shrew (Rychlik 1997, Churchfield and Rychlik 2006). As the proportion of putative water shrew faeces genetically confirmed to belong to the Eurasian water shrew (i.e. *Neomys fodiens*) was low (34%), only the presence or non-detection of the species confirmed by molecular analyses was considered, and not the number of collected faeces.

Statistical modelling

A statistical modelling approach was used to assess the relative contribution of the six categories of abiotic and biotic factors on the local distribution of the Pyrenean desman. First, we ensured that the explanatory variables were not highly correlated (Spearman rank correlation $|p| < 0.7$ for all pairs of variables). All quantitative abiotic variables were log-transformed to approach normality,

except the variables derived from a principal component analysis (PCA) (i.e. CURRENT, GRAINS, HVEG, Table 1).

Eighty-six percent of putative Pyrenean desman faeces collected and analysed by molecular tools were confirmed to have been produced by the species. Given this high identification rate, the total number of Pyrenean desman faeces counted in each sampled site was used as a surrogate for the intensity of habitat use by the Pyrenean desman. This assumption was based on the study by Nores et al. (1998) in which the density of faeces was much higher in areas where Pyrenean desman individuals were trapped (13.3 faeces per km) compared to areas where they were not (3.3 faeces per km). The number of Pyrenean desman faeces was thus the response variable in the following statistical modelling.

Generalised linear models (GLMs) were fitted using a quasi-Poisson family distribution for errors and a log-link function as overdispersion was present in the response variable. First, six models were computed for each category of explanatory variables described above (hereafter called “models by category”, see Table 1). All models were tested for significance and their explanatory performances were compared using the proportion of explained deviance. Second, an automated model selection procedure was applied to a model including all the 20 selected abiotic and biotic variables (hereafter called “full models”). The quadratic terms of all quantitative variables were also included in models to allow non-monotonous responses. To reduce the risk of over-fitting due to the small number of observations relative to the number of explanatory variables, the maximum number of explanatory variables included in the candidate models was set to six. This number seems to be a good compromise between the number of observations, the number of explanatory variables and the required computing time. Automated model selection (Calcagno and de Mazancourt 2010) was based on the ranking of Akaike information criterion (AIC) values corrected for small sample size and overdispersed data, QAIC_c (Burnham and Anderson 2002). An exhaustive screening of candidate models and comparison of QAIC_c enabled to eliminate the least significant variables (i.e. those which did not reduce the QAIC_c of models). Models with $\Delta\text{QAIC}_c < 2$ in comparison with the model with the lowest QAIC_c value were retained as the best candidate models and tested for significance. The best candidate models were used to calculate the model-averaged relative importance of explanatory variables (Burnham and Anderson 2002) as the sum of Akaike models’ weights in which the variable is included, rescaled between 0 and 1. The Akaike weights and percentages of explained deviance of the

best models were also used to assess models' explanatory performance.

All statistical analyses were conducted in R 3.3.2 (R Development Core Team 2014) using the *ade4*, *car* and *MuMIn* packages.

Results

Among the 464 faeces collected in the 65 sampled sites that were analysed using molecular genetics tools, the presence of the Pyrenean desman and the Eurasian water shrew was confirmed in 58 and 39 sites, respectively. The Pyrenean desman was detected in five sites alone, in 11 sites with the Eurasian water shrew only, in 20 sites with the dipper only and in 22 sites with both species. The number of Pyrenean desman faeces used as the response variable in the statistical models equalled (i) the total number of faeces observed in the field for the 58 sites where the presence of the Pyrenean desman was confirmed by molecular analyses, or (ii) zero in the seven sites where the identity of the Pyrenean desman was not confirmed by molecular tools.

Figure 2: Explanatory power (% of deviance explained) of GLMs relating the number of Pyrenean desman faeces to a single category of abiotic or biotic variables ("models by category"). See Table 1 for details about the variables belonging to each category.

