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The American mink *Neovison vison* is a widely distributed species in Spain, for which important basic ecological data are lacking. We studied mink den site selection in a location in central Spain during the winter of 2008/09 using snow-tracking and direct observation. Dens were located more frequently in tree roots (51.9%; N = 79) inside emergent vegetation (15.2%) and on human-created enbankments (15.2%). Human buildings and burrows were scarcely used (< 10%). The logistic regressions we carried out supported that minks used secondary river beds for shelter more often than their availability in the environment should predict. These secondary areas are characterised by their low width and the frequent presence of small streams (of < 1m width). In rough terms, this pattern could be a way of avoiding potential interactions with other animals sharing the same habitat, and also for thermoregulation while resting. On the basis of the wide habitat niche, ecosystem management as a measure of mink control appears to be ineffective.

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The American mink *Neovison vison* is a semi-aquatic mustelid originating from North America and which was successfully introduced into countries abroad due to extensive farming for the fur trade (Dunstone 1993, Macdonald & Harrington 2003). From the newly created population focus in the proximity of fur farms, animals expand towards the nearby water systems and hence colonise new areas (Dunstone 1993). The establishment of an American mink population appears to be driven by availability of prey and abundance of suitable den sites (Dunstone 1993, Larivière 1999).

Refuges are a first magnitude ecological resource whose selection involves a complex trial evaluation by free-ranging animals (Berryman & Hawkins 2006) playing a central role in mustelid populations (Birks et al. 2005). Usually, carnivore denning behaviour depends upon predation risk and thermoregulatory issues (Birks et al. 2005).

Mink dens are placed under boulders, tree roots and in rabbit burrows (Birks & Linn 1982, Dunstone 1993, Yamaguchi et al. 2003). Patterns of den site selection change across different habitats, with holes and trees being important in freshwater areas, whereas in coastal environments, mink shelter mainly in rabbit warrens (Dunstone 1993). The location of den sites of a single animal changes over successive days, and may not be the same one within a daily activity period (Birks & Linn 1982, Dunstone 1993, Zabala et al. 2007, Harrington & Macdonald 2008). Occupation of the same den by a male and a female is a rare event (Dunstone 1993).

Spain contains four large non-connected centres of the American mink, all of which originate from farm escapes, and their current status is largely unknown (Ruiz-Olmo et al. 1997). Central Spain supports one of the larger populations of the species, which became established in the early 1970s,

even though minks had been observed regularly in the field since 1956 (Ruiz-Olmo et al. 1997).

Studies on the biology and ecology of this species in central Spain are focused on their distribution and feeding biology (e.g. Bueno 1996, García et al. 2009a), and data on spatial ecology and other aspects of the biology of the species remain poorly known even today (but see Melero et al. 2008, García et al. 2009b). Furthermore, the few studies on habitat preferences which exist frequently rely upon scat-based sampling which seems to be un-correlated with mink presence or abundance (Bonesi & Macdonald 2004).

In this article, we assess patterns in the occurrence of den sites of the American mink in a locality of central Spain.

Material and methods

Study area

Our study was carried out from November 2008 to February 2009 (i.e. during the winter of 2008/09), on a 14 km long stretch of the river Tormes, in the vicinity of the city of Salamanca (province of Salamanca in central Spain). The coordinates of the centre point of our study area were: 40°57' 21.83"N, 5°39' 31.42"W (800 m a.s.l.). Typically, the climate is Mediterranean continental with cold winters (mean monthly temperature of 4.4 °C), and a mean monthly rainfall of about 40 mm, whereas summers are hot (22°C) and rainfalls rare (10 mm).

In the area, the river Tormes fits well with the morphology and dynamics of flood-plain rivers, i.e. very wide across (maximum about 320 m) and without any significant slope. These features allow the area to be structurally complex, with frequent islands and banks covered by a mediterranean riparian forest. The forest is mainly composed by willows *Salix* spp., poplars *Populus* spp. and blackberries *Rubus* spp. Emergent vegetation for-mations of different species (common reed *Phrag-mites autralis*, bulrush *Thypa* spp. and branched bur-reed *Sparganium erectum*) are frequent. Usual-ly, the riparian forest has been impacted by human recreative activities as a consequence of the prox-imity to the city of Salamanca. Up to 20% of our study area is highly impacted by these activities. In these areas, the riparian forest has been removed and replaced by artificial boulder-made embank-ments that hold several human buildings, e.g. mills.

