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Recent range contractions in the globally threatened Pyrenean desman highlight the importance of stream headwater refugia

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Abstract

Freshwater ecosystems are among the most threatened in the world, and downstream reaches of stream networks are often the most affected by multiple anthropogenic stressors. In these circumstances, many species currently restricted to stream headwaters may represent remnants of once larger populations, though this is generally difficult to ascertain due to limited historical data. Here, we document a case of range contraction into headwaters of the globally threatened Pyrenean desman *Galemys pyrenaicus*, a species endemic to South-western Europe, from surveys carried out in 1993–96 and 2014–15. Geostatistical logistic mixed models were used to relate the probabilities of desman occurrence and extinction to environmental variables in 74 sites within two watersheds in NE Portugal, while controlling for linear (Euclidean) and hydrologic spatial dependencies. In 1993–96, desmans were recorded at 85.1% of sites, and their presence was positively related to stream order and slope. In 2014–15, desmans were only found at 31.1% of sites, and their presence was positively related to slope steepness and negatively to stream order and maximum temperature of the warmest month. The extinction rate across periods was 63.5%, and the probability of extinction increased with stream order and declined with slope steepness. Maps of predicted distribution and extinction indicated that while the species was widely distributed in the first period, it largely disappeared thereafter from the main rivers and the largest tributaries, persisting mostly in stream headwaters in more mountainous areas. The severe decline found in ours and other studies suggests that the conservation status of the Pyrenean desman may need to be upgraded, and highlights the importance of headwater streams for its conservation. Furthermore, this study supports the view that headwater streams may be increasingly important for biodiversity conservation in modified river systems, favouring the permanence of remnant, albeit fragmented populations.

Introduction

Freshwater species are undergoing extinctions at unprecedented high rates (Vörösmarty *et al.*, 2010; Collen *et al.*, 2014), due to multiple anthropogenic drivers (Strayer & Dudgeon, 2010; Vörösmarty *et al.*, 2010; Collen *et al.*, 2014). However, these drivers do not act homogeneously across the stream network, often concentrating in downstream reaches where there are higher human population densities, and therefore more intensive water and land uses, infrastructures and exotic species (Zwick, 1992; Leprieur *et al.*, 2008; Vörösmarty *et al.*, 2010; Filipe *et al.*, 2017). As a consequence, species associated with downstream reaches may become extinct or otherwise confined to more preserved upstream reaches (Zwick, 1992; Allan, Palmer & Poff, 2005; Meyer *et al.*, 2007; Grant *et al.*, 2010; Isaak *et al.*, 2015, 2016), where in turn they may be at risk due to small population sizes and loss of connectivity (Corn & Bury, 1989; Fagan, 2002; Lowe, 2002; Labonne *et al.*, 2008; Williams *et al.*, 2009). However, lack of historical surveys often makes it hard to understand whether a species is confined to headwaters due to its ecological requirements, or whether headwaters are just remnants of once larger distributions, particularly in the case of small, rare or otherwise elusive species. These uncertainties have important consequences for selecting priority areas for conservation and restoration (Scheele *et al.*, 2017).

The Pyrenean desman *Galemys pyrenaicus* is an ecologically unique semiaquatic mammal, which is one of the two surviving species of the Tribe Desmanini (Mammalia, Soricomorpha, Talpidae) (He *et al.*, 2016). This globally threatened species is endemic to South-western Europe, where it is restricted to the Pyrenees, northern and central Spain, and northern Portugal (Fernandes *et al.*, 2008), where it is often associated to fast-flowing, shallow and well-oxygenated waters (e.g. Queiroz *et al.*, 1998; Charbonnel *et al.*, 2015). The Pyrenean desman is one of the less-studied European mammals (Charbonnel *et al.*, 2015), despite evidence for significant range reductions in Portugal (Quaresma, 2001), Spain (Gisbert & García-Perea, 2014) and France (Charbonnel *et al.*, 2016). Reasons for these declines are unclear, but they are possibly related to water pollution, reduced stream flows, habitat loss and fragmentation, predation by the invasive American mink *Neovison vison*, and climate change (Queiroz, Bertrand & Khakhin, 1996; Fernandes *et al.*, 2008; Morueta-Holme, Fløjgaard & Svenning, 2010; Charbonnel *et al.*, 2015, 2016). However, these threats have been identified primarily through expert judgement and from habitat associations derived from snapshot surveys, with no empirical study evaluating what factors drive local extinctions. Also, no study has yet quantified whether extinctions follow a clear spatial pattern, and whether headwaters or other areas within the stream network, if any, can represent important refugia for species persistence (e.g. Grant *et al.*, 2010).

In this study, we address these issues, by analysing the changes in Pyrenean desman distribution over a 20-year period in two Mediterranean watersheds. Specifically, we: (1) carried out a thorough survey of desman occurrence in 2014–15, revisiting occupied and unoccupied sites that had been sampled in 1993–96; (2) modelled the distribution in each time period, and the extinction probability across time periods, in relation to landscape-scale environmental variables; and (3) used distribution models to produce maps of species distribution across the stream network in each time period, and of extinction probability across periods. Results are used to discuss conservation strategies for the Pyrenean desman and other species restricted to headwaters.

