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Ecology and feeding behaviour of Common otter in the lower Mondego river valley

Dissertação de Mestrado em Ecologia,
orientada pelo Professor Doutor José Paulo Sousa e pela Doutora Joana Silva Alves (Universidade de Coimbra)
e apresentada ao Departamento de Ciências da Vida da Faculdade de Ciências e Tecnologia

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Dissertação apresentada à Universidade de Coimbra para cumprimento dos requisitos necessários à obtenção do grau de Mestre em Ecologia, realizada sob orientação científica do Professor Doutor José Paulo Sousa (Departamento de Ciências da Vida, Universidade de Coimbra) e da Doutora Joana Silva Alves (Centro de Ecologia Funcional)

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Resumo

A lontra comum (*Lutra lutra* L.) é um predador tipicamente piscívoro que está atualmente classificado como quase ameaçado na Europa. Este mamífero enfrenta muitas ameaças, como a fragmentação do seu habitat, a poluição dos cursos de água e a baixa disponibilidade de presas. No entanto, o seu estatuto de conservação em Portugal foi recentemente alterado para "Pouco Preocupante", facto que coincidiu com o aumento da dispersão de espécies introduzidas com potencial invasor no país. Após este fenómeno, a população de lontras em Portugal aumentou, e foi descrita como sendo uma das poucas populações a prosperar na Europa. Neste estudo pretende-se obter dados concretos sobre a ocorrência, abundância e atividade das lontras no Baixo Mondego, assim como compreender a evolução do seu comportamento alimentar após a introdução de espécies exóticas.

Os resultados obtidos resultam de métodos de foto-armadilhagem e de recolha e contagem de excrementos, e sua posterior análise laboratorial. Verificou-se que a lontra está bem distribuída no vale do Baixo Mondego, com maior ocorrência nos principais pauis (principalmente no Paul de Arzila) e na área a sul do vale, próxima dos campos de arroz. Os resultados apontam também para a plasticidade do comportamento alimentar da espécie, uma vez que foram detetadas alterações nos padrões de ocorrência e de composição alimentar de acordo com a sazonalidade e a abundância de alimento. Verificou-se que no inverno a lontra apresenta uma maior abundância em Arzila, movimentando-se no verão para a área envolvente do rio Arunca, composta maioritariamente por culturas de arroz com maior volume de água, o que proporciona uma maior disponibilidade de presas. No inverno, as presas principais foram as várias espécies de peixe, contrastando com a dominância no consumo de lagostim no verão, revelando um comportamento alimentar oportunista.

Após a introdução e expansão do lagostim, a dieta da lontra passou a ser quase exclusivamente constituída por esta espécie invasora. Atualmente, a sua dieta apresenta um menor consumo de lagostim, e um maior recurso às espécies de peixe. A evolução da composição da dieta de lontra poderá estar relacionada com uma diminuição na abundância de lagostim, consequência da sua predação por várias espécies de aves e mamíferos, incluindo a lontra.

Face aos resultados, a lontra pode estar a desempenhar um papel de espécie guarda-chuva através do controlo de espécies invasoras, como o lagostim, do qual beneficiam as espécies nativas. Este controlo de pragas permite que as espécies nativas recuperem nos seus efetivos populacionais, aumentando a biodiversidade dos ecossistemas ripícolas, para além de contribuírem para a redução dos danos causados às culturas de arroz por parte do lagostim, fornecendo assim um serviço de ecossistema, com benefícios diretos para as populações humanas e economia local.

Palavras-chave: Comportamento alimentar, espécies invasoras, *Lutra lutra*, plasticidade adaptativa, predador, *Procambarus clarkii*

Abstract

The Common otter (*Lutra lutra* L.) is typically a piscivorous predator that is currently classified as near threatened in Europe. This mammal species faces many threats, such as habitat fragmentation, pollution of water courses and low availability of prey. Nevertheless, its conservation status in Portugal has recently been changed to Least Concern, which was coincident with the increase of introduced species throughout the country. After this phenomenon, the population of otter in Portugal increased, and it was described as one of the few populations thriving in Europe. On the present study, it is intended to obtain robust data on the occurrence, abundance and activity of otter in the lower Mondego river valley, as well as to understand the evolution of their feeding behaviour after the introduction of exotic species.

The results obtained are the outcome of camera-trap methods and counting and collection of spraints, and its subsequent laboratory analysis. It was verified that otter is well distributed in the valley, with higher occurrences in the main marshes (mainly in Arzila Marsh) and in the south area of the valley, around the rice fields. The results also point to the plasticity of this species in terms of feeding behaviour, since were detected changes in their occurrence patterns and diet composition according to the seasonality and abundance of prey. It was verified that in winter the abundance of otter is higher in Arzila, followed by their movement in summer for the surrounding areas of the Arunca River, when the fields of rice hold larger amounts of water, which provides a greater availability of prey. In winter, the main preys were the several fish species, contrasting with the dominance in the consumption of crayfish in the summer, revealing an opportunistic feeding behaviour of otter.

After the introduction and expansion of crayfish, otter's diet became almost exclusively constituted by this invasive species. Currently, the otter presents a lower consumption of crayfish, and a greater use of fish species. The evolution of the composition of the diet of otters may be related to a decrease in the abundance of crayfish, which can be a consequence of its predation by several species of birds and mammals, including Common otter.

Based on our results, the Common otter may be playing a role as umbrella species through the control of invasive species, such as crayfish, from which the native species benefit. This pest control allows the populations of native species to recover, increasing the biodiversity of the riparian ecosystems, as well as contribute to the reduction of damages caused to rice crops by crayfish, and thus providing an ecosystem service which direct benefits to human populations and the local economy.

Key-words: Adaptive plasticity, foraging behaviour, invasive species, *Lutra lutra*, predator, *Procambarus clarkii*

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1. Introduction

The Common otter (*Lutra lutra* L.) is a semi-aquatic mammal with special adapted features that allows the species to have efficiency whether inside and outside of water (Pfeiffer and Culik 1998). Its morphological characteristics allow it to be an effective carnivore that is highly specialized in fish. It inhabits in riparian habitats near unpolluted water courses, in places with abundant vegetation, shelters and refuges (Chanin 2003). In freshwater habitats, the species acquires a nocturnal behaviour since it feeds on diurnal prey. This commonly happens in Portugal and most of Europe (Kruuk 2006). This mammal feeds mainly on fish, but can also feed occasionally on amphibians, invertebrates or even small reptiles and passerines (Silveira and Reis 1991, Martins et al. 2002). Some of the fish present in its diet are anguilids, centrarquids, cyprinids, mugilids and salmonids, according to their availability in the environment (López-Nieves and Casal 1984, Beja 1996, Gourvelou et al. 2000, Clavero et al. 2003, McCafferty 2005, Freitas et al. 2007, Sales-Luís et al. 2007).

The populations of this species are subject to several kinds of threats, from the fragmentation of habitat to all kinds of anthropogenic pressures, such as exploitation or construction of linear infrastructures, like dams and reservoirs. One of the major threat's Common otter is facing in Europe is the deterioration of their habitats, which associated to pollution, accidents, harassment, and fish availability, are contributing to the decline of otter populations around the world, and particularly in Europe (Foster-Turley et al. 1990).

The declining of the populations of Common otter in Europe is a topic that has been addressed many times since its start (Chanin and Jefferies 1978, Cortés et al. 1998, Conroy and Chanin 2000). In global terms, this species of otter presents a wide distribution, occurring throughout Europe and part of Asia. Nevertheless, its European Conservation status has changed from Vulnerable to Near-Threatened in 2004 because of trend of the populations decline in general (Cabral et al. 2005, Roos et al. 2015). Despite this global trend, in Portugal there are some populations that are widespread and thriving, occurring in several types of habitats (Trindade 1994), from streams to water reservoirs (Pedroso et al. 2004, Pedroso and Santos-Reis 2006), near rice fields

(Martins et al. 2002) or in coastal areas (Beja 1995). This population is considered as one of the most viable in southern Europe (Foster-Turley et al. 1990) and the national conservation status has changed to Least Concern in 2005 (Cabral et al. 2005).

The occurrence or absence of otters in a certain area may be the reflect of many factors, such as the overall fish biomass, the water course quality, the pollution, or the presence of infrastructures. However, the distribution of otters is linked not only to a single factor, but to a combination of them all (LaFontaine et al. 1998). Due to its sensibility to environmental pollution, and to changes in their habitats, the otter has been indicated as a good bioindicator of water quality and conservation of riparian habitats (Ruiz-olmo et al. 1998). However, it is important to highlight that its sensibility may be a consequence of the availability of fish, since polluted environments are normally associated with a decrease, or even absence, of fish diversity and abundance (Collares-pereira and Cowx 2004, Leitão et al. 2007). Food availability is perhaps the main determining factor for otter's occurrence, since these animals are food-limited mammals, and an association between the availability of food and the presence of otter has been addressed several times (LaFontaine et al. 1998, Ruiz-Olmo et al. 2001, Chanin 2003). It was also stated that the numbers of individuals using the water courses fluctuates according to the abundance of prey, in a classic predator-prey relationship (Ruiz-Olmo et al. 2001).

The thriving of the populations in some areas is assumed to be happening due to the high plasticity of otters' foraging behaviour (Krawczyk et al. 2015), since otters are able to adjust their habitat use depending on the food availability, more than due to the presence of potential threats, whether natural or human-made (Krawczyk et al. 2011, Martínez-Abraín and Jiménez 2015). This leads to an occupation of suboptimal habitat types (Pedroso et al. 2004, Martínez-Abraín and Jiménez 2015).

Many factors are interfering with the populations of the native fish that constitute the main prey for otters. The seasonal droughts in the streams, the habitat degradation, the industrial pollution and the introduction of exotic species contribute to a marked variability in the composition of river communities, and constitute the most serious conservation problems for Portuguese endemic fish (Pires et al. 1999). Ecologically, this constitutes a serious issue in terms of conservation of the populations of otter.

