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It is the ambience, not the menu. Prey availability does not drive habitat selection by the endangered Pyrenean desman

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Abstract

1. The Pyrenean desman (*Galemys pyrenaicus*) is an endangered stream-dwelling insectivore endemic to the Iberian Peninsula and the north of the Pyrenees. It favours riffles over slow habitats such as runs or pools, yet it is still unclear whether habitat preference is based on prey availability or on other factors, such as mechanical constraints on its hunting habits.
2. Desman diet and prey selection were analysed along the pristine Elama Stream and the slightly modified Leitzaran Stream in the Basque Country, Spain.
3. In each stream the prey consumed were identified by metabarcoding 94 desman faeces, and prey availability characterized from 10 Surber samples taken in each habitat type (riffles, runs and pools). Invertebrates were sorted and identified, the biomass of each taxon was calculated, and their DNA was also extracted, amplified using PCR, and sequenced to build a reference database.
4. Prey availability and diet varied between streams. Desmans positively selected shredders and invertebrates that live on the substrate, and selected against taxa that live in fine sediment, taxa with hard shells or those that are highly mobile. The diet of desmans was more selective in the Elama, the stream with better ecological status. Food availability did not differ among habitats, and therefore does not explain the preference of desmans for riffles; rather, mechanical constraints to deal with buoyancy and physical habitat heterogeneity were hypothesized to be the reason behind the habitat preference.
5. The results suggest that promoting the formation of riffles in streams can improve the situation of some desman populations living in degraded habitats.

KEYWORDS

behaviour, endangered species, feeding, hydropower, invertebrates, mammals, river, stream

1 | INTRODUCTION

Understanding the trophic ecology and foraging strategies of endangered animals is important for predicting their population dynamics and developing effective conservation programmes (Sinclair, Fryxell, & Caughley, 2006). Since foraging behaviour

largely depends on the composition, abundance and distribution of food resources (Waggitt et al., 2018), the relationship between habitat and food availability is a key issue in conservation biology (Goiti, Garin, Almenar, Salsamendi, & Aihartza, 2008; Karanth, Nichols, Kumar, Link, & Hines, 2004; Shuterland & Green, 2004).

The Pyrenean desman (*Galemys pyrenaicus*, É. Geoffroy Saint-Hilaire, 1811, Eulipotyphla, Talpidae, Figure 1) is a semi-aquatic insectivorous generalist mammal (Biffi, Gillet, et al., 2017; Biffi, Laffaille, et al., 2017; Hawlitschek, Fernández-González, Balmori-de la Puente, & Castresana, 2018) that lives in mountain streams. It is endemic to the northern Iberian Peninsula and the Pyrenees, but its distribution area has been severely reduced during recent decades, currently being listed as Vulnerable by the International Union for Conservation of Nature (Fernandes, Herrero, Aulagnier, & Amori, 2008). As previously foreseen by some authors (Biffi et al., 2016), the last and most complete radio-tracking work with the desman (Esnaola, González-Esteban, Elozegi, Arrizabalaga-Escudero, & Aihartza, 2018) showed that it has a strong preference for fast-flowing facies (riffles) over slower habitats (runs or pools). That later study described the habitat requirements of desmans at the microhabitat level and showed a strong preference for riffles, thus providing critical information to help its conservation and management. It is unknown, however, whether the observed microhabitat preference is the consequence of prey availability in riffles or other factors – that is, whether it selects its foraging areas ('the restaurant') as a function of the 'ambience' (the physical characteristics) or the 'menu' (the available prey). Indeed, the information lacking on desman foraging ecology limits the capacity to manage this species adequately (MAGRAMA, 2013; Némoz & Bertrand, 2008).

Visual identification of prey remains in desman faeces or stomach contents (Bertrand, 1992; Castién & Gosálbez, 1995; Santamarina, 1993) has shown that desmans feed on a broad spectrum of benthic invertebrates. More recently, DNA metabarcoding of faeces (Bohmann et al., 2014) allowed identification of their prey, including soft-bodied animals, to species level. Several authors have applied these methods to desmans: Biffi, Gillet, et al. (2017) and Biffi, Laffaille, et al. (2017), in the north of the Pyrenees, identified 156 prey genera from 91 families and 30 orders; subsequently, Hawlitschek et al. (2018) identified a total of 140 prey genera in the Iberian

Peninsula. These studies confirmed that desmans mostly eat Ephemeroptera, Plecoptera, Trichoptera and Diptera, although the results might have been affected by primer bias (Esnaola, Arrizabalaga-Escudero, González-Esteban, Elozegi, & Aihartza, 2018; Piñol, Senar, & Symondson, 2019) and other methodological constraints, such as storage conditions, DNA extraction procedures, sequencing technology and bioinformatic thresholds (Alberdi et al., 2019; Divoll, Brown, Kinne, McCracken, & O'Keefe, 2018; Oehm, Juen, Nagiller, Neuhauser, & Traugott, 2011).

To test whether the preference of desmans for riffles (Esnaola, González-Esteban, et al., 2018) is determined by food availability or other factors, prey availability was characterized in three microhabitats (riffles, runs and pools) and compared with the diet determined from desman faeces. All of the samples were collected simultaneously with the radio-tracking study of habitat selection performed by Esnaola, González-Esteban, et al. (2018). The first hypothesis was that desmans prefer riffles because of their higher prey availability (Dewson, James, & Death, 2007). The study was carried out in two streams with different ecological statuses to assess the flexibility in desman diet. The study also aimed to test which prey are selected by the desman. The results from this work should provide crucial information for conservation managers and guide future management actions to improve the protection of the threatened Pyrenean desman populations.

2 | MATERIALS AND METHODS

2.1 | Study area

The study was conducted in two mountain streams in the northern Iberian Peninsula (Basque Country; Figure 2). Both streams are located in Special Areas of Conservation (Official Journal of Navarre, 2015; Official Journal of the Basque Country, 2015) within the European Union Natura 2000 network, protected under the



FIGURE 1 The Pyrenean desman (*Galemys pyrenaicus*) in the Elama Stream. Photo by Jorge González-Esteban

