UNIVERSIDADE DE ÉVORA

Mestrado em Biologia da Conservação

Dissertação

Comparação de métodos de avaliação do uso do habitat pela lontra (*Lutra lutra* L.): Indícios de presença e rádio-telemetria

Autor:
Hugo André Henriques Leitão Franco Zina

Orientador:

Professor Doutor António Mira

Co-Orientador:

Dr. Lorenzo Quaglietta

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Resumo

Neste estudo aferiu-se a utilidade da prospecção de indícios de presença para avaliar áreas de maior actividade de lontra. O trabalho realizou-se no sul de Portugal numa zona com clima marcadamente Mediterrânico, tendo-se comparado a ocorrência e número de dejectos com a actividade avaliada com recurso a rádio-telemetria. Seguiram-se 6 lontras tendo sido realizados transectos pedestres para pesquisa de dejectos nas manhãs imediatamente seguintes ao rádio-seguimento. Comparou-se a presença de dejectos frescos e a abundância total de dejectos com as localizações dos animais e a influência nesta relação, da estação do ano, do sexo, da abundância de locais de marcação e da quantidade de precipitação. Para tal utilizaram-se várias combinações de Modelos Lineares Generalizados Mistos. Globalmente obteve-se uma relação significativa e positiva entre as localizações de lontra e a presença de dejectos frescos. A abundância total de dejectos esteve igualmente relacionada significativamente com as localizações, no entanto, esta relação é influenciada pelas outras co-variáveis. Este método apresenta-se útil para avaliar de forma expedita o uso do habitat pela lontra no Mediterrâneo, podendo ser usado com baixos custos na em acções de gestão e conservação

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Abstract

This study evaluated the utility of presence signs surveys to assess areas of otters activity. The work took place in southern Portugal in an area with Mediterranean climate. We compared the occurrence and number of spraints with the otter activity measured through radio-telemetry. Six otters were radio tracked and pedestrian surveys searching for spraints were performed during the following morning in the area used by the otters. We compared the presence of fresh and total abundance of spraints with the locations of animals and evaluated the influence season, sex, plenty of places to mark and amount of precipitation on this relationship. For this purpose we used various combinations of Generalized Linear Mixed Models. Overall we obtained a significant and positive relationship between the locations of otter and the presence of fresh spraints. The total abundance of spraints was also significantly correlated with the otter locations; however, this relationship was influenced by other co-variables. We concluded that otter presence signs surveys is a useful method to assess expeditiously otter habitat selection in the Mediterranean, and can be used with low costs in management and conservation programs.

Introdução Geral

A lontra euro-asiática (*Lutra lutra* Linnaeus, 1758) é um mamífero carnívoro de médio-grande porte, pertencente à família *Mustelidae*, e sub-família *Lutrinae* (Kruuk 2006). Outrora apresentava uma distribuição alargada que se estendia da Europa Ocidental e Norte de África até à China, Japão e Sudeste Asiático (Mason & Macdonald, 1986; Kruuk, 2006). Contudo, durante todo o século XX apresentou um acentuado declínio levando inclusive à sua extinção em alguns países asiáticos e europeus. Este declínio levou à classificação da sua população mundial com estatuto de conservação desfavorável – Quase Ameaçado (Ruiz-Olmo *et al*., 2008). A situação das populações de lontra na Península Ibérica, e particularmente em Portugal, é bem diferente das congéneres europeias. A distribuição de lontra em Portugal é alargada a toda a extensão do território continental, com excepção das grandes áreas urbanas de Lisboa e Porto (Trindade *et al.,* 1998). A vasta maioria das suas populações são saudáveis, ocorrendo em grandes densidades, o que torna a população portuguesa uma das mais viáveis da Europa (Macdonald & Mason, 1982; Trindade *et al.,* 1998; Conroy & Chanin, 2000).

A lontra é uma espécie difícil de observar devido à sua natureza esquiva e crepuscular, como tal, grande parte do conhecimento sobre este mustelídeo advém de estudos que têm como base indícios indirectos de presença, como dejectos, pegadas, etc. Para tal contribui o carácter comportamental destes animais, uma vez que tendem marcar, com dejectos e secreções anais, sítios proeminentes como rochas, pedras e raízes, tornando fácil a detecção dos indícios presença (Conroy & French, 1991; Kruuk, 2006). A maioria dos estudos efectuados refere-se à avaliação da presença de lontra em determinada região ou país (*ver*: Mason & Macdonald, 1986; Trindade, 1996; Trindade *et al.,* 1998), ou relacionando a abundância de dejectos com preferência e selecção de habitat (Macdonald & Mason, 1983; *ver*: Mason & MacDonald, 1987; Prenda & Granado-Lorencio, 1995). Contudo, a utilização dessa relação não é aceite de forma pacífica, tendo sido criticada por não ter em consideração aspectos como as naturais variações sazonais no comportamento de marcação das lontras, os diferentes tipos de habitat, bem como restrições metodológicas inerentes a diferentes unidades de amostragem (Kruuk *et al.*, 1986; Kruuk & Conroy, 1987). Por outro lado, defensores desta abordagem metodológica defendem que, utilizando amostragens estatisticamente robustas, a abundância de dejectos pode ser usada como um indicador geral de selecção de habitat (Mason & Macdonald, 1987; 1991). Este debate, que conta já com três décadas, mantém-se contudo actual, na medida em continuam a ser realizados estudos de modo a aferir a real utilidade da metodologia de amostragem de lontra com base em indícios de presença (*ver*: Ruiz-Olmo *et al.,* 2001; Guter *et al.,* 2007; Gruber *et al.*, 2007). Apesar de haver indícios de uma correlação positiva entre os dejectos frescos e índices de actividade da espécie (Guter *et al.,* 2007), é necessário precaução ao relacionar ausência de dejectos com ausência de actividade, ou extrapolar conclusões relacionando a abundância de dejectos com estimativas populacionais (Ruiz-Olmo *et al.,* 2001).