In Portugal, several exotic fish species have been introduced over the last decades. Many of these species have potential traits that allow them to become successful invaders. These characteristics include great capacity of survival, reproduction and excellent tolerance to inhospitable conditions within the environment, having dispersed throughout practically all Portuguese territory (Ribeiro et al. 2008).

At the end of the 17th century, the species *Carassius auratus* L. and *Cyprinus carpio* L. have been introduced, one for ornamental and the other for food purposes, respectively (Ribeiro et al. 2009). The invasion process of these species, introduced so long ago, remains stable at a certain stage. Although the apparent stability and restricted range of the populations, it is important to keep present that any favourable change on the environment may lead a species to become a widespread invader at any time (Clavero and Villero 2014).

In the 1960s and 1970s, two other species were introduced in Portugal, the pumpkinseed *Lepomis Gibbosus* L. for ornamental purposes, and the largemouth bass *Micropterus salmoides* Lacepède for fishing purposes (Ribeiro et al. 2009). These species have had a solid impact on trophic levels, and have interfered with the biodiversity of the native species due to its predation abilities (Godinho and Ferreira 1998, Almeida et al. 2012).

Later, in 1979, it was discovered a new species that would become one of the most problematic invasive species in Portugal, the red swamp crayfish *Procambarus clarkii* Girard, which occurrence in was first described by Ramos and Pereira 1981. The first occurrence of crayfish in the Mondego river valley was registered in 1987 (Anastácio 1993). This crayfish has a large niche breadth and presents high trophic diversity (Correia 2002), being an opportunistic omnivore that has competed with, and reduced several different populations of aquatic species, including amphibians, molluscs, macroinvertebrates and fish (Cruz et al. 2006, Global Invasive Species Database 2017).

Many studies on the diet of Common otter have shown a relation between the availability of food and the consumption of certain prey items, according to the seasonal population structures and fluctuations of prey species (Beja 1996, Correia 2001, Ruiz-Olmo et al. 2001, Clavero et al. 2003). This carnivore is able to change its diet in response to the increase in the number of invasive species in the ecosystems, and all these invasive species have been reported in studies on Common otter diet as preys that have large contributions in its diet, despite its slight preference for the consumption of eel *Anguilla anguilla* L. and barbel (López-Nieves and Casal 1984, Beja 1996, Ruiz-Olmo and Palazón 1997, Gourvelou et al. 2000, Correia 2001, Ruiz-Olmo et al. 2001, Martins et al. 2002, Ruiz-olmo et al. 2002, Pedroso and Santos-Reis 2006, Sales-Luís et al. 2007, Novais et al. 2010).

As Common otter is an opportunistic top predator (Ruiz-olmo and Jiménez 2008, Ritchie et al. 2012), it has an important role in maintaining the balance within the ecosystem through top-down control of invasive species (Roemer et al. 2009), regulating the populations of preys (Ritchie et al. 2012), and therefore, preserving the biodiversity of freshwater ecosystems, as well as preserving its own species. The importance of the bottom-up control is relevant, since the availability of preys is not constant over time, and may also have an impact in the abundance of otter. Conservation strategies for the protection of Common otter acquire an added importance because of its role as an umbrella species, since its conservation will bring stability to other populations of native species whose niche has been gradually occupied by exotic and invasive species (Ritchie et al. 2012).

Our aims in this study rely on the need to acquire more information on the abundance and occurrence of Common otter along the lower Mondego river basin, as well as, to understand the feeding behaviour of this species. We intend to understand whether the food resources used are mostly dominated by invasive or native species, and how this has evolved over time by analysing results from studies done immediately after the introduction of crayfish in Mondego river valley (1989), 15 years after (2004), and comparing those with the ones from this study.

Therefore, we hypothesise that it will be notorious the effect of invasive species on the feeding behaviour of Common otter, and that the occurrence of this top-predator along the Lower Mondego river valley will be related to the abundance of crayfish. Thus, it is expected that a high abundance of invasive species in the environment will translate into a high occurrence of invasive species in otter's diet, proving the opportunistic feeding behaviour of the studied species. Due to the close relationship between the presence of invasive species in the environment and their occurrence in the otter's diet, it is expected that a higher density of invasive species will be reflected as an increase in the abundance of otters along the lower Mondego river valley, revealing an adaptive habitat selection.

2. Materials and methods

2.1 Study area

The study area is the lower Mondego river valley, that has an area of about 250km^2 and belongs to the Mondego river basin. The valley is located in the central region of Portugal, between the city of Coimbra and Figueira da Foz (Fig.1). The climate of this region is predominantly Mediterranean (Archibold 1995), and the majority of the area is used as intensive agricultural fields, producing mainly rice and corn. The area has already suffered many different modifications along the years, as well as changes on the water course, modifications in the drainage system and designations of field structures, resulting in a reduction of the extension of its riparian vegetation (Martins et al. 2002).

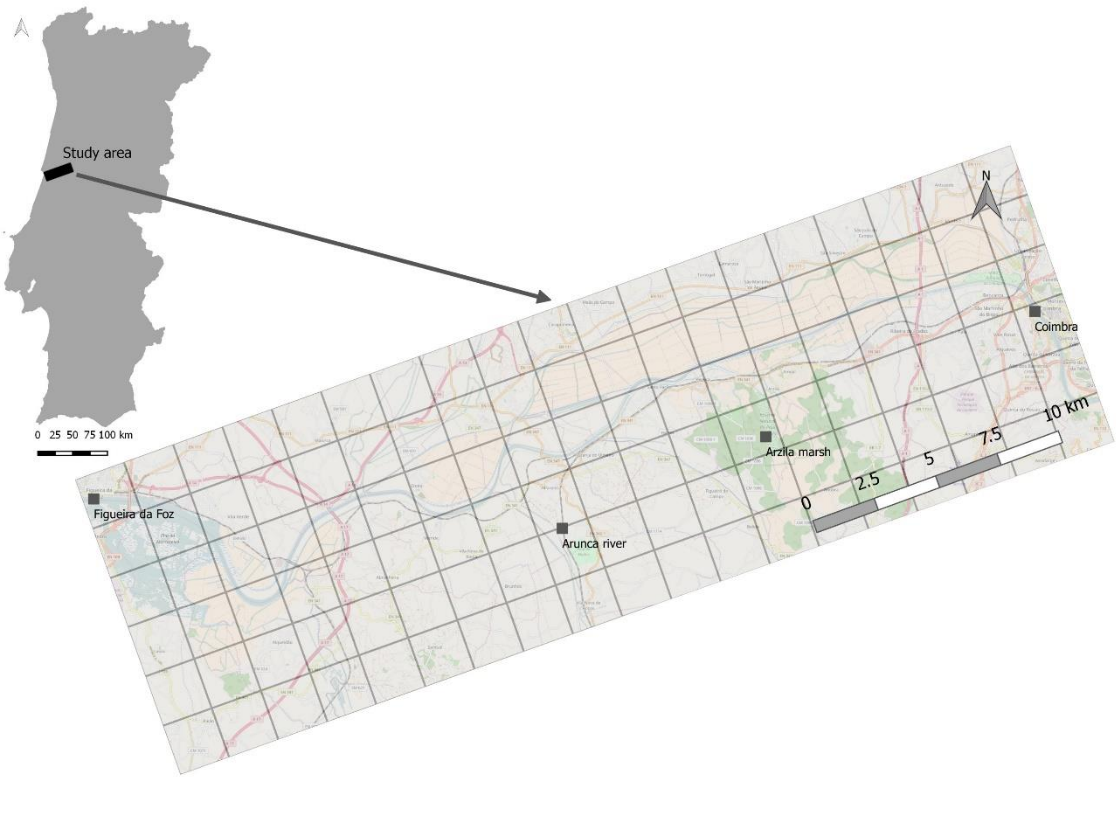

Fig. 1 – Map of the lower Mondego river valley.

The lower Mondego river valley comprises several important protected areas, such as Arzila, Taipal and Madriz Marsh, belonging to the National System of Protected Areas, and established by Ramsar Convention, Habitats Directive (92143/CEE) and Birds Directive (79/409/CEE).

An initial survey was carried out in the all area to determine the study sites that would be used as sub-areas of study. Two study sites were selected, being the first the south tributary river of Mondego River, denominated Arunca River (40º08'N, 8º39'W), and the second the Arzila Marsh. The first site was selected due to its suitability of the presence of a resident otter sub-population, since it is mostly constituted by rice fields, which have high follow of water during most of the year. The second one study site, the Arzila Marsh (40º10'N, 8º33'W), has a well-known occurrence of otters (Martins et al. 2002, Pinto 2004), allowing a short timescale collection of otter spraints.

2.2 Data collection

The field prospection and gathering of otter evidences occurred along the lower Mondego river valley. The prospection was made using a grid of 2km by 2km, and in each of the quadrants, transects of 100m were walked to detect signs of presence of otter. These transects were visited in January and June, and all evidences of otter, i.e. footprints and spraints (faeces) were registered. The collection of samples was conducted by following the IUCN method (IUCN 1990) to determine the distribution and occurrence of otters. Additional surveys were conducted near Pranto River and the marshes of Taipal and Madriz to determine the presence and relative abundance of Common otter. All the collected information was georeferenced and analysed in QGIS, 2.18.11 version.

The diet composition of the otter was assessed through the faecal analysis of the spraints collected in the Arunca River and Arzila Marsh. The samples of Arunca River were collected at the same time of each field prospection, in January and June (winter and summer season, respectively). In Arzila, the spraints were collected at a monthly basis, between September 2016 and June 2017, along two transects, defined within the Marsh, along the watercourse. The first transect, downstream, was 550 meters long, and the second transect was 270 meters long to upstream.

