Diet of the White Stork (Ciconia ciconia) in a heterogeneous Mediterranean landscape: the importance of the invasive Red Swamp Crayfish (Procambarus clarkii)

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Dieta da Cegonha-branca *(Ciconia ciconia)* numa paisagem Mediterrânica heterogénea: a importância de uma espécie invasora, o Lagostim-vermelho-do-Louisiana *(Procambarus clarkii)*

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ABSTRACT

Limited quantitative data are available on food habits of the White Stork *(Ciconia ciconia)* in Mediterranean environments, particularly in ricefields where a relatively new food resource, the invasive Red Swamp Crayfish *(Procambarus clarkii)*, is abundant. We studied the diet of the White Stork in a heterogeneous landscape (Central Portugal) in order to compare the importance of the Red Swamp Crayfish as a food resource in a dominant agricultural/ricefield area in relation to a predominant woodland/agricultural area. White Storks´ diet was analysed spatially (two sites) and seasonally (winter, spring, summer) using pellets (n = 122) collected between December 2012 and July 2013. Overall, from 1570 prey items identified, crayfish was the second most frequent and abundant prey in the diet (frequency of occurrence, FO = 79.5%; numerical frequency, NF = 22.9%, respectively), only surpassed by coleopterans (FO = 94.3%; NF = 57.7%). However, in terms of consumed biomass (global PB) crayfish dominated the diet (PB = 44.0%), representing 1.8 times the consumed biomass of coleopterans (PB = 24.2%). Consumption of crayfish was higher in the site with highest abundance of ricefields (NF: 32.0% vs. 17.7%; PB: 51.3% vs. 38.4%). Although no significant seasonal variations were detected in terms of the number of crayfish consumed by storks, consumed crayfish biomass was significantly higher in summer in relation to other seasons. Our findings suggest that in Mediterranean heterogeneous areas the White Stork feeds upon a wide range of prey taxa though, when available, coleopterans along with Red Swamp Crayfish dominate the diet.

Keywords: Feeding ecology, Mediterranean, pellet analysis, Red Swamp Crayfish, White Stork

RESUMO

O estudo dos hábitos alimentares da Cegonha-branca *(Ciconia ciconia)* em ambientes mediterrânicos carece de informação quantitativa, particularmente em áreas de arrozais onde um recurso alimentar relativamente novo, o Lagostim-vermelho-do-Louisiana *(Procambarus clarkii)*, é abundante. Analisámos a dieta da Cegonha-branca numa paisagem heterogénea no centro de Portugal com o intuito de comparar a importância desta espécie de lagostim invasor como recurso alimentar numa área dominada por culturas agrícolas e arrozais em relação a uma área predominantemente florestal/agrícola. A dieta da Cegonha-branca foi analisada espacialmente (dois locais) e sazonalmente (inverno, primavera e verão) a partir da análise de regurgitações (n = 122) recolhidas entre Dezembro de 2012 e Julho de 2013. De um total de 1570 presas identificadas, o Lagostim-vermelho-do-Louisiana foi o segundo recurso alimentar mais frequente e abundante na dieta (frequência de ocorrência, FO = 79.5%; frequência numérica, NF = 22.9%, respectivamente), unicamente excedido pelos coleópteros (FO = 94.3%; NF = 57.7%). Contudo, em termos de biomassa o lagostim dominou a dieta (PB = 44.0%) representando 1.8 vezes a biomassa consumida dos coleópteros (PB = 24.2%). O consumo de Lagostim-vermelho-do-Louisiana foi significativamente maior no local com maior percentagem de cobertura de arrozais (NF: 32.0% vs. 17.7%; PB: 51.3% vs. 38.4%). Embora não tenham sido detectadas variações sazonais significativas no consumo do lagostim em termos numéricos, o lagostim teve uma contribuição para a biomassa consumida significativamente maior no verão face às outras estações. Os resultados deste trabalho sugerem que nesta área mediterrânica heterógena, a Cegonha-branca alimenta-se de um vasto leque de presas, porém, quando disponíveis, os coleópteros e o Lagostim-vermelho-do-Louisiana dominam a dieta.

Palavras-chave: regurgitações, Cegonha-branca, ecologia alimentar, Lagostim-vermelho-do-Louisiana, Mediterrâneo

Introduction

The White Stork *(Ciconia ciconia)* is a large migratory species, being widely distributed and inhabiting a variety of open and agricultural habitats (Alonso et al. 1991, Hancock et al. 1992). This species is considered a generalist and opportunistic predator and its diet has been well documented throughout its distributional range in Europe (Mužinić & Rašajski 1992, Antczak et al. 2002, Tsachalidis & Goutner 2002). Several studies revealed that the White Stork feeds upon a wide range of prey including invertebrate and vertebrate species (Melendro et al. 1978, Antczak et al. 2002, Kosicki et al. 2006, Cheriak et al. 2014). Earthworms, orthopterans, coleopterans, and small mammals (predominantly voles in Eastern Europe) seem to be primary food resources throughout the breeding range of the White Stork. On the other hand, small fish, birds, reptiles, amphibians, and molluscs are sporadically consumed, being referred as complementary food resources (Antczak et al. 2002, Tsachalidis & Goutner 2002, Vrezec 2009, Catry et al. 2010). The diet of White Storks seems to be shaped by landscape use, prey availability and climatic conditions of each geographical region (Johst et al. 2001, Tsachalidis & Goutner 2002, Ciach & Kruszyk 2010, Chenchouni et al. 2015, Chenchouni 2017).

