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Original article

A comparison of methods for estimating common vole (*Microtus arvalis*) abundance in agricultural habitats

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ABSTRACT

Rodent outbreaks cause significant crop damages in agricultural areas worldwide, but routinely monitoring large areas at low cost remains a challenge. The common vole *Microtus arvalis* has recently colonized the agricultural plains of the northern Iberian Plateau, an area where it has started to produce population outbreaks with important impacts in agriculture, the environment and human health. Vole monitoring has become of prime importance to implement preventive management measures to control populations. In order to find a simple and reliable vole monitoring method to be applied in large areas, we compared abundance estimates derived from three methods: capture–mark–recapture (CMR), single capture events (SCE) and presence/absence of vole activity signs (VAS) during three seasons and on the main agricultural habitats in the study area. We show that an activity index based on the presence of fresh droppings and/or clippings had a similar performance to SCE in a large sample of plots ($n = 222$) across habitats and seasons. Data obtained with both methods (SCE, VAS) were also well correlated with those obtained with CMR, despite a limited sample size ($n = 23$ CMR plots). We suggest that the VAS method, which is a cheaper and easier alternative to trapping methods, provides a promising tool for scientists and managers to implement large scale monitoring of common vole in agricultural areas.

1. Introduction

Estimating population size or abundance accurately is crucial for population ecology, conservation and management (Engeman, 2005; Krebs, 1999; Witmer, 2005). It enables the study of factors that explain temporal or between-population variation in abundance, and is a basic tool for adequate population management, either of endangered species that need to be protected or of pest species that must be controlled (Tellería, 2004; Witmer, 2005). Obtaining reliable measures of abundance is often a methodological challenge, and there is a constant need for developing or validating better, simpler or cheaper methods for ecological and management studies (Krebs, 1999; Tellería, 2004; Witmer, 2005).

Multiannual population cycles (great and regular population size fluctuation) have been observed and extensively studied in common voles, particularly at northern or central latitudes (de Redon et al., 2010; Delattre et al., 1999; Imholt et al., 2011; Lambin et al., 2006). Vole cycles seem to have dampened or even disappeared in some northern European regions (Cornulier et al., 2013). In contrast, common vole *Microtus arvalis* (Pallas 1778) outbreaks have recently appeared in southern Europe (Viñuela et al., 2010). In the Iberian Peninsula, the distribution of the common vole was originally restricted to mountain ranges in the periphery of the northern Plateau. In the last 30 years, common voles have colonized the agricultural landscapes of the Duero basin, where recurrent population outbreaks appeared since the early 1980s (Luque-Larena et al., 2013).

During outbreaks common voles are considered a pest species in these agricultural areas, causing significant and costly crop damages (Jacob and Tkadlec, 2010), as well as tularemia outbreaks (Vidal et al., 2009). This prompted the use of extensive chemical control campaigns that impacted negatively on other species (Olea et al., 2009; Sanchez-Barbudo et al., 2012). Consequently, their economic, environmental and human health importance has increased exponentially. Monitoring common vole abundance over time in large-scale areas appears crucial for adequate management and outbreak forecasting.

Trapping methods are considered the "golden rule" for estimating small rodent abundance, as they provide estimates of

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population density (mark–recapture methods) or abundance (single capture methods), as well as detailed information about the captured individuals (sex, reproductive status, condition). These methods are reliable, but require a lot of resources, both material and human, and are time consuming, especially mark–recapture methods based on several days of continuous trapping (Tellería, 2004; Witmer, 2005). This makes them less suitable for continuous monitoring of large areas. Indirect methods are usually faster and easier to use, but may be subject to some bias (Tellería, 2004; Witmer, 2005). An alternative to vole trapping is the use of indirect methods based on the presence of vole activity signs (e.g., droppings and latrines, burrows, footprints or vegetation clippings). In central Europe, indirect methods based on re-opened burrow entrances have been used to reliably monitor common vole populations (Lisicka et al., 2007; Tkadlec et al., 2011). Alternative methods based on the presence/absence of vegetation clippings and/or droppings have also been succesfully used for estimating the abundance of several vole species, including the common vole (Delattre et al., 1999; Lambin et al., 2000; Madders, 2003; Terraube et al., 2011; Wheeler, 2008), although in some species such indirect methods had been proved inappropiate (Gervais, 2010).

The aim of this study was to compare three alternative methods for estimating common vole abundance in agricultural areas of Castilla-y-Leon, NW Spain, where M. arvalis outbreaks spread over an area of 5×10^5 ha (Luque-Larena et al., 2013; Viñuela et al., 2010). Two methods were based on vole captures: capture–mark–recapture (CMR), and single capture events (SCE). The third method was based on the presence/absence of vole activity signs (VAS), such as fresh droppings and clippings as signs of feeding activity. We first tested how the latter two simpler methods performed as compared with the most accurate but time consuming method (CMR). Secondly, we evaluated how the simpler two methods (SCE and VAS) performed in terms of describing differences in vole abundance between seasons (spring, fall or winter), main vole habitats (alfalfa, cereal or fallow), and location within the sampled field (edge vs. interior). We discuss the relative values and limitations of these methods for large scale population monitoring.

2. Methods

2.1. Study area

We conducted the study in Castilla y León, NW Spain, an autonomous region located in the northern plateau of the Iberian Peninsula and divided into 9 provinces (Fig. 1). It holds almost the entire catchment of the Duero River and includes a central agricultural plain surrounded by mountain ranges dominated by woodlands and pasturelands. The central plains are dedicated to agriculture (ca. 3.7 million ha), mostly winter cereals (mainly barley and wheat), alfalfa, sunflower, sugar beet, peas and maize. The study area was located in "Tierra de Campos", between the provinces of Palencia, Valladolid and Zamora (Fig. 1), an area heavily affected by common vole outbreaks over the last two decades (Luque-Larena et al., 2013).