All the spraints found were stored in plastic bags and labelled. It was also registered the number of spraints in the same place, GPS coordinates and other relevant environmental information. Subsequently, the collected spraints were frozen and stored for later analysis. All the information was registered in a database for further statistical analysis.

2.3 Diet composition

The laboratory analysis to identify the species present in the spraints started by drying the spraints at 60ºC for 48 hours, and posterior weighted to obtain the dry weight of each sample. Each spraint was placed within a container with detergent and water, and left overnight to remove the mucilaginous component of the spraints (Kingston et al. 1999, Gourvelou et al. 2000). After complete dissolution of the mucus, the samples were washed on three sieves of 0.5mm, 1mm and 2mm mesh, to remove all detergent residues as described by Kingston et al. 1999 and Sales-Luís et al. 2007. Three sieves were used to facilitate the separation of the remains by size, and therefore, enabling an easier separation of the prey remains present. Afterwards, the feeding remains were dried once again, at 60ºC for 48h (Kingston et al. 1999, Taastrùm and Jacobsen 1999, Novais et al. 2010).

Afterwards, the remains were sorted using a magnifying glass (x10-20), and the fish species were identified using reference collection and dichotomous keys for scales and fishtail vertebrae (Prenda et al. 1997, Novais et al. 2010). When identification was not possible, the fish remains were classified as unidentified fish ("Fish NI"). The identification of crayfish was reasonably simple due to its characteristic colour and structure of the exoskeleton (Sales-Luís et al. 2007).

2.4 Crayfish captures

To compare the abundance of the crayfish in the study sites with the occurrence of crayfish in the diet of otter, 10 crayfish traps were set monthly in Arzila Marsh (from December 2016 to June 2017). The traps were set at 100 meters interval along the downstream and upstream transects. Furthermore, crayfish traps were also set in the south and north area of the lower Mondego river valley. The traps were left overnight, and all the crayfish captured were counted and stored in the following morning (Sales-Luís et al. 2007).

2.5 Camera-trap surveys

One Bestok camera-trap was used to collect photos and videos of the otters. The recordings were made from March to May in Arzila Marsh. The main goal was to register the specific behavioural patterns of the species in our study area.

The camera was placed in a fixed-point, selected based upon the existence of otter evidence. A fish bait was placed near the camera to attract the individuals. The camera was set to take 1 photo (with 12MB resolution) and 1 video per minute per contact. The date and time were recorded in all photos and videos, and used for data analysis.

2.6 Statistical analysis

The behavioural activity patterns were estimated by fitting kernel density functions (Ridout and Linkie 2009) to temporal activity patterns of otters extracted from camera-trap data.

The diet composition was expressed in terms of percentage of dry weight (%DW), percentage of biomass (%BM) (calculated based on the digestibility coefficient described by Fairley et al. 1987), frequency of occurrence (FO) and relative frequency of occurrence (RFO). These frequencies were calculated as follows:

$$\%DW = \frac{Weight\ of\ each\ prey\ item}{Total\ weight\ of\ prey\ items} \times 100$$

$$\%BM = \frac{(Weight\ of\ each\ prey\ item\ \times Digestibility\ coefficient)}{Total\ (Weight\ of\ each\ prey\ item\ \times Digestibility\ coefficient)} \times 100$$

$$FO = \frac{Number\ of\ occurences\ in\ spraints}{Total\ spraints} \times 100$$

$$RFO = \frac{Number\ of\ prey\ items\ in\ each\ food\ category}{Total\ prey\ items} \times 100$$

Multivariate analysis was performed to evaluate the differences in the diet composition between the months, sites and seasons. Multivariate techniques are used because they detect and represent the underlying structure of the data, and have the capability to discriminate different groups. These analyses consisted of one ordination method, more specifically principal component analysis (PCA), and a permutation multivariate analysis of variance (PERMANOVA). The statistical analyses were performed using Canoco 5 and Primer 6 & Permanova+ software.

3. Results

3.1 Occurrence, abundance and activity of Common otter in the lower Mondego river valley

Based on a relative abundance of otter's signs found in each quadrant, it was possible to discriminate locations of unconfirmed, possible or confirmed occurrence of otters along the lower Mondego river valley. Otters were not randomly distributed among the study area. Results show that the number of indirect signs of otter's occurrence was higher in the south part of the river valley. Locations with confirmed otter occurrence were mostly situated near the rice fields or in protected areas, such as Arunca river area and the Taipal and Arzila Marsh, respectively (Fig.2).

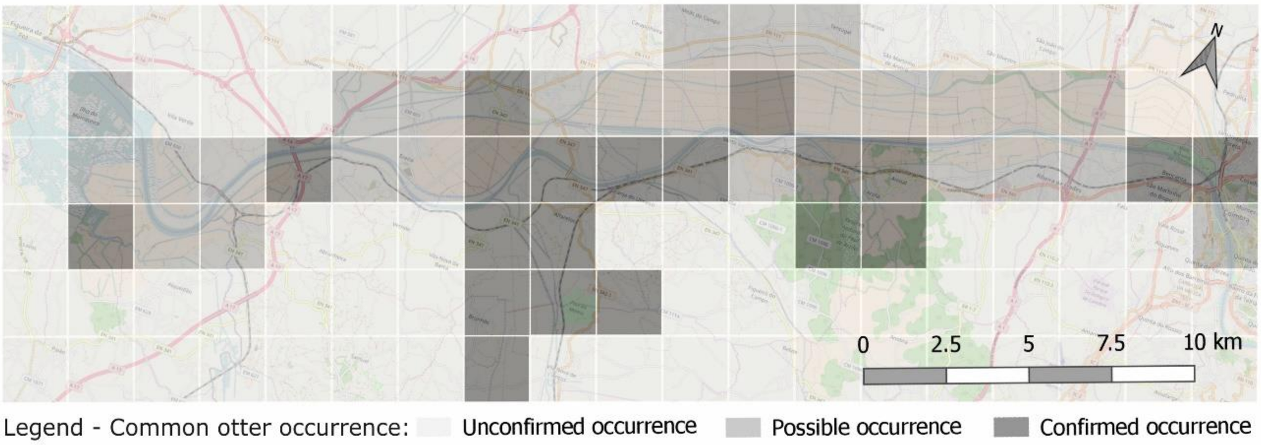

Fig. 2 – Map of occurrence of Common otter in the lower Mondego river valley.

In Arzila, otter's behaviour is mostly nocturnal, with activity occurring between 18:00h and 6:00h. The sunset and early night periods (19h to 21h) seem to be the hours with the highest activity, indicating a crepuscular behaviour (Fig.3).

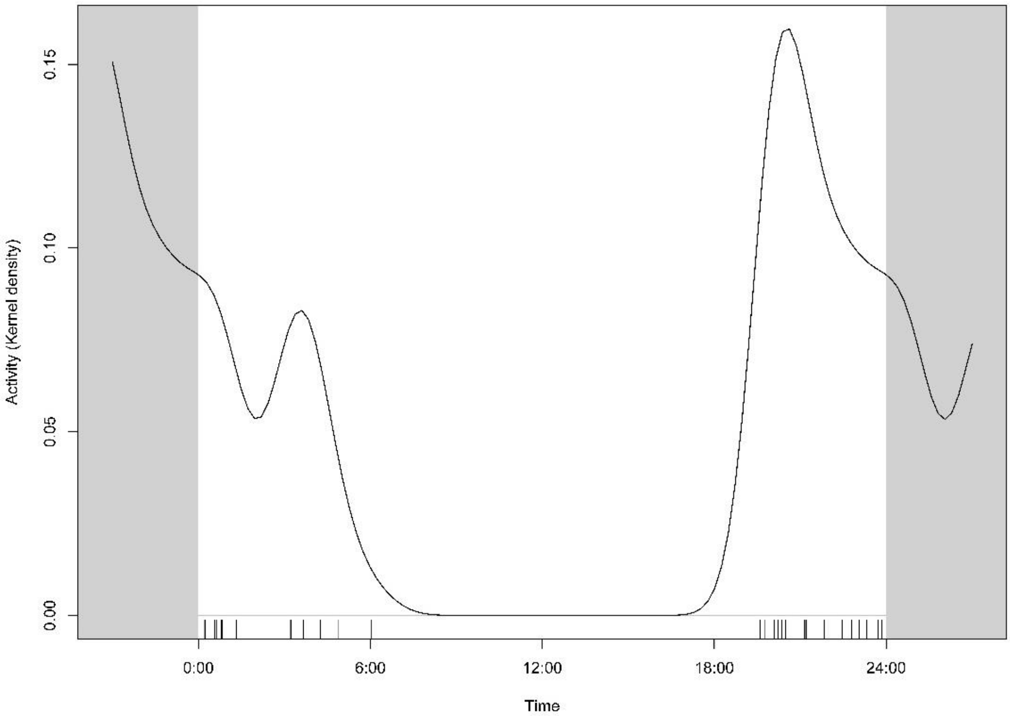

Fig. 3 – Activity pattern of Common otter in Arzila Marsh.

Regarding the abundance of otter signs (mostly spraints), in Arzila a higher number of spraints was found during winter (N=53), while in the Arunca River, the highest number was found during summer (N=33). Considering results from the monthly sampling performed in Arzila Marsh, the figure 4 shows that the months with the highest number of spraints were January and February. After March, the number of spraints decreased, to a minimum of 11 spraints in June (Fig.4).

