Clear as daylight: analysis of diurnal raptor pellets for small mammal studies

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Abstract

Clear as daylight: analysis of diurnal raptor pellets for small mammal studies.— Non–invasive approaches are increasingly investigated and applied in studies of small mammal assemblages because they are more cost–effective and bypass conservation and animal welfare issues. However, pellets of diurnal raptors have rarely been used for these purposes. We evaluated the potential of marsh harrier pellets (*Circus aeruginosus*) as a non–invasive method to sample small mammal assemblages, by comparing the results with those of sampling using Sherman live–traps and pitfalls. The three methods were applied simultaneously in an agricultural–wetland complex in NW Portugal. Estimates of species richness, diversity, evenness, abundance, and proportion of each species within the assemblage showed significant differences between the three methods. Our results suggest that the use of marsh harrier pellets is more effective in inventorying small mammal species than either of the two kinds of traps, while also avoiding any involuntary fatalities associated with the sampling of small non–volant mammals. Moreover, the analysis of pellets was the most cost–effective method. Comparison of the two trapping methodologies showed involuntary fatalities were higher in pitfalls than in Sherman traps. We discuss the advantages and flaws of the three methods, both from technical and conservational perspectives.

Key words: Animal welfare, *Circus aeruginosus*, Pitfalls, Pellets, Sherman traps, Small mammals

Resumen

Claro como el agua: análisis de las egagrópilas de aves rapaces diurnas para los estudios sobre pequeños mamíferos.— Los métodos no invasivos se investigan y se aplican cada vez más en los estudios de comunidades de pequeños mamíferos, ya que son más rentables en cuanto a sus costos y evitan los problemas relacionados con la conservación y el bienestar animal. Sin embargo, las egagrópilas de aves rapaces diurnas rara vez se han utilizado para estos fines. En este trabajo se evaluó el potencial que tienen las egagrópilas del aguilucho lagunero (*Circus aeruginosus*) como un método no invasivo para estudiar las comunidades de pequeños mamíferos, mediante la comparación de los resultados con los obtenidos en las trampas de tipo Sherman y las de caída (*pitfall*). Los tres métodos se utilizaron simultáneamente en un complejo formado por tierras agrícolas y humedales en el noroeste de Portugal. Las estimaciones de la riqueza, la diversidad, la uniformidad y la abundancia de especies y la proporción de cada una de ellas dentro de la comunidad mostraron diferencias significativas entre los tres métodos. Nuestros resultados sugieren que la utilización de las egagrópilas del aguilucho lagunero es más eficaz para inventariar las especies de pequeños mamíferos que cualquiera de los dos tipos de trampas, al mismo tiempo que evita la muerte involuntaria de animales asociada con el muestreo de pequeños mamíferos no voladores. Además, el análisis de las egagrópilas fue el método más rentable. Entre los dos métodos de captura, la muerte involuntaria de animales fue mayor en las trampas de caída que en las trampas de tipo Sherman. Se discuten las ventajas y los inconvenientes de los tres métodos tanto desde una perspectiva técnica como conservacionista.

Palabras clave: Bienestar animal, *Circus aeruginosus*, Trampas de caída, Egagrópilas, Trampas de tipo Sherman, Pequeños mamíferos

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Introduction

Species inventories are usually the very first step towards biodiversity conservation in a certain region and the basis for integrative and effective management strategies (Begon et al., 2005).

Small, non–volant mammals are highly diverse and play a major role in ecosystem structure and function worldwide. With a wide range of reproductive, locomotion and foraging strategies, small mammals are responsible for the maintenance of several interactions among wildlife communities, namely by promoting seed dispersal (Adler, 1995), or by constituting key prey for several groups of vertebrates (Carey & Johnson, 1995). Additionally, due to their sensitivity to environmental changes (Pardini et al., 2005), non–volant small mammals are excellent models for the study of ecosystem processes and patterns, and an important group to consider where the protection of ecological values is a concern (Converse et al., 2006).

Studying small mammals usually requires an effective capture plan to achieve a realistic assessment of the assemblies in accordance with the purpose of the work (Voss et al., 2001). Snap–trapping and live–trapping, with Sherman or Tomahawk traps, are the most commonly used methods to capture most small mammal species (Gurnell & Flowerdew, 2006), and supplementary surveys, such as pitfall trapping or active search are used for insectivorous or burrowing species (Voss et al., 2001). Differences in behavior, habitat use, diet, body size and use of vertical strata seem to significantly influence the effectiveness of traps (Sealander & James, 1958; Williams & Braun, 1983). It therefore seems that no single method will effectively yield an adequate sample of the species richness in an area (Voss et al., 2001). Besides efficiency and techniques, trapping small mammals also raises concerns related to ethics and animal welfare (Powell & Proulx, 2003; Putman, 1995), and to the ecological effects of involuntary or voluntary fatalities, the latter in removal–trappings. Acknowledging the usefulness of small mammals as bioindicators in terrestrial ecosystems, research and inventories of small mammal populations and assemblies have significantly increased in recent years. The potential associated fatality rates caused by hypothermia, discomfort or distress (Putman, 1995) may disrupt local populations and consequently, metapopulations (Sullivan & Sullivan, 2013), which taken to an extreme could result in conservation issues, such as monitoring in programmes dealing with sensitive or rare species.