Materials and methods

Study site and species

The study was conducted in NE Portugal, within the Sabor and Tua (N 41°09′–42°06′, W 7°37′–6° 16′) watersheds (Fig. 1), which encompasses the Long-Term Ecological Research (LTER) site of Baixo Sabor. The area comprises a wide range of environmental conditions regarding elevation (99–1676 m), total annual precipitation (568–1266 mm) and mean annual temperature (6.3–15.5°C). Climate is mainly Mediterranean, with precipitation concentrating in October to March, and being virtually absent during the hot summer months (June-August). Water flow is highly seasonal, with smaller streams often drying out or being reduced to a series of disconnected pools in summer, though the main watercourses and some tributaries are permanent (Ferreira *et al.*, 2016). In the 1990s, the desman was widespread in Northern Portugal, including the study area, where it was found throughout the Sabor and Tua watersheds (Queiroz *et al.*, 1998).

Desman surveys

The study was based on comparisons of Pyrenean desman presence/absence between two time periods, using as baseline the 1993–1996 national survey of the species (Queiroz *et al.*, 1998), complemented with information from a few additional sites surveyed in 2000 (C. M. Quaresma, unpubl. data). The 1993–1996 survey was made by experienced observers, who visited 74 sites in the Tua and Sabor watersheds, selected based on accessibility and the presence of ecological conditions potentially suitable to the species (Queiroz *et al.*, 1998). Most sites were in small streams, with shallow and flowing waters, abundant rocks and potential shelters (Queiroz *et al.*, 1998), which were spread across the two watersheds (Fig. 1). At each site, observers defined a stream sector with a maximum extent of 600 m, which was carefully surveyed for detecting desman's faeces. During surveys, observers waded slowly along the stream bed, searching also along the margins, and using a flashlight in shady areas and to look into cavities. The survey was stopped after detecting faeces unequivocally attributed to the desman, normally corresponding to latrines or fresh isolated faeces characteristic of the species (Queiroz *et al.*, 1998). In case of doubt, faeces were taken to the laboratory for microscopic examination to detect desman distinctive hairs, thereby avoiding misidentifications and thus minimizing the incidence of false positives (Queiroz *et al.*, 1998). However, the possibility of misidentifications could not be completely ruled out, though this was unlikely given the experience of the observers. The possibility of false negatives could also not be discarded, but the observation that detections occurred 88% of times in the first 200 m of the 600-m transect suggests that detectability was high (Queiroz *et al.*, 1998). This is in line with a recent study using a similar survey method, which estimated a detection probability of one given occupancy by desmans in 500-m stream reaches (Charbonnel *et al.*, 2014). To further minimize false negatives, no surveys were carried out in rainy periods and when there were high flows, because these reduce species detectability (Queiroz *et al.*, 1998; Charbonnel *et al.*, 2015).

From May to September 2014, and from May to November 2015, we revisited all 74 sites surveyed during the baseline survey, including both sites occupied by the species and sites where the species was not detected at the time. This was part of a larger study thoroughly covering the Sabor and Tua watersheds, during which we surveyed a total of 144 sites. At each site, surveys were made in a ≤600 m transect by two observers, following procedures similar to those in the baseline survey. To assure methodological consistency, observers were trained by one of the researchers undertaking