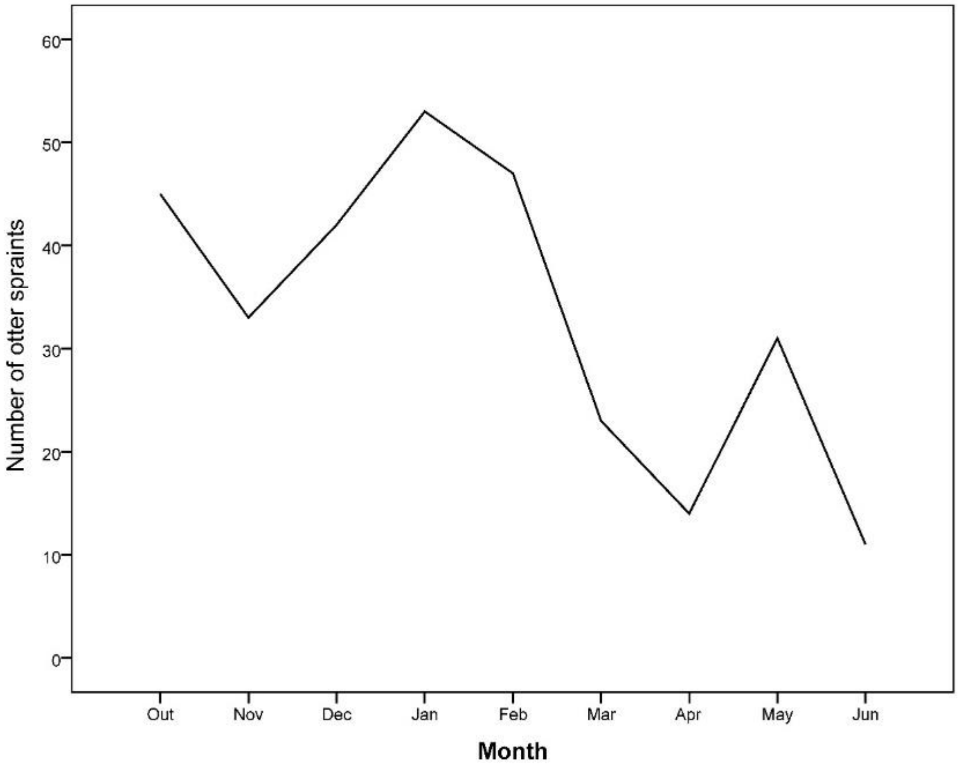

Fig. 4 – Number of collected otter spraints in each month, in Arzila Marsh.

3.2. Feeding behaviour of Common otter in the lower Mondego river valley

A total of 118 spraints was collected from Arunca River and from Arzila Marsh in winter and summer, containing nine prey items (table 1).

Table 1 – Frequency of occurrence (%FO) and relative frequency of occurrence (%RFO) of the prey items found in otter spraints collected in the two study areas (Arunca River and Arzila Marsh) in winter and summer.

	Arunca River				Arzila Marsh			
	winter (N=21)		**summer** (N=33)		**winter** (N=53)		**summer** (N=11)	
	%FO	%RFO	%FO	%RFO	%FO	%RFO	%FO	%RFO
Invertebrates								
Red swamp crayfish (*P. clarkii* Girard)	42.86	18.00	100	62.26	45.28	30.77	100	73.33
Fish (total)	90.48	82.00	51.52	37.74	83.02	69.23	36.36	26.67
Native Fish								
Barbel (*L. bocagei* Steindachner)	4.76	2.00	0.00	0.00	7.55	5.13	0.00	0.00
Brown trout (*S. trutta* L.)	23.81	10.00	0.00	0.00	11.32	7.69	0.00	0.00
European eel (*A. anguilla* L.)	4.76	2.00	0.00	0.00	0.00	0.00	36.36	26.67
Thin-lipped grey mullet (*L. ramada* Risso)	42.86	18.00	0.00	0.00	24.53	16.67	0.00	0.00
Introduced Fish								
Goldfish (*C. auratus* L.)	19.05	8.00	6.06	3.77	16.98	11.54	0.00	0.00
Largemouth bass (*M. salmoides* Lacepède)	61.90	26.00	3.03	1.89	20.75	14.10	0.00	0.00
Pumpkinseed (*L. gibbosus* L.)	38.10	16.00	45.45	28.3	3.77	2.56	0.00	0.00
Wild common carp (*C. carpio* L.)	0.00	0.00	0.00	0.00	7.55	5.13	0.00	0.00
Fish NI	0.00	0.00	6.06	3.77	9.43	6.41	0.00	0.00

The winter, otter's diet was dominated in terms of frequency of occurrence by fish preys, both native and introduced, in both study sites. In Arunca River and Arzila Marsh there was a prevalence of introduced fish over the native, being the thin-lipped grey mullet the most frequent of the native species and the largemouth bass of the introduced species (table 1).

In summer, the consumption of crayfish was higher than the consumption of fish preys. In the Arunca River, no native species of fish were consumed in summer, while in Arzila, no introduced fish species were consumed by otters in this season (table 1), leading to the differences obtained between the two study sites for summer season ($t = 3.037; p = 0.001$).

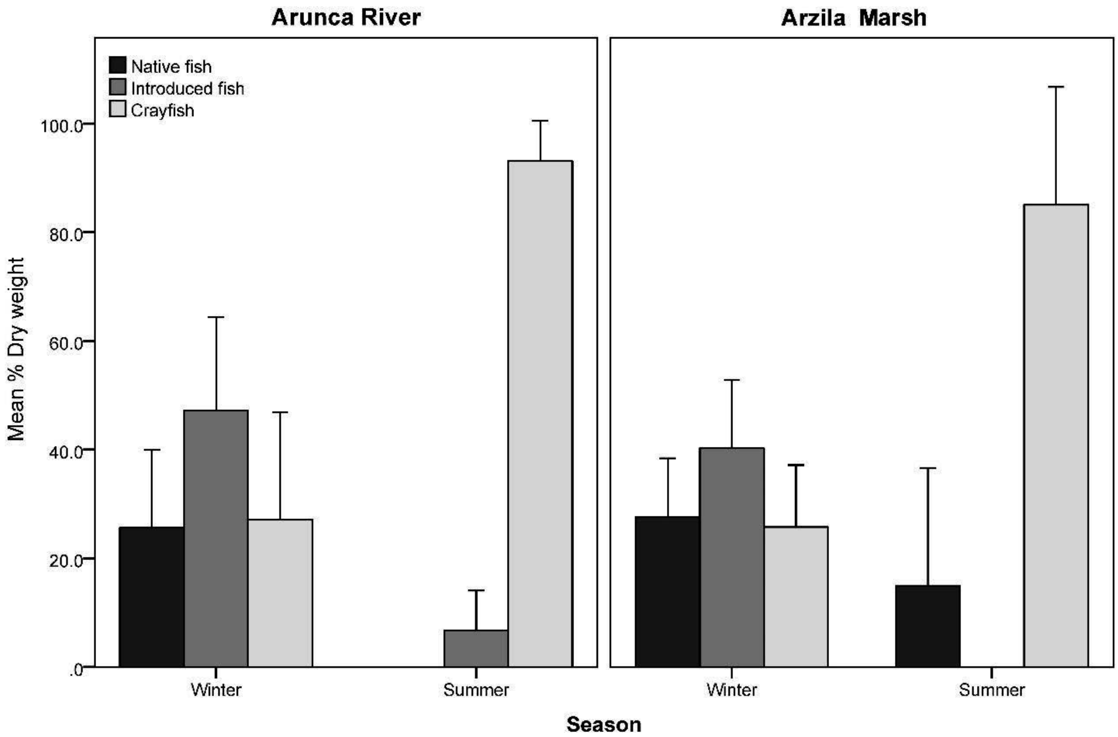

Fig. 5 - Mean percentage of the dry weight of Native fish, Introduced fish and crayfish consumed by otters in winter and summer in the two sites (Arunca River and Arzila Marsh).

The mean percentage of dry weight of each food category consumed by otters revealed that there were significant differences between seasons ($pseudo - F_{(1,112)} = 7.5153$; $p = 0.001$) and between the study sites ($pseudo - F_{(1,112)} = 2.0458$; $p = 0.032$). However, the interaction between the factors was not significantly different ($pseudo - F_{(1,112)} = 1.6705$; $p = 0.104$) (Fig.5).

In winter, diet compositions in the two study sites were not significantly different ($t = 0.95454; p = 0.51$), contrarily to summer, where significant differences were found between the two study sites ($t = 2.475$; $p = 0.008$).

Regarding the analysis of the percentage of biomass, the results agree with those obtained with the % of dry weight, showing significant differences between the two study sites also in summer ($t = 2.5624$; $p = 0.006$).

3.3 Temporal feeding behaviour of Common otter in the Arzila Marsh

From the 558 occurrences found in Arzila Marsh, 376 samples were collected and analysed. The most frequent prey item found in the diet in Arzila Marsh was the crayfish (RFO=53.94%), while fish species represent 46.06% of all prey items. However, according to the values of biomass, globally the percentage of fish biomass consumed (58.88%) overcomes the biomass of the crayfish (41.12%). Within the category of the native fish, it is noticeable the frequent occurrence of the European eel (RFO=7.89), followed by the barbel (RFO=4.84%). Regarding the introduced fish, it is noticeable the higher occurrence of the wild common carp (RFO=8.06%) and goldfish (RFO=7.35%). In terms of percentage of biomass, it can be observed the higher contribution of barbel and goldfish for the otter's diet, with 11.33% and 12.64%, respectively (table 2).

Table 2 – Diet composition of Common otter in Arzila Marsh in terms of Percentage of dry weight (%DW), Percentage of biomass (%BM), Frequency of occurrence (%FO) and Relative frequency of occurrence (%RFO).