A avaliação da preferência de habitat pode ser realizada através da aplicação de outras metodologias, como a rádio-telemetria, a qual permite saber com uma margem de erro mínima a localização do animal, assim como o tempo que este despende em determinada zona (*ver*: Durbin, 1998; López-Martín *et al.,* 1998). No entanto, esta metodologia é uma técnica completamente contrária à prospecção de índicos de presença, na medida em que é altamente intrusiva e de elevados requisitos logísticos. A rádio-telemetria requer a captura e manuseamento dos animais, sendo necessária a colocação de um rádio-transmissor que, no caso da lontra, devido a constrangimentos anatómicos, tem de ser inserido na cavidade intra-peritonial através de intervenção cirúrgica. Findado o processo de captura e marcação do animal, segue-se a tarefa de obter as localizações sendo, para isso, necessária uma logística rigorosa. Estas particularidades tornam a rádio-telemetria uma técnica exigente, necessitando de inúmeros recursos financeiros, logísticos e humanos.

Dada a actual conjuntura mundial, urge cada vez mais encontrar soluções eficazes e de baixo custo, de modo a maximizar os métodos usados em biologia da conservação, principalmente quando os recursos disponíveis são limitados (Myers *et al.,* 2000; Franco *et al*., 2006). Com base nesta premissa, o presente trabalho propõe-se a aferir a utilidade da avaliação de habitat obtida através de uma metodologia baixo custo, baixos requisitos logísticos e de fácil aplicação – a prospecção de indícios de presença de lontra, comparando-a com as reais localizações de indivíduos, obtidas através da rádio-telemetria. Para tal, será comparada a presença e abundância de dejectos com as localizações de lontra, e avaliada a influência que variáveis como o sexo do animal, a estação do ano, a pluviosidade e disponibilidade de locais de marcação, possam ter nesta relação.

Este estudo foi inserido no projecto OPA – *Otter Project in Alentejo*, numa parceria entre a Unidade de Biologia da Conservação da Universidade de Evora e a Universidade de Roma "La Sapienza", resultante do projecto de doutoramento do Dr. Lorenzo Quaglietta.

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Introduction

Otters (*Lutra lutra*) are semi-aquatic carnivores from the Mustilidae family with a large historical global distribution that extended from Southwestern Europe and Northern Africa to Southwest Asia and Japan (Mason & Macdonald, 1986, Kruuk, 2006). However, during the last century otter populations have undergone serious declines and are currently very rare or extinct in several countries (Macdonald & Mason, 1994; Conroy & Chanin, 2000; Kruuk, 2006). In the Iberian Peninsula otter distribution is somewhat different in both countries. In Spain, distribution is irregular (Barbosa *et al.,* 2001), with healthier populations occurring mostly in the Northern and Northeastern parts of the country (Conroy & Chanin, 2000; Barbosa *et al.,* 2003). Otter populations in Portugal are widespread and thriving throughout the country (Macdonald and Mason, 1982; Trindade *et al., 1*998; Conroy & Chanin, 2000) and can be found in coastal habitat, mountain freshwater habitats and Mediterranean freshwater habitats, only being absent in the most heavily populated areas of Lisbon and Oporto (Trindade *et al.,* 1998). This makes Portugal one of the best countries to study otter populations in their natural habitat.

Due to the otter's generally nocturnal or crepuscular, as well as solitary and elusive behaviour, studying this species is considerably challenging. For this reason, most of the information obtained on this species arises from the use of indirect methods (Guter *et al.,* 2007) such as spraint surveys. Spraints are otter scats and are also the most obvious evidence of its presence (Kruuk, 2006). Otters tend to spraint on prominent places and vantage points such as rocks along the water's edge, boulders, roots and can use these places over a long period of time (Conroy & French, 1991; Kruuk, 2006). Nonetheless, it is important to keep in mind that several factors influence sprainting behaviour such as season, habitat, prey availability, sex and reproductive cycle (Macdonald & Mason, 1987; Conroy & French, 1991; Prenda & Granado-Lorencio, 1995; Ruiz-Olmo & Gosálbez, 1997; Prigioni *et al.,* 2005).

Otters survey techniques were developed during the 1970's and were endorsed throughout the scientific community (Mason and Macdonald, 1986). With this method several studies were performed linking otter presence, abundance and habitat selection to presence and number of spraints (Macdonald & Mason, 1983; see Mason & MacDonald, 1987; Prenda & Granado-Lorencio, 1996). However, Kruuk *et al.,* (1986) did not find clear evidence of a relation between sprainting activity and otter activity. This question brought forward a discussion between those who questioned the survey method to assess activity (Kruuk & Conroy, 1987) and those who defended it (Mason &

Macdonald, 1987;1991). Reviewing this discussion, Mason and Macdonald (1987) stated that as long as large sample sizes that can be used in statistical comparisons are provided, spraint density may be used as a broad indicator of otter population status. However, in these types of analyses, conclusions and extrapolations should always be considered with caution, whilst keeping in mind: a) type of habitat (comparisons should be made between similar habitats); b) seasonal fluctuations of otter marking; c) sampling unit (Kruuk *et al.,* 1986; Kruuk & Conroy, 1987). Nowadays this discussion is still appropriate and lively. In recent years, research has been conducted in order to understand if one can extrapolate habitat selection and otter activity with spraint survey methods either by using old methods such as visual census (Ruiz-Olmo *et al.*, 2001) or by exploring new methods applying technology advances (Guter *et al.,* 2007). However, Ruiz-Olmo *et al.,* (2001) warned of the impossibility to estimate otter numbers based on spraint numbers alone and that an absence of spraints does not necessarily imply otter absence and a lack of correlation between otter numbers and presence signs. On the other hand, Guter *et al.,* (2007) showed a positive correlation between sprainting activity correlates with otter visitations. Another possible answer to this problem was found by Gruber *et al*., (2007) that developed an accurate visitation rate estimators via indirect signs, although this method was developed in a commercial fish farm. In spite of some improvement techniques and new arguments brought forward into this discussion, much has yet to be done.