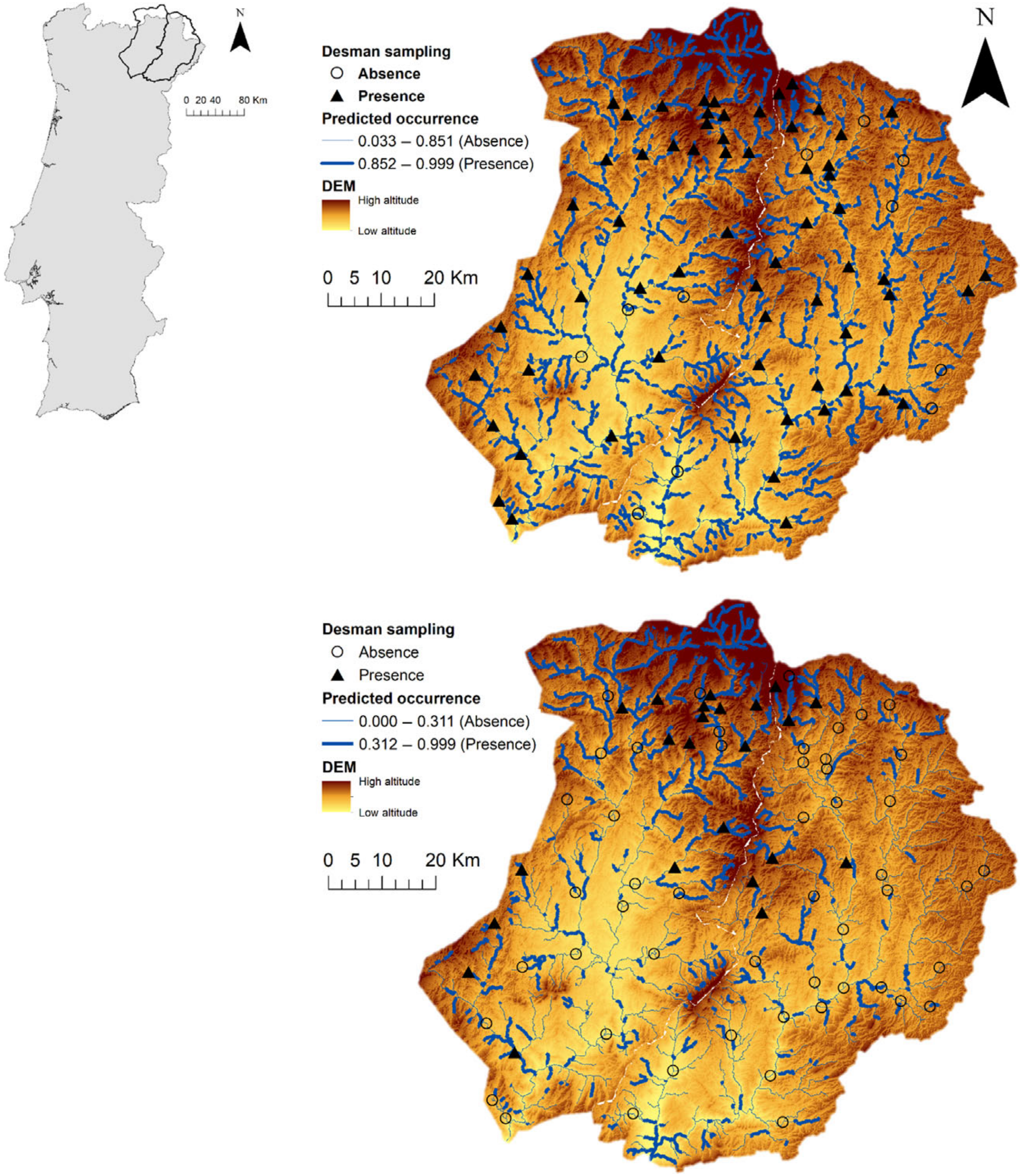

Figure 1 Location of the study area in NE Portugal (upper left panel), and maps of predicted distribution of the Pyrenean desman (*Galemys pyrenaicus*) in two watersheds in 1993–96 (upper panel) and 2014–15 (lower panel). In each map, we show the location of the 74 sites where the species was surveyed, indicating whether the species was recorded (closed triangles) or not (open circles). The digital elevation model hillshade shows the topography of the study landscape. Thresholds for predicted occurrences were set equal to the observed species prevalence.

surveys in the same area in 1993–96 (C.M. Quaresma). The survey always covered the first 200 m where desmans were generally detected in the baseline survey (Queiroz *et al.*, 1998), though we continued up to a maximum of 600 m until at least three faeces visually attributed to the species were detected. Surveys took in average 2.58 h/site (±1.18;

0.12–7.92). All faeces attributed to desman were georeferenced in the field using a GPS, collected, stored in individual vials with 96% ethanol, and taken for molecular identification in the laboratory. Collected faeces were also scored in the field to prioritize molecular identification, based on smell, colour, texture and deposition site (Queiroz *et al.*, 1998; C.M. Quaresma, pers. comm.; L. Quaglietta, pers. obs.), ranging from those unlikely (1) to those very likely (10) to belong to desman. We took a very conservative approach and collected from each site also faeces that departed significantly from the desman archetypal to minimize false negatives. Molecular methods were used because they provide a reliable and objective approach to attribute faeces to a given species, even when there are no hairs or other diagnostic features. Care was taken when collecting and storing faeces to reduce the possibility of contamination that might lead to false positives. By minimizing both false positives and false negatives, these procedures were expected to enhance comparability between survey periods despite differences in the observers involved and in methodological details.

Molecular analysis

From each site, we selected for molecular identification at least the five faecal samples scored in the field as the most likely to belong to desman, though in some cases this was not possible because of smaller sample sizes. Faecal samples were analysed progressively from the most likely to the least likely to belong to desman, until at least one tested positive for the species. This approach was taken to reduce costs while maximizing the chances of species detection. The molecular analysis followed procedures detailed in Supporting Information. Briefly, DNA was extracted using commercial DNA stool kits, and amplified and sequenced using a small fragment of the cytochrome *b* gene, with desman-specific primers (278 bp - Igea *et al.*, 2013) and an universal primer set (360 bp - Kocher & White, 1989; Palumbi, 1996). The desman-specific amplification was repeated whenever necessary to minimize false negatives. All extraction and amplification protocols were performed in a physically isolated laboratory, properly conditioned to avoid risk of DNA contamination (Gilbert *et al.*, 2005). Sequence similarity searches were then performed using GenBank BLAST system for species identification.