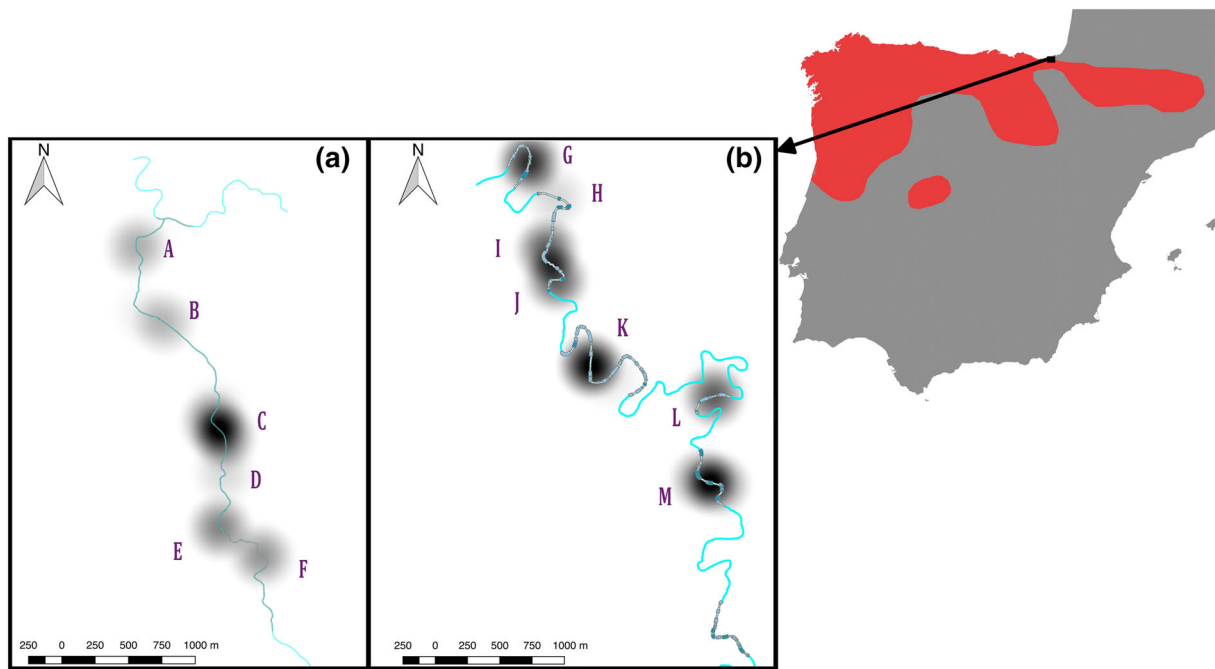


FIGURE 2 Study area: (a) Elama Stream and (b) Leitzaran Stream. Faecal samples were clustered in sampling sites (A–M) according to a heat map of their occurrence along the prospected stream stretches. The distribution of the Pyrenean desman in the Iberian Peninsula is displayed in red (Fernandes et al., 2008)

European Habitats Directive (Council of the European Communities, 1992). The presence of desman was one of the reasons for designating these sites as Special Areas of Conservation. Desman populations are isolated (Government of Navarre, 2015) and in decline (Basque Government, 2012); thus they are subject to specific conservation strategies (MAGRAMA, 2013) and are included within the framework of priority actions of the Natura 2000 network (Basque Government, 2019; Government of Navarre, 2015). The streams studied are at ‘good ecological status’ according to the European Water Framework Directive (Council of the European Communities, 2000), but they face contrasting environmental pressures. The Elama (Navarre) is a second-order headwater stream in Artikutza draining an uninhabited basin of 1,415 ha over schist that has been managed strictly as a nature reserve since 1919, resulting in an extensive cover of beech and oak forests (Castro, 2009). At present, there is no forestry activity in Artikutza. Only 25 authorized vehicles have access to the land every day, and the scarce human activity in the territory is related to conservation, research and rural tourism. On the other hand, the Leitzaran (Gipuzkoa) is a fourth-order stream draining a basin of 12,402 ha over limestone, slate and sandstone. Contrasting with the Elama, there are two towns in the headwaters of the Leitzaran Stream with 3,150 inhabitants in total, after which the stream enters a long, uninhabited valley ca. 25 km in length where, together with rural tourism, forest activities and hydropower diversion schemes are the main human activities (Izagirre, Argerich, Martí, & Elosegi, 2013).

The research was carried out in the same reaches and periods as the radiotracking survey published by Esnaola, González-Esteban, et al. (2018), on a 4 km long section of the Elama (from 1°48′36″W

43°12′40″N to 1°48′4″W 43°11′14″N; 330 m mean altitude; 7.08 m mean width) and a 10 km long section of the uninhabited stretch of the Leitzaran (from 1°57′26″W 43°8′57″N to 1°56′9″W 43°6′55″N; 290 m mean altitude; 12.42 m mean width). The difference between section lengths resulted from the lower density of desmans in Leitzaran.

2.2 | Desman diet

Faeces of the Pyrenean desman were searched along both streams (Figure 2) in September and October 2016, by building artificial shelters especially designed for this species (González-Esteban, Esnaola, & Aihartza, 2018). These shelters were used for greater efficiency when collecting samples as without them it was not possible to find faeces in the Elama, and they were mainly found in the Leitzaran. A total of 188 droppings (94 per stream) were collected, stored in tubes containing 98% ethanol and frozen at -80°C until DNA extraction.

DNA extraction from desman faecal samples, PCR amplification and high-throughput sequencing were performed as described in Esnaola, Arrizabalaga-Escudero, et al. (2018), combining the use of both Gillet (Gillet et al., 2015) and Zeale (Zeale, Butlin, Barker, Less, & Jones, 2011) primer sets to identify the broadest range of prey species consumed and the predator itself. To build a customized database, DNA was also extracted from the invertebrates collected in both streams, amplified and sequenced at the Analytical Services (SGIker) of the University of the Basque Country, UPV/EHU. More methodological details are given in Supporting Information S1 and S2 (Table S1).

The results on diet were expressed as the frequency of occurrences (FOO) and the percentage of occurrences (POO) of each prey taxon for each stream separately. The FOO of a prey taxon represents the proportion of scats where it was detected, and the POO represents the number of occurrences of each taxon divided by the total number of occurrences of all taxa, expressed in percentages (Deagle et al., 2019).

2.3 | Prey availability

Aquatic macroinvertebrates were sampled in September and October 2016 with a Surber net (30 × 30 cm; 1 mm mesh) across the three habitat types (Overton, Wollrab, Roberts, & Radko, 1997) identified in the previous radiotracking study (Esnaola, González-Esteban, et al., 2018): 'riffles' with fast, turbulent water, uneven surface level and white water; 'runs' with near-laminar flow and even depth; and 'pools' with slow flow on river-bed depressions. Ten samples were collected per habitat and stream, making a total of 60 samples. These were stored in plastic jars and preserved with 70% ethanol until identification.

In the laboratory, macroinvertebrates were sieved with a 2 mm mesh, sorted, counted, measured and identified under a dissecting microscope. The taxonomic resolution was to genus or species level for most taxa, except for some Diptera, Coleoptera, Oligochaeta, Hirudinidae and Nematoda, which were identified to family or order level (Supporting Information S2, Tables S2 and S3). Prey biomass (g AFDM m⁻², or grams of ash-free dry mass per square metre) was calculated from size-mass regressions (Supporting Information S2, Table S4).

2.4 | Statistical analyses

Sampling completeness was assessed for both diet and invertebrate availability by building taxon accumulation curves using the iNEXT online platform (Chao, Ma, & Hsieh, 2016). Differences in dietary composition between streams were analysed using permutation-based MANOVA (PerMANOVA; Anderson, 2001) at both species and family level, and taxa driving these differences were identified with a correspondence analysis (CA). PerMANOVA was implemented using the function *procD.lm* of the *geomorph* package for R (Adams, Collyer, Kaliontzopoulou, & Sherratt, 2017).