The number of Pyrenean desman faeces per sampling sites ranged from 0 to 71 (mean: 14 ± 19 faeces).

Overall, "models by category" explained between 0.3 and 30.6% of the total deviance in the number of Pyrenean desman faeces (Figure 2). Variables describing the topography, riverbed and water chemistry explained the highest proportion of deviance in Pyrenean desman faeces abundance while those describing the pedology and riverbank were very poor explanatory factors.

The model selection procedure identified five models as plausible candidates to best explain the abundance of Pyrenean desman faeces according to QAIC_c ranking (Table 2). Those models explained between 44.4 and 52.1% of the variability in the number of Pyrenean desman faeces with Akaike weights between 0.13 and 0.33 (Table 2). Eight variables were selected with different combinations in the five best candidate models. They included one riverbed variable (HUMIMP), three topography variables (NARROW, STRAH, SLOPE), two biotic variables (CINCLUS, NEOMYS) and two water chemistry variables (CONDUC, AMMON). Consistent with the "models by category", the variables describing the riverbanks or pedology were not selected in any of the best candidate models. The relative importance (i.e. sum of Akaike models' weights) of the selected best variables ranged from 0.19 to 1 and was the highest for HUMIMP and NARROW (Table 3).

The influence of HUMIMP and CONDUC was most often non-monotonous and bell-shaped indicating that the number of Pyrenean desman faeces collected was higher at low and intermediate values (Table 3). The negative sign of the estimates for NARROW, the presence of the Eurasian water shrew (NEOMYS 1) and AMMON suggested that these variables were negatively correlated with the number of Pyrenean desman faeces in streams. In contrast, there were more Pyrenean desman faeces detected in streams where the dipper was also detected (CINCLUS 1). The number of Pyrenean desman faeces was the highest in streams with Strahler order equalling two or three (STRAH 2, STRAH 3). Last, the variable SLOPE

Table 2: Best candidate GLMs ($\Delta\text{QAIC}_c < 2$ compared to the model with the lowest QAIC_c) explaining the number of Pyrenean desman faeces in streams.

Models	QAIC _c	ΔQAIC_c	Weights	Explained deviance (%)
AMMON + NEOMYS + HUMIMP + I(HUMIMP ²) + NARROW + STRAH	85.56	0.00	0.33	52.11
HUMIMP + I(HUMIMP ²) + NARROW + STRAH + CONDUC + I(CONDUC ²)	86.66	1.10	0.19	50.96
HUMIMP + I(HUMIMP ²) + CONDUC + I(CONDUC ²) + CINCLUS + SLOPE	86.71	1.15	0.19	45.14
AMMON + NEOMYS + HUMIMP + I(HUMIMP ²) + NARROW + CINCLUS	86.97	1.40	0.16	44.87
HUMIMP + I(HUMIMP ²) + NARROW + CONDUC + I(CONDUC ²) + CINCLUS	87.45	1.88	0.13	44.37

See Table 1 for details about explanatory variables. ² is the quadratic term of the variable.

Table 3: Model-averaged estimates and relative importance of variables computed from the five best candidate GLMs (see Table 2) relating the abundance of Pyrenean desman faeces to abiotic and biotic factors.

Variable	Estimate	SE	p-Value	Number of models including the variable	Relative importance
HUMIMP	1.21	0.32	<0.001	5	1.00
I(HUMIMP ²)	−0.28	0.08	<0.001	5	1.00
NARROW	−0.77	0.28	0.007	4	0.81
STRAH 2	2.06	0.80	0.011	2	0.52
STRAH 3	1.61	0.81	0.050	2	0.52
STRAH 4	0.91	0.89	0.320	2	0.52
CONDUC	4.44	2.19	0.047	3	0.51
I(CONDUC ²)	−0.51	0.23	0.030	3	0.51
AMMON	−2.00	0.69	0.004	2	0.49
NEOMYS 1	−0.60	0.27	0.029	2	0.49
CINCLUS 1	1.06	0.45	0.020	3	0.48
SLOPE	0.51	0.20	0.015	1	0.19

See Table 1 for details about explanatory variables. ² is the quadratic term of the variable. SE, standard error.

has the smallest value of relative importance in models (0.19, Table 3), suggesting a limited contribution of this variable to the number of Pyrenean desman faeces at this scale.