Throughout the rest of the site, the forest was in a relatively good conservation condition.

The red fox *Vulpes vulpes*, common genet *Genetta genetta*, least weasel *Mustela nivalis* and the feral cat *Felis catus* share the riparian habitat with the mink (García & Mateos 2009, García et al. 2009a). The Eurasian otter *Lutra lutra* is also present in the river Tormes, but only during winter (García et al. 2009a). The riverine habitat houses a high diversity of suitable prey for the American mink. From mink scat analysis (P. García, unpubl. data), it is known that American crayfish *Procambarus clarkii* and different fish species make up the basic prey in the area. Amphibians and rabbits are relatively scarce throughout the site.

For further details on the habitat, see García & Mateos (2009) and García et al. (2009a, 2009b).

Location and habitat attributes of den sites

For the purpose of our study, den sites were defined as any site where a mink rests or hides (Kruuk et al. 1998, Birks et al. 2005). Due to the dates of the field work (winter 2008/09) none of the sites analysed were natal dens.

Resting sites were located by use of direct ob-servation and snow-tracking. When possible, direct observations are the best way to assess the population biology of semi-aquatic mustelids (e.g. Kruuk 2006), though being dependent upon the presence of a diurnal activity pattern. The studied population shows important daylight activity (Gar-cía et al. 2009b), enabling the study of some aspects of the mink biology from direct observations.

Vigils were carried out after and during nightfall and at dawn using binoculars and/or telescope from sites where a relatively wide area could be watched (bridges or other elevations in the landscape). However, in some particular situations, when there was high vegetation cover, the surface watched was smaller, so the sampling effort was increased to improve the effectiveness of the method. Observers stayed relatively hidden at the observation points thereby avoiding interferences in the behaviour of mink. The distance between observers and minks during vigils was 32.8 ± 2.3 m (mean $\pm$ SE; range: 2.0-80.0 m; N = 73). The overall sampling effort in the study period was 256 hours of vigilance (aver-age: 12.19 hours/km).

Complementarily, snow-tracking was carried out over three consecutive days during 10-12 December 2008. This is an adequate method for evaluating the distribution and habitat preferences of carnivores

(Sidorovich et al. 1996, Alexander et al. 2005) which provides similar results to those obtained from radio-tracking (Alexander et al. 2005). Tracks of minks in the snow were intensively searched for along the entire length of the study area, from the water edge to 20-40 m in the banks.

Our search area for den investigation covered the banks up to about 40 m from the water edge. We did this because minks in Salamanca rarely move > 10 m from the water (García et al. 2009b); moreover, the snow-tracking of mink trails performed in our study indicated that minks move close to water (< 10 m).

The two methods we used have an important source of potential bias, because detectability of dens could be a function of the visibility (direct observations) or accesibility (snow tracking) rather than of the presence/absence of dens. We tested the potential effects of this survey variable to evaluate if it had an influence and whether to include it as a covariate in the analyses. Accessibility and/or visibility was ranked in six levels (0 = none, 1 = poor, 2 = low, 3 = scarce, 4 = high, 5 = very high) and was evaluated in each den site location (N = 79; see section Results) and at 25 points within the study area where dens were not detected. Comparisons of these results did not show statistically significant differences (Mann-Whitney U-test: U = 824.00, df = 1, P = 0.205), so the methods used appeared to be adequate for the goals of our study.

Once a den was located, we assessed the characteristics of the surrounding habitat and the type of den (e.g. hole, boulders or tree roots). We measured 11 variables related to the habitat preferences of the mink (Table 1) within a radius of 10 m from the entrance of the den. We employed this distance because it could allow for an adequate evaluation of the cover of different types of vegetation (Sutherland 2000). We measured cover data in Braun-Blanquet ranks (Sutherland 2000) to avoid potential subjectivity. By means of the Random Point Generator tool available at Geo Mid-Point web page (http://www.geomidpoint.com/random/), we randomly selected 52 points separated by ≥ 50 m to evaluate the habitat availability on the 14-km long stretch in our study area. Afterwards, all these points were characterised in the field by measuring the same ecological variables as for den sites (see Table 1) within a radius of 10 m from each point. Therefore, these sites would represent the available habitat for minks in the area. From snow-tracking (Sidorovich et al. 1996) and individual-

Table 1. Description and measurement of attributes used for characterising den sites of the American mink in Salamanca, Spain. B-B indicates Braun-Blanquet cover categories.