Environmental and spatial variables

Each survey site was characterized using seven environmental variables potentially affecting desmans (e.g. Queiroz *et al.*, 1998; Barbosa, Real & Vargas, 2009; Charbonnel *et al.*, 2015), which were extracted in a Geographic Information System (GIS, ArcGis 10.2, ESRI 2013) from the digital maps listed in Table 1. We only used landscape-scale variables that could be easily extracted from digital maps, because we wanted to map predicted distributions across the entire watershed. Stream characteristics were described using Strahler's stream order as a proxy for habitat size and heterogeneity (Filipe *et al.*, 2017), and slope and flow accumulation were calculated from a 25 m resolution DEM (European Commission, DG Enterprise and Industry) using Spatial Analyst (ArcGIS 10.2, ESRI 2013). We also estimated the proportion of the watershed draining into each site with slopes >10° using Spatial Tools for the Analysis of River Systems (STARS) toolbox version 2.0.0 (Peterson & Ver Hoef, 2014), to test for slope effects at larger than site spatial scale, and the in-stream distance of the site to the nearest river source. We also considered climate variables extracted from WorldClim (Fick & Hijmans, 2017). As these were strongly intercorrelated, we only retained the maximum temperature of the warmest month, which may be related to availability of surface water during the dry Mediterranean summer, and thus to affect desman occurrence (Barbosa *et al.*, 2009; Charbonnel *et al.*, 2016). To confirm that there was no multicollinearity in the variables used in modelling, we computed Variance Inflation Factors using the package car (Fox *et al.*, 2017).

Spatial data necessary to account for spatial autocorrelation (see below) were obtained in a GIS using the Sabor and Tua watershed networks extracted from CCM2 (Catchment Characterization and Modelling database; Vogt *et al.*, 2007), and the layer of survey sites. Spatial estimates were made using the Spatial Tools for the Analysis of River Systems (STARS) toolbox version 2.0.0 (Peterson & Ver Hoef, 2014) for ArcGIS 10.2 (ESRI 2013).

Data analysis

We modelled presence/absence of desmans in 1993–96 and in 2014–15, and local extinctions between the two surveys, in relation to environmental variables. The extinction model considered sites where the species persisted (i.e. double presences), and sites where the species was detected in 1993–96 but not in 2014–15. Modelling was based on a recent geostatistical approach designed to deal with spatial dependencies across dendritic ecological networks (Ver Hoef, Peterson & Theobald, 2006; Peterson & Ver Hoef, 2010; Ver Hoef & Peterson, 2010; Peterson *et al.*, 2013), which was previously shown to improve species distribution modelling in stream networks (Filipe *et al.*, 2017). Geostatistical models are similar to conventional linear mixed models, with three spatial autocorrelation components specified in random errors. These components describe spatial dependencies between sites as functions of either straight-line distances (Euclidean model) between sites, hydrologic distances between sites connected by the water flow (tail-up model), or hydrologic distances irrespective of water flow connection (tail-down model).

The fixed component of the geostatistical models corresponded to a logistic function linking either the occurrence (presence/absence) or the extinction (extinction/persistence) of desmans to environmental variables. To build the fixed component of each model, we tested all combinations of environmental variables and selected those minimizing the model AIC. The random component was specified considering the full autocovariance structure and the same function types across models, which provides the greatest flexibility

Table 1 Description and summary statistics of environmental variables used to model the probability of occurrence and the probability of extinction of the Pyrenean desman *Galemys pyrenaicus* in two watersheds of Northeast Portugal.

Variable	Description	Source	Mean ± SD (Min_Max)
Stream order	Strahler's stream order	Extracted from CCM2 (catchment characterization and modelling database) (Vogt *et al.*, 2007)	2 ± 1 (1–6)
Slope	Slope of the site	Calculated from a 25 m resolution DEM (European commission, dg enterprise and industry) using Spatial Analyst (Arc GIS 10.2, ESRI 2013)	10.7 ± 7.8 (0–73.4)
Slope steepness	Proportion of slope > 10° in the contributing area of each reach (Peterson & Ver Hoef, 2014)	Calculated using the Spatial Tools for the Analysis of River Systems (STARS) toolbox version 2.0.0 (Peterson & Ver Hoef, 2014)	48.7 ± 16.8 (2.9–98.9)
Flow Accumulation	Accumulated weight of cells flowing into each downslope in a raster grid	Calculated from a 25 m resolution DEM (European Commission, DG Enterprise and Industry) using Spatial Analyst (Arc GIS 10.2, ESRI 2013)	$5.3 \times 10^4 \pm 3.0 \times 10^5$ (0-0-4.6 × 10^6)
Distance to source	Distance to the nearest river source measured along the river network	Calculated from CCM2 using Spatial Analyst (Arc GIS 10.2, ESRI 2013)	$3.3 \times 10^3 \pm 2.7 \times 10^3$ (0–1.5 × 10^4)
Temperature	Maximum temperature of warmest month	Extracted from WorldClim (~1950–2000)	28.3 ± 1.6 (21.5–31.1)