In view of this, radio-tracking presents itself as a very useful tool to correctly assess habitat selection. This technique requires tagging the animal with a radio transmitter. Due to the otter's cylindrical body and short neck, the radio transmitter must be inserted into the body which results in a highly intrusive technique. This is a costly method and is therefore seldom used. However, there are some studies making use of radio-tracking otters in order to assess their habitat preferences (Durbin, 1998; López-Martín *et al.,* 1998).

Improving the reliability and validating conclusions achieved with low cost methods, such as spraint survey techniques is of vital importance, as there is an increasing need to maximize conservation biology results with limited resources (Myers *et al.,* 2000). Several studies on birds and mammals aim to improve sampling methods by comparing different methodologies, in order to achieve more effective results with lower budgets (Ostrand *et al.,* 1998; Barea *et al.,* 2007; Franco *et al.,* 2007).

This study aims to identify the usefulness of spraint surveys to assess otter presence and activity, by comparing otter presence signs surveys with otter radio-tracking results.

The presence and abundance of spraints in rivers and reservoir banks shall be compared with otter radio-tracking locations. An important factor influencing this study is the severe Mediterranean climate which could influence these relationships. Thus, surveys and analysis are done on dry and wet seasons. We hope to contribute to a methodology assessment which can be useful in otter conservation throughout the Mediterranean basin.

Methods

Study area

The study was conducted in the Alentejo region in southern Portugal, on the outskirts of the city of Évora (alt. 283; lat 38°34'N long 007°54'W). This region is predominantly characterized by a Mediterranean climate consisting of long summer droughts with precipitation usually concentrated in autumn, winter and early spring. The mean temperature in 2008 was 13,7º C, with a maximum of 37º C recorded in July and a minimum of -2º C recorded in January. Mean annual rainfall was 400-500mm, which over 80% fell during the wet season, from October to April (www.cge.uevora.pt). The landscape is dominated by the characteristic man made habitat known as *montado,* composed of short herbaceous formations and old-growth oaks (helm oaks *Quercus rotundifolia* and cork oaks *Quercus suber*). This semi-natural habitat is used for extensive agriculture with seasonal crops and cattle.

Two river basins were surveyed is this study: Sado basin and Guadiana basin. Both basins present acceptable water quality with most pollution threats coming from agricultural untreated residues (Trindade *et al.,* 1998). The watercourses generally have a small width (<10 meters) and contain riparian vegetation composed of willow *Salix sp.,* alder *Alnus glutinosa*, narrowleaf ash *Fraxinus angustifolia* and brambles *Rubus sp.*. In the Alentejo region, small reservoirs are a common feature throughout the landscape, being used for water storage by farmers and cattle producers. These structures assume special relevance for otters during the dry season when most watercourses are depleted (Pedroso & Santos-Reis, 2006). The fish communities in the study area is dominated by exotic species, such as black bass (*Micropterus salmoides*)*,* pumpkinseed (*Lepomis gibbosus*)*,* common carp (*Cyprinus carpio*) and mosquitofish (*Gambusia holbrooki*), mainly present in the reservoirs but invading almost all the watercourses (Quaglietta, *in prep*). Another frequent exotic prey species for otters is the american crayfish (*Procambarus clarkii*) (Beja, 1996; Pedroso & Santos-Reis, 2006), which is abundant in most streams and reservoirs of the study area and throughout the Alentejo region.

Otter radio-tracking

A total of six animals (Table 1) were radio tracked for this study, between the 16th of June and the 12th of December 2008 (more in Quaglietta, *pers. comm.*). During this period, 23 radio tracking nights – cycles - were done. The locations of each animal were triangulated every 15 minutes, starting at dusk (generally 1 hour before sunset) and continuously until sunrise. Due to the rapid movements of these animals and difficulties in optimal signal reception, some cycles present gaps or are incomplete. The activity of the animal was recorded along with its location, with the signal fluctuations being easily distinguished when the animal was resting or in activity.

Table 1 – Study animals used. Legend: F – Female; M – Male.

ID otter	Capture date	Age at capture	Number of cycles
F1	15-06-2007	Adult	5
F3	13-12-2007	Adult	5
F4	17-12-2007	Adult	4
M3	28-09-2007	Sub-adult	2
M4	10-05-2008	Sub-adult	3
M5	04-09-2008	Sub-adult	4

Otter spraint surveys

The surveys were performed the morning after the radio-tracking cycle was finished. The operator surveyed the area where the radio-tracked animal was recorded during the previous night. Otter spraints play a key role in scent marking, thus otters tend to mark on prominent places (Macdonald & Mason, 1994; Kruuk, 2006). Thus, prominent places (e.g., roots, boulders, rocks, etc.) in stream banks, riverbeds and on reservoir banks were especially taken into account and carefully searched. Spraints were surveyed in both banks in rivers and in a 15 meters distance to water on reservoirs banks. When a spraint was found, its GPS location was taken, as well as marking place, distance to water/bank and estimated age. Age was ranked into two categories: Fresh – when the spraint was very humid and soft with food remains often aggregated which we considered as being from the previous night; Old - all the others. For an experienced operator it’s possible to distinguish between this to categories also by changes in colour, texture and odour. Although surveys were performed by an experienced operator one cannot assume that all spraints were found, and that all the sprains located belonged to the radio-tracked animal, however, we can assume that it was highly probable. An additional variable was collected, Sprintability (SA), which concerns the percentage of possible marking sites for otters in the stretch of river or reservoir bank surveyed. SA was ranked into four categories: Null – when there weren’t prominent places such as sandy reservoir banks; Low – when there were scarce prominent places, such as reservoir banks with a few boulders or rivers with high volume of water with only some roots emerged; Medium – regular presence of prominent places such as rocky riverbanks; High – Constant prominent places present such as a dry up river.

