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Spatial ecology of a freshwater turtle in a temporary pond complex

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À ciência. Ao caminho que ainda falta percorrer. À vida.

"It was the masterful and incommunicable wisdom of eternity laughing at the futility of life"

Jon Krakauer, Into the Wild

Spatial ecology of a freshwater turtle in a temporary pond complex

Filipe Alexandre Cabreirinha Serrano

Abstract

Connectivity is currently a central issue in landscape management and planning for the conservation of wildlife species occupying sparse habitat patches. The European pond turtle (*Emys orbicularis* L., 1758) is an endangered species that has important populations in several pond systems in the SW Alentejo region of Portugal. About 56% of the ponds of this region have been destroyed from 1991 to 2009, primarily as a consequence of the agricultural intensification. This destruction possibly has severe consequences for the European pond turtle, namely in the interpond connectivity of the overall pond system. A population of *E. orbicularis* living in a pond system in the surroundings of a small locality (Longueira-Almograve) has been regularly surveyed since 2003. A total of 595 captures, totalling 205 individuals, were performed during this period. Data on interpond movements was used to estimate movement probabilities among pairs of ponds separately for females, males and juveniles. The relationship between these movement probabilities and three measures of interpond distances – linear, least cost and accumulated cost distance – were tested through Mantel tests. Movement probabilities tended to be more correlated with accumulated cost distance estimates, although a significant relationship with linear distances was also found. Females had a higher proportion of successive movements between different ponds than males. The variation of the movement between pairs of ponds with distance was modelled by fitting a negative exponential function. This function was used to predict the interpond movement probabilities among all the ponds of the system, including non-sampled or undersampled ponds. Based on these probabilities, defining several alternative threshold probabilities to settle different degrees of connectivity among ponds, several graph-based attributes were then estimated in order to rank the ponds in terms of their importance to the overall functional connectivity for the European pond turtle in this landscape.

Keywords: Movement, connectivity, *Emys orbicularis,* graph analysis, least cost distance

Ecologia espacial de uma tartaruga-de-água-doce num complexo de charcos temporários

Filipe Alexandre Cabreirinha Serrano

Resumo

A conetividade é uma propriedade central na gestão e planeamento da paisagem para a conservação de espécies que ocupam parcelas de habitat dispersas. O cágado-de-carapaça-estriada (*Emys orbicularis* L., 1758) é uma espécie de tartaruga de água doce considerada em perigo em Portugal, que mantém importantes populações em diversos sistemas de charcos na Costa Sudoeste Alentejana. Cerca de 56% dos charcos desta região foram destruídos entre 1991 e 2009 em consequência da intensificação agrícola no perímetro de rega do Rio Mira. Esta destruição teve certamente importantes consequências para o cágado-de-carapaça-estriada, nomeadamente ao nível da perda de conetividade entre charcos de todo o sistema da região. Uma população de *E. orbicularis* que ocorre num sistema de charcos nas imediações de uma pequena localidade (Longueira-Almograve) tem sido regularmente amostrada desde 2003. Um total de 595 capturas, totalizando 205 indivíduos, foram realizadas durante este período. Os dados de movimentos entre lagoas foram usados para estimar a probabilidade de movimentos entre lagoas, separadamente para fêmeas, machos e juvenis. A relação entre essas probabilidades e três medidas de distância entre lagoas – distância linear, de menor custo e de custo acumulado – foi testada através de testes de Mantel. As probabilidades de movimento tenderam a apresentar uma maior correlação com a distância de custo acumulado, apesar de se ter encontrado uma relação significativa também com a distância linear. A proporção de movimentos sucessivos entre lagoas diferentes foi significativamente superior nas fêmeas. A variação da probabilidade de movimentos com a distância foi modelada através do ajustamento de uma função exponencial negativa. Esta função foi utilizada para prever a probabilidade de movimentos entre todos os charcos do sistema, incluindo charcos não amostrados ou subamostrados. Diversos atributos baseados em grafos espaciais foram estimados, definindo diferentes probabilidades críticas para estabelecer diferentes graus de conetividade entre charcos. Esta metodologia permitiu ordenar a importância dos charcos em termos da sua contribuição para a conetividade funcional global do sistema para *E. orbicularis*.

Palavras-chave: Movimentos, conetividade, *Emys orbicularis*, análise de grafos, distâncias de menor custo

Introduction

With the increase of human population and the consequent human movement into wilderness areas and industrialization, a decline in biodiversity is becoming noticeable (Pimentel et al., 1992; Kindlmann & Burel, 2008). To support this increasing population, natural habitat must be converted into one of productive use. Land conversion not only results in a decrease in the amount of original habitat but also influences the ability of organisms to move between remaining patches of natural habitat (Bowne et al., 2006; Roe & Georges, 2008).

At the present moment *ca.* 45% of surface area of the European Union is covered by farmland (FAOSTAT, 2014) and, with half the species dependent on agricultural related habitats, Europe's agricultural ecosystems are of critical significance for conservation purposes (Stoate et al., 2009). With the regulations imposed by the successive CAP reforms (European Common Agricultural Policy) two agricultural trends have been observed (Ferreira, 2012): one of abandonment and one of intensification, with the first being responsible for landscape degradation, increased risk of erosion, wildfires and increased potential colonization of invasive species (Stoate et al., 2009) and the latter reducing the number of invertebrates present in agricultural landscapes thus affecting the higher taxa feeding on them, through pesticides application (Stoate et al., 2001), and greatly contributing to the decline of grassland biodiversity (Stevens et al., 2004).