for representing multiple types of autocorrelation simultaneously (Ver Hoef & Peterson, 2010), and enhances consistency to the comparisons among models and map results, respectively. Therefore, based on preliminary screening of autocovariance functions, we specified random components considering a Cauchy function for the Euclidean model, and a linear-with-sill function for the tail-up and the tail-down models. To deal with confluences in tail-up models, we used watershed areas to weight the relative influence of the branching upstream segments (e.g. Ver Hoef *et al.*, 2006; Ver Hoef & Peterson, 2010).

Models' discrimination ability was estimated from the overall prediction success, as well as from the area under the receiver operating characteristic curve (AUC) (e.g. Václavík, Kupfer & Meentemeyer, 2012) and Cohen's kappa. For 2014–15, we also considered prediction success based on an independent dataset of 70 sites surveyed that were not included in distribution modelling. Prediction success was estimated considering species prevalence (i.e. the proportion of presences in the total number of sites surveyed in either 1993–96 or 2014–15) as threshold for predicted presences (Liu *et al.*, 2005), and the proportion of extinctions observed as threshold for predicted extinctions. We performed all analyses in the R version 3.2.3 (R Development Core Team, 2015), using SSN (Ver Hoef & Peterson, 2015), pROC (Robin *et al.*, 2011) and irr (Gramer *et al.*, 2012) packages.

Mapping

We used universal kriging (Cressie, 1993) based on the geostatistical models to produce maps of predicted distribution in 1993–96 and 2014–15, as well as maps of predicted extinction across periods. The two survey periods were mapped separately to assess variation in distribution patterns over time. To produce the maps, we first divided in a GIS the two watersheds in 4101 segments according to the following criteria: (1) each first order stream was one segment; (2) one segment in higher order streams was the reach between two successive tributaries; and (3) long reaches were divided, so that all segments were <1000 m. We then computed the centroid of each stream segment, and measured all environmental variables at that location, which were assumed to be constant across the entire segment (e.g. Ferreira *et al.*, 2016; Filipe *et al.*, 2017). The predicted probabilities for each segment were computed considering the geostatistical model, the values of the environmental variables, and the spatial dependencies. To produce binary maps describing predicted presence/absences and extinction/persistence, we used as thresholds the prevalence and the proportion of observed extinctions (Liu *et al.*, 2005).

Results

In 1993–96, desmans were detected in 63 (85.1%) out of 74 sites surveyed (Fig. 1). A total of 43.1% of presence sites were confirmed through microscopic examination of faecal samples. In 2014–15, we collected faecal samples at 68 of the 74 sites surveyed; in the remaining six sites there were no faeces attributable to desman. A total of 298 faecal samples were analysed for molecular species identification, of which 40% could not be used due to degraded DNA, 48.6% were attributed to other species, and only 11.4% were identified as desman (Table S1). The proportion of correct identifications was remarkably higher (92.7%), however, when considering only faecal samples confidently assigned in the field to desman (score ≥ 8; $n = 55$). Molecular analysis confirmed the presence of desmans at 31.1% of the sites surveyed. The extinction rate between the two surveys was 63.5%, with the species disappearing from very large sectors of the Tua and Sabor rivers (Fig. 2). There were no statistically significant differences in extinction rates between sites confirmed through microscopic examination (69.2%) and through field identification only (59.4%) (Fisher's exact test, $P = 0.596$).

The geostatistical model built for 1993–96 indicated that the probability of desman occurrence was positively influenced by stream order and slope (Table 2). Although the best environmental model included a significant negative effect of the maximum temperature of the warmest month, this variable had to be discarded due to convergence problems when fitting the full mixed model. For 2014–15, the geostatistical model indicated that occurrence probability was positively related to slope steepness and negatively to stream order and maximum temperature of the warmest month (Table 2). The geostatistical model for desman extinction indicated that the probability of a species disappearing from a site increased with stream order and declined with slope steepness. In the three models, the proportion of variation explained by the environmental component was 9–19%, while variation accounted for the spatial autocovariance ranged widely from 0% to 81% (Table 3). Model discrimination ability was moderate for the 1993–96 survey, but high for the 2014–15 survey and for the probability of desman extinction (Table 3). The overall prediction ability of the 2014–15 model based on the independent dataset was 80.0%, though it was higher for absences (83.6%) than for presences (66.7%).