Attending to all these constraints, and adding to the logistics and costs associated with trapping schemes, non–invasive approaches are increasingly investigated and applied (De Bondi et al., 2010; DeSa et al., 2012; Torre et al., 2013). When studies seek to examine aspects of assemblage composition, the most common non–invasive methods have long been the analysis of owl pellets, particularly from widespread and common species, such as *Tyto alba* (Torre et al., 2004, Rocha et al., 2011) and *Strix aluco* (Balčiauskienė, 2005; Petty, 1999), due to their generalist diets and close foraging ranges (Torre et al., 2013). However, pellets of diurnal raptors have rarely been used for these purposes, perhaps due to the relative difficulty in finding suitable amounts of pellets or in identifying prey remains, as many raptor species decapitate their prey before ingestion (Balfour & Macdonald, 1970) or are able to digest the skeletal parts of mammalian prey (Glue, 1970). Pellets of diurnal raptors were used by Santos et al. (2009) and Scheibler & Christoff (2007) but in both cases only as a complementary method for the inventory of small mammals in their study areas, specifically salt ponds of Aveiro, Portugal, and in agricultural areas of southern Brazil.

The marsh harrier (*Circus aeruginosus*) is a diurnal, medium–sized bird of prey whose distribution ranges from Europe and central Africa to central Asia, the northern parts of the Middle East and the Indian subcontinent (BirdLife International and NatureServe, 2014). It occurs in a wide range of habitats, from wetlands to agricultural areas (Cardador et al., 2011) and other human–shaped environments (Vandermeer, 2010). Marsh harriers mostly roost (Moreno et al., 2014) and nest (González, 1991) in paludal vegetation. They also use perches (Kitowski, 2007), under which it is common to find pellets.

Marsh harriers usually forage in open agricultural areas, particularly on the edge of ponds of fresh or brackish water, using the raid as the main hunting technique (Clarke et al., 1993). However, they have a high foraging plasticity, allowing them to use habitats that are not accessible to other birds of prey (Kitowski, 2007). The diet of marsh harriers is usually characterized as generalist (Strandberg et al., 2008) and influenced by seasonal and local conditions (Witkowski, 1989; Cardador et al., 2012), but most studies list small mammals as their primary prey (González, 1991; Alves, 2013). Lagomorphs (Schipper, 1973) and birds (Mateo et al., 1999; Clarke et al., 1993) may also be important prey.

Here we aimed to evaluate the potential of the analysis of marsh harrier pellets as a non–invasive method to determine the composition of small mammal assemblages, by comparing the results with those of two other methods, Sherman live–trapping and pitfalls, applied simultaneously in the same mosaic of habitats. We discuss advantages and drawbacks of the three methods, both from technical and conservational perspectives. Although other authors have made some considerations about the viability of diurnal raptor pellets as a technique to sample small mammals (*e.g.,* Andrews, 1990), to our knowledge this is the first study specifically designed to compare the efficiency of this method with that of other widely used capture methods.

Methods

Study area

This study was developed in Baixo Vouga Lagunar, located approximately between 8º 32' 57'' W and 8º 41' 32'' W; and 40º 49' 43'' N and 40º 41' 32'' N, in NW Portugal. The study area occupies about 12,205 ha and encompasses important ecosystems integrated in the Natura 2000 Network (PTZPE0004, PTCON0061), featuring one of the most important Portuguese wetlands (Ria de Aveiro). The climate is Mediterranean with strong Atlantic influences, and it has an annual mean temperature of about 15ºC. The average annual humidity is 77% and the average annual rainfall is 1,387 mm, with a shortage of rainfall in the summer. The system represents a complex agriculture–wetland mosaic, integrating a variety of natural and human–altered habitats (fig. 1), such as pastures (2.35% of the area), rice fields *Oryza sativa* (0.98%) and maize *Zea mays* (28.13%); and 'Bocage' (7.09%), a typical and rare landscape unit which consists of hedgerows of trees (*e.g., Salix alba*), shrubs (*e.g., Rubus ulmifolius*) and ditches that compartmentalize farmlands and pastures. The wetlands are composed of reedbeds of *Phragmites australis* (4.50%), saltmarshes of *Spartina maritima* (12.79%) and rushes of *Juncus maritimus* (6.67%) (Alves et al., 2014). This region also houses high faunal richness (*e.g.*, amphibians, birds (Special Protection Area for Birds PTZPE0004), and bats (Mendes et al., 2014). According to national bird censuses, the study area shelters 11 to 12 resident pairs of breeding marsh harriers, which corresponds to about 17% of the breeding population in the country (Rosa et al., 2006). The biological richness of Baixo Vouga Lagunar attracts ecotourists, and the region receives 25000+ visitors per year.