One of the major problems that arises with agricultural development is the homogenisation of parcels (Curado et al., 2011), as they simplify and reduce the number of resources (be it food or refuge, among others) and specially condition the requirements to satisfy biodiversity's changing needs as organisms complete their life cycles (Firbank, 2005; Tscharntke et al., 2005). In order to maximize profit, farmers convert all of the available land in crops or pastures, eliminating biotic components such as temporary ponds in the Mediterranean region, albeit considered as a conservation priority in the EU Habitats Directive (92/43/CEE). Temporary ponds are seasonal wetland habitats that are flooded in winter or beginning of spring and dry out for a variable period of time during summer. They are therefore subjected to extreme and unstable ecological conditions and present a high variability of parameters such as size, shape, depth, taxa diversity and flooding period duration (Zacharias & Zamparas, 2010; Pinto-Cruz et al., 2011). They play an important role in conserving aquatic biodiversity, as they are especially rich in endemic and endangered species (Zacharias et al., 2007), serving as early warning for effects of long-term and large-scale alterations (Zacharias & Zamparas, 2010). Temporary ponds also serve as fundamental elements in connectivity between aquatic habitats, being of special significance in fragmented landscapes

by acting as stepping-stones to amphibians (Curado et al., 2011) and freshwater turtles (Gibbons, 2003; Bowne et al., 2006; Roe & Georges, 2007). In fact, they are essential to the life cycle completion of many *taxa* that have specialized in the regime of alternation between wet and dry phases (Cancela et al., 2008). Despite their importance, these water bodies are prone to destruction due to their small size and disregarded importance and, even if not destroyed, an increase in isolation (and thus lack of connectivity) can occur as the surrounding landscapes changes (Curado et al., 2011).

Some species of freshwater turtles are among the *taxa* that depend on temporary ponds. These organisms are mainly susceptible to landscape changes and habitat destruction since their life history traits make it problematic for them to abide increased mortality (Congdon, 1993). Their demographic strategy implies high adult survivorship and high reproductive output with low egg and hatchling survivorship as to maintain viable populations (Congdon et al., 1993). As a consequence of these ecological traits, roughly 45% of freshwater turtle taxa are listed worldwide as threatened in the 2000 IUCN Red List of Threatened Species (Turtle Conservation Fund, 2002) making them one of the most threatened clades (Spinks et al., 2003; Proulx et al., 2014).

The European pond turtle (*Emys orbicularis* L., 1758) is one of the two autochthonous freshwater turtle species in the Iberian Peninsula (Pereira et al., 2011). Despite its wide geographic range, ranging, west to east, from Portugal to the area of the Aral Sea (Kazakhstan) and, north to south, from Denmark to northern Africa (Kuzmin, 2002), and being considered globally as "Lower risk/Near Threatened" (Tortoise & Freshwater Turtle Specialist Group, 1996) its populations tend to be extremely fragmented and in many countries (*e.g.* Portugal) the species is classified as "Endangered" (Cabral et al., 2005). Its major threats are the alteration and destruction of wetlands, namely temporary ponds (Keller, 1997), and road mortality (Cabral et al., 2005). This species usually lives in metapopulations or patchy populations, as do most freshwater turtle species (Joyal et al., 2001; Bowne et al., 2006; Howeth et al., 2008; Enneson & Litzgus, 2009), hence movement between water bodies is essential for maintaining a long-term stable population (Ficetola et al., 2005; Enneson & Litzgus, 2009; J. D. Congdon et al., 2011; Fortin, 2012). As pointed out by Roe & Georges (2008) animals inhabiting temporary water bodies have to select coping strategies such as aestivating and migrating to other wetlands that can support them and their requirements, with these behaviours being influenced by local and landscape factors and differing with sex and ontogenetic stage. The spatial distribution of aquatic turtles may in that way reflect partially the availability of suitable habitats and food resources (Lue & Chen, 2008) and this knowledge of movement patterns is fundamental when understanding life history and ecology (Litzgus & Mousseau, 2004; Kindlmann et al., 2005; House et

al., 2010; Rayfield et al., 2010) and ultimately designing conservation measures (Litzgus & Mousseau, 2004; Millar & Blouin-Demers, 2011; Fortin et al., 2012). Differences between sexes and age classes in the movement patterns should be taken into account (Fortin et al., 2012). Several studies report terrestrial movement by freshwater turtles of both sexes (Cadi et al., 2004; Litzgus & Mousseau, 2004; Bowne et al., 2006; Cadi et al., 2008; Griffin, 2008; House et al., 2010; Rasmussen & Litzgus, 2010). Males are hypothesized to perform long-range movements as a means to increase mating opportunities whereas females undergo long-distance migration when nesting (Morreale et al., 1984; Gibbons, 1986; Litzgus & Mousseau, 2004). Yet Gibbons (1986) argues that freshwater turtle males make more frequent and longer migrations, with it being true in some studies (Morreale et al., 1984; Meeske & Mühlenberg, 2004) while others report that more adult females than males (Sexton, 1959; Litzgus & Mousseau, 2004; Aresco, 2005) and more adults than juveniles travel between ponds (Sexton, 1959). Other studies show no significant difference in home range or movements (Meeske & Mühlenberg, 2004; Crockett, 2008; Pittman & Dorcas, 2009; Millar, 2010; Ryan et al., 2013) and so the case might be that these findings are context-dependent and that all the biotic and abiotic factors that play a role in freshwater turtle migrations are still far from fully understood (Bowers & Matter, 1997; Bowne et al., 2006; Roe & Georges, 2008).