The maps of desman distribution predicted from the geostatistical models showed a marked geographic pattern, which clearly changed over time (Fig. 1; Supporting Information Fig. S1). In 1993–96, desmans were widely distributed across the study area, though the distribution appeared more continuous in the Tua than in the Sabor watershed. In contrast, in 2014–15 the species was largely confined to headwater streams, albeit with a larger distribution still found in the Tua than in the Sabor watershed. These distribution shifts are clearly captured by the map of predicted extinctions across time periods, which showed that the species disappeared from vast areas, particularly in downstream reaches and in the Sabor watershed (Fig. 2; Supporting Information Fig. S2).

Discussion

This study documents a major decline in the occurrence of Pyrenean desman in Northeast Portugal in the last two decades, suggesting a strong range contraction into headwater streams. This is supported by the results of geostatistical modelling, which suggests that the species is currently confined to lower order streams with high slope steepness and lower maximum temperature of warmest month, while extinctions occurred in higher order streams and in streams

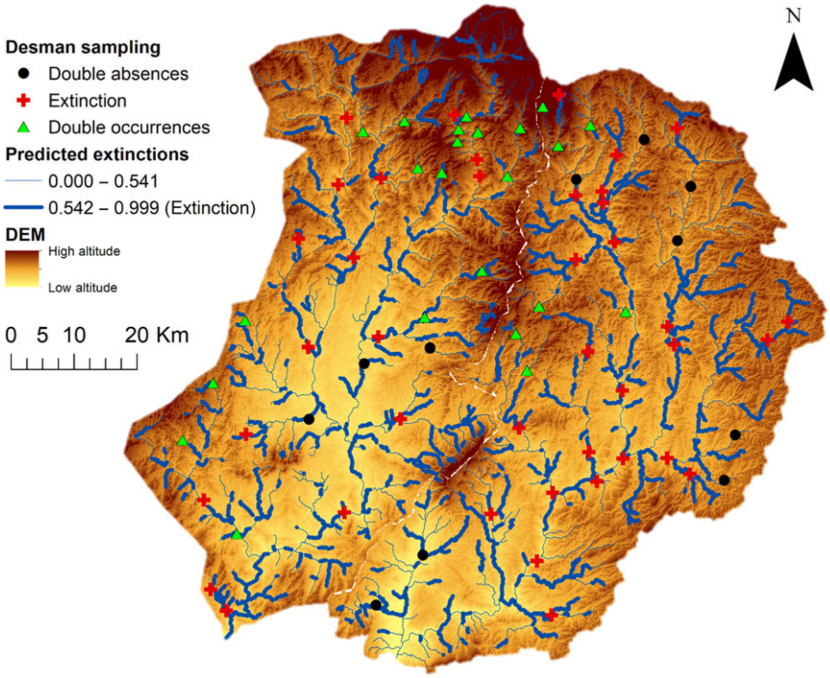

Figure 2 Map of predicted extinctions of the Pyrenean desman (*Galemys pyrenaicus*) between surveys carried out in 1993–96 and 2014–15. In the map, we show the location of the 74 sites surveyed in both periods, and whether we recorded double absences, double presences, or extinctions (i.e. shifts from presence to absence between the two surveys). The digital elevation model hillshade shows the topography of the study landscape. The threshold for predicted extinctions was set equal to the observed species extinction frequency.

Table 2 Parameter estimates of the fixed component of geostatistical models relating the probabilities of occurrence and extinction of the Pyrenean desman *Galemys pyrenaicus* to environmental variables.

	1993–96				2014–15				Extinction			
Parameters	*B*	SE	*t*	*P*	*B*	SE	*t*	*P*	*B*	SE	*t*	*P*
Intercept	−0.29	0.87	−0.34	0.734	24.8	8.13	3.04	0.003	1.65	1.36	1.21	0.229
Stream order	0.36	0.29	1.25	0.215	−0.49	0.36	−1.37	0.18	0.91	0.34	2.65	0.010
Slope	0.26	0.11	2.42	0.018	-	-	-	-	-	-	-	-
Slope steepness	-	-	-	-	0.09	0.03	3.22	0.002	−0.09	0.03	2.84	0.006
Temperature	-	-	-	-	−0.10	0.03	−3.33	0.001	-	-	-	-

Distribution models were built separately for the survey periods of 1993–96 and 2014–15 in two watersheds of Northeast Portugal, and the extinction model refers to the local disappearance versus persistence between survey periods. For each model, we indicate the regression coefficients (*B*), the standard error of the estimates (SE), and the corresponding *t*- and *P*-values.

Table 3 Percentage of variance explained and prediction ability statistics for models relating the probabilities of occurrence (1993–96 and 2014–15) and extinction of the Pyrenean desman *Galemys pyrenaicus* to environmental variables, in two watersheds of Northeast Portugal.