2.2. Density estimates from trapping and capture–recapture of marked individuals (CMR)

We used Sherman LFTA traps (8 cm × 9 cm × 23 cm; Sherman©) and a "grid" trapping design (square grid of 5 × 5 traps, each separated by 15 m). One side of the grid corresponded to the field edge, with the rest of traps inside the field (Fig. 2a). Each trap was baited with apple and a mixture of canned tuna and flour, also hydrophobic cotton was provided when the temperatures were low. The traps were left open for four days, and were checked twice a day,

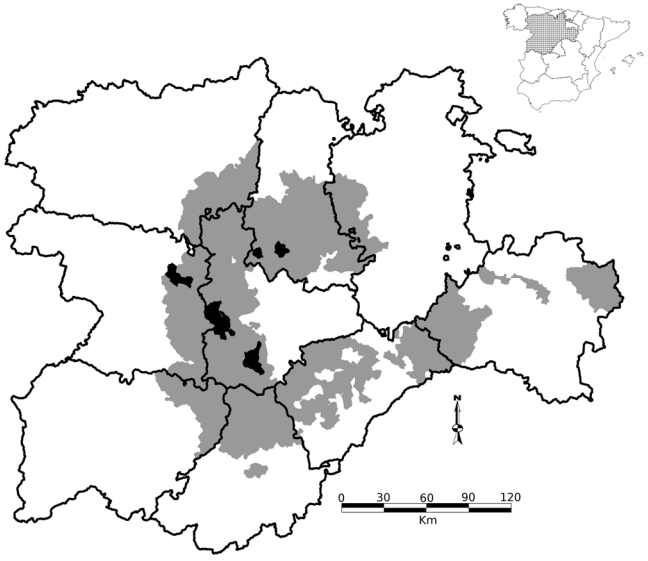

Fig. 1. Location of the study area in Castilla y León, NW Spain. Black lines = provincial boundaries. Black areas = study areas; Grey areas = areas heavily affected by the last common vole outbreak of 2007 (distribution data for summer 2007 is based in data from the Instituto Tecnológico Agrario de la Junta de Castilla y León, JCCM, Spain http://es.wikipedia.org/wiki/Plaga_de_topillos_en_Castilla_y_Leon_de_2007). Upper righter corner map: location of Castilla y León in Spain.

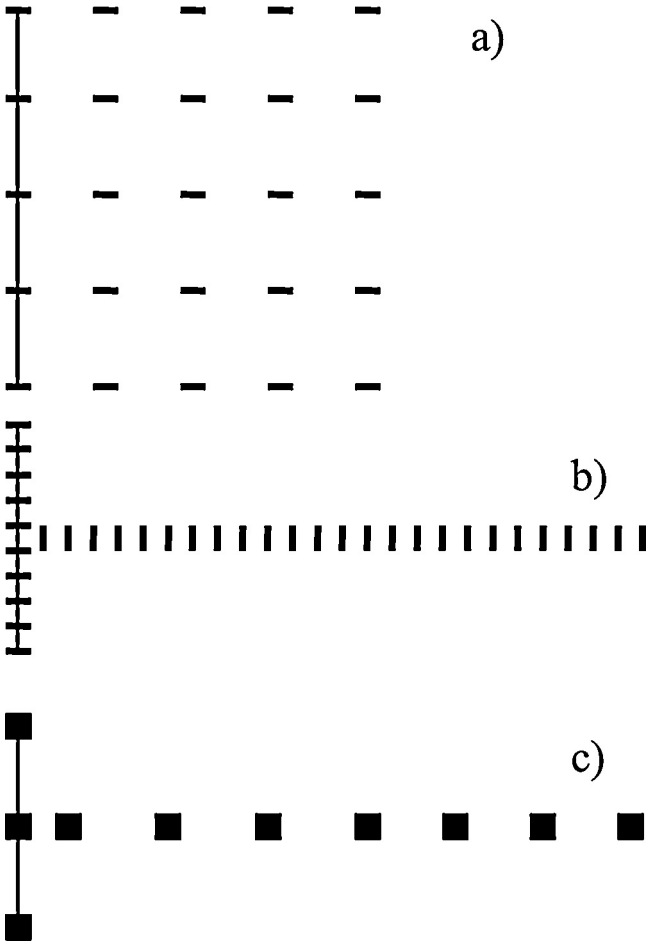

Fig. 2. Sampling designs used for each method. (a) "Grid" design for CMR trappings (25 traps each 15 m from the nearest one). The black line represents the edge of the plot. (b) "*T*" design for SCE vole trappings (traps every 2 m; 10 traps are in the edge and 25 are inside the plot); (c) "*T*" design for the VAS method. Vole signs are searched for in 30 cm × 30 cm squares distributed on the edge of the plot (3 squares) and inside the plot (7 squares) along a line of 50 m.

before sunset and after sunrise (with a total of six checks during the 4-days trapping session). This design and methods are comparable to the only long-term vole monitoring project in Spain (Fargallo et al., 2009).

During the trapping sessions, species other than common vole were caught, the commonest being wood mouse *Apodemus sylvaticus* (Linnaeus, 1758), algerian mouse *Mus spretus* (Lataste, 1883) and greater white-toothed shrew *Crocidura russula* (Hermann, 1780). To mark the captured voles we used a fur-clipping code (Gurnell and Flowerdew, 2006). Immediately after marking and recording basic data such as weight and sex, voles were released at the capture site (Fargallo et al., 2009).