This raises many questions regarding how habitat preferences may influence freshwater turtle migrations and especially how connectivity affects them. To answer these questions, long-term monitoring of turtle populations is usually required because adverse situations sometimes occur over long periods before the effects on a population become detectable (Bowne et al., 2006). It is also important to report interpatch movement rates (Zeller et al., 2012) and its importance to the population since there is a lack of information regarding movement between habitat patches, especially in fragmented landscapes and because these migrations act as a response to the system's isolation and connectivity (Bowers & Matter, 1997; Bowne & Bowers, 2004).

Connectivity is a measure of how landscapes facilitate movement and thus is a vital component of a species persistence in a heterogeneous landscape (Taylor et al., 1993; Bowne et al., 2006; Rayfield et al., 2010; Fortin et al., 2012). Without connectivity, survival and genetic flow decrease (Pardini et al., 2005; Proulx et al., 2014) as do mobility, home range size and breeding success (Nikolakaki, 2004). It may be especially low in agricultural landscapes (e.g. croplands) (Sheperd & Swihart, 1995; Gustafson & Gardner, 1996) since it decreases in disturbed land use classes and increases with natural and undisturbed habitat (Millar, 2010). It can be either *functional connectivity* when it is measures the responses of an organism dispersal through its matrix or *structural connectivity* when analysed solely on its landscape attributes and

not relating it to animal movement (Bowne et al., 2006; Baguette & Dyck, 2007; Kindlmann & Burel, 2008) To put into practice effective and resource-optimal conservation measures it is important to understand the factors affecting connectivity and what are the consequences on population structure, abundance and movements (Johnson & Gaines, 1990; Taylor et al., 1993; Mönkkönen & Reunanen, 1999; Rayfield et al., 2010; Zeller et al., 2012; Fortin et al., 2012). (Russell, 1999)

This study targets to describe the movements performed by a freshwater turtle species, *Emys orbicularis*, and to relate them with a heterogeneous land matrix, understanding how it affects its movement patterns. This species was chosen as a model organism because of its discrete habitat requirements, relative ease to mark and follow, large range size and high longevity. In addition, this species may provide valuable information on freshwater turtle spatial ecology that might be applied to other species with similar ecological requirements since little is known about the importance of pond connectivity for conservation of turtle metapopulations (Pereira et al., 2011). We aim to answer the following questions: i) Do movement rates vary between sex and age class? ii) Do least cost distances relate stronger to the probability of movements between pairs of ponds than linear distances? iii) Does the probability of movements decrease with distance between ponds? This information will then be used to develop a graph-based connectivity model that will help to establish management priorities to maintain or enhance the overall functional connectivity of the system for *Emys orbicularis*.

Materials and methods

Study area

The study was carried out within the Natural Park of Sudoeste Alentejano and Costa Vicentina located on the coastal plain of southwest Portugal (37º 30' N, 8º57' W) (Beja & Alcazar, 2003) (Fig. 1). Climate is typical Mediterranean with oceanic influence. Aridity increases southwards, with annual mean temperature increasing from 15 to 16º C, and annual precipitation going from 650 to 400 mm, of which >80% falls in the October– March period (Ferreira, 2012).

The landscape is flat throughout most of its extension with some tree cover composed of small woods, windbreaks and stream valleys. Agriculture and livestock farming are the most significant land uses; with extensive cultivation of winter cereals on a cereal–fallow rotation basis, and beef cattle, respectively. Since

the early 90's there has been an increase of irrigation systems and, as a consequence, an increase in the use of fertilizers and chemical-based products such as pesticides.

Temporary ponds are scattered in this landscape, occupying shallow depressions and varying greatly in terms of depth, water volume, permanence and the associated biotic factors. These ponds are intimately associated with changes in the hydrological regime, filling in the winter and drying in the summer. Some of these are deepened by farmers and used as reservoirs transforming them in permanent water bodies (Beja & Alcazar, 2003). This affects the functioning of the pond's biotic components, becoming more susceptible to exotic species as are the Louisiana crayfish (*Procambarus clarkii*), the pumpkinseed sunfish (*Lepomis gibbosus*) and the small-mouth bass (*Micropterus salmoides*). During the extent of this study twenty ponds were considered in the capture-mark-recapture overview, all of them included in the surrounding agricultural fields of Longueira-Almograve.

Fig. 1 – Study area location

Capture

From May 2003 to June 2005 and then from April 2010 to July 2013 (all sampling sessions done by Pedro Segurado, with the exception of June and July 2013 when the sampling was done by the author) turtles were captured either by hand or by traps baited with sardine placed perpendicularly along the pond

shoreline. Traps were checked and baited on a daily basis and captured turtles were individually marked by notching the marginal scutes (Keller, 1997).

Biometric data was recorded for each individual at each capture event, including carapace length (CL), measured with a Vernier calliper to the nearest millimetre, and weight (W), measured with a Pesola scale.

The age class of the individuals was classified according to the carapace length, following the criteria used by Keller (1997): juveniles CL < 115mm; subadult males CL < 120 mm, adult males CL > 120 mm; subadult females CL < 130mm, adult females CL > 130mm. Adult and subadult individuals were sexed based on the differentiation of secondary sexual characters. Males attain smaller sizes (Berry & Shine, 1980) and have smaller and more concave plastrons with a longer precloacal tail.