Variable	Categories	Values
Pool cover (stretch of river with standing water)	B-B: < 1 %	0
	1-5 %	1
	6-25 %	2
	26-50 %	3
	51-75 %	4
	76-100 %	5
Island cover	BB (see above)	B-B (see above)
Tree cover	BB (see above)	B-B (see above)
Scrub cover	BB (see above)	B-B (see above)
Helophytic cover	BB (see above)	B-B (see above)
Rock/boulder cover	BB (see above)	B-B (see above)
Stream/tributary abundance (< 1 m wide)	None	0
	Rare	1
	Scarce	2
	Frequent	3
	Abundant	4
	Very abundant	5
Water depth	< 1 m	0
	1-2 m	1
	2-3 m	2
	3-4 m	3
	> 4 m	4
Width (of the stretch measured from the two closest banks in a straight line)		0.3-320.32 m
Bank slope		0-95%
Human activity	None	0
	Rare	1
	Scarce	2
	Frequent	3
	Abundant	4
	Very abundant	5

based identification during vigils (using throat spots; Dunstone 1993), we estimated the mink population during our study period to be approximately seven animals.

Statistical analysis

We employed Detrended Correspondence Analysis (DCA) as a preparatory approach prior to further analysis. DCA performs the assessment of the environmental gradient in the area as derived from the ecological variables measured (Jongman et al.

1995; see Table 1 for ecological variables). Axis I of the DCA resulted in 4 SD, which means that the response of minks to this ecological gradient is Gaussian-like (Jongman et al. 1995).

Therefore, the attributes we used to characterise the dens were initially analysed using a Multiple Correspondence Analysis (MCA) in order to remove the potential covariance between the variables (Jongman et al. 1995).

The variables with a relatively high value (see section Results and Table 1; the value was subjectively set at nearly 0.10) for one of the factors obtained from the MCA and with the low values for the remaining factors were considered as the variables which explained the greater amount of variance of all variables included in the MCA. Indeed, these were not related to other selected variables.

Variables selected in the MCA procedure were included in a logistic regression (LR) as independent variables, using the matrix of presence of dens and the randomly selected points (values one and zero in the matrix, respectively) as dependent variables. Each variable was initially tested alone and its significance to the den site distribution pattern was evaluated (Hosmer & Lemeshow 2000, Pearce & Ferrier 2000). The variables with statistical significance in the Wald's test ($P < 0.05$) were selected for inclusion in the final logistic model.

The adjustment and the reliability of the LR models were tested by means of the Pearson χ^2 test, the Wald's test, and the classification rates obtained from the model (Hosmer & Lemeshow 2000). Moreover, the Area Under the ROC Curve (AUC) and the Akaike's Information Criterium (AIC) were measured to evaluate the performance of the LR analysis (Hosmer & Lemeshow 2000, Pearce & Ferrier 2000). The AUC could vary between zero and one. The closer the value gets to one, the better the estimated LR model is. In the case of the AIC, the better model is the one with the lowest AIC value.

This statistical procedure is commonly used as an approximation to habitat use in ecological studies and it is particularly adequate in methodological designs as used in our study (Hosmer & Lemeshow 2000, Pearce & Ferrier 2000).

We used the χ^2 test to test for differences in the proportions of the den types used by minks.

Statistical measurements were made using S-PLUS 8.0 (S-PLUS Enterprise Developer), MyStat 12.0 (Systat Software Inc.) and PAST (Hammer et al. 2001).

Results

During the study period, we found 79 den sites used by minks; i.e. an average of 5.64 dens/km surveyed. American minks preferentially used dens in tree roots ($\chi^2 = 99.59$, df = 5, P = 0.001), and especially of willows *Salix* sp. (51.9% of dens). Dens were also found situated within the aquatic emergent vegetation growing on the banks (15.2%) and in human-created embankments of large boulders (15.2%). Different man-made buildings (10.1%) and holes in sand or soil (6.3%) were also used for resting. In only one case, a den site was placed in a natural rock formation (1.3%). There was no evidence of an intra- or interspecific shared use of a single den.