Prey availability was compared between streams at three levels: potential prey availability (including all sampled invertebrates), availability of consumed prey (only taxa found in the diet) and the availability of positively selected prey (only the taxa that were positively selected, i.e. that occurred more frequently in the diet than in invertebrate samples, with a threshold of 20% difference). Taxon richness of potential prey availability was compared using two-way ANOVA (stream × habitat), considering taxa at species and family level. The density (individuals m⁻²), biomass (g AFDM m⁻²) and average body mass (g AFDM) of each measure of availability were also compared

with ANOVA, after normalizing by log₁₀ transformation. Average body mass (g AFDM) per prey taxon was calculated by dividing biomass by density (Flores, Larrañaga, Díez, & Elosegí, 2011).

To assess prey selection, potential prey availability, consumed prey availability and positively selected prey availability were compared with the FOO of each taxon in the diet. For that, prey availability was characterized in three ways, but no significant differences were found when using any of the methods tested, so the most conservative method – availability weighted by the mean desman activity (Esnaola, González-Esteban, et al., 2018) – was used (Supporting Information S3). Prey selection was estimated for each stream and for both streams together, showing the difference in FOO of each taxon between diet and availability. To do this, prey availability was also expressed in the FOO of each prey taxon for each stream, representing the proportion of Surber samples where each prey taxon was detected in each stream. A threshold of 20% difference was arbitrarily set to determine prey selection as positive or negative.

Positively selected prey taxa were used in order to have a more conservative approach towards prey availability, looking for differences between habitats that could explain the spatial ecology of the desman (its preference for fast-flow habitats; Esnaola, González-Esteban, et al., 2018). Estimations of available density, biomass and average body mass of prey were made for both streams and the values compared with ANOVA.

The significance level of the tests was set at $P < 0.05$. Statistical analyses were performed using R 3.4.3 (R Core Team, 2014).

3 | RESULTS

3.1 | DNA sequences

DNA was successfully extracted and amplified from all samples, except for one faecal sample from the Leitzarán Stream that did not produce enough reads during sequencing. In this way, 94 faeces from the Elama and 93 from the Leitzarán were analysed. In total, 4,896 operational taxonomic units (OTUs) were obtained with the Gillet primer set and 844 OTUs with the Zeale primer set. From them, 64 Gillet and 75 Zeale OTUs passed the filters for diet analysis and were assigned to 41 taxa (23 families) with Gillet, and 46 (33 families) with Zeale. Both primer sets shared 13 families. Gillet amplified several OTUs corresponding to the desman itself in all of the samples, confirming that all the faeces belonged to this species.

3.2 | Diet

Taxon accumulation curves reached an asymptote, and sample completeness was >95%, confirming that the sample size used (number of faeces) was large enough to describe the diet of desmans in both the Elama and Leitzarán streams (Supporting Information S2, Figure S1).

The diet was mostly composed of Insecta and Malacostraca (FOO being 100 and 93%, respectively), with Ephemeroptera, Diptera,

Amphipoda and Trichoptera the most common orders (FOO 99, 94, 92 and 88%, respectively; Figure 3; Table 1; Supporting Information S2, Table S5). The most frequently consumed prey families were Heptageniidae, Gammaridae, Baetidae and Simuliidae in both streams (FOO 94, 92, 91 and 90%, respectively), and the most common genera (FOO >70%) were *Baetis*, *Ecdyonurus* and *Echinogammarus* in the Elama and *Baetis*, *Echinogammarus* and *Epeorus* in the Leitzaran.

Dietary composition differed significantly between streams, both at the species level ($F = 58.24$; d.f. = 1; $P = 0.001$) and family level ($F = 16.88$; d.f. = 1; $P = 0.001$). Odontoceridae, Perlidae, Leptophlebiidae and Astacidae contributed the most to these differences (CA graph dimension 1, Supporting Information S2, Figure S2), with the first three mostly occurring in the Elama Stream and the Astacidae in the Leitzaran Stream (Figure 4).

3.3 | Prey availability

Taxon accumulation curves with abundance and presence-absence data reached an asymptote, and sample completeness was >80% (Supporting Information S2, Figure S3, Figure S4). Heptageniidae (FOO being 68%) and Perlidae (FOO 62%) were the most abundant taxa in the Elama Stream (FOO >50%), and Gammaridae (FOO 96%), Baetidae (FOO 80%), Hydropsychidae (FOO 72%), Heptageniidae (FOO 68%), Athericidae (FOO 55%) and Leuctridae (FOO 55%) in the Leitzaran Stream (Table 2; Supporting Information S2, Tables S2 and S3).

Invertebrate taxon richness was higher in the Leitzaran than in the Elama (Figure 5), the differences being statistically significant at both the species level ($F_{1,53} = 4.41$; $P = 0.041$) and family level ($F_{1,53} = 6.59$; $P = 0.013$). Differences between habitats and the interaction between streams and habitats were not significant.

The density of potential prey ($F_{1,53} = 28.60$; $P < 0.001$), their biomass ($F_{1,53} = 7.62$; $P = 0.008$) and their average body mass ($F_{1,53} = 52.31$; $P < 0.001$) differed significantly between streams, with higher density and biomass in the Leitzaran and higher average body

mass in the Elama (Figure 6a–c). Differences among habitats and among habitats within each stream were not significant. In contrast, density ($F_{1,53} = 28.6$; $P < 0.001$), biomass ($F_{1,53} = 7.81$; $P = 0.007$) and average body mass ($F_{1,53} = 73.85$; $P < 0.001$) of consumed prey differed significantly between streams, with the density being higher in the Leitzaran Stream and biomass and average body mass higher in the Elama Stream (Figure 6d–f). Again, differences among habitats and the interaction between streams and habitats were not significant.

3.4 | Prey selection

- Combining both streams more taxa were positively selected – six families comprising Simuliidae and Psychodidae (Diptera), Baetidae and Heptageniidae (Ephemeroptera), Odontoceridae (Trichoptera) and Gammaridae (Crustacea) – than negatively selected – four families comprising Planorbidae (Gastropoda), Goeridae (Trichoptera), Perlidae (Plecoptera) and Athericidae (Diptera) – (Table 2).
- Overall, positive selections were of greater magnitude than negative selections. Desmans positively selected more taxa in the Elama – 11 families comprising the six mentioned in (a) together with Hydropsychidae (Trichoptera), Limnephilidae (Trichoptera), Leptophlebiidae (Ephemeroptera), Chironomidae (Diptera) and Leuctridae (Plecoptera) – than in the Leitzaran – four families comprising Simuliidae and Psychodidae (Diptera), Heptageniidae (Ephemeroptera) and Rhyacophilidae (Trichoptera).
- Desmans negatively selected more taxa in the Leitzaran – seven families comprising the four mentioned in (a) together with Naididae and Lumbricidae (Oligochaeta) and Polycentropodidae (Trichoptera) – than in the Elama – four families comprising Goeridae (Trichoptera), Perlidae (Plecoptera) and Athericidae and Limoniidae (Diptera).