Interpond movements

The total number of ponds where each individual was caught was compared between sexes and age classes (females, males, juveniles) using Kruskal-Wallis tests followed by the pairwise multiple comparison test after Kruskal-Wallis implemented in the kruskalmc function of the pgirmess package for R, version 3.0.2. (R Core Team, 2013). To control for the effect of the total number of captures, we compared instead the residuals of the linear regression between the number of captures and the number of ponds where the individuals were captured.

The movement rate among successive captures, as measured by the proportion of total consecutive captures carried out between different ponds, was also computed and compared between sexes and age classes using chi-square tests. If an individual was captured consecutively in the same pond it was assumed that it had not moved to other ponds.

The probability of turtle movements between each pair of ponds (A,B) was computed as the mean proportion of individuals of pond A and B that was also caught, respectively, in pond B and A, weighted by, respectively, the sampling effort at pond A and B. This may be translated by the following expression:

$$P_{a,b} = \frac{[(N_{a,b}/N_a) \times E_a] + [(N_{a,b}/N_b) \times E_b]}{(E_a + E_b)}$$

where $P_{a,b}$ is the probability of movement between pond a and b, $N_{a,b}$ is the number of individuals caught in both ponds, N_a and N_b is the number of individuals caught, respectively, in pond a and b, and E_a and E_b is the sampling effort (number of trapping days), respectively, in pond a and b. This probability was computed for all individuals and separately for each sex and age class for comparative purposes.

The probability of turtle movements between each pair of ponds was then compared, through Mantel tests (Mantel, 1967), to several measures of interpond distances: linear (Euclidean) distance, least cost distance and accumulated cost distance. The Least cost distance and Accumulated cost distance computation was performed with the Pathmatrix extension for ArcView 3.x (Ray, 2005), and involved first the creation of a movement resistance surface based on land use polygons, since we expected that the spatial location of least-cost links would be related with the spatial pattern of the habitat and matrix land use types and with the relative traveling cost values of each (Rayfield et al., 2010).

Seven land use classes were identified in the study area (ponds, meadows, grazing fields, dunes, croplands, forests and urban areas), along with four linear structures (sand roads - no traffic but human use and presence; dirt roads - low traffic; asphalt roads - high traffic and agricultural ditches) (Fig. 2). Each landscape element was digitized to a Geographical Information System using Quantum GIS version 2.0.1 Dufour (Project Development Team, 2012) and aerial images from the software Google Earth (version 7.1.2.2041). The outer limits of the ponds were used as borders between aquatic and terrestrial environments.
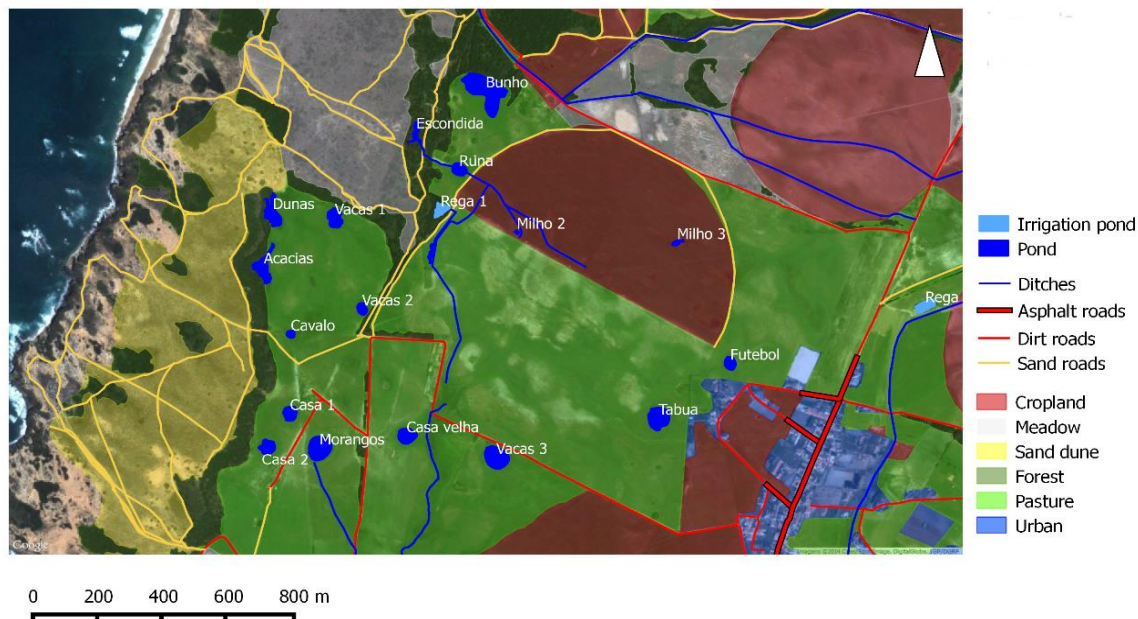

Fig. 2 – Land use map

To each of these landscape elements, a degree of resistance to turtle movement was attributed based on the literature Bowne et al., 2006; Rayfield et al., 2010) and expert judgment. Resistance values ranged from 0 (pond) to 20 (asphalt roads). In order to assess the sensitivity to the subjective selection of values

between the minimum and the maximum, different set of resistance values with contrasting distributions were considered (Table 1). We considered two contrasting set of values: a set where values were compressed around the medium value of 10 (compressed scenario – scenario 2) and a set where values were closer to either the minimum (0) or the maximum (20) values (contrasting scenario – scenario 3).