Recently, the appearance of new food resources, such as rubbish dumps, has produced considerable shifts in the feeding habits (e.g. foraging behaviour; Tortosa et al. 2002, Ciach & Kruszyk 2010, Gilbert et al. 2016) and diet composition of White Storks (Peris 2003). Likewise, the spread of the invasive Red Swamp Crayfish (*Procambarus clarkii*; hereafter referred as "crayfish") has been suggested to be an important driver of observed dietary changes of the White Stork (Correia 2001, Tablado et al. 2010), as well as a major cause for the establishment and increase of White Stork wintering populations in the Iberian Peninsula (Tablado et al. 2010, Catry et al. 2017). This invasive crayfish was introduced in southwestern Europe from North America in the 1970s, and is now widespread in wetlands (e.g. ricefields) across Portugal and Spain, where it became an abundant new food resource exploited by White Storks. For example, in Portugal, in a freshwater marsh located in the Tagus river basin, White Storks show a high consumption of crayfish, which is available all over the year (Correia 2001). In Spain, the crayfish is also an important prey item in White Storks' diet in ricefield areas (Negro et al. 2000, Tablado et al. 2010, Sanz-Aguilar et al. 2015), a typical habitat where this invasive species is often abundant (Anastácio et al. 2009). However, available information is still insufficient to fully understand the relationship between White Storks and crayfish, namely concerning a quantitative assessment of crayfish contribution to White Stork's diet considering simultaneously the contribution of other food resources.

Here, we aimed to describe and compare the diet of the White Stork at two sites within a Mediterranean area characterized by a heterogeneous landscape: one site dominated by woodland with agricultural patches, and the other dominated by mixed agricultural habitats, with a high percentage occupied by ricefields (another site). Specifically, we aimed to (1) quantify the proportion and biomass contribution of crayfish in the diet of White Storks in relation to other food resources and (2) evaluate possible spatial-seasonal variations of crayfish consumption by White Storks.

Methods

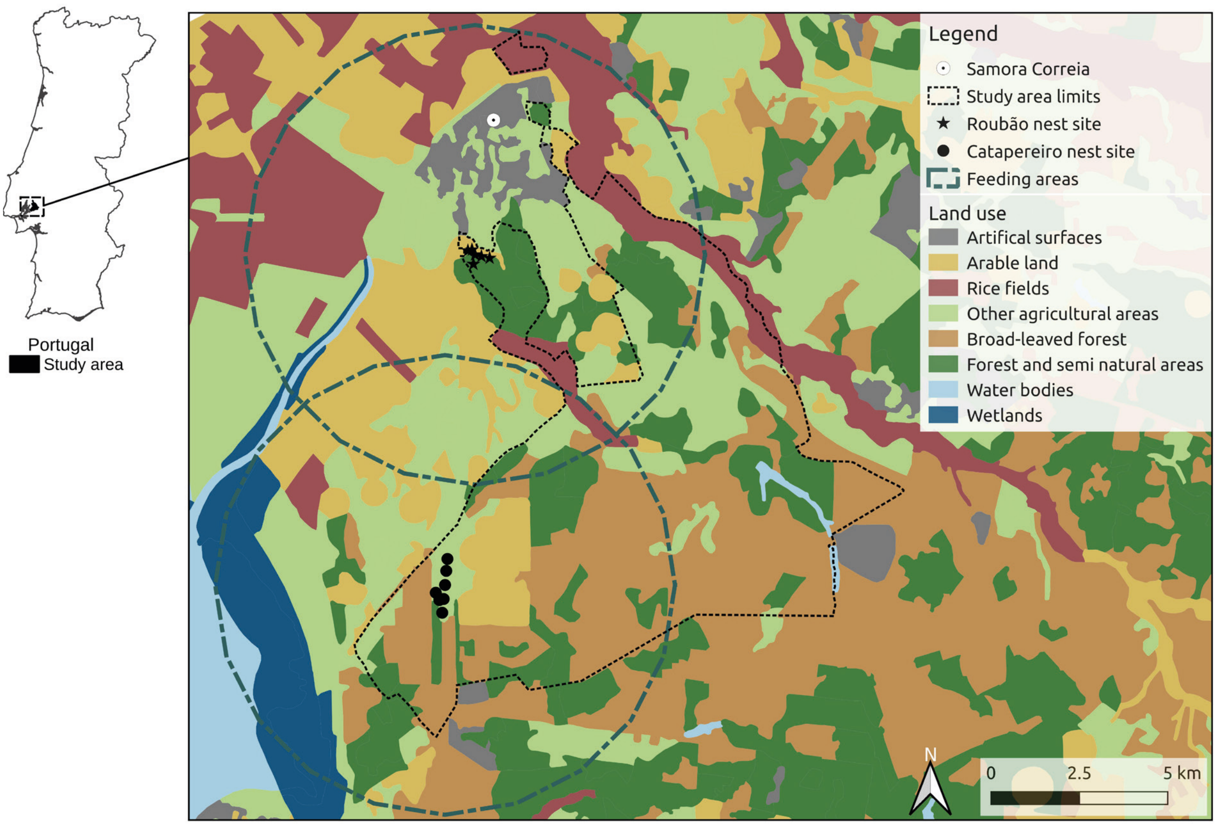
Study area

The study was carried out in Charneca do Infantado (Figure 1), within the estate "Companhia das Lezírias S.A", which is the largest Portuguese agroforestry farmstead (38° 52' N, 08° 51' W; Central Portugal), located on the left margin of the Tagus River. The area is characterized by a landscape mosaic with high abundance of cork oak woodlands, pine forests and agricultural lands, such as ricefields and pastures.