In summary, the FOO in the diet of all positively, opportunistically and negatively selected taxa (Table 2), averaged 4.49, 2.83 and 0.25,

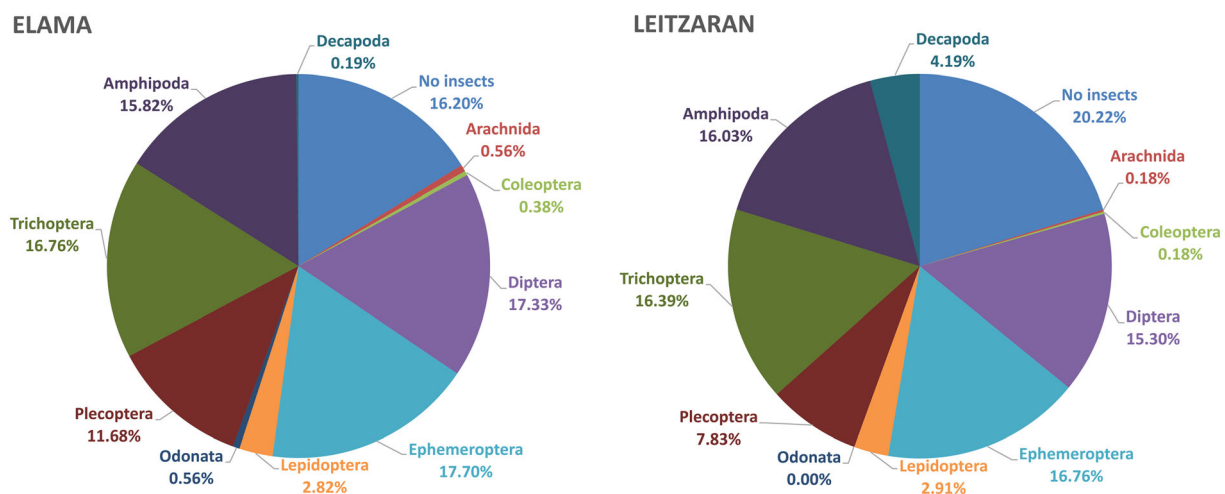


FIGURE 3 Pyrenean desman diet composition. Percentage of occurrences (POO) of each prey group, in both streams

TABLE 1 Comparison between the present study and previous dietary studies

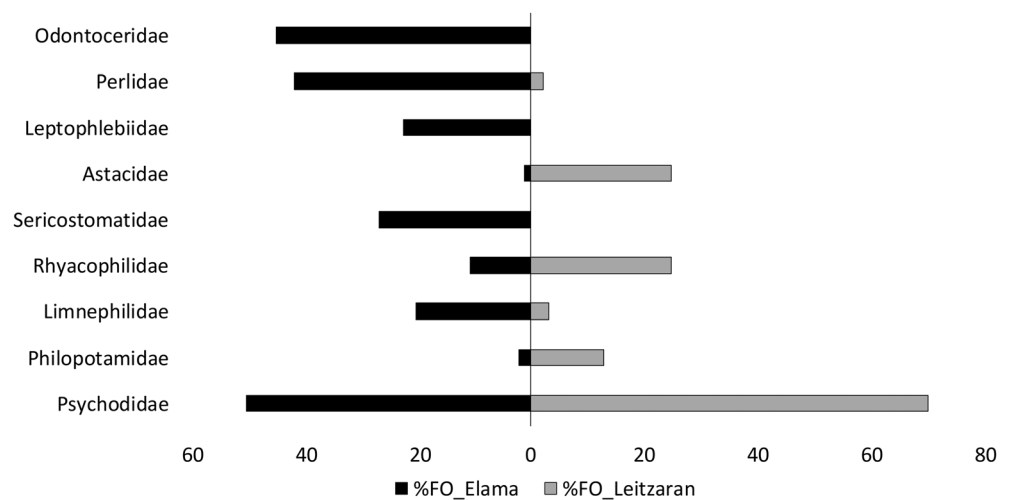
Class	Order	Study area Family	Present study						Biffi, Gillet, et al., 2017	Biffi, Laffaille, et al., 2017	Hawlicschek et al., 2018
			Elama			Leitzarlan			North Pyrenees	North Pyrenees	North-west Iberian Peninsula
			Primers								
			G	Z	G+Z	G	Z	G+Z	G	G	F and M
Arachnida			0	3	3	1	0	1	2	8	0
Clitellata	Haplotaxida	Lumbricidae	0	0	0	0	0	0	1	6	0
Diplopoda			0	0	0	0	0	0	4	22	0
	Glomerida		0	0	0	0	0	0	2	16	0
		Doderiidae	0	0	0	0	0	0	0	8	0
		Glomeridae	0	0	0	0	0	0	2	11	0
	Polydesmida		0	0	0	0	0	0	1	7	0
Insecta			100	100	100	100	100	100	100	93	100
	Coleoptera		1	1	2	1	1	1	14	14	56
		Carabidae	0	1	1	0	0	0	7	1	0
		Scirtidae	1	0	1	0	0	0	6	8	0
	Diptera		16	100	98	12	99	90	46	66	100
		Anthomyiidae	0	0	0	0	0	0	3	5	0
		Blephariceridae	0	0	0	0	0	0	1	7	0
		Chironomidae	13	51	53	10	41	44	15	42	66
		Empididae	1	3	3	0	9	9	0	2	0
		Limoniidae	0	10	10	0	4	3	1	2	0
		Mycetophilidae	0	2	2	0	6	5	0	4	0
		Psychodidae	2	51	50	0	76	70	3	8	0
		Simuliidae	1	96	94	2	94	86	15	18	0
		Stratiomyidae	0	0	0	0	0	0	11	1	0
		Tipulidae	0	7	6	0	0	0	5	4	0
	Ephemeroptera		100	43	100	99	60	99	87	71	100
		Baetidae	87	0	87	95	0	95	57	42	0
		Ephemerellidae	2	1	3	0	0	0	37	2	0
		Ephemeridae	4	0	4	0	0	0	0	8	0
		Heptageniidae	98	41	98	90	60	90	59	61	0
		Leptophlebiidae	22	2	22	0	0	0	5	1	0
	Lepidoptera		0	16	16	0	19	17	1	3	0
		Erebidae	0	7	6	0	7	6	0	1	0
		Geometridae	0	7	6	0	11	10	0	2	0
	Neuropteroida		0	0	0	0	0	0	0	0	12
	Orthoptera		0	0	0	0	0	0	7	0	0
		Anostomatidae	0	0	0	0	0	0	7	0	0
	Plecoptera		62	21	66	33	44	46	85	53	37
		Leuctridae	22	21	28	30	44	45	30	9	0
		Nemouridae	4	0	4	1	0	1	49	26	0
		Notonemouridae	0	0	0	0	0	0	6	0	0
		Perlidae	41	0	41	2	0	2	26	33	0
		Perlodidae	0	0	0	0	0	0	33	5	0
		Pteronarcyidae	0	0	0	0	0	0	5	6	0