To calculate the population size we used the Schnabel Method and formula (1)

$$\widehat{N} = \frac{\sum_t (C_t M_t)}{\sum_t R_t} \quad (1)$$

where C_t is the total number of individuals caught in the sample t, R_t is the number of individuals already marked when caught in sample t, M_t is the number of marked individuals in the population just before sample t is taken and $\widehat{N}$ is the estimated population size. When $C_t/\widehat{N}$ and $M_t/\widehat{N}$ were less than 0.1, we used another formula (formula 2) which provides better density estimates than formula (1) under these circumstances (Krebs, 1999).

$$\widehat{N} = \frac{\sum_t (C_t M_t)}{\sum_t R_t + 1} \quad (2)$$

Since we estimated the vole population in 0.5625 ha, we multiplied by 1.78 to obtain population densities in voles per ha. Some voles died during CMR trappings (8.7% of all captured voles died; mean 15.25% of all captured voles per trapping; $\sigma = 16.75\%$), so in order to take this into account when comparing with density estimates obtained for other methods used after the CMR, we corrected the density estimate by subtracting the number of voles that died. From the CMR trapping method, we thus obtained the following density estimates:

(1) Number of *M. arvalis* per hectare, hereafter MApH (see Table 1).
(2) Corrected number of *M. arvalis* per hectare, hereafter cMApH (MApH minus the number of voles that died during CMR trapping multiplied by 1.78).

2.3. Abundance estimates obtained from single capture trapping events (SCE)

We used the same type of traps for SCE, but in this case they were checked and removed ca. 24 h after setup. The trapping design consisted of two lines of traps forming a "*T*", with a total of 35 traps spaced 2 m between each other, with the shorter line (10 traps) being on the edge of a field (field margins/road or track ditches) and the longest line (25 traps), perpendicular to the former, inside the field (Fig. 2b). We collected data separately for edges and fields since edges are considered a favourable habitat for small mammals in agricultural landscapes (Butet et al., 2006; de Redon et al., 2010). Each trap was baited with apple or carrot. When the temperatures were low, hydrophobic cotton was provided inside traps to increase vole survival.

From SCE trappings, we obtained the following abundance estimates (Table 1):

(3) Number of *M. arvalis* captured per trap, hereafter MApT (calculated excluding traps that captured species other than *M. arvalis*).
(4) Number of small mammals other than common voles captured per trap, hereafter OMpT (also calculated using only the traps available for capturing).

For each trapping event, we calculated an overall estimate per plot (for all 35 traps; MApT and OMpT) as well as one for the edge ($MApT_{edge}$; $OMpT_{edge}$) and for inside the field ($MApT_{field}$; $OMpT_{field}$) separately.

2.4. Abundance estimates derived from vole activity signs (VAS)

We inspected 10–15 squares of 30 cm × 30 cm in each plot and carefully looked for signs of activity of *M. arvalis* in them. Each square was placed every 5–7 m along a transect (Fig. 2c). The same observer (DJ) looked for signs of activity during the study. As signs, we recorded for each square the presence/absence of fresh droppings and fresh clippings (Madders, 2003; Terraube et al., 2011). Fresh droppings from *M. arvalis* were distinguished from older ones by their colour (greenish instead of light brown–grey) and softer texture when opened with a nail. Common vole droppings are 3–5 mm long with rounded tips on both ends and differ from those of mice (whose droppings have pointy tips) or shrews (long, black droppings, slightly like crude oil). Fresh clippings consisted of cuts and little mounts of green vegetation, a common sign of vole foraging (Wheeler, 2008).

We also used a "*T*" sampling design to search for signs of presence, with a shorter transect on the field edge (20 m long) and a longer one perpendicular to the former inside the field (50 m long; see Fig. 2c). Initially we used a total of 10 squares (2 on the field edge and 8 inside the field). We subsequently modified the design using 3 squares on the field edge and 7 inside, to finally increase

Table 1
Definitions, acronyms, sample size (*N*) and summary results of the methods used to estimate *M. arvalis* abundance.

Method	Acronym	Meaning	*N*	Max/Min	Mean
CMR (capture–mark–recapture)	MApH	*M. arvalis* per ha, captured, calculated using the Schnabel method	23	125.91 1.78	47.82
SCE (single capture event)	MApT*	*M. arvalis* captured per trap	230	0.58 0	0.056
	OMpT	Small mammals other than *M. arvalis* captured per trap	230	0.67 0	0.075
VAS (vole activity signs)	PSpS*	Proportion of squares with either fresh *M. arvalis* dropping or fresh clippings	233	1 0	0.18
	Dps*	Proportion of squares with fresh droppings of *M. arvalis*	233	1 0	0.12
	CpS*	Proportion of squares with fresh clippings	233	0.8 0	0.13

*Acronym without suffix: overall index (for both edge and field).
*Acronym with "Edge" suffix: refers to estimate using only traps (SCE) or squares (signs presence/absence) located on the edge (field margin; see Fig. 2).
*Acronym with "Field" suffix: refers to estimate using only traps (SCE) or squares (signs presence/absence) located inside the field (see Fig. 2).
*Acronym with "c" prefix: refers to a correction to the density obtained substracting dead voles during CMR trapping.

Table 2
Summary of the number of different plots (sample size) used for the vole sampling method ("*T*" trapping designs for both SCE and vole presence signs methods; "Grid" sampling for the CMR method), according to season and habitat (crop type). Numbers in brackets refer to the number of plots that have been sampled more than once.