Table 1 – Cost of resistance to movement scenarios

	Pond	Ditch	Sand road	Meadow	Pasture	Dune	Cropland	Dirt road	Forest	Urban	National road
Scenario 1 - Equal intervals	0	2	4	6	8	10	12	14	16	18	20
Scenario 2 - Extreme-concentrated intervals	0	1	2	3	4	5	16	17	18	19	20
Scenario 3 - Center-concentrated intervals	0	6	7	8	9	10	11	12	13	14	20

Analysis of interpond connectivity

To assess the importance of each pond to the overall system connectivity we used a graph based approach (Pascual-Hortal & Saura, 2007; Pereira et al., 2011; Decout et al., 2012) to measure several connectivity parameters within the pond system of the study area. Graphs are a set of nodes connected by links used to attain pairwise relations between different objects (Urban et al., 2009). Here it aims to establish the relation between suitable patches of habitat (represented by nodes) interconnected via ecological corridors (represented by links). This allows to understand how these patches are connected and to rank the nodes according to their contribution to the overall system's connectivity (Saura & Torné, 2009).

To evaluate the importance of each pond to the functional connectivity of the pond system, we computed the following measures (see e.g. Baranyi, Saura, Podani, & Jordán (2011) and Urban et al. (2009)): Betweenness Centrality (BC), Closeness Centrality (CC), Maximum Cohesion (MC) and the contribution to the Probability of Connectivity (dPC). The BC represents the number of times a node is contained in the shortest pathways between all pairs of nodes divided by the total number of shorter paths between all pairs of nodes. It assesses how much that node is involved in the flow of organisms through the landscape (Baranyi et al., 2011). The CC is the inverse of the mean topological distance (i.e. number of links) between a node and the remaining reachable nodes, measuring how close the node is to the remaining nodes. The MC is the measure of cohesion of the most cohesive block to which each node is allocated. This measure

is based on a hierarchical cohesive blocking method implemented in the cohesive.blocks function of the igraph library (http://igraph.org/r) for R. The BC, CC and MC indices are measures of the structural connectivity because they only consider topological relationships between nodes. Lastly, the PC is defined as the probability that two points randomly placed within the landscape fall into habitat areas that are reachable from each other (interconnected) given a set of n habitat patches and the links (direct connections) among them (Saura & Pascual-Hortal, 2007). This index takes into account habitat attributes (e.g. area or quality for a given species) and probabilities of movement between patches, and therefore, unlike the former indices, it has a strong functional component, i.e., it considers behavioural or ecological aspects of specific individuals or taxa. The contribution of each node to the overall PC (dPC) of the network was computed by systematically removing each of the nodes from the landscape and evaluating their individual impact.

To compute the PC it was necessary to associate a probability to each link between each pair of ponds. This probability was modelled using the probability of turtle movements between each pair of ponds. We fitted a negative exponential function of the interpond distance (Urban & Keitt, 2001; Saura & Pascual-Hortal, 2007), from which all interpond movement probabilities (p_{ij}) were calculated as:

$$p_{ij} = a \times e^{-b \times d_{ij}}$$

where d_{ij} is the interpond distance (m) and a, b the coefficients to be estimated. This probability tends asymptotically to zero and is equal to a when $d_{ij}=0$. This curve was fitted using the nls function of the R software, version 3.0.2. (R Core Team, 2013).

The parameters BC, CC and MC were computed, respectively, using the igraph library (http://igraph.org/r) for R and PC was computed with Conefor Sensinode 2.2 (Saura & Torné, 2009).

Results

Population size and structure

A total of 595 captures were performed throughout the whole study period (Table 2). Among these captures 205 individuals were identified (juveniles n=62, males n=78 and females n=65).
Parameters as the "average number of ponds visited", "standard deviation", "% captures in a single pond" and "average number of captures per individual" were also calculated as shown on Table 2.

Table 2 – Parameters of capture events

	Juveniles	Females	Males	Total
Total captured individuals	62	65	78	205
Total captures (incl. recaptures)	205	162	228	595
Average number of ponds visited	1.213	1.514	1.432	1.386
Standard deviation	0.461	0.864	0.755	0.693
% captures in a single pond	80.9	65.7	68.2	71.6
Average number of captures per individual	3.306	2.492	2.923	2.902

The sex ratio was 1.2:1 and, while juveniles account for roughly only 30% of the population, they represented the class with highest number of average captures per individual (3.306) and proportion of captures in a single water body (80.9%). The juvenile class also recorded the least average number of
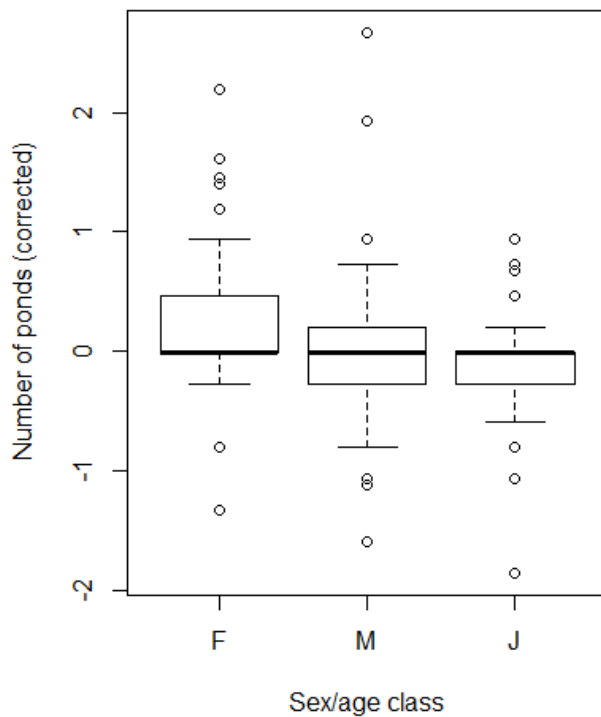

Fig. 3 – Boxplot showing differences between sexes and class ages in the total number of ponds (corrected for the total number of captures – see Materials and Methods section) where the individuals were caught.

ponds visited (1.213). Comparatively among sexes, males were captured the most (228 in 595) whereas females were captured in more ponds (average value of 1.514) but accounted for less average number of capture per individual than the other classes (2.492).