TABLE 1 (Continued)

Class	Order	Study area Family	Present study						Biffi, Gillet, et al., 2017	Biffi, Laffaille, et al., 2017	Hawlicscek et al., 2018
			Elama			Leitzaran			North Pyrenees	North Pyrenees	North-west Iberian Peninsula
			Primers						G	G	F and M
			G	Z	G+Z	G	Z	G+Z	G	G	F and M
	Trichoptera		61	75	87	75	46	88	64	64	46
		Hydropsychidae	48	27	51	73	7	73	47	54	0
		Limnephilidae	2	20	20	0	4	3	19	15	0
		Odontoceridae	0	46	45	0	0	0	3	12	0
		Philopotamidae	0	2	2	6	13	13	0	4	0
		Polycentropodidae	0	4	4	0	6	5	1	0	0
		Rhyacophilidae	0	11	11	0	27	25	7	7	0
		Sericostomatidae	27	0	27	0	0	0	2	5	0
Malacostraca			89	1	89	95	27	97	18	23	0
	Amphipoda		89	0	89	95	0	95	17	21	0
		Gammaridae	89	0	89	95	0	95	17	21	0
	Decapoda	Astacidae	0	1	1	0	27	25	0	1	0
		Number of samples	94	92	94	93	85	93	287	184	41

Note: Results expressed in frequency of occurrences (FOO). Taxa present in <5% of the faecal samples in all studies were removed. Primers: G, Gillet et al. (2015); Z, Zeale et al. (2011); F, Folmer, Black, Hoeh, Lutz, & Vrijenhoek (1994); M, Meusnier et al. (2008). The data used in the analyses of the present study have been highlighted in bold, i.e. the data obtained through Gillet and Zeale primers.

FIGURE 4 Frequency of occurrences (FOO) of the prey families that most influenced dietary differences between both streams. These taxa contributed more than 1% to the first dimension of the CA, which separates sites belonging to each stream. Taxa were ordered from the highest (top) to the lowest contribution values (bottom)



respectively. These values were 6.37, 0.88 and 0.57 for the Elama, and 2.71, 4.43 and 0.08 for the Leitzaran.

There were significant differences between streams in the density of positively selected prey taxa ($F_{1,49} = 33.1$; $P < 0.001$), biomass ($F_{1,49} = 4.80$; $P = 0.033$) and average body mass

($F_{1,49} = 38.14$; $P < 0.001$). Available density was higher in the Leitzaran Stream, but available biomass and average body mass were higher in the Elama Stream (Figure 7a–c). Differences among habitats and the interaction between streams and habitats were not significant.

TABLE 2 Diet selection at the family level for each stream and for both streams together

Family	Elama			Leitzarar			Total		
	Diet (FOO)	Avail. (FOO)	Dif.	Diet (FOO)	Avail. (FOO)	Dif.	Diet (FOO)	Avail. (FOO)	Dif.
Simuliidae	0.936	0.064	0.87	0.860	0.115	0.75	0.898	0.089	0.81
Psychodidae	0.500	0.000	0.50	0.699	0.000	0.70	0.599	0.000	0.60
Baetidae	0.872	0.064	0.81	0.946	0.804	0.14	0.909	0.434	0.48
Heptageniidae	0.979	0.679	0.30	0.903	0.678	0.23	0.941	0.679	0.26
Odontoceridae	0.447	0.000	0.45	0.000	0.000	0.00	0.223	0.000	0.22
Gammaridae	0.894	0.445	0.45	0.946	0.960	-0.01	0.920	0.702	0.22
Hydropsychidae	0.511	0.241	0.27	0.731	0.716	0.02	0.621	0.478	0.14
Limnephilidae	0.202	0.000	0.20	0.032	0.000	0.03	0.117	0.000	0.12
Leptophlebiidae	0.223	0.000	0.22	0.000	0.015	-0.01	0.112	0.007	0.10
Geometridae	0.064	0.000	0.06	0.097	0.000	0.10	0.080	0.000	0.08
Astacidae	0.011	0.000	0.01	0.247	0.099	0.15	0.129	0.049	0.08
Chironomidae	0.532	0.335	0.20	0.441	0.498	-0.06	0.486	0.416	0.07
Erebidae	0.064	0.000	0.06	0.065	0.000	0.06	0.064	0.000	0.06
Leuctridae	0.277	0.073	0.20	0.452	0.547	-0.10	0.364	0.310	0.05
Mycetophilidae	0.021	0.000	0.02	0.054	0.000	0.05	0.038	0.000	0.04
Noctuidae	0.043	0.000	0.04	0.022	0.000	0.02	0.032	0.000	0.03
Empididae	0.032	0.064	-0.03	0.086	0.000	0.09	0.059	0.032	0.03
Syrphidae	0.000	0.000	0.00	0.043	0.000	0.04	0.022	0.000	0.02
Lonchopteridae	0.011	0.000	0.01	0.032	0.000	0.03	0.021	0.000	0.02
Scatopsidae	0.000	0.000	0.00	0.032	0.000	0.03	0.016	0.000	0.02
Linyphiidae	0.032	0.000	0.03	0.000	0.000	0.00	0.016	0.000	0.02
Cordulegastridae	0.032	0.003	0.03	0.000	0.000	0.00	0.016	0.002	0.01
Aturidae	0.000	0.000	0.00	0.011	0.000	0.01	0.005	0.000	0.01
Diptera	0.000	0.000	0.00	0.011	0.000	0.01	0.005	0.000	0.01
Gyrinidae	0.000	0.000	0.00	0.011	0.000	0.01	0.005	0.000	0.01
Carabidae	0.011	0.000	0.01	0.000	0.000	0.00	0.005	0.000	0.01
Chloropidae	0.011	0.000	0.01	0.000	0.000	0.00	0.005	0.000	0.01
Oniscidae	0.011	0.000	0.01	0.000	0.000	0.00	0.005	0.000	0.01
Pediciidae	0.011	0.000	0.01	0.000	0.000	0.00	0.005	0.000	0.01
Scirtidae	0.011	0.000	0.01	0.000	0.000	0.00	0.005	0.000	0.01
Glossosomatidae	0.011	0.003	0.01	0.000	0.000	0.00	0.005	0.002	0.00
Philopotamidae	0.021	0.064	-0.04	0.129	0.084	0.05	0.075	0.074	0.00
Rhyacophilidae	0.106	0.322	-0.22	0.247	0.030	0.22	0.177	0.176	0.00
Lumbriculidae	0.000	0.000	0.00	0.000	0.001	0.00	0.000	0.001	0.00
Dytiscidae	0.000	0.000	0.00	0.000	0.003	0.00	0.000	0.001	0.00
Asellidae	0.000	0.003	0.00	0.000	0.000	0.00	0.000	0.002	0.00
Leptoceridae	0.000	0.003	0.00	0.000	0.000	0.00	0.000	0.002	0.00
Ephemereillidae	0.032	0.040	-0.01	0.000	0.000	0.00	0.016	0.020	0.00
Nemouridae	0.043	0.067	-0.02	0.011	0.000	0.01	0.027	0.033	-0.01
Cucurlionidae	0.000	0.000	0.00	0.000	0.015	-0.01	0.000	0.007	-0.01
Sphaeriidae	0.000	0.003	0.00	0.000	0.015	-0.01	0.000	0.009	-0.01
Rhagionidae	0.000	0.040	-0.04	0.000	0.001	0.00	0.000	0.021	-0.02
Sericostomatidae	0.266	0.316	-0.05	0.000	0.000	0.00	0.133	0.158	-0.03
Nematoda	0.000	0.064	-0.06	0.000	0.000	0.00	0.000	0.032	-0.03
Tipulidae	0.064	0.131	-0.07	0.000	0.000	0.00	0.032	0.065	-0.03