The MCA indicated three attributes related to the variability in the data (Table 2). Two of these were characteristics of the water course entity (the width and the relative abundance of streams < 1 m wide) in the surroundings of the point. The third variable, the slope of the bank, could define the major structure of river banks; generally speaking, low slopes are correlated with a high development of the riparian vegetation. The remaining variables were not selected in this step of the analysis (see Table 2).

The width of the river negatively correlated with the presence of sheltering sites (Wald test = 9.12, df =1, P = 0.003; see Tables 3 and 4 for the other parameters), and the abundance of tributaries provided a significantly positive association to den use (Wald test = 8.076, df = 1, P = 0.001; see Tables 3

Table 2. Results of the Multiple Correspondence Analysis (MCA) for the analysed attributes. The variables selected for the logistic regression to explain mink denning habitat selection are shown in italics.

Variable	Factor 1	Factor 2
Pool	0.017	0.016
Island	0.001	0.017
Arboreal cover	0.002	0.017
Scrub cover	0.005	0.017
Helophytic cover	0.027	0.006
Rock cover	0.049	0.008
Tributaries	*0.101*	*0.006*
Depth	0.008	0.018
Width	*0.260*	*0.690*
Bank slope	*0.505*	*0.184*
Human activity	0.024	0.021

Table 3. Adjustment parameters for the Logistic Regression (LR) models using the variables selected in the MCA. The best fitted model is emphasised in italics.

Model	AIC	AUC	χ^2 (df)	P	Wald (df)	P	% correct
1: TRIBUTARIES	167.17	0.58	10.47 (1)	0.004	8.08 (1)	0.004	60.8
2: WIDTH	168.46	0.68	9.68 (1)	0.002	9.12 (1)	0.003	66.9
3: BANK SLOPE	176.04	0.58	2.10 (1)	0.148	2.03 (1)	0.154	60.8
4: TRIBUTARIES + WIDTH	*163.79*	*0.67*	*170.32 (96)*	*0.000*	*-*	*-*	*99.2*

and 4). These two variables also showed high classification rates with 60.8% for tributaries abundance and 66.9% for width of the river (see Table 3). Bank slope, despite being selected in the MCA, did not provide a significant model (Wald test = 2.03, df = 1, P = 0.154; see Table 3).

The AIC value for model 4 (including abundance of tributaries and width of river) was the lowest of all models tested (AIC = 163.79). This model was highly significant (χ^2 = 170.32, df = 96, P = 0.000), and had the highest rates of correctly classified cases (99.2%). Equations for all LR models tested are presented in Table 4.

Discussion

Den sites are a vital element within mink ranges, animals' movements being restricted to their vicinity (Gerell 1970, Birks & Linn 1982, Dunstone 1993, Yamaguchi et al. 2003). American mink in Salamanca used a wide range of places for sheltering, but preferred tree root systems, and we found only little evidence of their use of holes (only 6.3% of 79 dens). Burrows and crevices have been found to represent 6-44% and scrub refuges 7-79% in different areas in Europe (Gerell 1970, Birks & Linn 1982, Dunstone 1993, Yamaguchi et al. 2003, Harrington & Mac-

Table 4. Parameters of the LR equations for the four models using the variables selected in the MCA. The best fitted model is emphasised in italics.

Model		Coefficient	SE
1: TRIBUTARIES	Constant	0.125	0.202
	Tributaries	0.410	0.144
2: WIDTH	Constant	1.217	0.325
	Width	- 0.007	0.002
3: BANK SLOPE	Constant	0.185	0.249
	Bank slope	0.009	0.006
4: TRIBUTARIES + WIDTH	*Constant*	*0.815*	*0.366*
	Tributaries	*0.357*	*0.155*
	Width	*-0.005*	*0.002*

donald 2008). Nevertheless, if we include crevices, burrows and rock piles in a unique category of underground sites (as done by Zabala et al. 2007), the number of dens in this category increases to 22.4%, which is a share at the same level as reported by Zabala et al. (2007) in northern Spain (26.3%).