The diet of the White Stork was assessed by analysing pellets collected underneath nests in two sites, Catapereiro and Roubão, separated by 8.4 km (Figure 1). The nests were located on the top of transmission electricity pylons: 18 nests in seven pylons at Catapereiro and 12 nests in six pylons at Roubão. Land use around nest sites was assessed and characterized in a buffer of 6.5 km (maximum distance of a foraging flight recorded in the area by visual estimation; E. Ferreira unpubl. data) around each nest site centroid by using the Corine land cover 2006 information (Caetano et al. 2009). In Catapereiro, the predominant land use comprises broad-leaved forest (25.9%) – mainly cork oak woodland – mixed agricultural areas (21.9%), forest and semi natural areas (18.2%) and arable land (18.1%). Here, the percentage of ricefields is low (3.6%). The Roubão nest site is mainly characterized by arable land (30.9%), mixed agricultural areas (29.0%) and ricefields (20.3%). Here, the percentages of broad-leaved forest (2.3%) and forest and semi natural areas (10.4%) are low. The remaining land use types (artificial surfaces, wetlands and water bodies) accounted individually for less than 10% of the land use at each nest site.

Figure 1 - Study area ("Charneca do Infantado") in Central Portugal showing details on the location of the sampled nest sites, feeding areas and main land use types.

Figura 1 - Localização da área de estudo ("Charneca do Infantado") em Portugal com destaque para a localização dos locais de ninhos amostrados, áreas de alimentação e principais classes de uso do solo.



Pellet Collection and Prey Identification

During the first visit to the study area we surveyed both nest sites and removed old pellets, which were not included in diet analyses. Afterwards, pellet collection took place once a month, from December 2012 to July 2013 (except March 2013), covering the presence of the White Stork in nesting areas during the whole breeding period. Only intact and fresh pellets found under the pylons with nests were collected. In the laboratory, pellets were soaked in water and washed through a 2 mm mesh sieve to disaggregate their content. Afterwards, food remains were identified using a binocular stereomicroscope with the help of identification keys, reference collections, and specialist consultation. Mammals were identified through microscopic hair analysis (Pinto 1978, Teerink 1991) and reptiles by the presence of scales and bone remains. Bird identification was based on microscopic analysis of feathers (Brom 1986) and insects from the presence of different body parts (e.g. heads, mandibles, legs, elytra and thorax) according to Chinery (1997). The crayfish – the only malacostraca species detected – was identified through fragments of body parts, namely grastroliths, uropods, rostrum and propodites of the chelae (Beja 1996, Correia 2001). Prey item remains were identified to the lowest possible taxonomic level and then the minimum number of individuals (MNI) was quantified for each prey taxa. We estimated MNI by counting the number of fragments/items recovered in each pellet corresponding to different individuals of a same given prey taxa (Chenchouni et al. 2015).

Data Analysis

Prey items were grouped into the following eight main prey categories: crayfish, orthopterans, coleopterans, other insects, reptiles, birds, small mammals, and lagomorphs. Diet composition was expressed as the frequency of occurrence (FO), numerical frequency (NF) and the percentage of consumed biomass (PB). FO was calculated for each main prey category as the number of pellets containing a prey item i / total number of pellets × 100 – being only determined for the global data set (data from the two sites across the three seasons combined). NF was calculated for each prey item identified as the minimum number of individuals (MNI) of a given prey item i / total number of prey items N × 100 (Chenchouni et al. 2015). PB was calculated for each prey item identified as the mean biomass of a given prey item i / total consumed biomass of all prey items × 100 – using mean individual live weights of the consumed prey as a proxy for ingested biomass (Supporting information, Table S1). NF and PB were determined for the global data set and then by site (Catapereiro and Roubão) and season (winter: from December to February; spring: from April to May; summer: from late June to July), wherein seasons represent different phases of a single breeding season. Considering the relatively low number of pellets per site (Table 1), we carried out seasonal analyses combining data from both sites. Chi-square tests for independence (x^2) with Bonferroni correction for multiple comparisons were used to test the significance of NF and PB differences in each consumed prey category between sites and seasons. Diet diversity was determined using the Shannon index (H’ = – Σpixlog pi where pi represents the proportion of each prey taxa in the diet; Shannon & Weaver 1949) at the family level, i.e. the most precise taxonomic level, since not all prey items could be identified to species level. All statistical analyses were performed in the software R 3.4.3 (R Core Development Team 2017).

Table 1- Diet composition of the White Stork *(Ciconia ciconia)* in the two study sites (Catapereiro and Roubão) throughout the study period (winter, spring and summer). N: number of individuals; NF (%): numerical frequency of prey in diet; PB (%): percentage of consumed biomass; N total: total number of individuals; N pellets: number of pellets collected from each site and per season; H': diet diversity according to Shannon index.