Data Analysis

The study area's hydrographical network was inserted in a Geographical Information System - GIS. Missing sections of the hydrographical layer were drawn using study area orthophotomaps and the network was then split into 100 meter sections identified numerically. Each section was later classified according to its SA. All the cycle locations and every spraint location were also inserted into the GIS. Concerning a possible bias effect on radio-tracking data, when the animals were inactive (resting) for periods longer than 30 minutes, only the resting locations when the animal first ceased activity and right before restarting activity, were considered. Every spraint and otter location for each corresponding pair cycle/spraint survey, was attributed to a section of the hydrographical network using the NEAR function in Spatial Analyst tools present in ArcGis 9.0 (ESRI). The results of this process were spraint and activity location counts per stream section for each pair cycle/spraint survey. In addition, three other variables were considered: animal sex (SEX), the mean value of precipitation during 7 days before the survey (RAIN) and season (SEASON). All surveys carried out from June to September were considered to be in the dry season, and the other were included in the wet season).

Generalized Linear Mixed Models (GLMMs) were used to ascertain if there is a significant relation between the presence and number of spraints, and the presence and amount of time spent by otters at a given river section. These models extend from Generalized Linear Models (GLM) by adding a random effect factor (RE) in the predictor (Zuur *et al.,* 2007). In our data we could not disregard the potential lack of independence that surveying the same animals several times had on data collection, therefore animals were ranked into 6 categories and fitted as a random factor. Another potential problem that can be addressed by mixed effects modelling is spatial autocorrelation, which in our data could be plausible. Therefore, we considered river section as a random factor as well.

Two response variables (Table 2) were considered: fresh spraints (FFSP) and all spraints (ALLSP). Due to the high number of zeros in FFSP (>60%), a binomial error distribution were used, whereas a Poisson error distribution were used for ALLSP. Both models were fit by Laplace approximation. The relationship between spraint presence and abundance with the real otter locations was assessed by specifying several different model for each dependent variable (FFSP and ALLSP, accordingly), where EXP means explanatory variable (SA, SEX, RAIN or SEASON):

1. Y = LOC + RE (Null model: probability of presence or abundance is related with otter activity locations, assuming lack of variation due to SA, SEX, RAIN or SEASON).
2. Y = LOC + EXP + RE (additive model: variation in probability of presence or abundance of spraints varies with (SA, SEX, RAIN or SEASON), but otter activity locations have no variation in relation to the same explanatory variable).
3. Y = LOC x EXO + RE (interaction model: differences, in otter activity among SA, SEX, RAIN or SEASON classes).

We tested the influence of SA, SEX, SEASON and RAIN (Table 2) on each dependent variable and compared the different models using the Akaike Information Criterion (AIC). The significance level was set at α = 0.05. GLMMs were carried out using R 2.12.1 (R Development Core Team 2010) free statistical software and the Lme4 package (Bates and Sarkar, 2006).

Table 2 – Variables used for the Generalized Linear Mixed Modelling.

Variable	Description	Data categories and/or units	Response variable	Fixed effect	Random effect
FFSP	Number of fresh spraints, per river section	Numerical	X		
ALLSP	Number of all spraints, per river section	Numerical	X		
LOC	Number of otter radio-tracking locations, per river section	Numerical		X	
SA	Classes of prominent sites for otter marking, per river section	1 - Null; 2 - Low; 3 - Medium; 4 -High		X	
SEX	Animal sex	0 - Female; 1 - Male		X	
SEASON	Season considering the volume of precipitation	0 - Rainy; 1 - Dry		X	
RAIN	Precipitation mean value in 7 days before the spraint surveys	Numerical		X	
ANIMAL	Animal identification	6 ranks			X
IDFID	Identification of each 100m river section	320 ranks			X

Results

Field work preliminary analysis

A total of 52,2 km of rivers, streams, reservoir banks and pond banks were surveyed after 23 radio-tracking nights, resulting in 536 otter radio-tracking locations and collection of 3853 spraints, 556 of which were fresh spraints (Table 3). We found most spraints after radio-tracking female otters, however, the number of fresh spraints per cycle/night was higher after a male cycle (Table 3). Despite the fact that fewer cycles were performed for males comparatively to females, the distance surveyed for males was longer, 27,3 km. Contrary to most Northern and Central European studies (Macdonald & Mason, 1987; Conroy & French, 1991) we found more spraints in the dry season, as confirmed by Index of Kilometer Abundance - IKA (Table 4). However this summer peak is usually recorded for the Mediterranean areas (Ruiz-Olmo & Gosálbez, 1997). Regarding the Sprainting Ability, most 100m sections of the surveyed area were ranked as Medium SA, presenting of prominent marking sites (Figure 1). Over 80% of the spraints were found in 100m sections ranked as Low or Medium SA (Figure **1** 1), whereas only 2,3% of the surveyed spraints were found in 100m sections ranked as Null SA (Figure 1)..

Table 3 – Length of otter survey transect, number of otter locations and spraints, according to sex.

Sex	Distance surveyed	Nº of cycles/surveys	Otter locations	FFSP	ALLSP	FFSP per cycle/survey	ALLSP per cycle/survey
Female	24.9 km	14	292	316	2366	22.6	169.0
Male	27.3 km	9	244	240	1487	26.7	106.2
Total	52.2 km	23	536	556	3853	24.2	167.5

Table 4 – Number of cycles and number of spraints collected, per season and cycle.