Prey items	%DW	%BM	%FO	%RFO
Invertebrates				
Red swamp crayfish (*P. clarkii* Girard)	71,38	41,12	80,05	53,94
Fish (total)	28,62	58,88	56,91	46,06
Native Fish				
Barbel (*L. bocagei* Steindachner)	5,51	11,33	7,18	4,84
Brown trout (*S. trutta* L.)	1,65	3,4	3,99	2,69
European eel (*A. anguilla* L.)	2,16	4,43	11,70	7,89
Thin-lipped grey mullet (*L. ramada* Risso)	2,23	4,58	5,05	3,41
Introduced Fish				
Goldfish (*C. auratus* L.)	6,14	12,64	10,90	7,35
Largemouth bass (*M. salmoides* Lacepède)	3,43	7,05	6,91	4,66
Pumpkinseed (*L. gibbosus* L.)	0,35	0,72	3,19	2,15
Wild common carp (*C. carpio* L.)	5,12	10,54	7,45	5,02
Fish NI	2,04	4,19	11,97	8,06

When analysing the mean percentage of dry weight of the three main categories of prey items (Native fish, Introduced fish and crayfish), it is possible to observe its fluctuations over time (Fig.6). In most of the months, the crayfish had an important presence in the diet, with its peak of abundance decreasing in the middle of November, reaching its minimum values in December, and then rising again until May. The presence of introduced fish showed the opposite pattern, having its peak of abundance in December and January, and being absent from April to June. Regarding the native species of fish, although lower in terms of absolute values, its presence was quite regular along the months, with a slight increase in November, December and January (Fig.6).

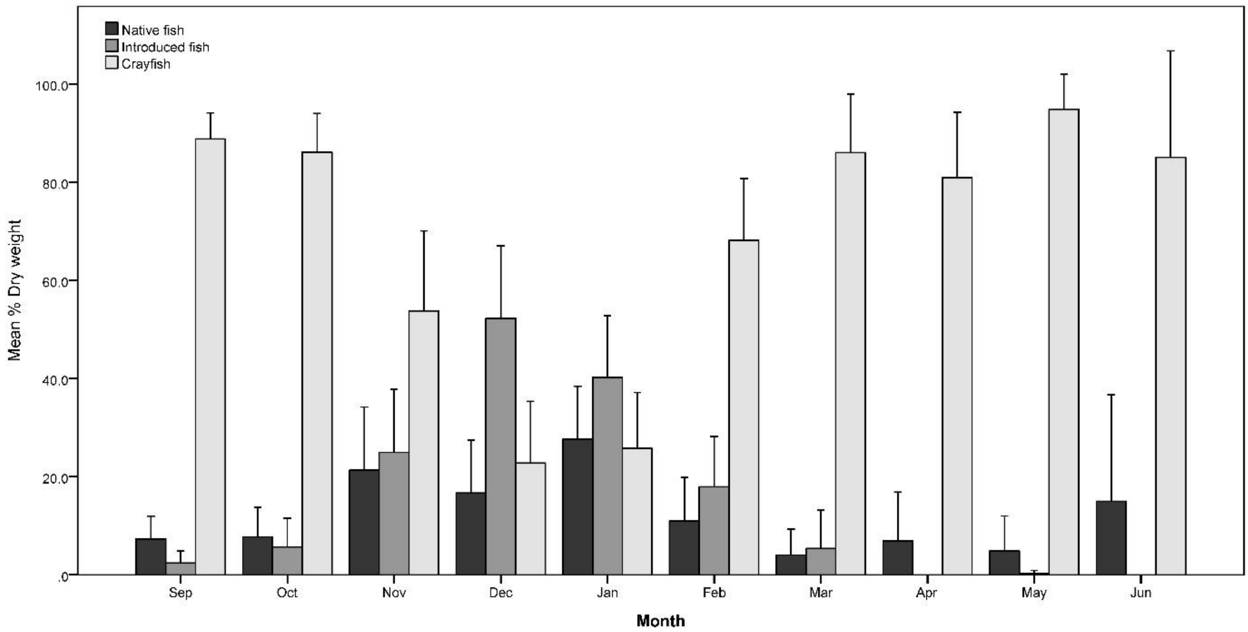

Fig. 6 - Mean percentage of dry weight of Native fish, Introduced fish and crayfish in otter's diet along the months in Arzila Marsh.

The PCA biplot explains 64.4% of the total variance in the diet composition. The first axis represents a positive gradient of abundance of crayfish (PClark), while the second axis represents the gradient of fishes' abundance (Fig.7). Analysing the variation of the diet composition and the preferences of species throughout the months in function of all the prey found, it is possible to see that red swamp crayfish is an important prey in warmer months (September, October and from March to June). In December, an extremely high contribution of the goldfish (CAurat) to the diet of otter was found, being the other species of fish more present in November and January (Fig.7).

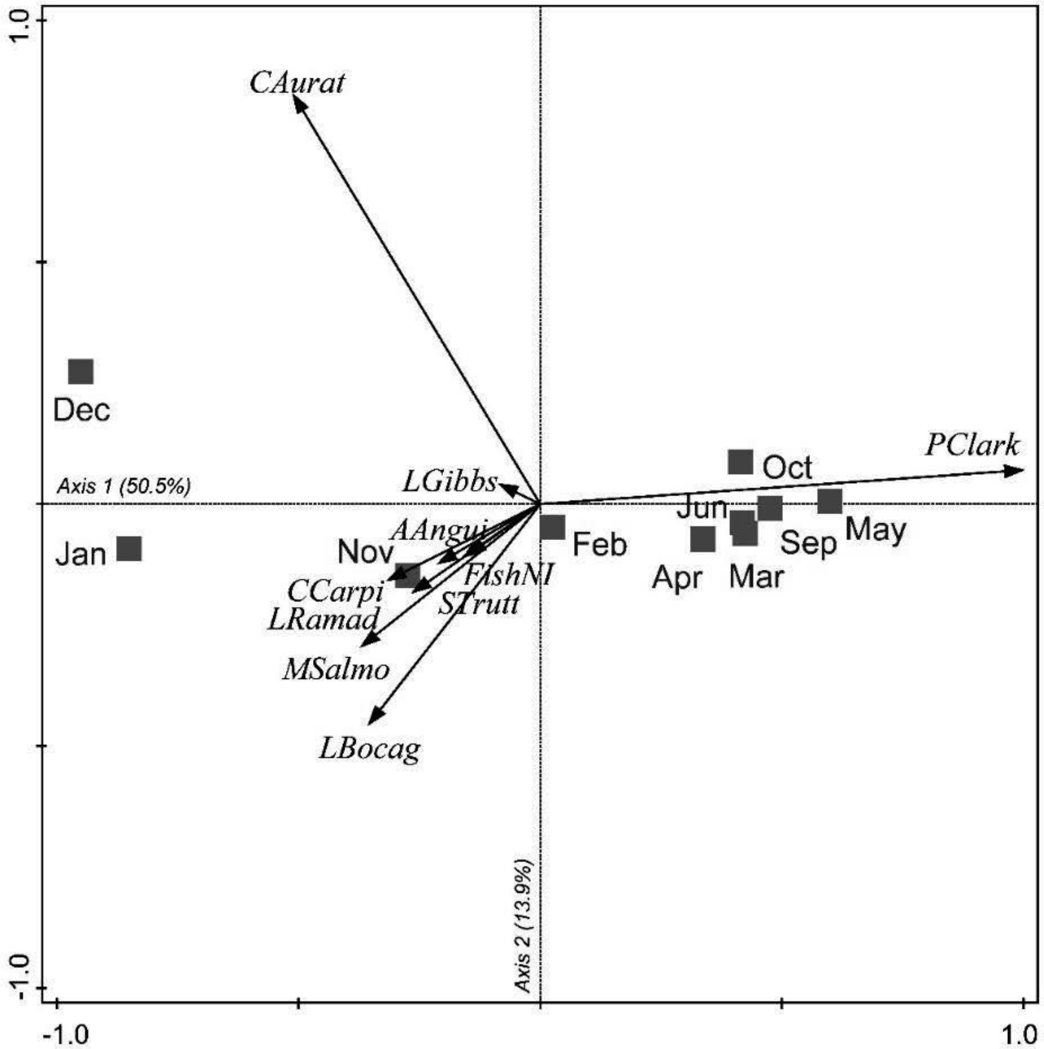

Fig. 7 - PCA biplot of diet composition of Common otter showing the differences between months. Legend: PClark – crayfish; CAurat – goldfish; LGibbs – pumpkinseed; AAngui – European eel; Strutt – brown trout; CCarpi – wild common carp; LRamad – thin-lipped grey mullet; MSalmo – largemouth bass; LBocag – barbel; FishNI – unidentified fish

The presence of the native fish species in the diet is reasonably regular along the months, although with abundances always lower than 20%. The consumption of barbel was higher between November and February, month when it starts to decrease (Fig.8a). Following the trend of fish species in general, brown trout and thin-lipped grey mullet are more relevant for otter diet during the winter months (Fig.8b & 8d). The European eel seems to be more relevant during the spring months, being the fish species more consumed during this period (Fig.8c).