Sampling type:	"*T*"			"Grid"		
Season: habitat:	Spring	Summer	Fall	Spring	Summer	Fall
Alfalfa	26	26(2)	37(3)	3	2(2)	3(3)
Cereal	25	24	15	0	0	0
Fallow	27	30	17(3)	5	5	3(3)
Vineyard	1	0	2	0	0	2
Total	230(8)	23(8)				

the total number of squares from 10 to 15, with 5 squares on the field edge and 10 inside the field. These modifications in the number of squares used were aimed at improving the reliability of our method particularly in the edges.

In order to obtain abundance estimates for each plot, we summed the presence (=1)/absence (=0) scores of specific signs or combinations of signs in each square and divided it by the number of sampled squares. From this method, we obtained the following estimates (Table 1):

(5) Frequency of squares with *M. arvalis* signs (fresh droppings and/or fresh clippings), hereafter positive presence per square index or PSpS.
(6) Frequency of squares with fresh *M. arvalis* droppings, hereafter dropping presence per square index or DpS.
(7) Frequency of squares with presence of fresh clippings, hereafter clipping presence per square index or CpS.

We calculated an estimate for each sampling plot (using all squares; DpS, CpS, PSpS), as well as one separately for the squares located in the edge (DpS_{edge}; CpS_{edge}; $PSpS_{edge}$) or inside the field (DpS_{field}; CpS_{field}; $PSpS_{field}$).

2.5. Sampling procedure and sample sizes

Vole abundance estimates were obtained from plots in fields of alfalfa, winter cereal and fallows (uncultivated lands with natural herbaceous vegetation or pastures), the three commonest habitats in the study area, as well as in vineyards (another crop with high economic value), at three different periods (spring, summer and fall) between the Fall of 2009 and the Fall of 2011. Each plot was sampled only once (i.e., in one particular period, but using two or three of the different trapping methods in the course of a few days, see below). The only exception was 8 plots used for CMR in two or three periods. Also, each plot was placed in a different field (except in the case of 3 alfalfa plots). Sample sizes per crop type and season are summarized in Table 2. Trapping was not performed on cereal plots to avoid damaging crops. No trapping was performed in the coldest winter months, when vole population density is at a seasonal low, in order to avoid vole mortality in traps (mortality rates during trapping increase at low temperatures, and a high mortality rate would make CMR data less reliable).

CMR and SCE trappings were conducted sequentially at the same place during the same week, with a 1-day interval without trapping between each method. The CMR method was performed first. VAS indices were also obtained at the same place, during the first day of trapping. When comparing the estimates from CMR and SCE, we used the cMApH index (corrected for the voles that died during CMR trappings), because SCE were conducted after the CMR trapping. When comparing the estimates from CMR and VAS, we used the (uncorrected) MApH index, since the VAS was done in the first day of CMR trapping.

2.6. Statistical analysis

We used R version 2.14.0 (R Development Core Team, 2008) and SAS version 9.2 (SAS, 2008).

We used Pearson correlations to compare abundance estimates obtained from different methods, using the rcorr function; Hmisc package (Harrell, 2012). We used the sciplot package for Fig. 4 (Morales, 2011).

SCE trapping data were analyzed using generalized lineal models with a binomial distribution. We used a two-vector response variable with the number of traps that captured *M. arvalis* out of the number of traps that were available for capturing (i.e. number of set traps minus the number of traps with captures of other species). When we detected over-dispersion, we used a quasi-binomial distribution.

CMR trapping data were analyzed using general linear mixed models (LMMs), with the response variable being log-transformed (log-transform MApH for VAS and log-transform cMApH for SCE). We included Plot identity as a random factor in order to take into account that some plots were sampled more than once in different seasons (see Table 2).

VAS data (DpS, CpS, PSpS) were analyzed using generalized lineal models with a binomial error distribution, also using a two-vector response variable with the number of squares with presence out of the number of squares sampled. When testing for a possible influence of the number of squares sampled in the edge (which ranged from 2 to 5), we performed separate analyses for samples with 2, 3 or 5 squares.

We used LMMs and piecewise regression (NLIN procedure) in SAS to model the relationship between CMR, SCE and VAS. Piecewise regression allowed us to determine potential changes in linear trends before and after possible cut-off points (e.g. above or below a threshold vole density), by estimating simultaneously four parameters (intercept, point at which the relationship changed, slope before that point and slope after that point).

To explore variation in vole abundance according to season, habitat and location in the field, we used GLM/ANOVA with PSpS and MApT as response variables, and Habitat (crop type), Season (spring, fall or winter), Location in the field (edge or interior) and two-way interactions (season × habitat; location × habitat; season × location) as explanatory variables. We restricted these analyses to these two methods as we had enough sample size for them ($N = 233$), but a more limited sample size for the Capture–Mark–Recapture (CMR) method ($N = 23$ from 15 different plots). As we had few plots from vineyard (Table 2), these data were not used in this analysis. Additionally, since the number of traps or squares sampled in edge and field varied, we included the number of traps or squares sampled (log transformed) as a weight in these analyses.

All tests are two-tailed and all data are expressed as means ± SEM.

3. Results

3.1. Comparisons between abundance estimates from different methods

Associations between the vole abundance estimates (at plot level, i.e. including both edge and field) obtained from different methods are summarized in Table 3. The MApT index was highly correlated with the cMApH and also correlated with OMpT. However, OMpT was not significantly correlated with cMApH (Table 3). The index PSpS, based on vole presence signs, showed the strongest correlation with common vole captures per trap (MApT) and with common voles per ha (MApH). It also correlated with an abundance

Table 3
Summary of associations (Pearson correlations) between M. Arvalis abundance estimates obtained from different methods (CMR, SCE, VAS). Values given are: Pearson correlations coefficients, sample sizes, ie number of plots (in brackets) and p. cMApH was used for MApT and OMpT only.