TABLE 2 (Continued)

Family	Elama			Leitzaran			Total		
	Diet (FOO)	Avail. (FOO)	Dif.	Diet (FOO)	Avail. (FOO)	Dif.	Diet (FOO)	Avail. (FOO)	Dif.
Lepidostomatidae	0.000	0.073	-0.07	0.000	0.000	0.00	0.000	0.037	-0.04
Planariidae	0.000	0.000	0.00	0.000	0.084	-0.08	0.000	0.042	-0.04
Hirudinidae	0.000	0.000	0.00	0.000	0.099	-0.10	0.000	0.049	-0.05
Hydrobiidae	0.000	0.000	0.00	0.000	0.100	-0.10	0.000	0.050	-0.05
Ephemeraeidae	0.043	0.006	0.04	0.000	0.149	-0.15	0.021	0.078	-0.06
Elmidae	0.000	0.000	0.00	0.000	0.115	-0.11	0.000	0.057	-0.06
Hydrophilidae	0.000	0.131	-0.13	0.000	0.000	0.00	0.000	0.065	-0.07
Naididae	0.000	0.000	0.00	0.000	0.196	-0.20	0.000	0.098	-0.10
Lumbricidae	0.000	0.040	-0.04	0.000	0.296	-0.30	0.000	0.168	-0.17
Polycentropodidae	0.043	0.006	0.04	0.054	0.446	-0.39	0.048	0.226	-0.18
Limoniidae	0.096	0.391	-0.30	0.032	0.099	-0.07	0.064	0.245	-0.18
Planorbidae	0.000	0.090	-0.09	0.000	0.354	-0.35	0.000	0.222	-0.22
Goeridae	0.021	0.381	-0.36	0.000	0.197	-0.20	0.011	0.289	-0.28
Perlidae	0.415	0.616	-0.20	0.022	0.418	-0.40	0.218	0.517	-0.30
Athericidae	0.043	0.292	-0.25	0.000	0.548	-0.55	0.021	0.420	-0.40

Note: Diet expressed in frequency of occurrences (FOO). Average availability of each taxon (Avail.) was weighted by the mean activity of desmans (see explanation in Supporting Information S3) and expressed also in FOO. Dif is the difference between both metrics. Positive and negative selections are highlighted in bold.

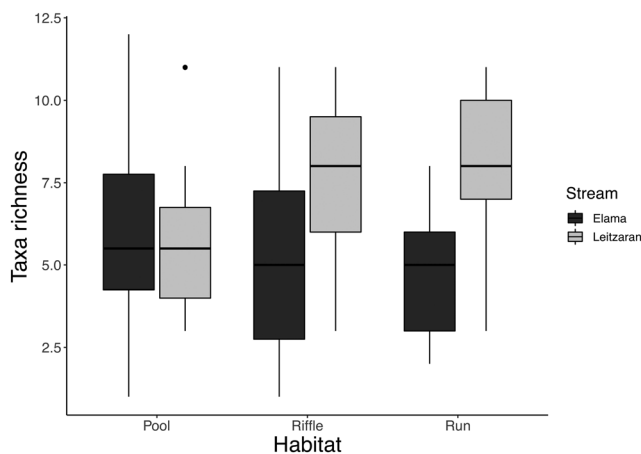


FIGURE 5 Taxon richness of potential prey per habitat and stream. The confidence limits were represented with the '1.5 rule': the whiskers extend to the most extreme data point which is no more than 1.5 times the length of the box away from the box

4 | DISCUSSION

This study completes the first research on the foraging requirements of the Pyrenean desmans simultaneously addressing the spatial ecology of two populations (Esnaola, González-Esteban, et al., 2018), their diet and prey availability in their foraging areas. The radiotracking study (Esnaola, González-Esteban, et al., 2018) did not explain the reasons behind their habitat preference, so it did not provide enough information to conserve and manage desman populations. The

present study aimed to fill that gap, and the headline result was that prey availability does not explain desman habitat selection.

4.1 | Diet

Desmans are specialist rheophilic predators with a strong preference for riffle habitat (Esnaola, González-Esteban, et al., 2018), but the results depict desmans as generalist foragers on a wide variety of prey, including 41 species, 23 families and nine orders of arthropods identified in their diet.

Traditional morphology-based diet studies of prey remains in guts, stomachs or faeces have described desmans as feeding mainly on Trichoptera, Ephemeroptera, Plecoptera, Crustacea (Amphipoda) and Diptera (Bertrand, 1992; Castián & Gosálbez, 1995; Santamarina & Guitián, 1988), and less frequently on Coleoptera, Annelida and Odonata (Puisségur, 1935; Richard & Viallard, 1969; Santamarina, 1992). Similarly, in the present work desmans mostly fed on Ephemeroptera (Heptageniidae and Baetidae), Diptera (Simuliidae), Plecoptera and Amphipoda (Gammaridae), their selection differing between streams, particularly regarding trichopteran Rhyacophilidae. The species-level identification of consumed prey achieved by molecular methods allows a more detailed characterization of the desman diet. It is worth noting that molecular methods confirmed the minor importance of soft-bodied prey groups, which could have passed unnoticed in morphological studies. Thus, potential prey such as Clitellata, Nematoda and Amphibia appeared at very low frequency (Biffi, Gillet, et al., 2017; Esnaola,