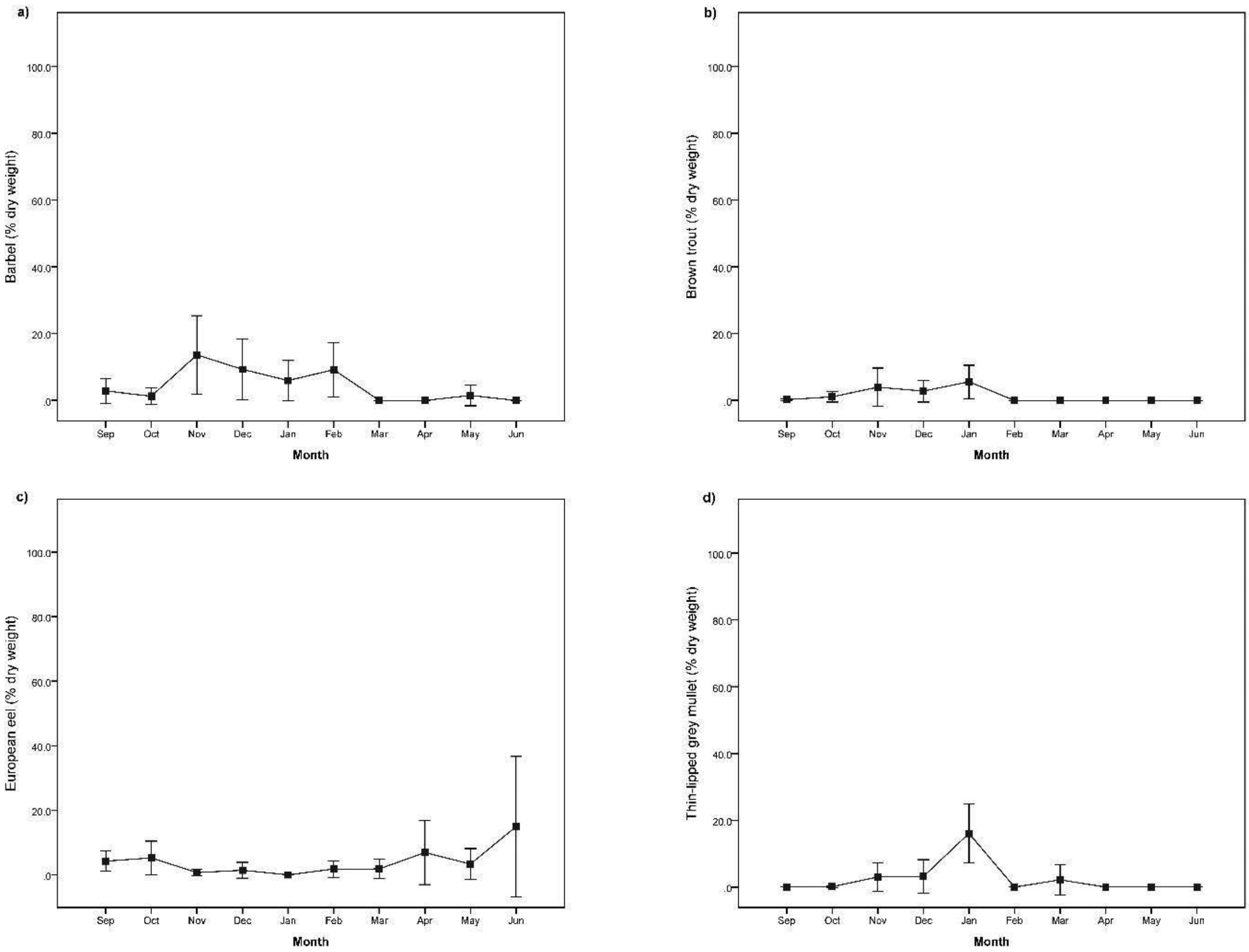

Fig. 8 – Mean percentage of dry weight of Native fishes in the otter's diet: a) barbel, b) brown trout, c) European eel and d) thin-lipped grey mullet.

Regarding the introduced fish species, in terms of percentage of dry weight, its abundance in the diet is in general higher than the one of native fish species, with peaks reaching 20% and higher. Temporally, goldfish, wild common carp and largemouth bass present its higher values from November to February, with a maximum in December or January (Fig.9a, b & c). The pumpkinseed was the only introduced fish species presenting such low abundance that is not possible to infer a temporal consumption pattern (Fig.9d).

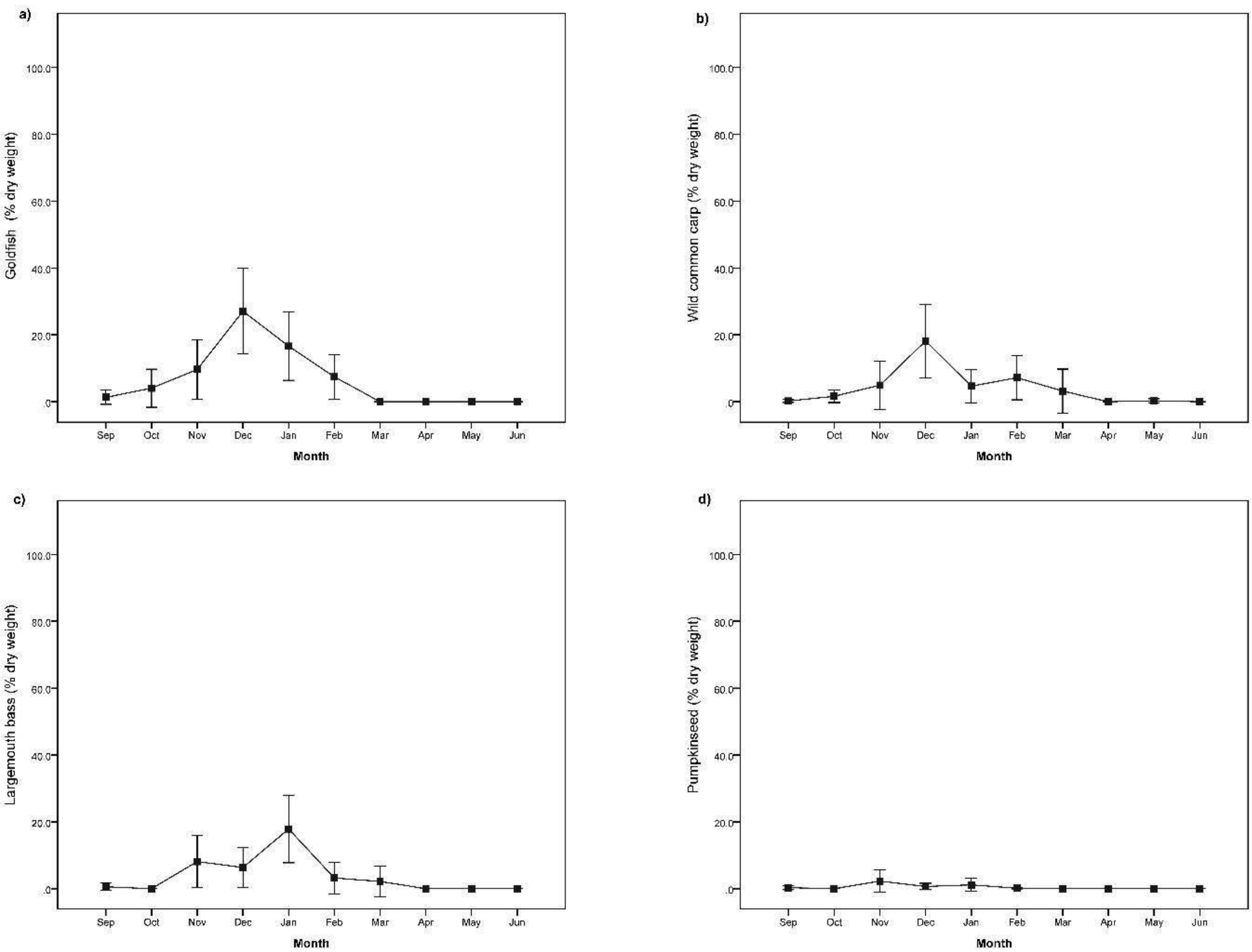

Fig. 9 - Mean percentage of dry weight of Introduced fishes in the otter's diet: a) goldfish, b) wild common carp, c) largemouth bass and d) pumpkinseed.

By analysing the percentage of dry weight of crayfish present in the otter diet over time, and comparing it to the graphics of the fish species in general, it is possible to highlight its higher values, reaching percentages higher than 95%. The lower percentages of crayfish occur in December and January (Fig.10), which represents the months when the percentage of fish in general was higher.

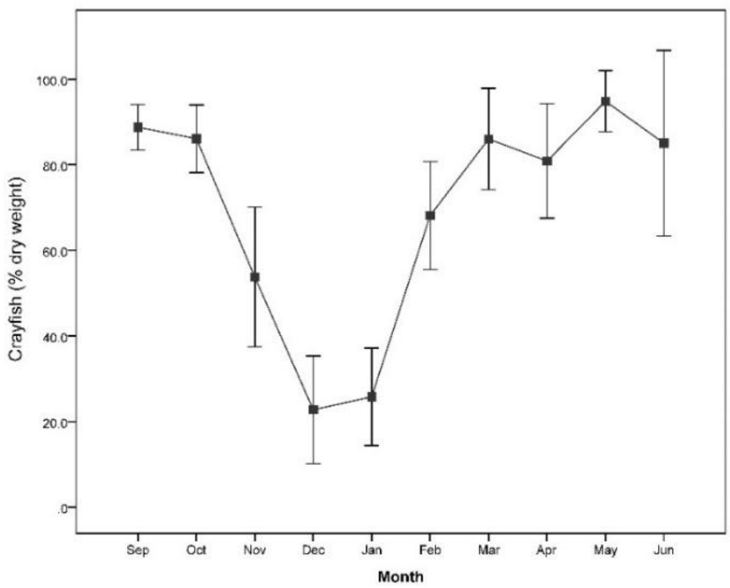

Fig. 10 - Mean percentage of dry weight of crayfish in the otter's diet.

Comparing the percentage of crayfish present in otter's diet and the number of crayfish captured, is possible to observe that its trends are similar, except for December, in which the number of crayfish captured is much higher than its consumption (Fig.11).