Method/estimate	Trapping-CMR	Trapping-SCE		Vole presence signs		
	MApH/cMApH	MApT	OMpT	PSpS	DpS	CpS
MApH	1					
MApT	**0.67 (22)** 6.9 e^{-4}	1				
OMpT	-0.04 (22) 0.85	**0.56 (230)** <2.2 e^{-16}	1			
PSpS	**0.66 (23)** 6.6 e^{-4}	**0.76 (230)** <2.2 e^{-16}	0.55 (230) <2.2 e^{-16}	1		
DpS	0.35 (23) 0.10	**0.70 (230)** <2.2 e^{-16}	0.52 (230) <2.2 e^{-16}	0.89 (233) <2.2 e^{-16}	1	
CpS	**0.68 (23)** 3.9 e^{-4}	**0.70 (230)** <2.2 e^{-16}	0.41 (230) 7.3 e^{-11}	0.89 (233) <2.2 e^{-16}	0.69 (233) <2.2 e^{-16}	1

index of small mammals other than common voles (OMpT, Table 3). Among the simpler VAS indices, the CpS index (Clipping per Square) had the best relationship with MApT and MApH. The other simple index, DpS (Droppings per Square), also had a good relationship with MApT and with OMpT but was not significantly related to MApH (Table 3).

In summary, the a priori most accurate estimate, based on trapping and CMR, and providing a density measure, strongly correlated with common voles per trap obtained from SCE, and with estimates derived from the presence/absence of vole activity signs except DpS (Table 3). Therefore, both MApT and PSpS can potentially be used as predictors of *M. arvalis* density.

A linear mixed model testing if vole density (log-MApH) was predictable from vole abundance signs (PSpS) was significant ($F_{1,7} = 15.26$, $p < 0.01$).

$$\text{MApH} = \exp\ [(1.79 \pm 0.39 + (3.34 \pm 0.86) \times \text{PSpS})] - 1$$

Using piecewise regressions we could identify two linear trends with a cut-off point at 0.55 (±0.14 PSpS index) and an intercept of 1.12 (±0.51) (NLIN procedure; $F_{3,19} = 9.24$, $p < 0.001$). The initial relationship between the index PSpS and the density of *M. arvalis* had a slope of +5.72 (±1.43) for densities up to 70 voles per ha, but after the cut-off point the slope decreased to +0.52 (±3.04). This model explained 59.34% of the variance, an improvement from the previous model indicating that the relationship between the PSpS index and vole density (MApH) was not strictly linear (Fig. 3). The predictive power was reduced for densities above 70 voles per ha (saturation at higher population densities and higher standard error than at low densities).

A linear mixed model testing if vole density (log-cMApH) is predicted from the SCE abundance index (MApT) was also significant ($F_{1,6} = 26.65$; $p < 0.01$).

$$\text{MApH} = \exp[1.26 \pm 0.4 + (8.68 \pm 1.68) \times \text{MApT})] - 1$$

When using a piecewise regression, we also identified two linear trends with a cut-off point at +0.22 (±0.05 MApT index) and a intercept of +0.49 (±0.49) (NLIN procedure; $F_{3,18} = 14.6$, $p < 0.001$). As in the previous case, the initial relationship between MApT and *M. arvalis* per hectare had a steeper slope +16.6 (±4.76) at lower densities (before a cut-off point at 62 voles per ha) and a lower slope afterwards (+0.06, ±3.56). This model explained 70.87% of the variance.

3.2. Variation in abundance estimates according to location (edge or field interior) and sampling effort

For two methods (SCE, VAS), we obtained separate vole abundance estimates for the fields and their edges (Table 4; Fig. 2). We evaluated how well the abundance estimates obtained from single trapping events (MApT) and from the vole sign presence (PSpS) correlated with each other using the edge and field data separately. The correlation between both abundance estimates was much stronger using data from inside the fields ($r = 0.76$, $n = 230$, $p < 0.001$) than from the edges ($r = 0.43$, $n = 230$, $p < 0.001$). These differences were influenced by the number of squares sampled in the edge. When sampling only two squares in the edges, there was no significant relationship with the captures ($r = -0.33$, $n = 18$, $p = 0.19$); when the number of squares sampled in the edge was three, the relationship was significant ($r = 0.34$, $n = 187$, $p < 0.001$) and finally the best result (strongest correlation) was obtained with five squares in the edge ($r = 0.63$, $n = 25$, $p < 0.001$). Even when weighting in our models the sampling effort (number of traps or squares sampled), we found a much better predictive power of $\text{MApT}_{\text{field}}$ based on $\text{PSpS}_{\text{field}}$ (GLM, family = quasibinomial); ($F_{1,228} = 247.18$; $p < 0.001$; deviance explained = 57.79) than of $\text{MApT}_{\text{edge}}$ based on $\text{PSpS}_{\text{edge}}$ ($F_{1,228} = 69.49$; $p < 0.001$; deviance explained = 33.42).

3.3. Variation in abundance estimates according to season, habitat and location in the field

We explored whether the variation in vole abundance according to season, habitat and location in the field was similar when using PSpS and MApT.