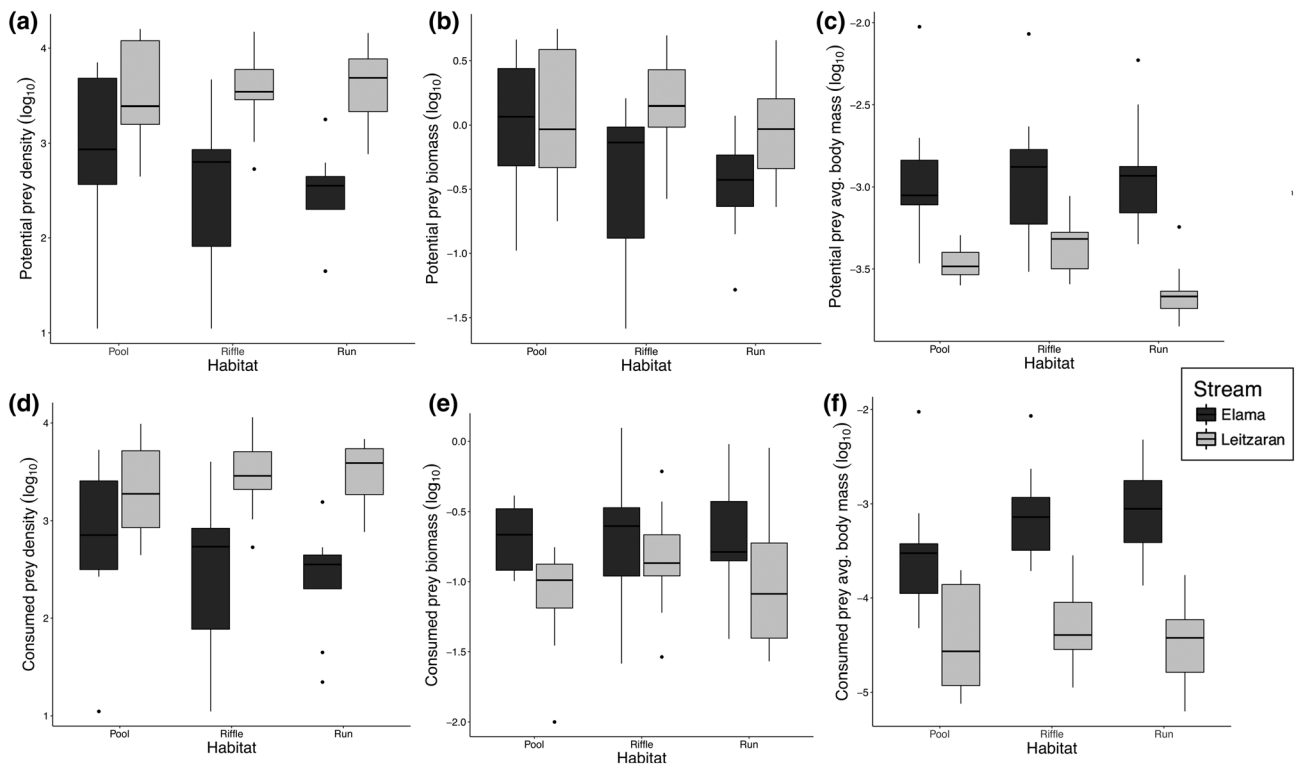


FIGURE 6 Potential and consumed prey density (a, d), biomass (b, e) and average body mass (c, f) of each habitat in each stream. The confidence limits were represented with the ‘1.5 rule’

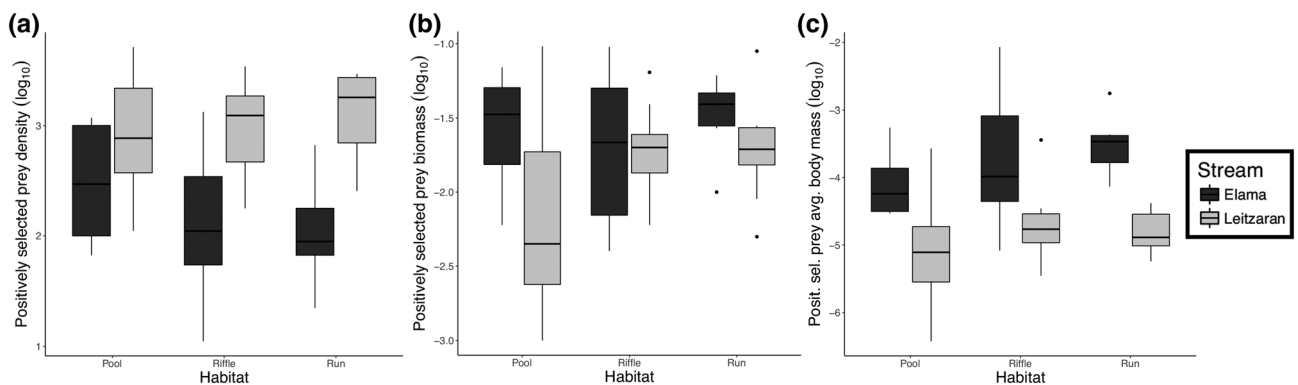


FIGURE 7 Positively selected prey density (a), biomass (b) and average body mass (c) of each habitat in each stream. The confidence limits were represented with the ‘1.5 rule’

Arrizabalaga-Escudero, et al., 2018). In contrast, freshwater crayfish (*Pacifastacus*) was a frequent prey in the Leitzaran, which had not been reported before for the desman.

Despite methodological differences (Alberdi et al., 2019) that make comparisons difficult among the five studies recently published on desman diet using DNA metabarcoding (Biffi, Gillet, et al., 2017; Biffi, Laffaille, et al., 2017; Esnaola, Arrizabalaga-Escudero, et al., 2018; Gillet et al., 2015; Hawlitschek et al., 2018), there are several noteworthy aspects for discussion. First, the present study found a lower number of prey taxa than Hawlitschek et al. (2018), Biffi,

Gillet, et al. (2017) and Biffi, Laffaille, et al. (2017), probably as the bioinformatic procedures in the present work were more conservative, discarding OTUs with less than 1% of reads (i.e. 98.7% of Gillet OTUs and 91% of Zeale OTUs; Da Silva et al., 2019) to avoid secondary predation, false positives and chimaeras (Alberdi, Aizpurua, Gilbert, & Bohmann, 2018; Alberdi et al., 2019; De Barba et al., 2014; Sheppard et al., 2005). Thus, most of the rare taxa detected by Biffi, Gillet, et al. (2017) and Biffi, Laffaille, et al. (2017) were most likely filtered out by the procedure of this work. In addition, Biffi, Gillet, et al. (2017) and Biffi, Laffaille, et al. (2017) probably underestimated

some of the most frequent (FOO >25%) prey groups identified in this study owing to primer bias (Esnaola, Arrizabalaga-Escudero, et al., 2018). In the present study, the dipterans Psychodidae, Simuliidae and maybe Chironomidae, the trichopterans Odontoceridae, Phylopotamidae, Polycentropodidae and Rhyacophilidae, the plecopterans Leuctridae, and Astacidae or Lepidoptera, were mainly amplified by Zeale primers (Table 1), confirming the importance of primer choice in metabarcoding studies (Elbrecht & Leese, 2017). On the other hand, there were some contrasts among the study sites, as well as with those studied by Biffi, Gillet, et al. (2017), Biffi, Laffaille, et al. (2017) and Hawlitschek et al. (2018), who surveyed much broader areas (Table 1). These contrasts probably reflect differences in faunal composition, a consequence of differences in local conditions or sampling periods, although this cannot be confirmed as other authors have not described prey availability at the microhabitat level. Whatever the reason for the differences, it is worth noting that the diet of desmans in the Elama, contrasting with the Leitzaran, resembles more closely those described by Biffi, Gillet, et al. (2017) and Biffi, Laffaille, et al. (2017) in the north of the Pyrenees.