Sampling and identification of small non–volant mammals

Once a month, we collected marsh harrier pellets (*n* = 75) near nesting sites and under perches used by the species, during the breeding season of 2012, *i.e.* from February to August. The closest distance between a pellet collecting site and a nest was ca. 200 m. Our collecting procedures did not seem disturbing to the birds, especially considering the regular touristic visits to the area. Using a telescope, we spotted the collecting sites through direct observation of the birds from nine observation points each covering a circular area with a radius of 1.5 km. Observations took place monthly in the first three months and lasted for two hours per observation point. Regular flooding around perches and roosts prevented us from collecting pellets throughout the whole year. We oven–dried the pellets at 60°C for a day and the dry content was then separated after moisturizing. Food items were identified and quantified through the presence of non–digestible remains. Since harriers tend to rip the meat off their prey rather than swallow the entire animal (Hosking, 1943; Balfour & Macdonald, 1970), mammals were identified based on cranial structures described in the literature (Gállego & López, 1982; Gállego & Alemany, 1985; Blanco, 1998a; Blanco, 1998b) but also on detailed features of the fur: cuticular print, core and cross section (Teenrik, 1991; Quadros & Monteiro–Filho, 2006; Valente, 2012). *Talpa occidentalis* was the only species identified solely through cranial structures; all other species were identified by both cranial and hair structures. We used the 'minimum number of individuals' analysis in order to reduce possible erroneous counting of the number of prey (Lyman et al., 2003).

Simultaneously we sampled small non–volant mammals in the study area using Sherman and pitfall traps. For each habitat in the study area (reedbeds, rushes, saltmarshes, Bocage and rice and maize fields) and whenever possible, three replicate of small mammal sampling sites were randomly distributed within the nine 1.5 km radius harriers sampling areas, as long as 1 km of minimal distance between sampling sites was assured, to maintain spatial independence. Small mammals sampling sessions took place every two months, in a total of three sampling rounds for the study period. Each small mammal sampling site consisted of a line of 30 Sherman live traps (17.5 x 6 x 6 cm) separated 10 m from each other and baited with a mixture of canned sardines and hamster food, and a line of four pitfalls (buckets ca. 30 cm deep, 5 L capacity) connected with a drift fence buried to prevent animals from passing under it. In the Sherman traps, cotton was provided as nesting material. Whenever possible, traps were set under the cover of stones, shrubs or herbs to provide camouflage and some thermal insulation. Both standardized methodologies were applied simultaneously, in order to minimize the effect of the selectivity of each method and collect more representative data on the composition of the small mammal assemblage. At each sampling round, traps were active for five consecutive nights and visited every early morning as was previously tested (Gurnell & Flowerdew, 2006). At each trap check, we provided dry bedding material and a new food supply. We ringed the collected animals individually and released them after identification.

Statistical analysis

Since the trapping methods did not allow the identification of all small mammals to the species level, we used genera accumulation curves to assess patterns of genera richness in the incidence matrix obtained with each sampling method (Gotelli & Colwell, 2001). We calculated Mao Tau and Chao 1 richness estimators (Chao et al., 2009; Torre et al., 2013) using the software EstimateS 9.0 for Windows (Colwell, 2011). The completeness of the inventory made with each method was assessed by fitting the Clench equation to the observed genera accumulation curve, using the quasi–Newton method equation (Soberón & Llorente, 1993). We used the same procedures to estimate species richness with the pellet sampling method.

For each method we assessed the assemblage structure using the number of identified genera, abundance (measured as the number of individuals captured in 100 night–traps or in 100 pellets [Mills et al., 1991]), and the proportion of each taxa within the assemblage, measured through the percentage of occurrence of each taxa, calculated as $\%O_i = n_i / N_+ \times 100$, where n_i is the number of individuals of species *i* and N_+ is the total number of individuals identified or captured with a given method. We also calculated indices of evenness (Pielou index) and diversity (Shannon–Wiener and Simpson index) for all three methods. We calculated two diversity indices to ascertain possible effects of rarely recorded taxa: Simpson's index is less sensitive to presence, giving more weight to common taxa (Simpson, 1949); Shannon–Wiener's index is more sensitive to rare taxa (Magurran, 2004). To calculate evenness and diversity indices, due to a high number of zeros, pellet collecting sites (*n* = 8; see map in fig.1) and habitat replicates (for trappings; *n* = 13) were considered as samples.

We searched for differences between methods in assemblage composition and abundance of small mammals, as well as in evenness and diversity indices, using analysis of variance (ANOVA) and controlling for the effects of sample size (Rahbek, 1997).

Cost comparison

For each sampling method we calculated the associated costs for total working hours and expenses. Working hours for each trapping method considered two people working in the field, and included all steps of the monitoring programme (installation, checking, animal handling and trap removal), totaling on average 24 six–hour–days per person and per sampling round for Sherman traps and 24 three–hour–days per person and per sampling round for pitfall traps. As for pellets, field work was performed by two people, and included spotting of pellet collection sites (on average five five–hour–days per person and per month), and pellet collection (on average one four–hour–day per person and per month). Pellets (lab work) were analysed by the same person and took on average of 20 five–hour–days per month. Expenses included fuel for field trips and supplies, such as bait and cotton for the traps and cover slips, and microscope slides for pellet analysis. The price of traps and lab equipment was not included in the budget as these items were already available in the research facilities.