Variation in MApT was significantly explained by Habitat, Season, Location and by the interaction Season × Habitat, but not by Location × Season or Habitat × Location (Table 4). Overall, estimated common vole abundance was greater in the fall than in spring or summer, in alfalfa than cereal or fallow (this difference being particularly high in the fall), and in the edge than inside the field (Fig. 4).

Variation in PSpS was explained by Habitat, Season, Location, and by the interactions Season × Habitat, and Location × Season, but not by the interaction Location × Habitat (Table 4). Relative differences in abundance between Habitats, Season and Locations derived from this index were similar to those derived from the single capture event method, but appeared greater with the former method (Fig. 4). The greatest difference was the estimated abundance of voles in edges in spring and fall (for all habitats), which was much higher with PSpS than with MApT (Fig. 4).

As habitat and season are important factors explaining the abundance of *M. arvalis*, we tested if the relationship between abundance estimates derived from the SCE and activity sign methods changed depending on those factors. We modelled the relationship between MApT and PSpS including the habitat and season, as well as their interaction with PSpS as explanatory variables. We found that PSpS ($F_{1,213} = 338.19$; $p < 0.001$), Habitat ($F_{2,213} = 6.37$; $p < 0.01$) and Season ($F_{2,213} = 5.27$, $p < 0.01$) were significant, but PSpS × Habitat ($F_{2,213} = 0.51$; $p > 0.05$), PSpS × Season ($F_{2,213} = 2.12$; $p > 0.05$) and

Table 4
Variation in abundance estimates according to season, habitat and location in the field according to MApT and PSpS.

Explanatory variables	Dependent variables	
	MApT	PSpS
Habitat	$F_{2,440} = 24.24$, $p < 0.001$ (1.03exp-10)	$F_{2,446} = 29.33$, $p < 0.001$ (1.08exp-12)
Season	$F_{2,440} = 25.59$, $p < 0.001$ (3.05exp-11)	$F_{2,446} = 35.6$, $p < 0.001$ (4.51exp-15)
Location	$F_{1,440} = 8.176$, $p < 0.01$ (0.0045)	$F_{1,446} = 32.7$, $p < 0.001$ (1.97exp-8)
Season × Habitat	$F_{4,440} = 9.13$, $p < 0.001$ (4.31exp-7)	$F_{4,446} = 7.01$, $p < 0.001$ 1.77exp-5)
Location × Season	$F_{2,440} = 1.91$, $p > 0.05$ (0.15)	$F_{2,446} = 6.33$, $p < 0.01$ (0.0019)
Habitat × Location	$F_{2,440} = 2.06$, $p > 0.05$ (0.13)	$F_{2,446} = 0.63$, $p > 0.05$ (0.53)

Habitat × Season ($F_{4,213} = 0.11$; $p > 0.05$) were not significant. Therefore, the slope of the relationship between MApT and PSpS did not differ between Habitats or Seasons, although the intercept varied, reflecting the differences in vole abundances between habitats and seasons.

4. Discussion

We have shown that abundance estimates based on vole activity signs (VAS) are comparable to those obtained from trapping methods at the vole densities found in this study. When comparing abundance estimates derived from VAS and the single capture events (SCE) in a large sample (223 plots) across habitats and seasons, we found that both these methods had similar predictive values, and that the simpler method similarly pick up variations in abundance between seasons or habitats. Abundance estimates derived from the SCE and VAS methods were also well correlated with abundance estimates derived capture–mark–recapture (CMR) data, despite a more limited sample (23 estimates from 15 different plots). CMR density estimates are considered the most accurate, but this method is the most expensive (more traps are needed) and time consuming (4-days of trapping per plot) to estimate rodent density. It may be argued that our sample size is small and that no CMR data were available for cereals, but the good correlations and high predictive value (explained variance) found in our sample strongly suggest that VAS and SCE are good reliable indicators of abundance, at least within the density range and habitats sampled.

Because the vole presence signs method is cheap, fast (it takes between 15 and 20 min per plot) and easy to implement, it offers interesting opportunities when a recurrent large-scale monitoring is needed. Although vole activity signs are relatively easy to detect, lack of consistency between different observers could be a source of error. In our study, the same observer performed all the VAS estimates, in order to ensure consistency. If different observers use the VAS method (as would be required for large-scale monitoring scenarios), appropriate training is recommended in order to ensure consistency. Different criteria used to distinguish between fresh and older droppings or clippings could also be source of error, especially since the rate at which those signs dry up most likely depend on variable conditions of temperature and humidity. Another possible source of error could be the misidentification of species from their droppings, due to the presence of other similar voles. In our study area, two other *Microtus* vole species are present (*M. duodecimcostatus* and *M. lusitanicus*) but both of them are much more subterranean than *M. Arvalis.* The other species present, *Arvicola sapidus*, lives near water courses; droppings and foraging signs from these species may be mistaken with those of *M. arvalis*, but this error would be negligible, given the scarcity of those species and the differences in the habitats that they occupy.