Tabela 1 - Composição da dieta da Cegonha-branca *(Ciconia ciconia)* descrita por local de estudo (Catapereiro e Roubão) e por estação do ano (inverno, primavera, verão). N: número de indivíduos; NF (%): frequência numérica de presas na dieta; PB (%): percentagem de biomassa consumida; N total: somatório do número de indivíduos; N pellets: número de regurgitações recolhidas por local de amostragem e estação do ano; H': valor do nicho trófico (índice de Shannon).

PREY TAXA	CATAPEREIRO									ROUBÃO								
	winter			spring			summer			winter			spring			summer		
	N	NF (%)	PB (%)	Nv	NF (%)	PB (%)	N	NF (%)	PB (%)	N	NF (%)	PB (%)	N	NF (%)	PB (%)	N	NF (%)	PB (%)
Class Malacostraca		21.8	28.9		13.6	34.9		20.9	45.3		32.6	68.9		37.3	46.5		28.5	53.7
Order Decapoda																		
Procambarus clarkii	24	21.8	28.9	62	13.6	34.9	91	20.9	45.3	15	32.6	68.9	76	37.3	46.5	91	28.5	53.7
Class Insecta		71.8	23.2		84.4	48.0		78.2	47.8		67.4	31.1		59.3	17.7		69.9	38.4
Order Odonata					0.2	0.2												
Odonatata NI				1	0.2	0.2												
Order Orthoptera		10.9	5.5		0.4	0.4		28.7	23.8					7.4	3.5		31.4	22.5
Gryllotalpa gryllotalpa	12	10.9	5.5				1	0.2	0.2							1	0.3	0.2
Orthoptera NI				2	0.4	0.4	124	28.4	23.6				15	7.4	3.5	99	31.0	22.3
Order Hemiptera					0.4	0.4								0.5	0.2			
Hemniptera NI				2	0.4	0.4							1	0.5	0.2			
Order Coleoptera		60.9	17.6		82.0	45.9		47.5	22.5		67.4	31.1		51.5	14.0		38.6	15.8
Carabidae																		
Calosoma maderae	6	5.5	1.6															
Carabus lusitanicus				1	0.2	0.1												
Carabus melancholicus				8	1.8	1.0	4	0.9	0.4							2	0.6	0.3
Carabus sp.				5	1.1	0.6	1	0.2	0.1				1	0.5	0.1			
Chlaenius olivieri				1	0.2	0.1	1	0.2	0.1				1	0.5	0.1	1	0.3	0.1
Cicindela campestris				3	0.7	0.4							1	0.5	0.1			
Poecilus kugelanni				1	0.2	0.1												
Scarites cyclops				5	1.1	0.6	2	0.5	0.2				2	1.0	0.3	1	0.3	0.1
Carabidae NI	3	2.7	0.8	64	14.1	7.9				1	2.2	1.0	13	6.4	1.7	17	5.3	2.2
Dytiscidae							31	7.1	3.4									
Dytiscidae NI	1	0.9	0.3	46	10.1	5.7	24	5.5	2.6	1	2.2	1.0	25	12.3	3.3	17	5.3	2.2
Histeridae																		
Histeridae NI	1	0.9	0.3	4	0.9	0.5	2	0.5	0.2				2	1.0	0.3	1	0.3	0.1

PREY TAXA	CATAPEREIRO									ROUBÃO								
	winter			spring			summer			winter			spring			summer		
	N	NF (%)	PB (%)	Nv	NF (%)	PB (%)	N	NF (%)	PB (%)	N	NF (%)	PB (%)	N	NF (%)	PB (%)	N	NF (%)	PB (%)
Silphidae																		
Silpha puncticollis				3	0.7	0.4										1	0.3	0.1
Silphidae NI	30	27.3	7.9	30	6.6	3.7	4	0.9	0.4				7	3.4	0.9	7	2.1	0.9
Dynastidae																		
Oryctes nasicornis	3	2.7	0.8															
Scarabaeidae																		
Bubas bison	2	1.8	0.5							4	8.7	4.0						
Bubas sp.				3	0.7	0.4												
Copris hispanus										1	2.2	1.0						
Onthophagus sp.										1	2.2	1.0						
Scarabaeidae NI										9	19.6	9.0						
Melolonthidae																		
Melolontha papposa							1	0.2	0.1	9	19.6	9.0	12	5.9	1.6			
Tenebrionidae																		
Akis sp.							1	0.2	0.1							13	4.1	1.7
Blaps sp.				1	0.2	0.1										1	0.3	0.1
Erodius sp.							2	0.5	0.2									
Pimelia sp.							3	0.7	0.3									
Sepidium sp.				6	1.3	0.7	7	1.6	0.8							4	1.3	0.5
Tenebrionidae NI				43	9.5	5.3	73	16.7	7.9				4	2.0	0.5	47	14.7	6.1
Chrysomelidae																		
Chrysomela sp.				3	0.7	0.4	2	0.5	0.2				2	1.0	0.3	1	0.3	0.1
Chrysomelidae NI				1	0.2	0.1	2	0.5	0.2									
Curculionidae																		
Curculionidae NI				2	0.4	0.2										3	0.9	0.4
Coleoptera NI	21	19.1	5.5	143	31.4	17.6	47	10.8	5.1	5	10.9	5.0	35	17.2	4.7	7	2.2	0.9
Insect larvae NI				6	1.3	1.1	9	2.1	1.5									
Class Reptilia		0.9	0.7		1.8	15.5		0.5	2.5								1.3	
Order Squamata		0.9	0.7		1.8	15.5		0.5	1.2								1.3	
Chalcides striatus				4	0.9	5.6	1	0.2	1.2							2	0.6	2.9
Psammodromus sp.	1	0.9	0.7															
Colubridae NI				4	0.9	9.8												
Reptilia NI							1	0.2	1.2							2	0.6	2.9