Results

In total, 429 small mammals of 11 species were recorded: seven rodents and four insectivore species (table 1). All eleven species were detected in marsh harrier pellets. Sherman traps captured five rodent and one insectivore taxa, and pitfall traps captured three rodent and two insectivore taxa. Six taxa were identified to species level only with pellets: *Arvicola sapidus*, *Mus musculus*, *Mus spretus*, *Sorex granarius*, *Sorex minutus* and *Talpa occidentalis*. Traps did not add any distinct species. Overall, pellets presented higher scores for the number of identified genera and species, evenness and diversity (table 1) than either of the two trapping techniques. The total number of captured individuals was highest with Sherman trap sampling (table 1).

The estimated species richness of small mammals using the pellet sampling was 11.33 ± 0.93; (*n* = 75; fig. 2A). The Clench equation showed strong adjustment to the species accumulation curve (r^2 = 0.9999), with a slope of 0.029, showing the proximity to an asymptote, and thus indicating that the sampling of small mammals with this method was quite complete and reliable (fig. 2A). Estimates of the number of identified genera (Sherman 5.00 ± 0.45, *n* = 39; pitfalls 5.00 ± 0.17, *n* = 39; pellets 8.00 ± 0.25, *n* = 75), Clench model adjustment to the genera accumulation curve (Sherman r^2 = 0.954; pitfalls r^2 = 1.000; pellets r^2 = 0.998) and slopes of the obtained curves (Sherman 0.004; pitfalls 0.033; pellets 0.015) showed that further field efforts would not result in a relevant increase of detected genera (fig. 2B, 2C, 2D). All evaluated assemblage composition parameters showed significant differences between methods: number of genera (F = 54.424, P < 0.0001), abundance (F = 30.548, P < 0.0001), and the proportion of each genus within the assemblage (F = 4.112, P = 0.017). Tukey post–hoc tests showed there were differences between pairwise comparisons, in the number of genera and abundance between Sherman and pitfall traps and between Sherman traps and pellets; and percentage of occurrence between Sherman traps and pitfalls. Sherman traps detected a greater number of genera per sample (2.103 ± 1.046 genera/sample) than pellets (0.7475 ± 0.617 genera/sample) or pitfalls (0.462 ± 0.482 genera/sample) (results expressed as mean ± standard deviation).

Indices presented significant differences between methods: Pielou (F = 15.685, P < 0.0001), Shannon–Wiener (F = 11.009, P < 0.0001), and Simpson (F = 3.742, P = 0.037), with pellets consistently scoring the highest values.

Involuntary fatalities associated with trapping methods were 0.51 and 1.79 individuals per 100 trap–nights with Sherman traps and pitfalls, respectively. The species showing highest fatality rates were *Crocidura russula* and *Microtus lusitanicus*.

Cost estimations showed that pitfall trapping was the fastest method, although pellet analysis was the cheapest. Sherman trapping was the most time–consuming and the most expensive method (table 2).

Discussion

The combination of sampling methods used in this study identified 11 species of small non–volant mammals in the study area, where 12 species are known to be present (considering rodents and shrews, and excluding squirrels, hedgehogs and bats; Bandeira et al., 2013). Comparing our results with the independent and long–term mammal study of Bandeira et al. (2013), pellets only failed to detect *Rattus rattus*, a scavenger species that prefers to live around human settlements (Ewer, 1971). We did not sample within or around urban areas, but based on previous observations, it is plausible to assume that areas in such close proximity to humans are avoided by the marsh harriers (Alves et al., 2014).

In the pellets we found remains of small non–volant mammal species known to be less common or rare, such as *Sorex minutus* and *Arvicola sapidus*, an aquatic species. The two Iberian endemisms, *Sorex granarius* and *Talpa occidentalis*, were also only recorded in pellet samples. When comparing the three methods alone, pellet analysis seemed to be most cost effective and efficient method for inventorying small mammals