We found that minks used human buildings for resting dens in 10.1% of cases in Salamanca, whereas UK mink populations used human buildings in just 7% of cases (Birks & Linn 1982, Dunstone 1993, Harrington & Macdonald 2008). In northern Spain, Zabala et al. (2007) found a similar rate of resting sites in buildings (i.e. 7%).

Our sample supports that American mink use secondary stretches of the river more than available. Low river width and high abundance of tributaries in our study area resemble the secondary stretches, which are also associated with the presence of islands within the river system. Data on factors affecting the spatial position of resting sites are few (Yamaguchi et al. 2003, Zabala et al. 2007). Attributes defining the position of a den seem to be related to bank vegetation and river morphology. Mink habitat use is negatively correlated to river width and positively with river depth and riparian forest cover (Dunstone 1993, Yamaguchi et al. 2003, Zabala et al. 2007, Melero et al. 2008).

Vegetation variables and depth of water were removed in the MCA, and did not appear to be related to the presence of sheltering sites. However, stretch width was one of the most significant habitat variables in Salamanca, which Zabala et al. (2007) also reported from other localities in the Iberian Peninsula.

Bases on denning behaviour models for mustelids (Birks & Linn 1982, Dunstone 1993, Birks et al. 2005, Kruuk 2006), it has been hypothesised that the spatial organisation of the sites is determined by some major ecological constraints on the populations. First, in sites such as our site in Salamanca, with high availability of resources, refuge places

could tend to occur in areas next to foraging sites (Gerell 1970, Dunstone 1993, Yamaguchi et al. 2003, Zabala et al. 2007). The lack of any sheltering sites connected to the pool and vegetation cover (theoretically important features in mink's foraging habitat; Dunstone 1993, Bonesi et al. 2000, Yamaguchi et al. 2003, Melero et al. 2008) seems to refute this key role.

Predation and interference risks are most likely important factors affecting den site selection (Dunstone 1993, Yamaguchi et al. 2003, Zabala et al. 2007, Harrington & Macdonald 2008). American mink are frequently killed by sympatric mammal species such as the Eurasian otter, fox *Vulpes* sp. or dog *Canis lupus familiaris* and also humans (Dunstone 1993, Larivière 1999), all of which are present in Salamanca (García & Mateos 2009). Hence, it is expected that features determining rest site selection will be riparian cover and/or denning in unexposed areas (Yamaguchi et al. 2003, Zabala et al. 2007). We did not observe this clearly in Salamanca, where the degree of exposure of a den was not a determinant (indirectly measured from riparian plant or boulder cover; see Tables 1-4) in the LR models tested. In contrast, covered dens were dominant in our sample from Salamanca (92.3%).

The presence of the Eurasian otter leads to a range of behavioural responses in the American mink, mainly to avoid unfavourable interactions with the otter (Bueno 1996, García et al. 2009a, 2009b, Harrington et al. 2009). In our study area in Salamanca, the Eurasian otter shows a marked preference to rest in the areas with high vegetation cover within the main drain bed (P. García, unpubl. data; through snow-tracking and direct observations). Thereby, the association of mink dens in Salamanca to areas out of the main drain bed could be a way of reducing potential outcompeting interactions with otters inhabiting the main channel. Harrington et al. (2009) researched the habitat-based segregation hypothesis in the river Thames, UK, but did not get enough evidence and discarded it. Interestingly, in Salamanca, there was a certain change towards diurnal activity by mink whenever otter was present (García et al. 2009b) as has also been shown by Harrington et al. (2009). In addition, our data from Salamanca may to some degree indicate a spatial segregation, which could emphasise the high adaptability of the American mink outside their native range. An alternative explanation could be the selection of dens for thermoregulation reasons (Birks et al. 2005), but we do not have enough data for testing this. Nevertheless, the distribution of den sites in Salamanca might be explained as a way of avoiding competitive and predatory interactions, perhaps with some sites probably selected for thermal isolation.

Resting places used by the feral mink exposed in our study have a potential application in the management of invasive populations. Trapping campaigns could increase its effectiveness by focusing the effort on areas near potential den sites. Mink control can also include habitat management (Macdonald & Harrington 2003), both by means of reducing the quality of the environment for its use as dens and by improving the suitability of species for potential competitors. In any case, the wide habitat niche of the mink and their flexibility, as supported by the findings in our study, make habitat manipulation a difficult form of mink population control.

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