PREY TAXA	CATAPEREIRO									ROUBÃO								
	winter			spring			summer			winter			spring			summer		
	N	NF (%)	PB (%)	Nv	NF (%)	PB (%)	N	NF (%)	PB (%)	N	NF (%)	PB (%)	N	NF (%)	PB (%)	N	NF (%)	PB (%)
Class Aves														1.0	20.0			
Order Anseriformes														1.0				
Anatidae NI													2	1.0	20.0			
Class Mammalia		5.5	47.1		0.2	1.6		0.5	4.4					2.5	15.7		0.3	2.1
Order Insectivora		0.9	1.4					0.5	4.4					1.0	2.8			
Crocidura russula	1	0.9	1.4										1	0.5	0.7			
Talpa occidentalis							1	0.2	2.7									
Insectivora NI							1	0.2	1.7				1	0.5	2.1			
Order Rodentia		3.6	12.8		0.2	1.6								1.5	12.9		0.3	2.1
Microtus sp.													1	0.5	2.1	1	0.3	2.1
Mus sp.	1	0.9	2.5															
Rattus sp.													1	0.5	9.1			
Rodentia NI	3	2.7	10.3	1	0.2	1.6							1	0.5	1.7			
Order Lagomorpha		0.9	32.9															
Lagomorpha NI	1	0.9	32.9															
N total	110			455			436			46			204			319		
N pellets	10			33			33			5			18			23		
Shannon index (H')	1.97			1.97			1.9			1.46			1.99			1.74		

Results

From a total of 122 White Stork pellets analysed, we identified and quantified 1570 prey items comprising 46 taxa belonging to 5 classes, 10 orders and 21 families (Table 1). Coleopterans (FO = 94.3%), crayfish (FO = 79.5%) and orthopterans (FO = 27.9%) were the prey categories more frequently found in pellets. Other prey categories, namely reptiles (FO = 11.5%), small mammals (FO = 8.2%), and other insects (FO = 4.9%) had a moderate frequency in pellets. Birds (FO = 1.6%) and lagomorphs (FO = 0.8%) were the least represented prey in pellets. Regarding the numerical frequency of prey in diet (global NF %), the crayfish was the second most consumed prey category (NF = 22.9%), only surpassed by coleopterans (NF = 57.7%). Indeed, the crayfish represented 1.4 times the consumption of orthopterans (NF = 16.2%) and 6.9 times the sum of other insects (NF = 1.2%), reptiles (NF = 1.0%), small mammals (NF = 0.9%), birds (NF = 0.1%) and lagomorphs (NF = 0.1%) together. In terms of biomass (global PB), crayfish (PB = 44.0%) dominated the diet of White Storks representing 1.8 times the PB of coleopterans (PB = 24.2%), 3.7 times the PB of orthopterans (PB = 11.9%) and 2.2 times the sum of PB of small mammals (PB = 6.5%), reptiles (PB = 5.3 %), birds (PB = 4.0 %), lagomorphs (PB = 3.3%), and other insects (PB = 0.8%) together.

Spatial Analysis

The crayfish was the second most consumed prey taxa (NF) at both sites, accounting for 32.0% of all prey consumed at Roubão and 17.7% at Catapereiro (Figure 2). The coleopterans dominated the diet at both sites, ranging from 45.5% at Roubão to 64.6% at Catapereiro, whereas the orthopterans, the third most important prey category, represented 20.2% of the diet at Roubão and 13.9% at Catapereiro. However, in terms of biomass (PB), crayfish represented the most important prey category at both sites (PB = 51.3% at Roubão; PB = 38.4% at Catapereiro), while coleopterans were ranked second (PB = 30.6% at Catapereiro; PB = 15.9% at Roubão), followed by orthopterans (PB = 12.4% at Roubão; PB = 11.5% at Catapereiro; Figure 2). The proportion of the other prey categories (other insects, reptiles, birds, small mammals and lagomorphs) varied among sites, however, together represented a low fraction of the diet: less than 5% of NF at each site; and, individually, each prey category accounted for less than 10% of PB at each site. We found significant between-site differences both on NF and PB mainly for the most consumed prey categories, with crayfish being significantly more consumed and represented in terms of biomass at Roubão, while coleopterans and other insects were more consumed and had a larger contribution to the consumed biomass at Catapereiro (Chi-square pairwise tests with Bonferroni correction significance at $P < 0.006$; Table 2). For the orthopterans, only significant spatial differences in terms of NF were detected, wherein this prey was more common in the diet at Roubão. No significant between-site differences on NF were found for reptiles, birds, small mammals, and lagomorphs. Nevertheless, the contribution of these prey (evidenced as secondary and occasional food items by NF) to the consumed biomass varied significantly between sites (Table 2). Diet diversity was higher at Catapereiro (H' = 2.20) than at Roubão (H' = 2.07), with species richness values of 43 and 34 prey taxa, respectively (Table 1).