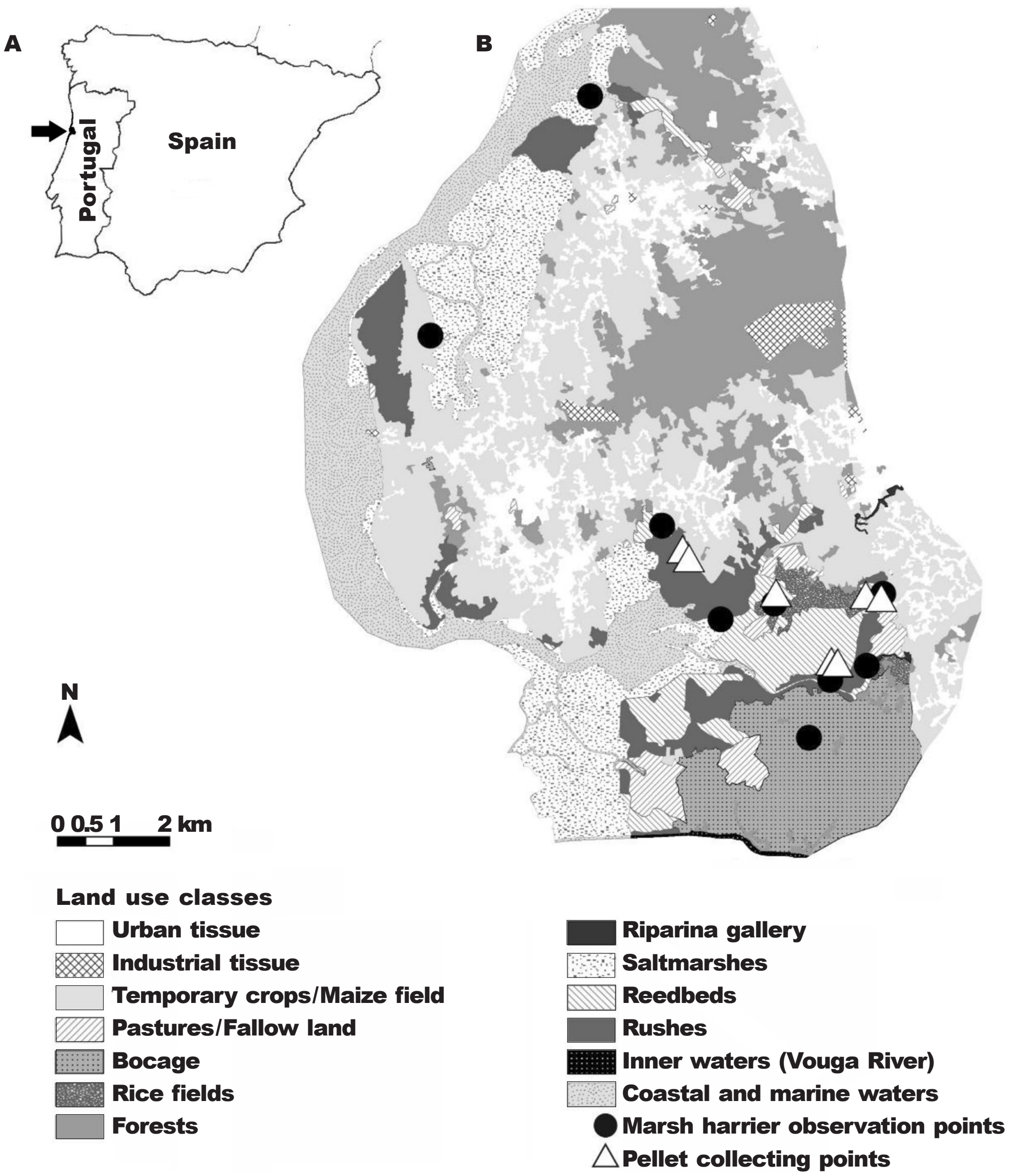

Fig. 1. Study area: A. Location of the study area in the Iberian peninsula; B. Land cover of the study area with marsh harrier observation points and pellet collecting sites.

Fig. 1. Área de estudio: A. Ubicación del área de estudio en la península ibérica; B. Cobertura del suelo en el área de estudio, con los puntos de observación de aguilucho lagunero y los puntos de recolección de las regurgitaciones.

in our study area. However, if we consider the two trapping schemes together the differences begin to fade. Nonetheless, in terms of species inventorying, our results indicate that even by analyzing a reduced number of pellets, information on the number of taxa detected can be significantly higher than that retrieved with large trapping efforts. Indeed, the species accumulation curve for pellet analysis indicates that 11 pellets were sufficient to reach five small mammals species, which is equivalent to the entire species count allowed by Sherman traps and pitfalls altogether throughout the whole study. At the genus level, trapping methods altogether yielded six genera, a score achieved with the analysis of 22 pellets.

Our field trapping did not always allow identification of some individuals to the species level, such

Table 1. Type of activity (N, nocturnal; D, diurnal, according to Blanco 1998a, 1998b), Portuguese conservation status (PT), international conservation status (IUCN), number of individuals (N), percentage of occurrence (%O), and abundance (A, measured as the number of individuals captured in 100 night–traps or in 100 pellets) of all species recorded with Sherman and pitfall trapping and with the analysis of marsh harrier pellets. Number of identified genera and species, number of captured individuals, abundance, Pielou's evenness index, Shannon–Wiener's diversity index and Simpson's diversity index are presented for each method. Indices were calculated with data to the genus level, allowing comparison between methods. Numbers in bold highlight the highest value obtained per considered parameter: * Iberian endemism.

*Tabla 1. Tipo de actividad (N, nocturna; D, diurna, de acuerdo con Blanco 1998a, 1998b), estado de conservación (PT); estado de conservación internacionañ (UICN), número de individuos (N), porcentaje de presencia (%O) y abundancia (A, medida como el número de individuos capturados en 100 trampas/noche o en 100 regurgitaciones), de todas las especies registradas con trampas de tipo Sherman y de caída y mediante el análisis de regurgitaciones de aguilucho lagunero. Para cada método evaluado se presentan el número de géneros y de especies identificados, el número de individuos capturados, la abundancia, el índice de uniformidad de Pielou, el índice de diversidad de Shannon–Wiener y el índice de diversidad de Simpson. Los índices se calcularon con los datos a nivel de género, lo que permitió comparar los métodos. Los números en negrita son los valores más altos obtenidos en los parámetros indicados: * Endemismo ibérico.*