Similar methods based on vole indices have been successfully used to estimate abundance (Madders, 2003; Terraube et al., 2011; Wheeler, 2008), and have been successfully tested for *M. arvalis* in France (Delattre et al., 1990). In the present study, amongst the activity sign indices considered, the PSpS index (presence of fresh clippings and/or droppings) appears to be the best. A main limitation of both the PSpS index and the MApT indices is that their accuracy seems to be reduced at high vole densities (above 70 voles ha for PSpS and 62 voles ha for MApT). A similar problem had been reported for methods based on counts of re-opened burrows in central Europe (Lisicka et al., 2007). However, the threshold level observed was higher for the VAS than SCE method, suggesting that the former could be a better alternative to the CMR method. The maximum vole density estimated in our samples was 126 voles/ha, a density similar to that found to reduce 8.7% alfalfa production

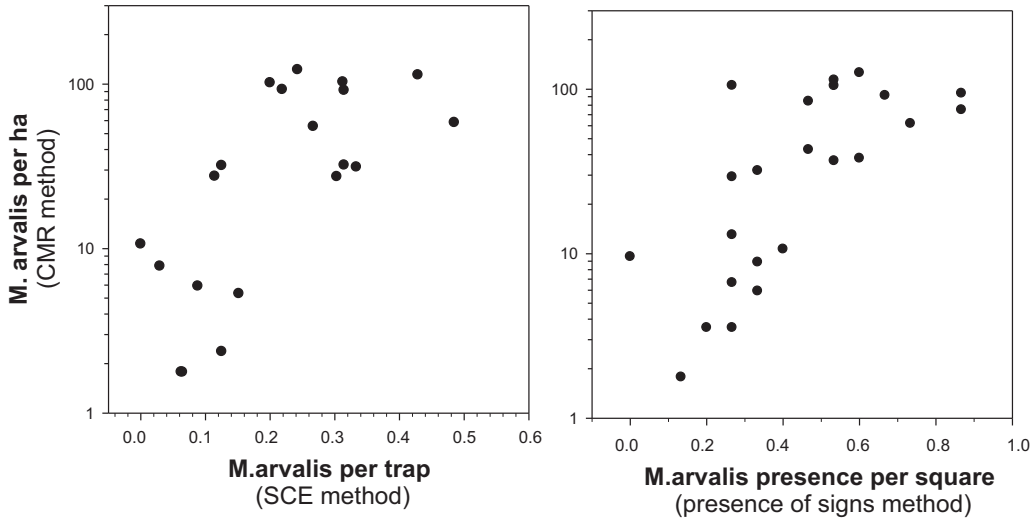

Fig. 3. Relationship between the vole abundance estimates obtained with the CMR method (*M. arvalis* per ha) and the SCE method (*M. arvalis* per trap, left) or from the VAS method (*M. arvalis* presence per square, right).

in Poland (Babinska-Werka, 1979). This density is still much lower than what has been observed during vole outbreaks, when vole density may exceed 1000 vole/ha in favourable habitats such alfalfa fields (Delibes, 1989; Jacob and Tkadlec, 2010; Vidal et al., 2009). Thus, additional work on this index might be necessary to solve this problem of saturation at high vole abundances. Increasing the number of sampled squares may increase the accuracy of the index, but this remains to be tested. In any case, the index would still be useful for large-scale research and monitoring within this range of densities, and to detect population increases before they cause crop damages, and therefore a valuable tool for outbreak forecasting and preventive control. Technical works in NW Spain consider that vole control campaigns should start whenever winter density is greater than 50 voles/ha (Arenaz, 2006). This "threshold density" for management decisions is therefore within the range of density for which the indirect monitoring method performed well. We did not collect data during winter, when minimum year round density is the rule in small rodent populations. However, given that the vole activity signs index appeared to be reliable for obtaining vole abundance estimates across the other three seasons, and especially since there are no abrupt changes between seasons that could affect sign detection (i.e. there is no continuous snow cover during winter), this method should be equally useful at this time of year.

We found some differences in results obtained with the two "simpler" sampling methods when comparing differences between seasons, habitats and locations (field vs edge). The most noticeable was the higher estimates derived from sign indices (PSpS) in spring and fall, especially in the edge (Fig. 4), as compared with those obtained from captures (MApT). This difference could be due to a greater food and cover availability for voles in spring and autumn (making them leave more presence signs), or be an effect of baiting the traps, which may be more attractive in summer when drought and farming practices make food scarcer, or when predation risk might be increased making voles seeking refuges such as traps (Garratt et al., 2012; Jacob and Brown, 2000). The difference could also reflect seasonal changes in foraging strategies, expected in highly variable agro-environments (Jacob and Hempel, 2003). If voles move less in spring and fall, the capturability might be reduced, but they would still leave activity signs.

The vole sign method worked better inside fields than in their edges, possibly because we had fewer squares sampled in edges. By sampling 5 squares in field edges, instead of 3 or 2, the performance of the index notably improved. Therefore increasing the number of squares in edges may improve density estimates. Field edges are not homogeneous habitats, since their structure is highly variable in agricultural landscapes, from a few centimetres to several metres wide, with varying vegetation density and cover that may affect our ability to accurately detect activity signs (Tellería, 2004; Witmer, 2005). A limitation of this method in edges might be the difficulty to find vole signs in wide edges with high cover density, but this problem is of lesser concern since using small squares makes comprehensive searches easy. Another issue could be that in that kind of high quality habitats voles may move less, as observed in other vole species (Renwick and Lambin, 2011), and may be thus less likely to enter traps. This could affect in particular our CMR method, given the distance between traps. Moreover, as field edges may be used by voles as dispersal corridors (Renwick and Lambin, 2011), part of the observed differences may be due to the activity of non-resident voles.