Discussion

In this study, novel categories of abiotic and biotic factors were tested and identified as important in explaining the local distribution of the Pyrenean desman, but with varying intensities. More specifically, these factors allowed identifying environmental conditions that are found in habitats that are lightly (i.e. few faeces) vs. heavily (i.e. many faeces) used by the species. When taken by category, drivers describing the topography, riverbed or water chemistry better explained the number of Pyrenean desman faeces than those describing biotic interactions, riverbanks or pedology. This result was confirmed when combining different categories of variables in a single model as the best candidate models included not only topography, water chemistry and riverbed variables, but also variables describing the biotic environment. This finding suggests that the abundance of Pyrenean desman faeces is likely driven by a mix of abiotic and biotic factors confirming previous studies focusing on other species (e.g. Jo et al. 2017). It is worth noting that the availability in deposit areas for faeces (DEPOS) did not explain the number of Pyrenean desman faeces, suggesting that the potential bias that could have arisen from differences in the availability of depositional areas for faeces between sampled sites was not a confounding factor here.

Novel factors identified as influencing the Pyrenean desman habitat preferences

To date, the influence of human activities on the Pyrenean desman has only been studied with indirect factors acting at large scale such as the distance to the nearest roads or towns (Barbosa et al. 2009, Williams-Tripp et al. 2012), the proportion of urbanised or agricultural lands (Biffi et al. 2016, Charbonnel et al. 2016), the human population densities near the river and the density of upstream obstacles to water flow (Charbonnel et al. 2016). Although their influence was reported to be contrasted in these previous studies, we found here that human pressures acting at a fine spatial resolution have a significant negative impact on the Pyrenean desman. Indeed, the proportion of human activities in the riverbed, which can be regarded as an index of human impact intensity, is the major driver of the number of collected Pyrenean desman faeces. Despite the highlighted bell-shaped response, the number of faeces starts decreasing when more than 20% of the site length is impacted by human activities. During faeces sampling, we observed a variety of human impacts including trampling by anglers, bathers and cattle grazing. Trampling likely affects not only the structure of riverbed and riverbanks, but also the diversity, structure and composition of aquatic macroinvertebrate communities, which are the main food resources for the Pyrenean desman (Biffi et al. 2017a). Indeed, trampling results in an increase in the drift of benthic invertebrates and organic matter, as well as in the clogging of the riverbed due to riverbank erosion (Evans et al. 2006, Hardiman and Burgin 2011, Escarpinati et al. 2014). We also observed that the quality of water was affected in some sites by the presence of cattle manure,

wastewater discharges and water abstraction systems. In a previous study, Biffi et al. (2016) found a lower influence of urban areas within the near floodplain on the presence of the Pyrenean desman. They suggested, as Peyre (1956), that the species may exhibit some tolerance to human disturbances if riverbed and riverbank conditions remain suitable. Thus, our study seems to confirm that all activities directly impacting the riverbed could have an adverse effect on Pyrenean desman populations like the regression observed in the Spanish Pyrenees after a dam release (Aymerich and Gosálbez 2015). The integrity of riverbed habitats at fine scale is thus an important factor making suitable conditions for the Pyrenean desman in streams.