		Conservation status		Sherman			Pitfalls			Pellets		
Taxa	Activity	PT	IUCN	N	%O	A	N	%O	A	N	%O	A
Rodentia												
Apodemus sylvaticus	N	LC	LC	79	22.44	1.35	1	5.26	0.13	8	13.79	10.67
Arvicola sapidus	N/D	LC	VU	–	–	–	–	–	–	2	3.45	2.67
Microtus agrestis	N	LC	LC	2	0.57	0.03	–	–	–	15	25.86	20.00
Microtus lusitanicus	N/D	LC	LC	33	9.38	0.56	–	–	–	7	12.07	9.33
Microtus sp.	–	–	–	–	–	–	9	47.37	1.15	–	–	–
Mus musculus	N	LC	LC	–	–	–	–	–	–	4	6.90	5.33
Mus spretus	N	LC	LC	–	–	–	–	–	–	6	10.34	8.00
Mus sp.	–	–	–	167	47.44	2.85	2	10.53	0.26	–	–	–
Rattus norvegicus	N	NA	LC	1	0.28	0.02	–	–	–	4	6.90	5.33
Eulipotyphla												
Crocidura russula	N/D	LC	LC	70	19.89	1.20	5	26.32	0.64	7	12.07	9.33
Sorex granarius	N/D	DD*	LC	–	–	–	–	–	–	3	5.17	4.00
Sorex minutus	N/D	DD	LC	–	–	–	–	–	–	1	1.72	1.33
Sorex sp.	–	–	–	–	–	–	2	10.53	0.26	–	–	–
Talpa occidentalis	N/D	LC*	LC	–	–	–	–	–	–	1	1.72	1.33

	Sherman	Pitfalls	Pellets
No. identified genera	5	5	**8**
No. identified species	5	2	**11**
Total captures	**352**	19	58
Abundance	6.02	2.44	**77.33**
Pielou eveness index	0.78	0.83	**0.84**
Shannon–Wiener index	1.26	1.33	**1.75**
Simpson diversity index	0.68	0.72	**0.80**