Season	Nº of cycles	FFSP	ALLSP	FFSP per cycle/survey	ALLSP per cycle/survey	IKA (spraints/Km)
Dry	15	433	2855	28.9	190.3	89.2
Wet	8	123	998	15.4	124.8	49,4

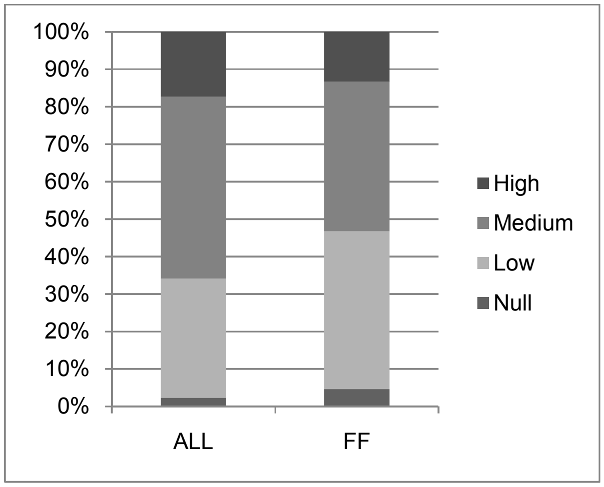

Figure 1 – Proportion of fresh and all spraints found on each spraintability class (right)..

Relationships between spraints and otter activity

The comparison between the fresh spraint presence models suggests that the null model, with only radio-tracking locations, is the most parsimonious (Table 5). All the additive and interaction models displayed much higher AIC scores (Zuur *et al.*, 2009). Moreover, none of the additive or interaction terms are significant meaning that the relationship between presence of spraints and otter activity in each of the 100m sectors does not depend on sex, season, spraintability classes or rainy events. For ALLSP abundance, all the interaction models have lower AIC and ,thus, are more parsimonious than the null model. The most parsimonious model is the interaction model between radio-tracking locations and season. However, the interaction terms in all models are highly significant. These results suggest that that the relationship between spraint abundance and otter locations is different between sexes and seasons, along spraintability classes and is influenced by the amount of rain (Table 6). In Figure 2, these differences are evident for sex and season as y regression slopes have opposite signs for each category. Average spraint numbers were higher per river section in females (9.5 ± 11.19) comparatively to males (5.4 ±11.35). A similar relationship was found in the dry season (8.9 ± 10.9) compared to the wet season (4.9 ± 11.7). For the amount of rain (Figure 3) the differences are related only to the magnitude of slope as the slope sign is the same for both classes. Overall amount of rain does not have any significant effect on the relationship between LOC and ALLSP (Table 6). However, when the same variable is categorized (0- no rain; 1- rain), there is a significant interaction among LOC and RAIN and after rainy days a strong inverse relationship between otter locations and number of spraints is revealed (Figure 3). SA classes show significant influence in abundance of spraints and also when interacting with cycle locations (Figure 4). The Medium class has the highest average number of spraints, also having the highest variation. All classes show higher variation value when compared to mean value.

Table 5 – Summary of models for predicting spraint presence and spraint abundance in relation to four different variables.

Model		AIC	Deviance	ΔAIC
Spraint Presence - FFSP				
Null model				
1A.	LOC	365.3	357.3	0
Additive models				
1Ba.	LOC + SA	622.2	608.2	256.9
1Bb.	LOC + SEX	618.5	608.5	253.2
1Bc.	LOC + SEASON	617.7	607.7	252.4
1Bd.	LOC + RAIN	617.1	607.1	251.8
Interaction models				
1Ca.	LOC x SA	620.6	600.6	255.3
1Cb.	LOC x SEX	618.5	606.5	253.2
1Cc.	LOC x SEASON	619.6	607.6	254.3
1Cd.	LOC x RAIN	617.4	605.4	252.1
Spraint Abundance - ALLSP				
Null model				
2A.	LOC	1920	1912	109
Additive models				
2Ba.	LOC + SA	1902	1888	91
2Bb.	LOC + SEX	1919	1909	108
2Bc.	LOC + SEASON	1845	1835	34
2Bd.	LOC + RAIN	1922	1912	111
Interaction models				
2Ca.	LOC x SA	1837	1817	26
2Cb.	LOC x SEX	1819	1807	8
2Cc.	LOC x SEASON	1811	1799	0
2Cd.	LOC x RAIN	1897	1885	86

Table 6 – Results of the Generalized Linear Mixed Modeling performed in order to evaluate the potential relationship between spraint presence and abundance (*significant at 0.05; **significant at 0.01;*** significant at 0.001).