The differences between sexes and age classes of the total number of ponds where individuals were caught, corrected for the total number of captures (Fig. 3), were significant (Kruskall Wallis test, K=8.78, p<0.02). According to the multiple comparison tests after Kruskal-Wallis (Table 3), there are significant differences only between females and juveniles.

Table 3 – Results of the Multiple comparison test after Kruskal-Wallis (TRUE indicates p < 0.05).

Comparisons	obs.dif	critical.dif	difference
F-J	32.36565	27.54859	TRUE
F-M	22.14805	27.94657	FALSE
J-M	10.21760	25.88337	FALSE

Interpond movements

Significant differences in the movement rates between males and females (Pearson's Chi-squared test with Yates' continuity correction, χ^2=5.835, p<0.02) and between adults and juveniles (Pearson's Chi-squared test with Yates' continuity correction χ^2=15.8744, p<0.001) were found. In average, the same females individuals were found more often in different ponds than males (Table 4). Consecutive captures of adult individuals were also more often made at different ponds than juvenile individuals (Table 4).

Table 4 – Number of successive captures of individuals at the same pond, at different ponds and the total number of successive captures. The resulting movement rates (proportion of successive movements performed between different ponds) is also shown.

	Females	Males	Juveniles	Total
Same pond	47	97	99	253
Different ponds	44	45	20	116
Total	91	142	119	369
Movement rate	0.48	0.31	0.16	0.31

The results of Mantel tests relating interpond movement probabilities with both linear and least cost path (Fig. 4) distances were overall similar (Table 5). A significant negative relationship between interpond movement probabilities and simple linear distances among ponds was found, either considering all the individuals, either separately for both sexes and juveniles (Table 5). However, overall, interpond movement probabilities showed stronger relationships with the accumulated cost distances, especially in

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scenario 1 (males p<0.007; females p<0.02), except for juveniles in which the most significant relationship (p<0.02) was found for the least cost distances (scenario 3). In general, scenarios 1 and 3 explained best the relationship between movement probabilities and interpond distances.

Table 5 – Results for each distance type and scenario

Distance type	Scenario	Class	R	p-value	c.l. 2.5%	c.l. 97.5%
Euclidian	-	Total	-0.460	0.002*	-0.573	-0.336
		Females	-0.288	0.036*	-0.411	-0.176
		Males	-0.328	0.019*	-0.420	-0.235
		Juveniles	-0.312	0.026*	-0.417	-0.168
Least cost	1	Total	-0.372	0.010**	-0.490	-0.262
		Females	-0.222	0.084	-0.332	-0.061
		Males	-0.221	0.088	-0.325	-0.131
		Juveniles	-0.311	0.028*	-0.457	-0.167
	2	Total	-0.300	0.030*	-0.421	-0.175
		Females	-0.170	0.144	-0.300	-0.013
		Males	-0.144	0.170	-0.244	-0.057
		Juveniles	-0.285	0.034*	-0.419	-0.072
	3	Total	-0.442	0.003**	-0.565	-0.314
		Females	-0.275	0.041*	-0.416	-0.139
		Males	-0.278	0.035*	-0.369	-0.184
		Juveniles	-0.340	0.014*	-0.479	-0.196
Accumulated cost	1	Total	-0.489	0.001***	-0.590	-0.412
		Females	-0.342	0.011*	-0.472	-0.241
		Males	-0.358	0.007**	-0.434	-0.279
		Juveniles	-0.278	0.034*	-0.378	-0.063
	2	Total	-0.431	0.001**	-0.540	-0.348
		Females	-0.308	0.020*	-0.438	-0.196
		Males	-0.302	0.017*	-0.384	-0.205
		Juveniles	-0.237	0.060	-0.359	-0.110
	3	Total	-0.464	0.001***	-0.566	-0.406
		Females	-0.299	0.029*	-0.417	-0.157
		Males	-0.329	0.017*	-0.437	-0.218
		Juveniles	-0.286	0.034*	-0.424	-0.120