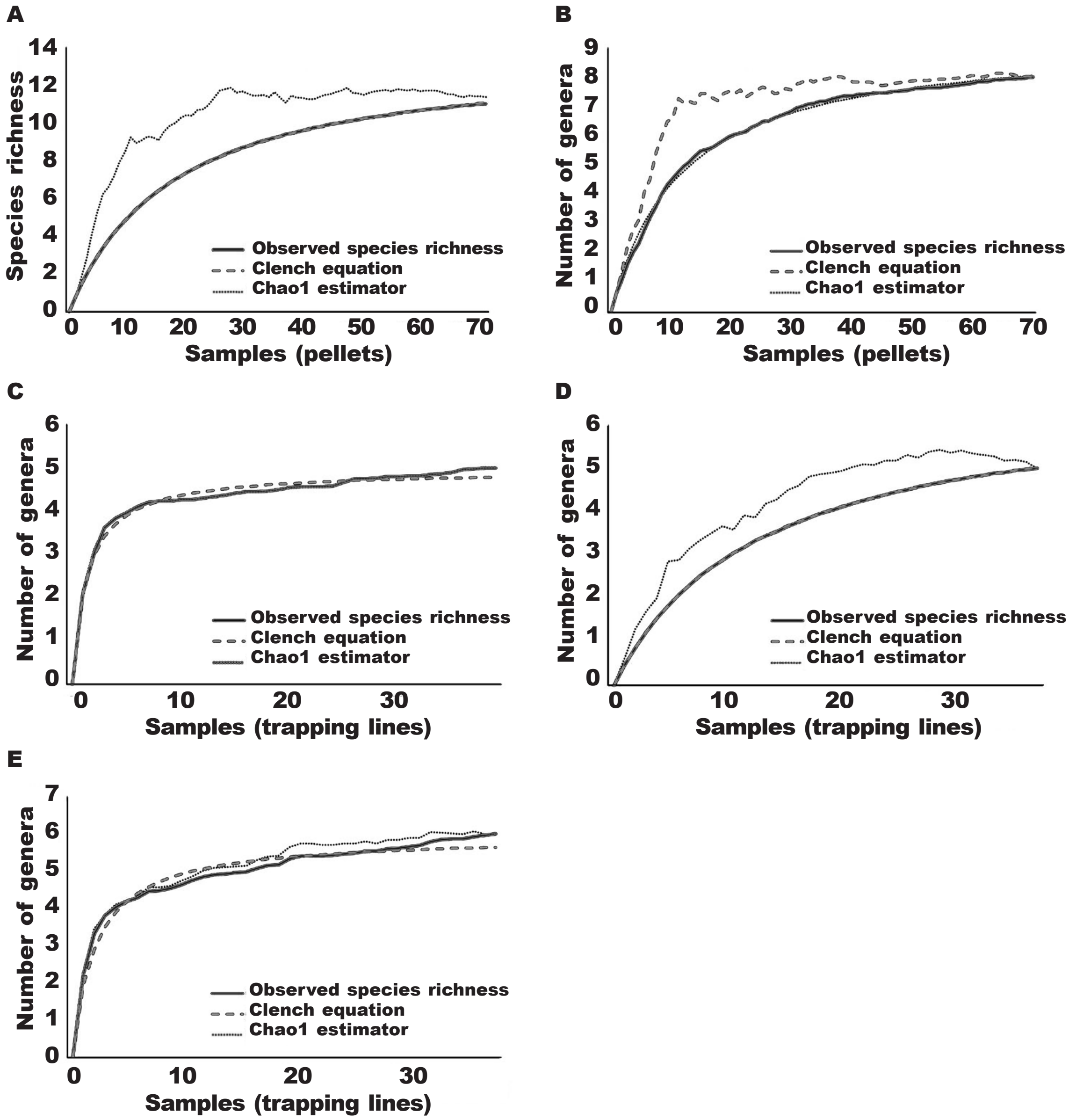

Fig. 2. A. Species accumulation curve and estimated number of species (Chao 1 estimator) for the small mammal assemblage preyed by marsh harriers; B–E. Genera accumulation curve and estimated number of genera identified through marsh harrier pellets (B), Sherman traps (C), pitfalls (D), Sherman and pitfall traps altogether (E). Observed data were fitted to the Clench equation to evaluate the completeness of the inventories.

Fig. 2. A. Curva de acumulación de especies y riqueza de especies estimada (estimador Chao 1) para el agregado de pequeños mamíferos cazado por los aguiluchos laguneros; B–E. Curva de acumulación de géneros y riqueza estimada de los géneros identificados a través de las regurgitaciones de aguilucho lagunero (B), las trampas de tipo Sherman (C), las trampas de caída (D) y las trampas de tipo Sherman y de caída juntas (E). Los datos observados se ajustaron a la ecuación de Clench para evaluar la exhaustividad de los inventarios.

as members of the genus *Crocidura*, which, when wet and anxious, may be difficult to carefully observe and distinguish. It is important to emphasize that no animals were intentionally sacrificed for later identification in the lab, so all morphometric analyses were done in the field and in live individuals, which were ultimately released. In fact, due to conservation constraints, the sacrifice of terrestrial vertebrates is

Table 2. Summary of the estimated cost for each sampling method (total working hours and total expenses): S. Sherman traps; Pt. Pitfall traps; Pl. Pellets.

Tabla 2. Resumen de la estimación de costos para cada método de muestreo (total de horas de trabajo y gastos totales): S. Trampas de tipo Sherman; Pt. Trampas de caída; Pl. Regurguitaciones.

	S	Pt	Pl
Total working (hours)	1,008	504	856
Total expenses (€)	1,596	1,211	365

neither ethically acceptable for the purpose of this study, nor legally permitted, so the identification of those individuals remained ambiguous. Conversely, all mammal remains present in marsh harrier pellets were identified to the species level, based on cranial and/or fur features.

Sherman traps tend to capture species with high capturability, oversampling these species and undersampling trap–shy species (Iriarte et al., 1989). Trap size and layout, among many other factors, influence their effectiveness (Smith et al., 1975). According to Boonstra & Krebs (1978), pitfalls are more efficient in sampling individuals of all ages among the small mammal assemblage but may fail to capture species with greater body size (M. Alves, personal observation). In our study area, it is possible that Sherman traps may have oversampled small mammal species that are more active on the surface, such as *Apodemus sylvaticus* and *Crocidura russula*, though presenting higher capturability rates than fossorial species, such as *Microtus* rodents, which may be undersampled. On the other hand, pellet sampling may be biased by the predator's ecological habits and preferences. Indeed, previous studies in the area have shown that during the breeding season, marsh harriers significantly prefer to forage on reedbeds (Alves et al., 2014), and to feed on *Microtus* species over other more abundant taxa (Alves, 2013). Also, marsh harriers may range over distances of up to 5,000 m from the nest during the breeding season (Cardador et al., 2009). Pellets may thus reflect a larger foraging area than that surveyed by trapping techniques; this may be useful when the objectives are to sample wide study areas.

Consistent differences in the estimates of species richness, abundance and proportion of species between the three methods suggest that supporting an assemblage study using only one method may lead to seriously biased results. Using various sampling methods combined is a way to overcome the biases of each method and obtain more complete information on the non–volant small mammal assemblage present in the study area. This is a well–established and recurring conclusion (Smith et al., 1975; Williams & Braun, 1983), even in studies evaluating indirect and non–invasive sampling methods (Jaksić et al., 1981). Marsh harrier pellets, for instance, proved to be particularly efficient for inventorying species richness. However, population parameters such as abundance are probably more accurate when calculated with direct approaches, such as capture–recapture schemes (Hopkins & Kennedy, 2004). Also, due to the large home range and ecological preferences of raptors, pellets may fail to provide accurate information on the microhabitat preferences of small mammals.