Model	Estimate	Std. Error	z value	P	Model	Estimate	Std. Error	z value	P
Spraint Presence - FFSP					**Spraint Abundance - ALLSP**				
Null model					*Null model*				
1A. LOC	0.090	0.020	4.437	**9.13e-06*****	2A. LOC	0.065	0.008	8.179	**2.86e-16 *** **
Additive models					*Addictive models*				
1Ba. LOC	0.305	0.066	4.638	**3.52e-06 *****	2Ba. LOC	0.064	0.008	8.113	**4.95e-16 *****
1Ba. SA (factor2)	0.626	0.582	1.076	0.282	2Ba. SA (factor2)	1.653	0.410	4.026	**5.67e-05 *****
1Ba. SA (factor3)	0.520	0.578	0.900	0.368	2Ba. SA (factor3)	1.908	0.405	4.716	**2.41e-06 *****
1Ba. SA (factor4)	0.136	0.614	0.222	0.824	2Ba. SA (factor4)	1.754	0.420	4.173	**3.00e-05 *****
1Bb. LOC	0.316	0.065	4.883	**1.04e-06 *****	2Bb. LOC	0.065	0.008	8.192	**2.56e-16 *****
1Bb. SEX(factor1)	-0.962	0.671	-1.433	0.152	2Bb. SEX(factor1)	-0.889	0.426	-2.087	**0.0369 ***
1Bc. LOC	0.330	0.066	5.013	**5.35e-07 *****	2Bc. LOC	0.082	0.009	9.511	**<2e-16 *****
1Bc. SEASON(factor1)	0.785	0.470	1.670	0.095	2Bc. SEASON(factor1)	0.763	0.093	8.226	**<2e-16 *****
1Bd. LOC	0.323	0.066	4.928	**8.32e-07 *****	2Bd. LOC	0.065	0.008	8.188	**2.65e-16 *****
1Bd. RAIN	40.815	21.297	1.916	0.055	2Bd. RAIN	-3.040	5.439	-0.559	0.576289
Interaction models					*Interaction models*				
1Ca. LOC:SA (factor2)	-1.016	0.523	-1.941	0.052	2Ca. LOC:SA (factor2)	-0.461	0.109	-4.218	**2.47e-05 *****
1Ca. LOC:SA (factor3)	-0.928	0.522	-1.778	0.075	2Ca. LOC:SA (factor3)	-0.310	0.108	-2.861	**0.004224 ****
1Ca. LOC:SA (factor4)	-0.385	0.641	-0.601	0.548	2Ca. LOC:SA (factor4)	-0.396	0.128	-3.106	**0.001896 ****
1Cb. LOC:SEX (factor1)	0.187	0.136	1.379	0.168	2Cb. LOC:SEX (factor1)	0.193	0.021	9.253	**< 2e-16 *****
1Cc. LOC:SEASON(factor1)	0.046	0.131	0.355	0.723	2Cc. LOC:SEASON(factor1)	-0.112	0.019	-5.802	**6.54e-09 *****
1Cd. LOC:RAIN	22.450	17.926	1.252	0.210	2Cd. LOC:RAIN	-11.990	2.400	-4.995	**5.89e-07 *****

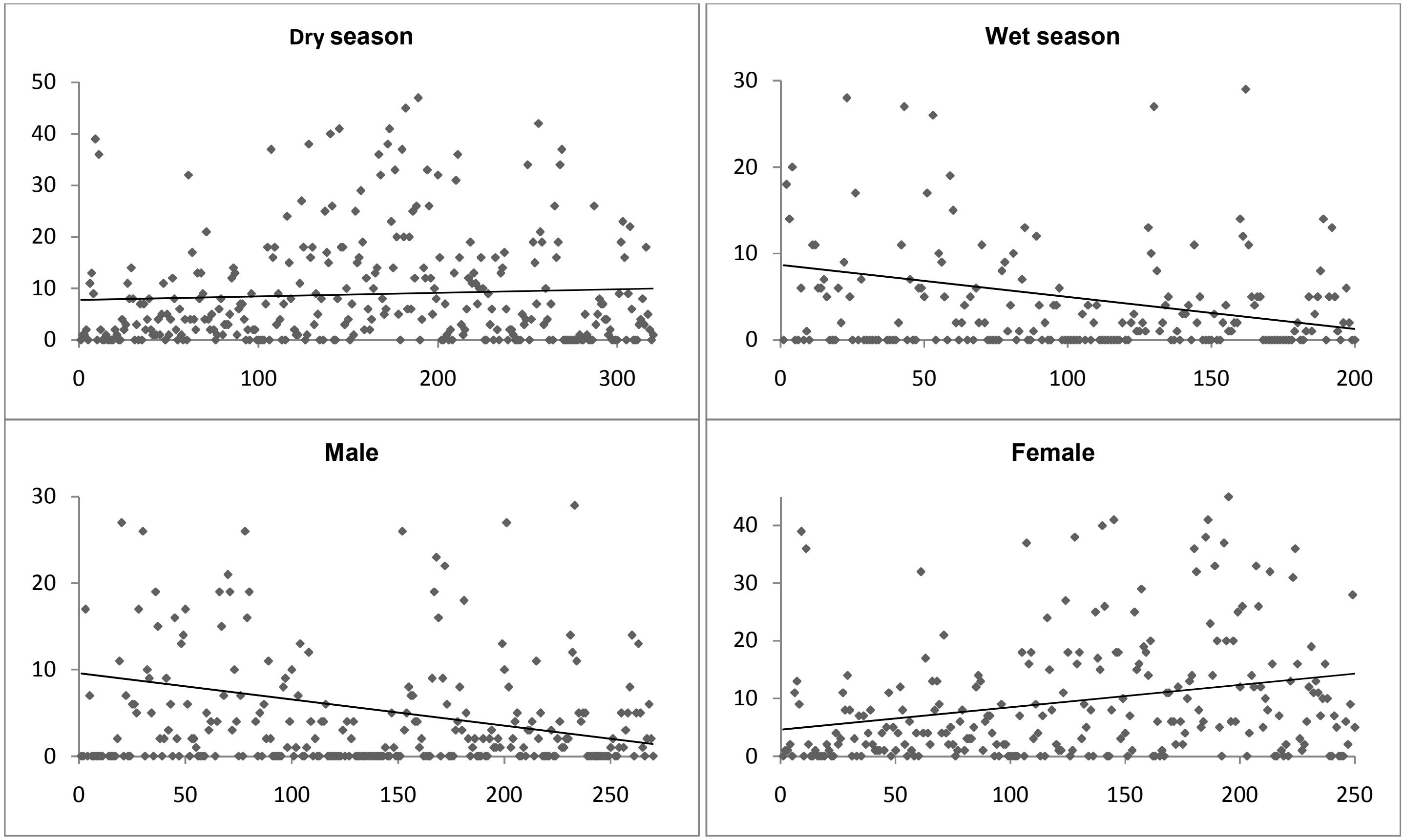

Figure 2 – Graphic analisys of otter locations (LOC - xx axis) and all spraints (ALLSP – yy axis) accordingly to SEASON (top) and SEX (bot).