4.2 | Prey selection

The estimated general patterns of prey selection coincided with previous studies, albeit with small differences. First, desmans seem to positively select conspicuous invertebrates that live on the surface of the substrate, such as Simuliidae, Baetidae and Heptageniidae. Bertrand (1992) also speculated that desmans would positively select benthic and rheophilic prey. Second, they seem to positively select shredding invertebrates linked to coarse organic matter, such as Gammaridae, Odontoceridae, Leptophlebiidae, Limnephilidae and Leuctridae. Castién and Gosálbez (1995) reported negative selection of gammarids, which they attributed to the sclerified tegument, but the results of the present study contradict their observation. This probably reflects a preference of desmans for accumulations of leaf litter, where the density of invertebrates, mainly large shredders, is higher than in other types of substrates (Flores et al., 2017). Third, desmans negatively select soft-bodied taxa inhabiting fine sediments, such as Athericidae, Limoniidae, Naididae and Lumbricidae. Fourth, they negatively select invertebrates with hard shells, such as Goeridae or Planorbidae, which might reflect lower interest in food items that are hard to process; similarly, Santamarina (1992) found that molluscs were also selected against. Fifth, desmans negatively selected predators such as Perlidae, Polycentropodidae and Rhyacophilidae. The reason for this negative selection is unclear, but it may be related to defence or escape strategies exhibited by these predators. Nevertheless, these are tentative interpretations that cannot be generalized, as some prey items with similar characteristics (e.g. Psychodidae) were positively selected. Remarkably, selection interacted with the location as Rhyacophilidae were negatively selected in the Elama and positively selected in the Leitzaran. The study of biological and ecological traits of prey could shed some light on this selection pattern.

Biffi, Laffaille, et al. (2017) also compared desman molecular diet composition with prey availability in the Ariège department (French Pyrenees). They concluded that the prey most frequently consumed corresponded to the most abundant taxa in streams, and suggested which prey characteristics are valuable or avoided by desmans, but their conclusions are not comparable with those of the present study for different reasons. They described desman prey availability in only one-third of the sites at which they described the diet (19 vs. 58 sites), and their study did not allow a detailed microhabitat-level description of the food that desmans had available. For these reasons we consider that those data are not adequate to infer trophic preferences and prey selection by desmans at the local scale.

4.3 | Location vs. selection

In the Elama Stream, desmans positively selected some shredding invertebrates that are usually associated with coarse organic matter, which did not happen in the Leitzaran Stream. We suggest that the habitat complexity of the Elama favoured the retention sites of coarse organic matter, where habitat stability, surface area and food resources for invertebrates are greater, and a higher biomass of prey accumulates (Flores, Díez, Larrañaga, Pascoal, & Elosegi, 2013). In these areas desmans encounter prey with a higher body mass (on average), which constitutes more profitable food. When the profitability of the main prey items falls below a critical threshold, it is expected that a generalist predator will switch to alternative, and more profitable, prey items (Křivan, 1996). Therefore, and taking into account that in the Leitzaran Stream the opportunistically consumed taxa had the highest FOO values in the diet, we suggest that desmans may select other prey in the Leitzaran because the preferred ones were absent, widening their trophic niche in the face of lower availability (Araujo, Bolnick, & Layman, 2011).

4.4 | Choosing a restaurant: the ambience or the menu?

In a previous study on the foraging ecology of the desman, Esnaola, Arrizabalaga-Escudero, et al. (2018) reported that riffles were their preferred habitat in both study areas, and suggested that this was a consequence of higher prey availability in those areas. The present work, however, showed no significant inter-habitat differences in prey availability, neither for prey consumed nor for prey positively selected. These results raise the question of why desmans forage preferentially in riffles: why do they choose that restaurant? This work shows that prey availability (the menu) cannot explain habitat selection by desmans, so it is likely that the physical characteristics of the habitat (the ambience) determine the spatial preferences of desmans. The fast flow velocity in riffles probably makes it easier for desmans to overcome their natural buoyancy and crawl on the bottom using their strong claws to take hold (Richard, 1986), as has been observed in other rheophilic species such as the

white-throated dipper (*Cinclus cinclus*) and the Pyrenean newt (*Calotriton asper*).

4.5 | Implications for conservation

Recent research (Esnaola, González-Esteban, et al., 2018) has highlighted the importance of fast-flowing habitats for desmans, showing that riffle availability affects the quality of the available habitat and the connectivity among populations. Maintaining a constant flow of water in the stream channel is therefore essential for desman conservation. Water diversion schemes, however, generate still water in the reservoir, fragmenting populations, and the bypass diverts the water, reducing the flow in the channel. On many occasions the diversion canal carries more water than the stream, and this can be an important cause of death for desmans (Elosegi, 2010). Thus, assessing how the riffle surface varies with the flow will be key to set appropriate environmental flows taking into account the requirements of the desman. Maintaining a minimum flow in the stream will not be enough, however, and removing obstacles that fragment desman populations and increasing riffle availability and stream heterogeneity (e.g. by adding dead wood in the channel; Flores et al., 2013) will also be essential. For instance, large dams have been removed to restore natural flow regimes and improve desman population connectivity both in the Artikutza Stream (e.g. the Enobieta dam; Yarzabal, Martín-Vide, Elosegi, & Díez, 2018) and in the Leitzaran Stream (e.g. the Inturia dam; Urquijo, 2018).

Although desman populations are occasionally monitored in the Elama and the Leitzaran streams there are no specific monitoring programmes for desmans, which will be essential to manage the species effectively. We recommend the regular monitoring of desman populations using artificial shelters (González-Esteban et al., 2018) and the study of their population dynamics, as well as the design and compliance of ecological flows that take into account the needs of desmans.

The desman could be considered as a flagship species (Melero, Aymerich, Santulli, & Gosálbez, 2014) or an umbrella species (Kalinkat et al., 2017), as its management protects an entire ecosystem: restoring stream connectivity according to the needs of the desman will also favour many other freshwater species that inhabit headwater streams in the Iberian Peninsula and the Pyrenees. The desman has been reported in 296 Natura 2000 sites (<https://natura2000.eea.europa.eu/>). Furthermore, several European projects have included the restoration of stream stretches to improve desman populations (LIFE Desman, 2014; LIFE Desmania, 2016; LIFE Irekibai, 2017), yet in the countries concerned the general population is unaware of the desman, and that undermines its protection. Given that context, scientific popularization will be essential to obtain the support of the general public needed for the conservation of the desman.

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SUPPORTING INFORMATION

Additional supporting information may be found online in the Supporting Information section at the end of this article.

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