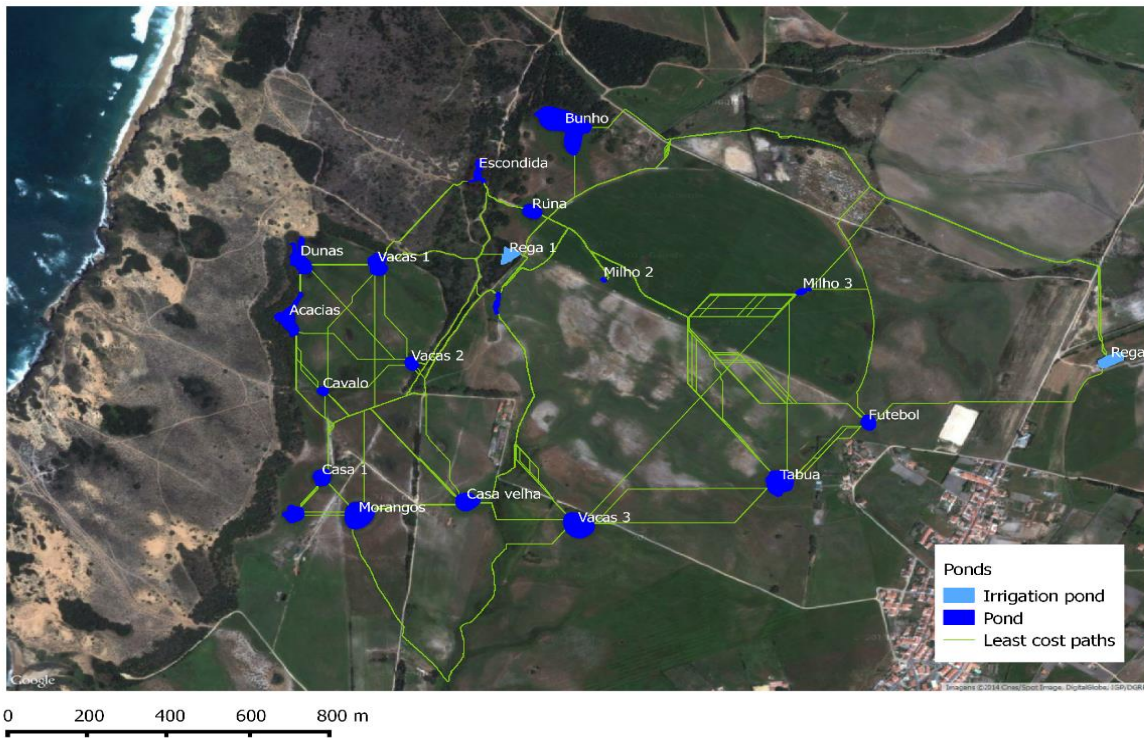* for p ≤ 0.05, ** for p ≤ 0.01 and *** for p ≤ 0.001.

Fig. 4 – Least cost paths for scenario 1

The fitted calibration curve describing the decrease of the probability of interpond movement with the linear interpond distance was very similar between sexes (Fig. 5a). Therefore, a single overall curve was fitted (Fig. 5b) and further used to extrapolate the probability of movements between unsurveyed ponds allowing to analyse the connectivity of the whole pond system.
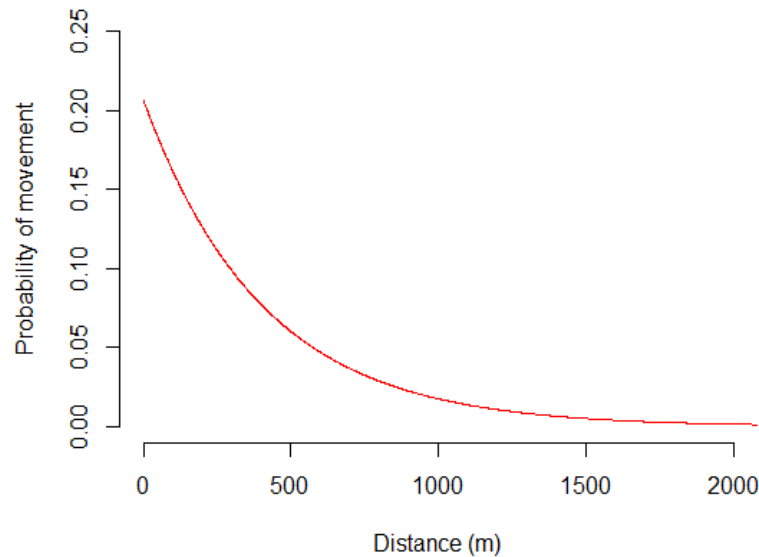
a)
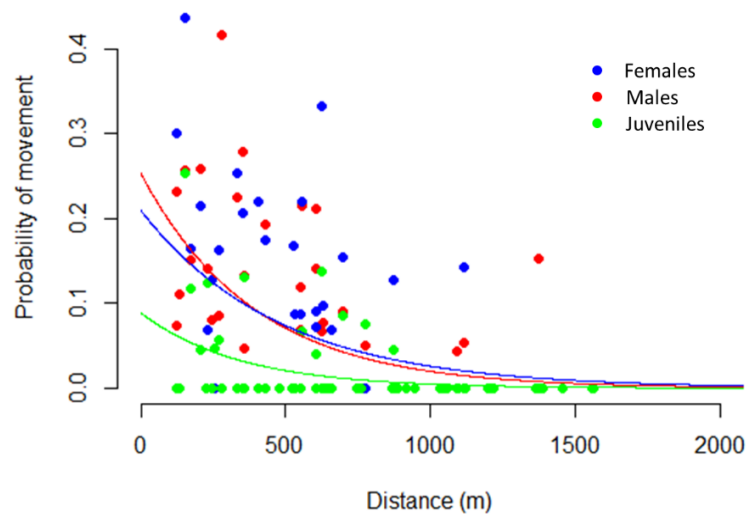
b)

Fig. 5 - Probability of interpond movement vs. Linear interpond distance (a - calibration curve for males, females and juveniles; b – final calibration curve).