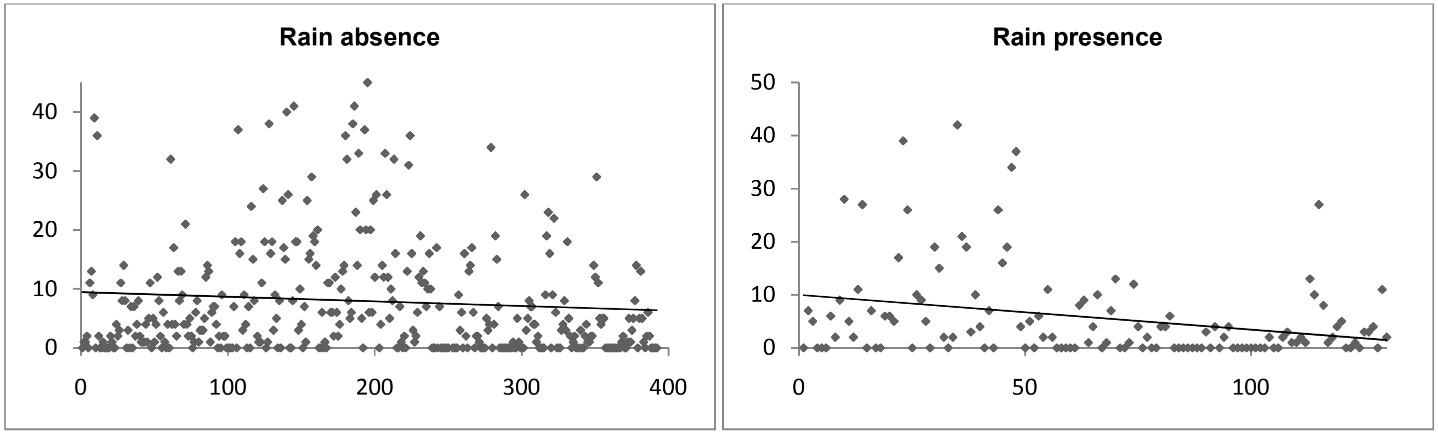

Figure 3 – Graphic analisys of otter locations (LOC - xx axis) and all spraints (ALLSP – yy axis) accourdingly to RAIN.

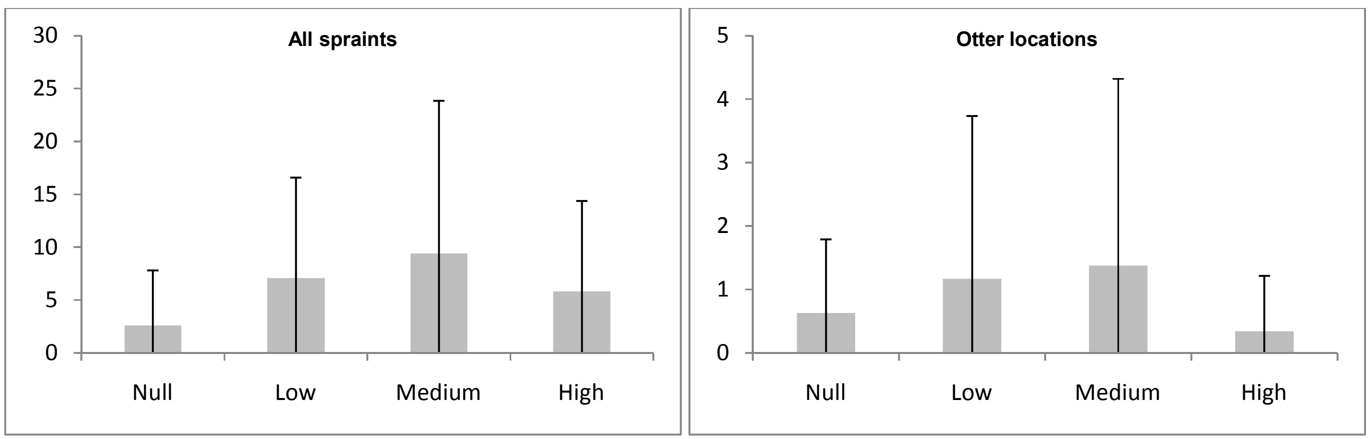

Figure 4 – Mean and standard deviation of number of otter spraints (left) and otter locations (right) in the four spraintability classes.

Discussion

This study aimed to investigate the usefulness of indirect surveys as a valid method to correctly assess presence and habitat use by otters. There has not been a true consensus on validating habitat use results obtained through spraint survey methodologies, since the mid 1980s (Kruuk *et al.,* 1986; Kruuk & Conroy, 1987; Mason & Macdonald, 1987; Mason & Macdonald, 1991). In order to address this problematic, we compared spraint survey data with radio-tracking otter locations, which is an unquestionably valid technique to assess habitat use. This enabled us to correlate spraint presence and abundance on river and reservoir stretches that we knew were used by radio-tracked otters, with otter locations. Although it was impossible to record all otter activity in every cycle, we were able to capture a fair amount of otter locations (536 locations, on average, more than 23 locations per cycle). Another important factor in this study is the influence of Mediterranean climate, as contrary results are often reported for northern or central European studies when compared with southern European ones. The seasonal marking pattern is one example of how different climates can influence results. Northern and central European studies found a regular pattern of increased marking activity in winter falling progressively to a low level in summer (Macdonald & Mason, 1987; Conroy & French, 1991). However, this is not true in Southern Mediterranean freshwater habitats, where broad fluctuations in marking activity are less evident, mainly depending on the region and year, and sometimes even showing summer peaks (Ruiz-Olmo & Gosálbez, 1997). For instance, our results showed a higher number of spraints found in the dry season, corroborating the difference in seasonal marking patterns between Mediterranean and northern European otters. This example emphasizes the need to be very cautious when extrapolating results to areas with very different natural conditions. Thus, our results should always be interpreted as being representative of Mediterranean climate.