Table 2- Comparison of the main prey consumed by White Storks (*Ciconia ciconia)* between study sites and seasons. Results refer to the chi-square tests (X^2) with Bonferroni correction for multiple comparisons testing the differences in numerical frequency and percentage of consumed biomass of each consumed prey category (*: significant results ($p < 0.006$); NA: Not applicable.

Tabela 2 - Comparação do consumo das principais categorias de presas encontradas em regurgitações de Cegonha-branca *(Ciconia ciconia)* entre locais de estudo e estações do ano. São apresentados os resultados dos testes de qui-quadrado (X^2) com correcção de Bonferroni para comparações múltiplas para a frequência numérica e percentagem de biomassa consumida. *: diferenças significativas ($p < 0.006$); NA: Não aplicável.

PREY CATEGORY	Catapereiro vs. Roubão				winter vs. spring				winter vs.summer				spring vs. summer			
	NF		PB		NF		PB		NF		PB		NF		PB	
	X^2	*p*	X^2	*p*	X^2	*p*	X^2	*p*	X^2	*p*	X^2	*p*	X^2	*p*	X^2	*p*
Crayfish	42.1	<0.001*	124.5	<0.001*	1.2	0.269	3.3	0.071	0.1	0.813	42.8	<0.001*	2.0	0.156	49.4	<0.001*
Orthopterans	10.7	0.001*	1.3	0.249	9.6	0.002*	18.2	<0.001*	32.8	<0.001*	172.1	<0.001*	183.8	<0.001*	652.2	<0.001*
Coleopterans	54.3	<0.001*	216.0	<0.001*	5.7	0.017	37.6	<0.001*	19.0	<0.001*	0.5	0.503	119.4	<0.001*	108.5	<0.001*
Other insects	8.0	0.005*	34.0	<0.001*	2.4	0.122	9.3	0.002*	1.9	0.171	7.7	0.006	0.3	0.596	0.5	0.476
Reptiles	0.6	0.438	69.8	<0.001*	0.4	0.538	68.3	<0.001*	0.04	0.841	27.4	<0.001*	0.6	0.427	46.5	<0.001*
Birds	3.5	0.061	406.3	<0.001*	0.5	0.491	94.5	<0.001*	NA	NA	NA	NA	2.3	0.130	341.6	<0.001*
Small mammals	0.3	0.605	30.2	<0.001*	5.0	0.026	7.3	0.007	11.7	<0.001*	97.5	<0.001*	1.5	0.226	76.6	<0.001*
Lagomorphs	0.6	0.451	199.0	<0.001*	4.2	0.040	867.0	<0.001*	4.9	0.028	938.0	<0.001*	NA	NA	NA	NA

Figure 2 - Proportion of the main prey categories in the diet of the White Stork *(Ciconia ciconia)* by site, expressed as the numerical frequency of prey in diet (NF) and percentage of consumed biomass (PB). Dark grey: Catapereiro; light grey: Roubão.

Figura 2 - Contribuição dos principais grupos de presas para a dieta da Cegonha-branca *(Ciconia ciconia)* nos dois locais de estudo, em termos da frequência numérica de presas na dieta (NF) e percentagem de biomassa consumida (PB). Cinzento-escuro: Catapereiro; cinzento-claro: Roubão.

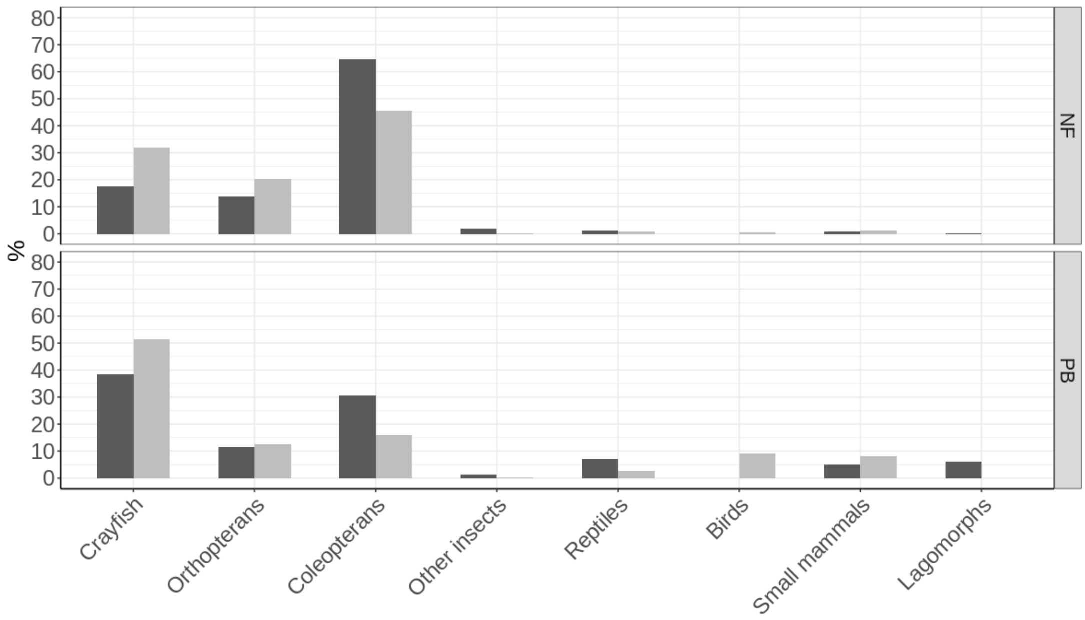
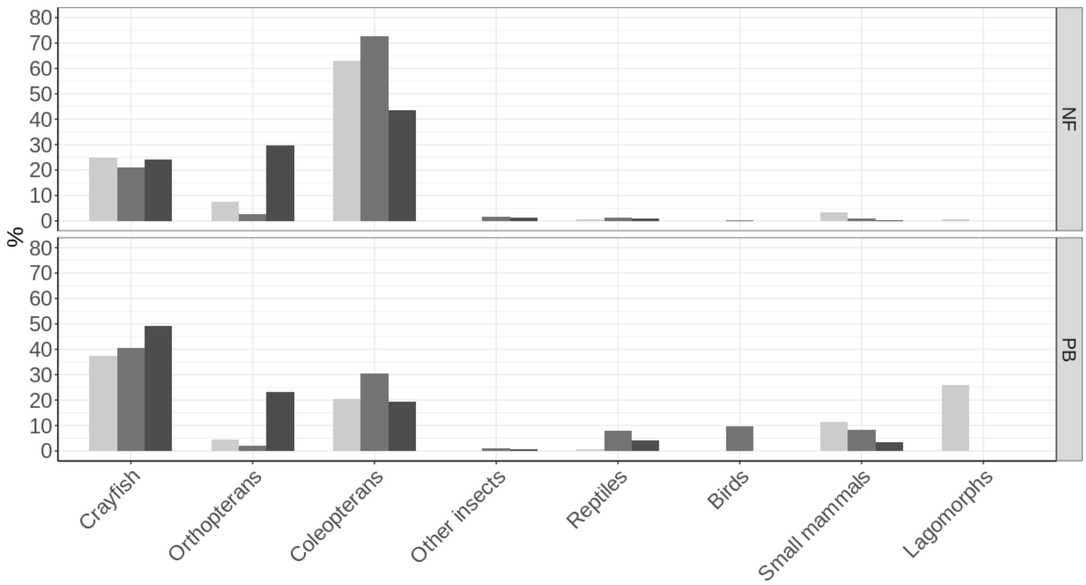