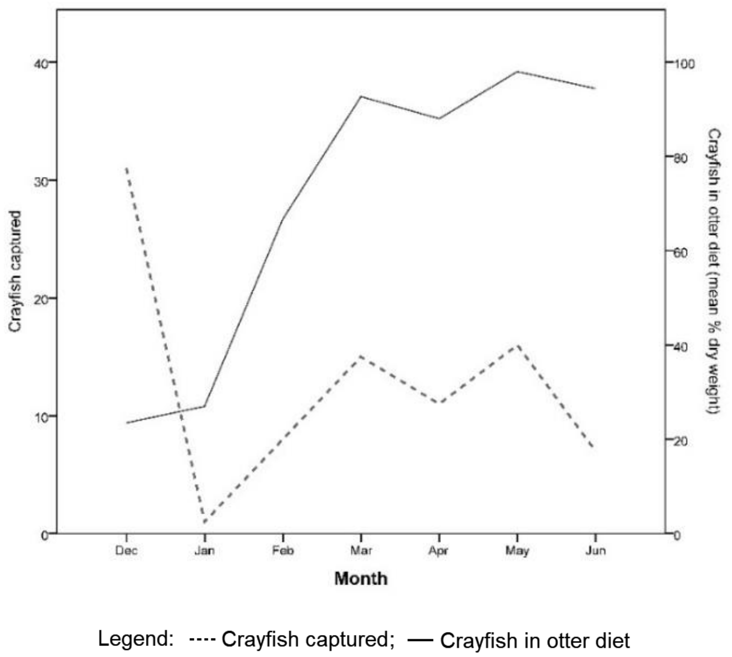

Fig. 11 – Comparison between the crayfish captured and the percentage of dry weight of crayfish present in otter's diet, in each month.

3.4 Comparative study on the feeding behaviour of Common otter in Arzila Marsh in the last three decades

Analysing the study of (Martins et al. 2002), conducted right after the first registered report of crayfish presence in Mondego valley (table 3 - 1989's results), is possible to observe that the diet of Common otter was dominated by fish, followed by amphibians and insects. European eel and the threespined stickleback were clearly the most frequent taxa (RFO=10.3% and 11.4%, respectively), being both native fishes. Regarding the consumption of introduced species, it was observed the occurrence of the Eastern mosquitofish (RFO=5.4%) and of wild common carp (RFO=2.7%) in their diet (table 3).

In 2004, 15 years after the first study on otter diet, the diet composition showed a reduction of the consumption of fish (RFO=27.5%) and a high frequency of crayfish (RFO=71.8%). Overtime, the frequency of amphibians and insects in the diet of Common otter reduced, being currently completely absent. Globally, it was clear the diminishing of the consumption of native fish by otters, such as European eel (RFO=5.0%) or threespined stickleback (RFO=0.0%). Regarding the introduced fish species, besides the disappearance of the Eastern mosquitofish from the diet, it is possible to observe an increase in the frequency of the other exotic species, such as the wild common carp (RFO=3.7%), and the appearance of goldfish, largemouth bass and pumpkinseed in otter's diet, representing respectively 1.0%, 0.5% and 4.9% (table 3).

In 2016, 27 years later, the total frequency of fish species in the diet of Common otter has increased, representing 46.1% of all prey items (table 3). Currently, the consumption of barbel, eel and thin-lipped grey mullet by otter increased, and a new prey item (brown trout) was found in the diet of Common otter (RFO=2.7%). Regarding the introduced fish, the occurrence of pumpkinseed decreased (RFO=2.2%), but all the other introduced species showed an increase in otter's diet. The red swamp crayfish decreased its relative frequency of occurrence from 71.8% to 53.9% and amphibians, insects, passerines and rodents were completely absent (table 3).

Table 3 – Comparison between the diet of Common otter in Arzila Marsh in the last three decades: a) after the introduction of crayfish (Martins et al. 2002); b) 15 years after (Pinto 2004); c) 27 years after (present study).

	1989[a] (N=159)	**2004**[b] (N=610)	**2016**[c] (N=376)
Prey items	%RFO	%RFO	%RFO
Fish (total)	45.2	27.5	46.1
Native Fish			
Barbel (*L. bocagei* Steindachner)	2.5	1.0	4.8
Brown trout (*S. trutta* L.)	0.0	0.0	2.7
Chub (*S. cephalus* L.)	4.4	0.0	0.0
Cobitis maroccana (*C. maroccana* Pellegrin)	1.6	0.0	0.0
European eel (*A. anguilla* L.)	10.3	5.0	7.9
Iberian nase (*P. polylepis* Steindachner)	5.0	1.4	0.0
Ruivaco (*A. oligolepis* Robalo)	1.9	0.1	0.0
Thin-lipped grey mullet (*L. ramada* Risso)	0.0	1.7	3.4
Threespined stickleback (*G. aculeatus* L.)	11.4	0.0	0.0
Introduced Fish			
Eastern mosquitofish (*G. holbrooki* Girard)	5.4	0.0	0.0
Goldfish (*C. auratus* L.)	0.0	1.0	7.3
Largemouth bass (*M. salmoides* Lacepède)	0.0	0.5	4.7
Pumpkinseed (*L. gibbosus* L.)	0.0	4.9	2.2
Wild common carp (*C. carpio* L.)	2.7	3.7	5.0
Fish NI	0.0	8.2	8.1
Invertebrates			
Red swamp crayfish (*P. clarkii* Girard)	0.0	71.8	53.9
Amphibians	22.0	0.1	0.0
Insects	20.8	0.0	0.0
Passerines	8.0	0.3	0.0
Rodents	0.0	0.4	0.0
Others	4.0	0.0	0.0

4. Discussion

The Common otter is a species that has been near-threatened all over Europe for the last 12 years due to the decline of its populations (Chanin and Jefferies 1978, Lodé 1993, Prigioni et al. 2007, Roos et al. 2015). However, in Portugal this tendency has been contradicted, and otter populations have started to recover since 1995 (Fialho 2016). The results from this study agree with this recovery trend, since it were found evidences of presence of otter throughout the lower Mondego river valley. This recovery is presumably associated with the presence and increase of invasive species, since its occurrence lead to an increased availability of food in the water course (López-Nieves and Casal 1984, Beja 1995, Correia 2001). The presence of introduced species in the lower Mondego river valley was confirmed, both through its presence in the diet composition of otter, and through the capture of crayfish in the study area.

During this study, in the north area of the Mondego river valley, the agriculture fields were mostly cultivated with maize, unlike the south area, which was mainly composed by rice fields. The crayfish is a species whose presence is highly associated to the rice cultures (Correia and Ferreira 1995, Correia 2002). Since our results show a higher occurrence of otters in the south part, proven by the higher number of spraints collected in the Arunca River, it is possible to infer that the distribution of otter may be associated to the occurrence of this invasive species. On the contrary, maize fields negatively influence the otters' occurrence, since they are associated to dry soils without high diversity of prey items for otters (LaFontaine et al. 1998).

It is also important to highlight the high occurrence of otters in the marshes, specially Arzila Marsh, as expected (Kruuk 2006). This site has characteristic features that make it suitable for otters. It has regular and stable water resources without significant changes in water flows, food resources are constant and easier to capture, without spending too much energy since the ditches are narrow. The displacement, either by water or by land, is facilitated because most of its activity occur in interface between water and land (Roos et al. 2015). The abundant riparian vegetation of marshes also presents indirect benefits for otters, since it increases availability of invertebrates, which in turn are predated by fish, leading to the increase of fish density at these sites (Mason and MacDonald 1982, Chanin 2003). On the other hand, the main arm of the river showed low occurrence of otter, maybe due to the much stronger and instable water flow, interfering with its prey populations (Mason 1995, Ruiz-Olmo et al. 2001).

Otters usually prey on less active and slow moving species (Wise et al. 1981, Chanin 2003, Roos et al. 2015). The otters' activity pattern observed during our study confirms the general activity patterns described for this species. The nocturnal behaviour observed by the camera-traps monitoring in Arzila Marsh is also related to the type of consumed preys, since most of the fish species identified in the otter's diet have a diurnal activity, these preys are less active during night periods (Spencer 1939, Swift 1964, Langley et al. 1993, Sánchez-Vázquez et al. 1996, Trancard et al. 2011, Warden and Lorio 2011).

Regarding the results obtained in Arzila, the number of collected sprains was not regular over time, with a decrease of its numbers in late spring/early summer. This fact could be related to the reduction of the river flow along this season, and the consequent decrease of the abundance of fish available in Arzila. Such environmental change may lead the otter population to move to areas with more water available, like the open rice fields, where the food resources available may be higher. The decrease in the abundance of otter in Arzila, and its possible transition to other areas with different habitat can also be pointed out by the changes on the feeding behaviour that was detected. After the winter months, the type of food items identified changed, becoming the diet basically composed by crayfish. As so, considering the marked drought periods experienced this year, it is possible that otters change its habitat use to forage in other areas of the Mondego river valley during the summer season, decreasing their time and activity in Arzila. This hypothesis is in agreement with the results found at the Arunca River, where the opposite pattern in the abundance of spraints was observed, with its number increasing in summer, and being lower in winter. As expected, otters seem to have a high plasticity in terms of foraging behaviour, selecting the habitats used accordingly with the availability of resources (Durbin 1998, Chanin 2003, Krawczyk et al. 2015).

On a shorter time scale, based on the monthly results from Arzila, it was detected a higher occurrence of almost all fish species in the diet of otter during the winter (López-Nieves and Casal 1984, Beja 1995, Taastrùm and Jacobsen 1999, Novais et al. 2010), which is in agreement with the season during which the fish species are more abundant and present higher population densities. The presence of these preys in the marsh area may be higher due to the greater flow through the ditch, which represent an improving in the capture of fish by otters. In contrast, the winter is the season with lowest occurrence of crayfish, both in the diet and in terms of abundance in the wild, confirmed by our results of crayfish captures, and in agreement with other studies (Beja 1996, Correia 2001).