We have found a positive relationship between fresh spraint presence in river and reservoir sections with otter activity from the previous night. A strong correlation between fresh spraints and otter visitations was also found by Guter, *et al.,* (2008) in northern Israel. Furthermore, our models revealed that this relationship is not influenced by otter sex, season, abundance of marking places or amount of rain. Thus, our study shows that otters tend to mark in river sections where they spend more time, contrary to the results obtained by Kruuk *et al.,* (1986). We also found a significant relationship between spraint abundance, considering all spraints found, and number of otter locations. However, this relationship was strongly influenced by all the other covariables. Although it is impossible and would be erroneous to assume that all spraints found the next morning belonged to the radio-tracked animal, our modelling showed that the animals' sex influences the relation between spraint abundance and otter locations, with the relationship being negative for males and positive for females. This can be explained by differences in the marking behaviour of each sex. Males tend to have larger home ranges (Kruuk, 2006) and a bigger vital area to mark. In our consideration, male spraints are usually smaller probably to achieve a higher marking capability. Thus, smaller spraints may be more easily destroyed and are less detectable. In fact, we found a higher number of fresh spraints following male activity cycles when compared with females, and a much lower number of all spraints were found following male activity cycles. This may explain why the relationship between activity and spraints is not straightforward in males.

Season and amount of rain also interacted significantly with spraint abundance and otter locations. Similarly to previous reports, our data showed a summer peak in otter activity, which can be related to a higher probability of spraints being removed during the wet season. In this season showers can be very intense resulting in relatively quick floods and in a high probability of spraints being washed away, thus influencing spraint counts. This fact is revealed by the negative relationship between the occurrence of rain and spraint abundance, whilst in absence of rain there is no clear trend between the number of spraints and otter locations. Moreover, it may also explain the opposite relationship between these two variables in the wet season, meaning that in wet season, using otter spraint surveys to evaluate space use may be misleading.

Abundance of prominent places was also taken into account, and as expected, has a strong relationship with spraint abundance. The abundance of spraints in spraintability classes was strongly significant when compared to the lower class. Thus, the absence of marking places inhibits otter marking activity. The importance of prominent places in otter sprainting activity is strongly related to otter scent communication (Kruuk, 2006). Our study shows that the relationship between spraint abundance and otter activity is strongly affected by the presence of adequate marking sites. In these circumstances we must be cautious as it can be erroneous to assume that low spraint abundance means low otter activity. We choose 100m section sampling unit arbitrary which can be influencing this interaction as suggested by Kruuk *et al.,* (1986) and Kruuk & Conroy, (1987). Further studies should be conducted in order to assess the influence of sampling size unit on the abundance of spraints according to abundance of prominent places and also in the relation to otter activity.

Overall, our results show that spraint surveys constitute an adequate technique to assess otter activity in a given area, despite the fact that absence of spraints does not necessarily imply otter absence as reported by Ruiz-Olmo and co-workers, (2001). The relationship between spraints and otter activity is particularly strong when considering abundance of all fresh and old spraints. However, our findings show that in spite of a significant relation between otter locations and spraint numbers, there are unquestionable factors that have a strong influence on that relation. Assessing otter habitat selection based only on indirect signs, without regarding factors such as season, amount of rain and sprainting ability, can lead to biased conclusions. Particular care must be taken when comparing regions or river sections with different availability of marking sites, as the number of spraints found is strongly influenced by them. Furthermore. one must take into account the differences in sprainting biology and behaviour according to sex.

Nevertheless, in terms of otter conservation and management and the need to set effective low cost methods that allow rough estimations of otter habitat selection, the evaluated survey techniques possess the basic requisites and appear to be suitable for these purposes in a Mediterranean environment.

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Considerações finais

A avaliação de diferentes metodologias de amostragem em campo, de modo a identificar a sua adequabilidade e validade enquanto instrumentos eficazes em Biologia da Conservação, apresenta-se de grande relevância, principalmente tendo em consideração a actual conjuntura mundial de crise económica. A necessidade de avaliar a presença de indivíduos de espécies prioritárias para a conservação como é o caso da lontra (*Lutra lutra*), que figura nos anexos II e IV da Directiva Habitats, bem como estimar os seus requisitos de habitat com metodologias eficazes e de baixo custo, é uma prioridade.

O presente trabalho demonstrou a existência de uma relação positiva entre troços de rio e margens de açudes utilizados intensamente pela lontra com a presença e abundância de dejectos deste mamífero nesses locais. Contudo, esta relação é influenciada pelo sexo dos indivíduos, pela abundância de sítios proeminentes para marcação, pela pluviosidade, bem como pela estação do ano. São ainda adiantadas algumas sugestões sobre o porquê desta interacção. Assim, de modo a extrapolar conclusões válidas no que à lontra diz respeito, a aplicação da metodologia da procura indícios de presença tem, necessariamente, de ter em conta a influência destas variáveis. Por outro lado, conclui-se também da relevância de conduzir estudos futuros, de modo a aferir a influência da unidade de amostragem na relação entre as localizações de lontra e os indícios de presença.

Em suma, a metodologia de prospecção de indícios de presença comprova-se útil na obtenção de uma estimativa geral das áreas de maior actividade de lontra. Desta maneira, obtém-se uma ferramenta válida e expedita que pode ajudar à conservação desta espécie carismática na bacia do Mediterrânico.

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