Contrary to our expectations, a higher number of Pyrenean desman faeces were found in streams flowing in the narrowest valleys. Yet, this typology of streams may favour sudden flooding events that are known to have negative consequences on aquatic and semi-aquatic species (Mate et al. 2013, Royan et al. 2014, Camana et al. 2016). The reported affinity of the Pyrenean desman for streams with numerous tributaries (Charbonnel et al. 2015) may balance this risk because tributaries may act as refuges in case of flooding and are more numerous in the narrowest valleys of the study area. The highlighted preference of the Pyrenean desman for streams flowing in narrow valleys could also result from the negative relationship between narrowness of valleys and human activities as narrower valleys with steep floodplains are less accessible, especially when they are forested, and are thus less exposed to human alterations than wider and flatter lands. Narrow valleys are nevertheless still sensitive to canyoning activities which are known to negatively impact aquatic communities (Hardiman and Burgin 2011). Moreover, narrow valleys prevent sun penetration that may ensure (i) a slower degradation of faeces, for instance, from flushing due to rainfall, and (ii) more stable and colder water temperature (Poole and Berman 2001). Charbonnel et al. (2015) positively related the presence of the Pyrenean desman to colder air temperature and cold-water conditions were considered as important in other studies (Peyre 1956, Richard 1976, Hernandez 1988, Aymerich and Gosálbez 2014). The combination of a better preservation of faeces and more suitable climate conditions for the Pyrenean desman may thus have improved the detection of faeces in the narrowest valleys, explaining that higher faeces abundances were found in these sites.

Water chemistry is an important factor explaining the presence of aquatic species and the composition of communities in streams (Camargo and Alonso 2006, Cheng et al. 2016, Pallottini et al. 2017). In this study, the water conductivity and concentration in ammonium ions were

highlighted to have a substantial influence on the local distribution of the Pyrenean desman. The number of Pyrenean desman faeces decreased with increasing ammonium concentration while the response to conductivity was bell-shaped peaking at rather low conductivity values ($\approx 100 \mu\text{S}/\text{cm}$). The detrimental effects of nitrates on freshwater ecosystems are well known (Vitousek et al. 1997, Camargo and Alonso 2006). Nitrate concentrations are used as indicators of poor water quality with negative influence on otters (Jo et al. 2017). In our study, the sampled sites exhibited small concentration in ammonium (i.e. between 0.1 and 0.2 mg/l) that may suggest low potential toxicity risk. Overall, faeces of the Pyrenean desman were more abundant in streams with very limited ion content. Thus, thresholds at which ion concentration starts to negatively affect the species may be lower than for other aquatic species. Based on the current knowledge, it is not possible to ascertain the way water pollution may affect the Pyrenean desman. For instance, pesticide cocktails or medicinal substances are able to cause renal or hepatic pathologies and to affect the growth, behaviour and reproduction cycles of aquatic organisms (e.g. Schwaiger et al. 2004, Kidd et al. 2007, Gandar et al. 2016). The accumulation of pollutants in tissues is also a threat to semi-aquatic mammals (Peterson and Schulte 2016). As the main prey of the Pyrenean desman (i.e. Ephemeroptera, Plecoptera and Trichoptera, Biffi et al. 2017a) is commonly used as an indicator of stream water quality (Usseglio-Polatera et al. 2000, Kibichii et al. 2015), there is an urgent need to investigate the direct (e.g. on biology from DNA to population dynamics) and indirect (e.g. on prey) potential effects of water chemistry on the Pyrenean desman.

Then, we found that two species co-occurring with the Pyrenean desman are significantly related to its local distribution although it was assessed roughly using simple binary variables (presence vs. no detection). The positive relationship between the presence of the dipper and the Pyrenean desman may indicate that a stream suitable for one species also seems to be suitable for the other one (Santamarina and Guitian 1988, Santamarina 1993, Bertrand 1994). However, it is worth noting that the dipper may leave presence signs in habitats that are only punctually crossed during its movements. Such habitats would thus not necessarily suit its ecological requirements but may only represent suitable corridors. By contrast, the selected models highlighted a negative correlation between the presence of the Eurasian water shrew and the number of Pyrenean desman faeces indicating that less Pyrenean desman faeces were detected in streams where the Eurasian water shrew was also present. This finding might suggest a higher competitive interaction between