Considering the general patterns of consumption of the different prey categories, it may be possible to infer that otter is exhibiting a preference for fish species, whenever it is available, and a shift towards crayfish when fishes are more scarce. Our results show that prey diversity is lower in the summer. Therefore, there is a need for the otters to search for additional food resources, such as the crayfish. By exploiting this energy source, there is no need to search for additional prey, like amphibians, mammals and insects (Chanin 2003, Kruuk 2006). Since it is known that otters are able to consume larger proportions of other types of prey according to its availability (Ruiz-Olmo et al. 2001, Kruuk 2006), our results about the use of crayfish as the main food source during most of the months prove its opportunistic feeding behaviour.

In terms of the occurrence of each fish category, it is possible to conclude that otters are regularly consuming native fish species. Although the overall percentage of dry weight of the native fish appears to be low in the diet, the regular consumption over the different months indicates that this prey category may be quite important for otter, since it constitutes a constant energy complement.

Some of the native fish consumed are described as having a status of concern due to their low population densities, such as the case of the European eel (Jacoby and Gollock 2014) and the brown trout (Cabral et al. 2005). If the same trend proved to be happening in the studied region that may explain its low occurrences in general in the otter's diet. Nevertheless, Common otter is using these fish species as their prey, and demonstrates a certain preference for some of them, such as the European eel and the barbel, as supported by previous studies (Adrián and Delibes 1987, Beja 1996, Ruiz-Olmo and Palazón 1997, Ruiz-Olmo et al. 2001, Ruiz-olmo et al. 2002, McCafferty 2005, Freitas et al. 2007). The mean percentages of dry weight of thin-lipped grey mullet and brown trout observed in the diet of otter were very low, but its importance has been described in otters diet from other study areas (Ruiz-Olmo and Palazón 1997, Taastrùm and Jacobsen 1999, Clavero et al. 2003, Novais et al. 2010). European eel and barbel are the native fish species more abundant in the diet. Regarding the pattern of the eel in the diet through time, similar patterns were described by Beja 1996 and Cerqueira 2005, which was explained by the higher abundance of this species at that time of the year (Beja 1996). In fact, our results are also in agreement to the results from fish monitoring in the Mondego River, where was detected a higher abundance of eel during summer (Almeida et al. 2015). Concerning the barbel, its preference by otters was as expected (Ruiz-Olmo and Palazón 1997, Ruiz-Olmo et al. 2001, Ruiz-olmo et al. 2002). Its consumption is maintained over several months, reducing in March, as described for cyprinids in the diet of otter in previous studies (Beja 1996, Krawczyk et al. 2011).

Regarding the consumption of introduced fish species, its proportion in the diet is globally higher when comparing with the native fish species, but less constant, due to its completely decrease after the winter season. They seem to have a major importance in otters' diet during the winter, but as soon as the crayfish abundance in the environment increases, the presence of introduced fish in the diet of otter ceases.

The cyprinids, goldfish and the wild common carp, are the most abundant introduced fish species in the diet. These two species appear very frequently in otters' diet according with other studies (López-Nieves and Casal 1984, Beja 1996, Gourvelou et al. 2000, Pedroso and Santos-Reis 2006, Krawczyk et al. 2011), as well as the largemouth bass (López-Nieves and Casal 1984, Novais et al. 2010). Each of this species presents a peak in the diet in the middle of winter, once again confirming a higher predation upon these species when they are most abundant. The pumpkinseed presents a constantly low presence in the diet, despite its possible high abundance in the study area. This can be justified by the fact that otters may have higher energy benefits by capturing a prey with a larger biomass, like the wild common carp (Pedroso and Santos-Reis 2006), eels or barbels (Sales-Luís et al. 2007), in detriment of the smaller preys.

The mean percentage of dry weight of crayfish in the diet was lower in winter, but quite high during the rest of the months. This may be explained due to the restricted environmental conditions verified in spring and summer, such as the lower flow of water that decreases the fish density in the marsh (Mason 1995), or the higher abundance of full-grown crayfish individuals leading to its easier predation (Correia 2001). Nevertheless, the consumption of crayfish observed during this study is related to its availability and population structure (Beja 1996, Correia 2001), as confirmed by our results by the consistency between the proportion of crayfish in the diet, and the numbers of crayfish found/captured in the study area.

Despite having the higher frequency of occurrence in the diet of otter, the crayfish represented a lower percentage of biomass, when compared with the total percentage of biomass of fish species. This means that despite its occurrence, it seems to be energetically less valuable as a prey item for otter (Freitas et al. 2007). This sustains the hypothesis that the Common otter is using the available food sources in the absence of its preferred prey items, proving once more their opportunistic feeding.

Looking at the evolution of the diet of otter over the past three decades until now, it seems that fish species are regaining importance as prey items for otter. After a major reduction in the occurrences of fish species in the diet of otters from 2004, where the occurrence of crayfish exceed 70% (Pinto 2004), currently the fish preys increased to values similar to the previous ones (Martins et al. 2002), before the exponential growth of crayfish in the lower Mondego river valley. Its overall increase in the diet happened either due to native and introduced fish species, although the diversity of native fish species was lower. The predation upon amphibians, insects and passerines was quite considerable (Martins et al. 2002), however after the inclusion of crayfish as a food resource, its consumption has decreased (Pinto 2004) until its complete disappearance in our results. This may be explained either by the collapse in the amphibian populations (Delibes and Adrián 1987, Cruz et al. 2006) and for the lack of need for additional energy sources.

The decrease of fish species richness in Mondego estuary has been addressed and associated with anthropogenic factors and intense eutrophication processes (Leitão et al. 2007). It is likely that these factors may continue to be interfering with the abundance of the fish species, resulting in the loss of fish species richness along the years.

The crayfish also has a negative impact in the invaded areas, interfering with the diversity of fishes and many other species. In the diet, crayfish had a major importance since its occurrence was higher than the one obtained for fish species. Currently, the reduction of crayfish consumption may be reflecting changes in the abundances of crayfish in the environment towards its populations' partial decline. The use of pesticides (Cabral et al. 1998) and predation by many top predators, from birds to mammals (Correia 2001), may be having crucial effects upon the control of the populations of this invasive species, decreasing it. As a top predator, Common otter may be exerting an extremely important role in terms of conservation of the native species present in the studied habitats, due to its predation on introduced fish species, and most importantly, on crayfish.

The present increased consumption of introduced fish species also draws our attention to the species considered naturalized, such as the goldfish and the wild common carp. The increase in the occurrence of these species in the diet may be reflecting their higher abundances in the environment. Since the designation of naturalized species has a delicate meaning, it should be taken into account that, at any moment, a naturalized species may become invasive (Clavero and Villero 2014), and so otters may be having a significant role in controlling these populations.

It has been noted that the otter makes great use of introduced species, either naturalized or invasive. Some of the more problematic invasive species that occur in the lower Mondego river valley, such as the red swamp crayfish, are a resource that is used not only by otters but also storks, herons, foxes and others (Correia 2001). Its use as a food resource may have contributed towards an increase in otter populations, while otter may also be contributing to the control of this pest in the lower Mondego river valley.

In conclusion, it is possible that the otter as top-predator is having an umbrella species effect, due to its effective activity as a pest controller, providing top-down control. By controlling the populations of the introduced species, the Common otter plays a vital role in ecosystem restoration of the lower Mondego river valley, by providing more chances for several native species to develop. Simultaneously, otters are providing an ecosystem service, resulting in the important increase of the river diversity wildlife, and valorisation of the rice crops, which benefits human populations and local economy.

5. General conclusions

With this study, our aims were towards understanding the ecology and feeding behaviour of Common otter, mainly in terms of abundance, occurrence and activity along the lower Mondego river valley, and determine what food resources may be used by them. It was also intended to evaluate if these resources were mostly dominated by invasive or native species, and how is this been evolving through the years in the lower Mondego river valley after the introduction of exotic species, some of which with invasive potential. One of these invasive species is the Red swamp crayfish, which is responsible for countless disturbances in the ecosystems, such as interference with trophic chains, destruction of rice crops and decrease of native species populations. It is known that since the introduction of this invasive species, the otter changed its feeding behaviour, incorporating it in its diet. Therefore, it became more evident the need to acquire more information about this top-predator.

Through the evaluation of otter occurrence, it was possible to determine that this species is present along the river valley, but it is more abundant in marsh and flooded areas. These areas may present different availability of prey depending on the season, and otters were able to change their habitat use according to food availability. This result allows to conclude that otter presents a high plasticity both in habitat use and feeding behaviour.

Regarding feeding behaviour, otter makes use of different food resources, depending on the season. The winter season was mostly dominated by fish species, both native and introduced, while in summer the crayfish was the main food item. These results proved that otters search for additional preys to complement their diet, depending on the availability of resources, proving its opportunistic behaviour.

Regarding temporal comparisons between diets, it was possible to understand the influence of the crayfish in the otter's diet. Before the expansion of crayfish, their diet was mostly composed by fish, and some additional preys such as amphibians, insects, passerines and small mammals. After the crayfish dispersal, the otters' piscivorous diet was replaced by crayfish, becoming its main prey item. Presently, the overall occurrence of crayfish in the diet decreased, meaning that crayfish abundance may have decreased in the study area. Fish consumption has increased, both native and introduced, according to the seasonality of each species, which may indicate the recovery of fish populations.

Otters may be playing an important function as top-down control of exotic species, especially towards the invasive ones, such as the crayfish. It is, therefore, assumed that the Common otter may have an important role as umbrella species by controlling potential pests, allowing native species in their habitat to thrive, while minimize the effects of the crayfish in the rice crops of the lower Mondego river valley.

Nevertheless, more studies are needed to understand which other factors may be responsible for this species' recovery in the country, as well as what are the mains threats that otters currently face in the lower Mondego river valley. More studies should also be conducted to raise awareness about the importance of otter, and to be able to implement stricter, realistic and more appropriate conservation measures to protect this important top-predator.

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