Models	Environmental	TU	TD	EUC	Nugget	Overall Prediction Success	AUC	Cohen's Kappa
1993–96	0.09	0.19	~0.00	~0.00	0.72	0.64	0.75	0.26
2014–15	0.19	0.81	~0.00	~0.00	~0.00	0.96	0.96	0.91
Extinction	0.12	0.24	0.57	0.08	~0.00	0.99	0.99	0.97

The percentage of variance explained is provided for the environmental component and three spatial components: Euclidian (EU), tail-up (TU) and tail-down (TD). The nugget is the variation unexplained in models. AUC is the area under the receiver operating characteristic curve.

with lower slope steepness. Together with other recent studies (Gisbert & García-Perea, 2014; Charbonnel *et al.*, 2016), our results suggest that the Pyrenean desman may have suffered a major degradation of its conservation status since its evaluation as globally vulnerable in 2008 (Fernandes *et al.*, 2008). The current situation requires continued population monitoring and urgent conservation actions to halt and reverse the ongoing negative trend. More generally, these results corroborate previous research highlighting the importance of headwater streams for the conservation of aquatic and semiaquatic species (Lowe & Bolger, 2002; Durance & Ormerod, 2007; Meyer *et al.*, 2007; Covich *et al.*, 2009; Grant *et al.*, 2010; Carrara *et al.*, 2012; Isaak *et al.*, 2015, 2016).

Our conclusions rely on the assumption that surveys accurately described desman distribution, and so changes in occurrence patterns were real rather than resulting from methodological artefacts. We believe this assumption is reasonable, because surveys involved experienced observers and comparable field efforts and methods (Queiroz *et al.*, 1998). In particular, the field team in 2014–15 was trained by one of the researchers surveying the same area in 1993–96, thereby enhancing methodological consistency. However, it cannot be totally ruled out the possibility of errors in field identifications in 1993–96 when no molecular validation was carried out, which might have resulted in some false positives, and thus to overestimates in the distribution and in extinction rates across periods, though there is evidence suggesting that this problem was not serious. First, doubtful desman faeces in 1993–96 were confirmed through microscopic examination, which should have eliminated identification errors in part of the sites (43.1%) where the species was detected (Queiroz *et al.*, 1998). Second, molecular data from 2014 to 15 suggest that 92.7% of the faeces considered highly likely to be from desman in the field actually belonged to the species. This suggests that error rates were also likely to be low in 1993–96, when only latrines and characteristic fresh faeces were taken to indicate the presence of desmans without microscopic validation. Third, the extinction rate estimated from sites validated through microscopic examination (69.2%) was higher than that estimated at sites with only field data (59.5%), albeit not significantly so, while the opposite would be expected if there were high misidentification rates. Finally, even if there was a misidentification rate of 10–20%, much higher than that estimated through molecular data, it would result in just 4–8 false positives in sites confirmed only in the field, while the species disappeared at 22 of such sites. In contrast to the false positives, results might have been influenced by false negatives in 2014–15, contributing to underestimate the species distribution and overestimate the extinction rates, though this is also unlikely. First, we took a very conservative approach in the field to minimize false negatives, collecting faecal samples for analysis even when they departed significantly from the desman archetypal. These faecal samples were analysed when there was no better evidence for the presence of desman in a given site, thereby increasing the detection chances. Second, the wider study involving surveys at 144 sites in the same area revealed much the same pattern as that reported here, with the species also restricted to the headwaters and remaining largely absent from the lower reaches and larger rivers (L. Quaglietta, unpubl. data). Finally, a recent study using a survey method similar to ours estimated a detection probability of one given occupancy by desmans in 500-m stream reaches (Charbonnel *et al.*, 2014). Overall, therefore, it is likely that our results reflect a true decline of the Pyrenean desman, in line with studies carried out in other locations in Spain and France (Gisbert & García-Perea, 2014; Charbonnel *et al.*, 2016).

The environmental effects detected in our study are largely in line with those reported previously, which suggest that the species is associated with areas with low temperature, high stream flow, and steep terrain (Barbosa *et al.*, 2009; Morueta-Holme *et al.*, 2010; Charbonnel *et al.*, 2015, 2016). These conditions are generally met in cold, fast-flowing mountainous streams that usually have higher stability in the availability of aquatic macroinvertebrates (Charbonnel *et al.*, 2015), which constitute the bulk of desman diet (Biffi *et al.*, 2017). It is noteworthy, however, that while in 2014–15 the species was associated with headwater streams (negative effect of stream order), the results from 1993 to 96 suggest an association with larger streams (positive effect of stream order), which may be favourable because they predictably maintain flowing water during the dry summer period (Ferreira *et al.*, 2016). Also, in 2014–15 the probability of occurrence increased in areas with lower temperatures in the warmest month, which are mainly found in more mountainous areas. Changes in the determinants of distribution are also apparent in the extinction model, which show that the species disappeared primarily from higher order streams and from streams with lower slope steepness, further suggesting that over two decades the species largely retreated to cooler, and mountainous headwater streams.