these two mammals than with the dipper due to their similar biology, use of similar aquatic and terrestrial habitats and similar food requirements (e.g. trophic resources: Santamarina 1993, Castién and Gosálbez 1999; habitats: Bertrand 1994, Greenwood et al. 2002, Keckel et al. 2014, Biffi et al. 2016). However, an assessment of actual competitive interactions would require the study of both habitat use and diet of the two mammals using the same source of data (e.g. intensity of habitat use or occurrence data) and with a balanced sampling of sites where only one species is present, which was not the case here. In the light of a recent study on the diet of both species (Biffi et al. 2017b), another more likely hypothesis to explain this negative correlation between the two mammals would be that the riverbed conditions suitable for the presence of the Eurasian water shrew may be less suitable for the Pyrenean desman because their use of microhabitats and ecological requirements within streams are different at fine resolution. The Eurasian water shrew is known to be particularly active at the ecotone of stream banks (Lardet 1988) where water flows slowly. It has indeed poor morphological adaptations to aquatic environment (e.g. no palmed feet, Puisségur 1935; short duration of dives, Lardet 1988, Mendes-Soares and Rychlik 2009) that may explain a lower use of habitats with fast current velocities than the Pyrenean desman. To date, the effect of the Eurasian water shrew on the Pyrenean desman, and *vice versa*, remains unclear and would deserve to be further and more finely investigated.

Remaining uncertainties regarding the Pyrenean desman local habitat preferences

Despite these original findings contributing to a better understanding of the drivers of the Pyrenean desman local habitat preferences, about half of the variability in the number of the species faeces remained unexplained. First, other abiotic or biotic drivers could be more important in shaping the Pyrenean desman distribution at this local scale than those included in this study. However, a large diversity of abiotic factors has already been explored here and in previous studies (e.g. Charbonnel et al. 2015, 2016, Biffi et al. 2016), suggesting that missing variables would rather correspond to biotic interactions such as predation or the availability of trophic resources. Regarding predation, short-term studies on the introduced American mink *Neovison vison* (Schreber, 1777) in Spain reported a substantial decrease in the range of the Mediterranean water shrew and the Southern water vole (García-Díaz et al. 2013). Similar observations have been documented

in Great Britain on another water vole *Arvicola amphibius* (Linnaeus, 1758) (Aars et al. 2001). A potential negative impact of the American mink on the Pyrenean desman has been suggested by several authors (Gisbert and García-Perea 2014, Pedroso and Chora 2014, Biffi et al. 2016, Charbonnel et al. 2016) but has never been properly investigated. Regarding trophic resources of the Pyrenean desman, studies on food selectivity and prey availability should also be further documented as the decline of prey in altered conditions may have negative consequences on species that consume them and cause local extinction of populations (Real et al. 2009).

Second, the moderate response of the Pyrenean desman to the high number of abiotic and biotic factors tested here and in previous studies could suggest that this species would be more tolerant to a wide range of environmental conditions than previously thought. However, Charbonnel et al. (2015, 2016) reported that the Ariège department, where this study was conducted, is one of the most suitable areas for the Pyrenean desman in the French Pyrenees. In this context, it can be difficult to highlight non-optimal environmental conditions for the species. Thus, stronger responses of the Pyrenean desman to environmental factors would be expected with sampling conducted in both suitable and unsuitable areas where it would occur less frequently.