Figure 3 - Proportion of the main prey categories in the diet of the White Stork *(Ciconia ciconia)* by season, expressed as the numerical frequency of prey in diet (NF) and percentage of consumed biomass (PB). The three levels of grey (from light to dark) represent winter, spring and summer, respectively.

Figura 3 - Contribuição dos principais grupos de presas para a dieta da Cegonha-branca *(Ciconia ciconia)* por estação do ano, em termos da frequência numérica de presas na dieta (NF) e percentagem de biomassa consumida (PB). Os três níveis de cinzento (do mais claro para o mais escuro) representam o inverno, a primavera e o verão, respectivamente.



Seasonal analysis

Crayfish was regularly consumed by the White Stork throughout the study period (Figure 3), being the second most important prey taxa in winter (NF = 25.0%) and spring (NF = 20.9%), and the third in summer (NF = 24.1%). Coleopterans were the most consumed prey across the three periods (NF ranging between 43.7% in summer to 72.5% in spring), while orthopterans were the second most important prey in summer (NF = 29.8%) and the third in winter and spring (NF = 7.7% and 2.6% respectively). Regarding the consumed biomass, crayfish was the most representative prey category across all studied seasons (PB ranging from 37.3% in winter to 49.2% in summer; Figure 3). Coleopterans and orthopterans were the second most important prey categories in spring (PB = 30.6%) and summer (PB = 23.2%), respectively. Lagomorphs and small mammals recorded noteworthy PB values during the winter (PB = 26.1% and PB = 11.3%, respectively). The remaining prey categories (other insects, reptiles, and birds) were not consumed across all seasons and represented a low fraction of the diet: together, accounted for less than 5% of NF in each season; and individually, each prey category accounted for less than 10% of PB in each season. No significant differences were detected on crayfish consumption (NF) among seasons (Chi-square pairwise tests with Bonferroni correction significance at $P < 0.006$; Table 2). There were, however, significant seasonal differences in terms of PB, with crayfish having a larger contribution to the consumed biomass in summer in relation to spring and winter. The proportion of orthopterans in diet and its contribution to the bulk of biomass was significantly different among all seasons, peaking in summer. For coleopterans, a significantly higher consumption occurred in winter and spring in relation to summer, while in terms of PB a significant higher contribution to the diet was detected in spring in relation to winter and summer. The proportion on diet of small mammals (both NF and PB) was significantly higher in winter than in summer, and, additionally, a higher contribution in terms of PB was detected in spring than in summer. No significant seasonal variations regarding NF were found for other insects, reptiles, birds and lagomorphs. However, the contribution of these prey (secondary and occasional prey in terms of NF) to the bulk of consumed biomass varied significantly among seasons (Table 2). The diversity of diet seasonally decreased (H' = 2.11, H' = 2.10 and H' = 1.86 for winter, spring and summer, respectively), while species richness showed no seasonal trend (19, 34 and 29 prey taxa, respectively for winter, spring and summer; Table 1).