Very few studies have used pellets of a diurnal raptor to study the assemblages of small non–volant mammals (but see Santos & Fonseca, 2009; Scheibler & Christoff, 2007). Most studies use pellets of common and widespread nocturnal birds of prey, such as *Tyto alba*, a generalist species that presents a relatively narrow home range, pellets that are very easy to find and collect, and prey remains that are easy to identify through cranial structures. However, owls mainly prey on small nocturnal mammals and may over or under sample some particular type of prey present in specific habitats that they may prefer or avoid, respectively (Torre et al., 2004). The same is true for marsh harriers, but these birds seem to forage on habitats that are inaccessible to other birds of prey, such as high crops and reedbeds (Kitowski, 2007). The predator's hunting time may bias the sampling results towards more nocturnal or diurnal species, but our data suggest this may not be a major issue, because though marsh harriers are mostly diurnal, their pellets contained relevant amounts of mammals described as predominantly nocturnal (*e.g., Apodemus sylvaticus* and *Microtus agrestis;* table 1). This may be due to the general activity patterns of small mammals, which are rarely exclusively nocturnal or diurnal. Even short periods of diurnal activity may represent hunting opportunities for fast raptors, such as the marsh harrier, that use the raid hunting technique. Furthermore, in wetlands and other open areas that lack nesting sites for owls —such as the Baixo Vouga Lagunar— owl pellets are not even an option. On the other hand, the marsh harrier is a widespread species in the region, easy to identify and spot for pellet deposit sites. Studies developed in the study area (Alves, 2013) confirmed the diet of marsh harrier as generalist and mostly (68%) constituted of small mammals. It was also confirmed that marsh harriers forage on reedbeds and saltmarshes —wetlands that can be quite difficult to sample with traps, due to regular flooding, tides (in salt marshes) and vegetation density— but also on crops, providing a general sampling of the small mammals of a vast number of habitats. Our results also show that marsh harrier pellets provided more complete information on small non–volant mammal richness, and potentially of evenness and diversity. Table 3 summarizes further advantages and disadvantages of the three methods assessed in our study.

Finally, it should be highlighted that when designing any study on the structure of animal assemblages the choice of methods must carefully consider not only the technical limitations, but also the purposes of the study and ethical and legal questions. The

Table 3. Comparative list of advantages and disadvantages of using Sherman traps, pitfalls and the analysis of marsh harrier pellets for small mammal studies, according to our study.

Tabla 3. Lista comparativa de las ventajas e inconvenientes de utilizar trampas de tipo Sherman, trampas de caída y el análisis de las regurgitaciones de aguilucho lagunero para los estudios sobre pequeños mamíferos, según nuestro estudio.

Advantages	Disadvantages
Marsh harrier pellets	
Low logistical requirements	Pellets are hard (or sometimes impossible) to collect, especially in flooded or hard–reaching areas
Cost–effective	Collection is more time–consuming than pitfalls
Less time–consuming than Sherman traps	Not suitable to estimate density
More positive identification of species	The quality of the results depends on the diet of the birds, which is influenced by environmental constraints (*e.g.* landscape features and prey availability)
Higher completeness and effectiveness in species inventory, especially in heterogeneous and wide areas	Does not provide information on the spatial ecology of small mammals.
Detection of species occupying habitats where trapping is not possible	May underestimate nocturnal and overestimate diurnal prey species
Higher potential to detect rare species	
Provides more accurate information at the species level, allowing better diversity and evenness calculations	
Non–invasive method for small mammals	
Sherman traps	
Lower mortality than pitfalls	Time–consuming and expensive. High logistical requirements
Allows the observation of gender, age and physical condition	Frequently sprung–but–empty
Allows capture–mark–recapture techniques	Not usable in all kind of habitats (*e.g.* wetlands)
Suitable for density estimation	Lower potential to detect rare species
Provides information on the spatial ecology of small mammals	Results biased towards trap–prone species
	May oversample species that live at the surface
	Trapping success largely depends on external conditions that influence animal activity
Pitfalls	
Less time–consuming than Sherman traps or pellet analysis	Time–consuming and expensive
Allows simultaneous and sequential multiple captures	High logistical requirements
Allows the observation of gender, age and physical condition	Very conspicuous apparatus, subject to vandalism or theft
Allows capture–mark–recapture techniques	Lower potential to detect rare species
Suitable for density estimation	Biased towards common species
More successful in sampling trap–shy species than Sherman traps	May oversample species that live at the surface
	May fail to capture larger animals
	High mortality rates, not suited for studies with endangered species
	Trapping success largely depends on external conditions that condition animal activity

efficiency of marsh harrier pellets —and also other non–invasive methods— showed that, in some cases, and depending on the objectives (for instance, presence–absence data), trapping is unnecessary, thereby avoiding disturbance and fatalities among small mammals (Powell & Proulx, 2003; Sullivan & Sullivan, 2013). Collecting pellets, in particular near nests, may disturb the birds (Fernández & Azkona, 1993), but if the study is well planned and takes the reproductive and spatial ecology and the habits of the species into consideration, impact can be minimized. In our study, pitfalls presented considerable involuntary fatality rates for small mammals, mostly due to the humidity in the buckets, causing the animals to die from hypothermia. This sampling approach cannot therefore be recommended for small non–volant mammals in wetlands or areas with high humidity levels. In regions that harbour endangered or rare species, we encourage researchers to seek for non–invasive methodologies, or, at least, to previously determine the least detrimental research protocol for wildlife, safeguarding animal and ecosystem welfare.

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