Third, we used the number of collected faeces as a surrogate for the intensity of habitat use by the Pyrenean desman. Although non-invasive methods such as faeces surveys are commonly used to study species habitat selection, the binary variable they provide (i.e. detection/non-detection of indirect signs of species presence) does not allow to assess the intensity of habitat use (Hull et al. 2016). To date, the habitat selection of the Pyrenean desman had only been studied using presence-absence data (Biffi et al. 2016) or detection histories (Charbonnel et al. 2015, 2016). To overcome this shortcoming, our study relies on the assumption that a higher intensity of habitat use by the Pyrenean desman would imply an increasing number of faeces collected in the field. This higher intensity of habitat use can be regarded in terms of (i) a longer time spent in a site, increasing in turn the probability of depositing faeces, or (ii) a higher abundance of individuals that can produce a higher number of faeces in a short time. This assumption seems reasonable regarding the relatively small home-range of the species (Melero et al. 2012, 2014) and is corroborated by the observations of Nores et al. (1998) in the field where a higher number of faeces were found in sites where the Pyrenean desman was captured compared to sites where it was not. It is however worth noting that habitats where few faeces were found

may still be valuable to the ecology of the species even if they are less frequently used than others. While many studies have documented a positive relationship between the number of faeces and habitat use for terrestrial species (e.g. Kellner and Swihart 2017), this relationship has been little studied or supported for semi-aquatic species (e.g. for otters: Gallant et al. 2007, Romanowski 2013, Day et al. 2016). Indeed, depositional areas in stream ecosystems are more affected by hydrological fluctuations that may reduce faeces persistence (Ruiz-Olmo and Gosálbez 1997). As faeces deposit behaviour remains poorly understood for the Pyrenean desman, caution should be taken when interpreting our habitat selection results. There is indeed no clue about a potential link between faeces deposit and age, sex, season, abundance of resources or population density. Consequently, further studies are required to clarify if the number of Pyrenean desman faeces is relevant to study its local distribution and ecological requirements at fine resolution. For instance, non-invasive genetic capture-recapture methods (i.e. microsatellite genotyping that allows to individually identify the animal producing the faeces: Lampa et al. 2015, Gillet et al. 2016) could be used to investigate the Pyrenean desman faeces deposit over several seasons in sites where faeces densities are known to be contrasted.

Conclusion and perspectives

This study not only provides novel insights into the habitat use and selection of the Pyrenean desman but also raises new issues for upcoming research. Our results first stressed the relevance of considering human impacts at the riverbed scale, river narrowness and water chemistry to explain the local distribution and intensity of habitat use by the species. Such variables should thus be targeted for conservation planning of the Pyrenean desman. Based on the highlighted negative influence of human disturbances on the riverbed, the conservation or restoration of the riverbed seems to be one of the major challenges for protecting this endangered species in Pyrenean streams. Not only the impacts of human activities such as canyoning, hydraulic works, bank alterations or trampling by cattle but also people should be further explored as they are known to influence the riverbed structure, aquatic communities and the ecological continuum of rivers (Aparicio et al. 2000, Evans et al. 2006, Hardiman and Burgin 2011, Pedroso et al. 2014). The traditionally used sampling technique for searching faeces (i.e. which consists of wading within the riverbed) could be replaced by less invasive methods such as environmental DNA detection. This method is more and

more used for aquatic species (e.g. Rees et al. 2014, Padgett-Stewart et al. 2016) and may overcome the difficulty of detecting the elusive Pyrenean desman with molecular tools used in this study when only degraded DNA is contained in small or old faeces (McInnes et al. 2017).

While the influence of the presence of species sharing the same food and habitats resources as the Pyrenean desman in streams remains unclear, it encourages further studies on this topic. A particular focus on biotic interactions including not only predators (e.g. potential influence of the American mink on the Pyrenean desman range contraction, Barbosa et al. 2009, Gisbert and García-Perea 2014, Pedroso and Chora 2014), competitors, prey (e.g. upward shifts of prey in response to global warming, Domisch et al. 2013) but also parasites and other pathogens (e.g. new emerging diseases affecting amphibians, Skerratt et al. 2007) will be required to fill the remaining gaps in our knowledge of the Pyrenean desman.

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