Reasons for the changes observed are unknown at present, but we note that the larger streams of the Tua and Sabor watersheds have been increasingly invaded by two exotic crayfish *Procambarus clarkii* and *Pacifastacus leniusculus* (Bernardo *et al.*, 2011; Filipe *et al.*, 2017), which may decrease habitat suitability for desmans, for instance by affecting macroinvertebrate populations (Lodge *et al.*, 2012). Climate change may also have played a role, as increases in extreme summer temperatures may reduce water availability (Allan *et al.*, 2005; Brooks, 2009) and aquatic prey abundance (Durance & Ormerod, 2007; Domisch *et al.*, 2013). Finally, although the introduced American mink *Neovison vison* has only reached the study area recently (L. Quaglietta unpubl. data), and therefore its population size should be still limited, potential contribution of increasing predation by the mink to desman decline should be assessed (Gisbert & García-Perea, 2014; Charbonnel *et al.*, 2016). Other factors often associated with desman declines are unlikely to be important in our study area, as to the best of our knowledge there has been no major degradation of water quality or large-scale destructions of the riparian galleries. Although two large hydroelectric dams have recently been built near the mouth of both Tua and Sabor rivers, they were still under construction when the study was carried out, and thus they affected only a very small portion of the study area.

Our results have important implications for the conservation of the globally threatened Pyrenean desman. First, they show that the species may be suffering a precipitous decline, which is in line with other studies documenting major declines across the species range (Gisbert & García-Perea, 2014; Charbonnel *et al.*, 2016). The conservation status of this species should thus be reassessed, as the Vulnerable status attributed in 2008 (Fernandes *et al.*, 2008) is likely to be outdated presently (see also Charbonnel *et al.*, (2014) and Gisbert & García-Perea, (2014)). This would be important to eventually raise the priority of the species and thus attract much needed resources for improving its conservation. Second, a comprehensive survey over large spatial scales is urgently needed, ideally at the global species level, because relatively small-scale studies such as ours may not be representative of the entire species range. Besides updating the species distribution and thus contributing to clarify its conservation status, a global distribution survey would be useful to identify conservation threats and priorities across the species range. Environmental DNA might constitute a powerful tool in this regard, helping reducing costs and minimizing detection errors of this rare species (e.g. McKelvey *et al.*, 2016). Third, our study clearly highlights the importance of headwater streams for the conservation of the Pyrenean desman, as these may act as refugia against anthropogenic stressors, such as climate change and invasive species. Conserving such refugia should thus be of utmost priority, requiring the maintenance of adequate conditions such as high water quality, well-developed riparian galleries, and free-flowing waters without impoundments and water extraction. Finally, it would be important to understand the patterns of connectivity among desman populations restricted to headwater refugia and to develop conservation strategies to offset this eventual problem, because several of these populations may currently be largely isolated, which could lead to stochastic extinctions due to small population sizes, and consequent risk of inbreeding and loss of genetic diversity (Corn & Bury, 1989; Zwick, 1992; Fagan, 2002; Lowe, 2002; Labonne *et al.*, 2008). Overall, our findings strongly support the need to increase conservation efforts for the unique but elusive Pyrenean desman, which may be coming close to critically endangered due to the joint operation of multiple anthropogenic stressors, including climate change and introduction of biological invaders. More generally, our results add to an increasing number of studies highlighting the importance of headwater streams for aquatic biodiversity, which may prove to be critical refugia for many species under the current trend for increasingly modified river systems (Zwick, 1992; Allan *et al.*, 2005; Meyer *et al.*, 2007; Covich *et al.*, 2009; Grant *et al.*, 2010; Carrara *et al.*, 2012; Domisch *et al.*, 2013; Isaak *et al.*, 2015, 2016).

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Supporting information

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

Figure S1. Location of the study area in NE Portugal (upper left panel), and maps of predicted probability of occurrence of the Pyrenean desman *Galemys pyrenaicus* in two watersheds, in 1993–96 (upper panel) and 2014–15 (lower panel).

Figure S2. Map of predicted probability of extinction of the Pyrenean desman *Galemys pyrenaicus* between surveys carried out in 1993–96 and 2014–15.

Data S1. Protocol used for species genetic identification of faecal samples.

Table S1. Summary results of the genetic analyses of the non-invasive samples (faeces) putatively assigned to the Pyrenean desman *Galemys pyrenaicus* in the field, including list of other species detected.

Graphical Abstract

The contents of this page will be used as part of the graphical abstract of html only. It will not be published as part of main article.

The Pyrenean desman *Galemys pyrenaicus* is a globally threatened, ecologically and phylogenetically unique semiaquatic mammal endemic of South-western Europe, and one of the less-studied European mammals. Based on long-term survey data and state-of-the-art non-invasive genetics and stream geostatistical mixed models, we documented severe desman range contraction in Northeast Portugal, particularly into headwaters, with a 54% naïve occupancy drop and a 63.5% extinction rate in the last two decades. Our results suggest that the conservation status of the Pyrenean desman should be upgraded, and show the importance of headwater refugia for the desmans, supporting the view that these habitats may be increasingly important for biodiversity conservation under the current scenario of increasingly modified river systems, favouring the permanence of remnant, fragmented populations. Photo Credit : ©Joel Sartore/ National Geographic Photo Ark