Vole abundance varied between agricultural habitats (alfalfa, cereal and fallow) and location within these habitats (edge and field). Both captures and the sign activity index indicated greater

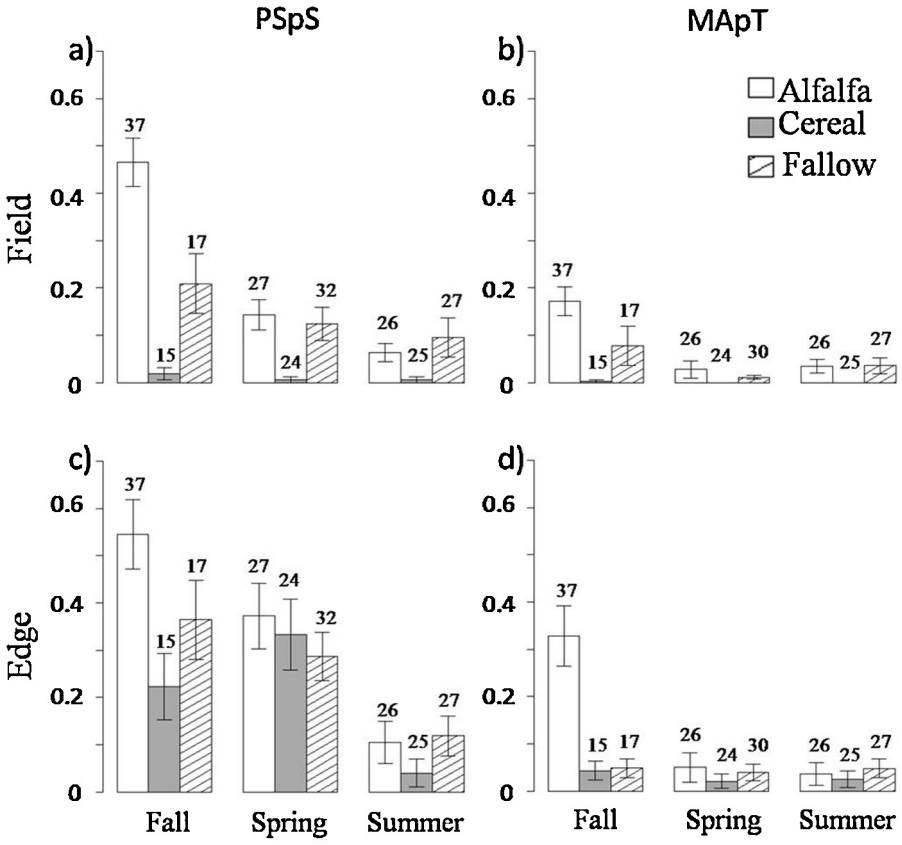

Fig. 4. Mean ± SEM vole abundances estimated with the VAS method (left; PSpS index) and the SCE method (right; MApT index) according to habitat and season. Numbers above bars are sample size.

abundances in the edges than inside the fields. Edges have been reported usually as an optimum habitat for small mammals in agricultural landscapes (Briner et al., 2007; Butet et al., 2006; de Redon et al., 2010; Spitz, 1977; Spitz and Bourllere, 1977). This may be due to the higher stability of the edge, usually not ploughed, allowing the persistence of burrows in a relatively undisturbed herbaceous habitat which provides food and cover. Common voles in central Europe live mostly in edges and rarely leave these (Briner et al., 2005), and agricultural areas with wide edges may have less crop damage problems during vole outbreaks (de Redon et al., 2010). Agricultural fields suffer other disturbances that could affect vole presence, such as harvesting. This could be particularly important in cereal fields, with harvesting occurring once in summer, and leaving the field almost bare of vegetation for several months, thereby explaining the lower vole density found in this habitat.

Of the three studied habitats, alfalfas held the highest vole abundances, both in edge and field. This herbaceous crop therefore appears as a main reservoir for voles, as reported elsewhere (Haim et al., 2007; Heroldova et al., 2007). Alfalfas provide high quality food all year round, and voles can maintain colonies there for many years, since there is no tillage for 3–8 years there while the crop is being cultivated. Alfalfas also offer protective cover against aerial predators (Jacob and Hempel, 2003), except when they are harvested (Haim et al., 2007).

5. Conclusions and applications

The Vole Activity Sign methods, and in particular the PSpS index with at least 5 points in the edge and 10 in the field, provides a promising tool from a monitoring point of view. It is a fast, cheap and reliable method for estimating vole abundance. It provides density estimates similar to those of other trapping methods, in particular the Single Trapping Event method for vole densities up to 70 voles per ha. It also compares well with estimates derived using a CMR method, although our sample size for this comparison was more limited. Testing the method with a larger sample size may be required to refine data fitting and improve reliability of estimates, particularly at higher vole densities. Additional work may also be required during winter and on cereal fields to improve data fitting into all seasonal scenarios. Nonetheless, the method offers new opportunities for managers and researchers to monitor vole population abundance in wide areas, as the sign method is relatively easy and requires fewer economic and human resources than trapping methods. The use of conventional trapping methods could be restricted to those areas where strong abundance increases are detected, in order to obtain more accurate information on the condition and reproductive status of voles, or whenever more detailed data are necessary. The vole sign index could be used as the main sampling tool for an early warning monitoring system, thus improving the adjustment of control campaigns to the real situation of vole populations, hereby improving the cost-efficiency of those campaigns and helping to prevent the economic, sanitary and environmental costs of ill-designed campaigns (Aldea-Mansilla et al., 2010; Olea et al., 2009; Vidal et al., 2009; Viñuela et al., 2010). The methodology we propose could facilitate the study of alternative outbreak control measures, for example the use of deep ploughing at certain times and areas, the increase in the frequency of harvestings in alfalfa crops, flooding crops in areas where it is possible (Haim et al., 2007; Jacob, 2003), detection of favourable habitats for voles where more intensive monitoring is required and study the possibility of reducing them (Butet et al., 2006) or implementing widespread biological control measures of the species (Haim et al., 2007; Paz et al., 2013; Pelz, 2002).

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