Discussion

White Storks in our study area feed upon a relatively wide range of prey, though a few specific food resources constitute the bulk of the diet. Regardless of the study site and season, coleopterans, crayfish, and orthopterans were the most consumed prey categories. Our results are similar to those from other dietary studies (based on pellet analysis) conducted in Europe, where insects (primarily coleopterans and orthopterans) were found to be the most frequent consumed prey (>80%), whereas vertebrates constituted only a small fraction (<10%) of the diet (Antczak et al. 2002, Tsachalidis & Goutner 2002, Miraglia et al. 2008, Vrezec 2009). On the other hand, in terms of consumed biomass, crayfish turn out to be the most representative prey among sites and across the studied seasons, whereas insects became less prominent on diet.

Following the introduction in Spain in the 1970s, craysfishes quickly spread across wetlands in the Iberian Peninsula, including ricefields (Geiger et al. 2005), becoming an abundant new food resource exploited by White Storks (Negro et al. 2000, Correia 2001). The consumption of this new prey promoted not only dietary changes but it also shaped the foraging behaviour of the White Stork in southwestern Europe (Correia 2001, Barbraud et al. 2002, Tablado et al. 2010, Sanz-Aguilar et al. 2015). In our study area, the crayfish was the second most important prey category for the White Stork in terms of number of individuals consumed and the most predominant in terms of biomass, being regularly consumed throughout the year. This is consistent with previously studies, suggesting that crayfish, where available, is an important dietary prey for White Storks.

Linking prey consumption with abundance and availability of prey is key to deepen on spatial-temporal diet variations and how predators exploit the available prey (e.g. Beja 1996, Correia 2001). Regrettably, in this study, diet analysis was not complemented with the assessment of ecological factors most likely to influence the diet of the White Stork, particularly the abundance and availability of prey (e.g. Correia 2001), which hinder and limit the extension of interpretations of the results. Nevertheless, the differences detected on crayfish consumption between sites, as well as its regular seasonal use by White Storks may be related to landscape structure and composition at each sampled site, though further investigation is required to test the potential effects of abundance and availability of prey on spatial-temporal diet variations. For instance, spatially, crayfish consumption is likely to be linked with the presence of ricefields, a major habitat for crayfish (Anastácio et al. 2009, Ramalho & Anastácio 2015). Specifically, the highest consumption of crayfish was recorded at Roubão, which is the site with higher abundance of ricefields nearby (20.3%), against 3.6% of ricefields at Catapereiro. Similar results were found by Tablado et al. (2010) in Guadalquivir marshes, in southwestern Spain. Accordingly, a greater presence of crayfish in the White Stork´s diet (expressed as percentage of crayfish in dietary samples) was recorded in areas mainly occupied by ricefields, rather than in natural marshland areas (Tablado et al. 2010). Although the White Stork is a generalist predator that can explore a variety of freshwater habitats, it tends to forage crayfish mainly in ricefields areas (Sanz-Aguilar et al. 2015). The spatial exploitation of the crayfish may also be influenced by the availability of other important prey in accordance to land use types. Specifically, coleopterans and orthopterans are abundant in arable land, mixed agricultural areas and broad-leaved forests, mainly cork oak woodland (Alonso et al. 1991, Galante et al. 1995, Tsachalidis & Goutner 2002, Silva et al. 2008). In fact, these habitats, which are also used by storks (Alonso et al. 1991, Johst et al. 2001, Catry et al. 2010), presented the highest difference in terms of land cover abundance between the two sites.

Regarding the seasonal consumption of crayfish by the White Stork, the continuous exploitation of this prey throughout all studied seasons is consistent with the few studies conducted in the Iberian Peninsula (Correia 2001, Tablado et al. 2010). Results of prey biomass consumption suggest that crayfish had a more important role in summer in relation to other seasons. However, in terms of numerical frequency our results indicate a regular seasonal pattern of consumption of crayfish, contrasting with the results from Correia (2001), which found seasonal differences on crayfish consumption by storks, with a lower predation intensity in winter and higher in summer. These patterns probably depend on crayfish abundance and availability to predators in accordance to hydrological cycle and water temperature of habitat types, which may be different between natural marshlands (found in Correia 2001) and ricefields – such as the case of this study – (Correia 1998, Anastácio et al. 2009, Ramalho & Anastácio 2015). Additionally, crayfish consumption may also be driven by the cost-benefit relation of foraging on other highly available food, particularly insects (as evidenced by the seasonal consumption of this prey). Notice, for example, that the White Stork apparently shifted from a diet mostly comprised by coleopterans in spring to a combined consumption of coleopterans and orthopterans in summer, which may be associated with peak density of these two prey taxa (Loureiro et al. 2009).

The establishment of crayfish populations has influenced the diet of several species of predators (e.g. *Lutra lutra*; Beja 1996, Barrientos et al. 2014), including the White Stork, resulting in dietary changes (Correia 2001, Tablado 2010), behavioural changes (e.g. increase of wintering population of storks; Catry et al. 2017) and demographic shifts (e.g. increase of local abundance of storks; Tablado et al. 2010). Moreover, the response of crayfish predators in relation to crayfish availability will likely continue to be strong in the absence of restrictive factors (e.g. nesting-site areas; Tablado et al. 2010). Thus, it is of great relevance to increase our knowledge on the potentially key role of the crayfish, considering the paradox trade-off of its positive effects vs. negative impacts on ecosystems (e.g. as a predator of amphibians and vector of diseases), as well as the driver of complex cascading effects on foods webs (Geiger et al. 2005). Specifically, broad-scale studies on this interaction, which assess the availability of prey species, may help to evaluate to which degree crayfish availability can lead to significant changes on populations of White Stork and other predators.

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