Analysis of interpond connectivity

To assess connectivity we analysed four metrics (Table 6). A probability threshold of 0.025 (probability below which a pair of ponds are considered disconnected) was used to compute BC, CC and MC. This probability is obtained through the calibration curve (Figure 5) since Mitrus (2010) refers that terrestrial movement above 1000 meters is unusual and it was used to highlight the most cohesive ponds and the ponds that bridge graph clusters. When no probability threshold is selected, all nodes are connected and these three measures are constant among nodes. Betweenness Centrality (Figure 6) returned the largest range of values with ponds such as "Milho 2", "Futebol" (and to a lesser extent "Rega 1" and "Vacas 3") having high scores and thus acting as main connectors in the pond system. Concerning Closeness Centrality the analyses yielded a very uniform array of results (Figure 7) and the degree of Maximum Cohesion was high for the most ponds (Figure 8), with "Futebol", "Tabua" and "Rega2" scoring the lowest. As for percentage of the variation in Probability of Connectivity (dPC) the top scorers were "Vacas 1", "Rega 1", "Milho 1" and "Vacas 2" whereas "Rega 2" and "Futebol" did not yield as high results as all of the remaining water bodies (Figure 9) "Rega 2" scored the lowest for all the analysed metrics, whereas "Milho 2" scored the highest except for dPC, where "Milho 1" attained even higher values.

Table 6 – Results of connectivity measures analyses (BC – betweenness centrality; CC – closeness centrality, MAXCO – maximum cohesion, dPC – variation of probability of connectivity after removing the pond).

| Id	Label	BC	CC	MAXCO	dPC
1	Acácias	1.08	0.03	9	8.56
2	Dunas	2.17	0.03	9	8.22
3	Ruína	5.51	0.04	9	9.2
4	Bunho	2.01	0.03	8	7.27
5	Morangos	0.56	0.03	9	8.3
6	Vacas 1	4.09	0.04	9	9.35
7	Casa velha	2.86	0.04	9	8.19
8	Casa 1	0.56	0.03	9	8.64
9	Casa 2	0.42	0.03	9	7.68
10	Escondida	0.97	0.03	9	8.58
11	Rega 1	9.66	0.04	9	9.81
12	Milho 1	4.88	0.04	9	9.89
13	Milho 3	5.76	0.03	4	5.66
14	Milho 2	32.91	0.04	9	8.44
17	Rega 2	0	0.02	1	3.19
18	Tabua	2.01	0.03	3	5.62
21	Vacas 3	9.07	0.04	9	6.89
22	Cavalo	3.59	0.04	9	9.2
23	Vacas 2	4.88	0.04	9	9.87
24	Futebol	18	0.03	3	5.15
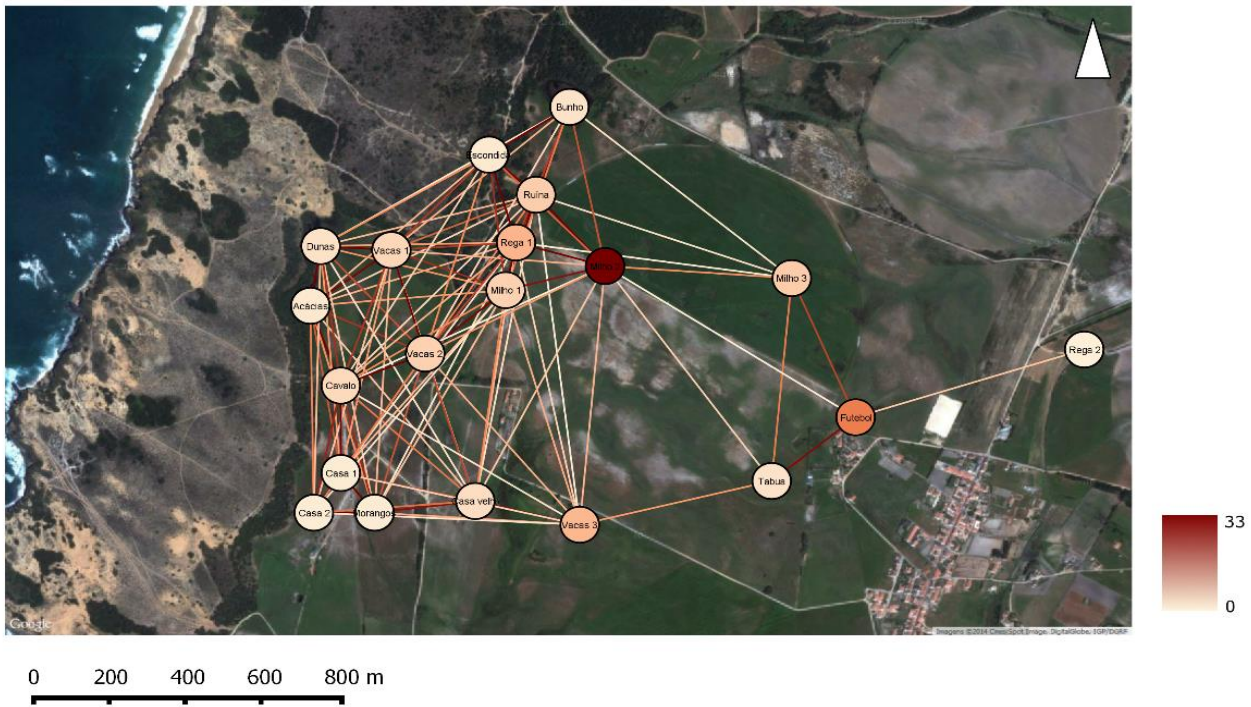
Figure 6 – Betweenness Centrality values for ponds in the study area
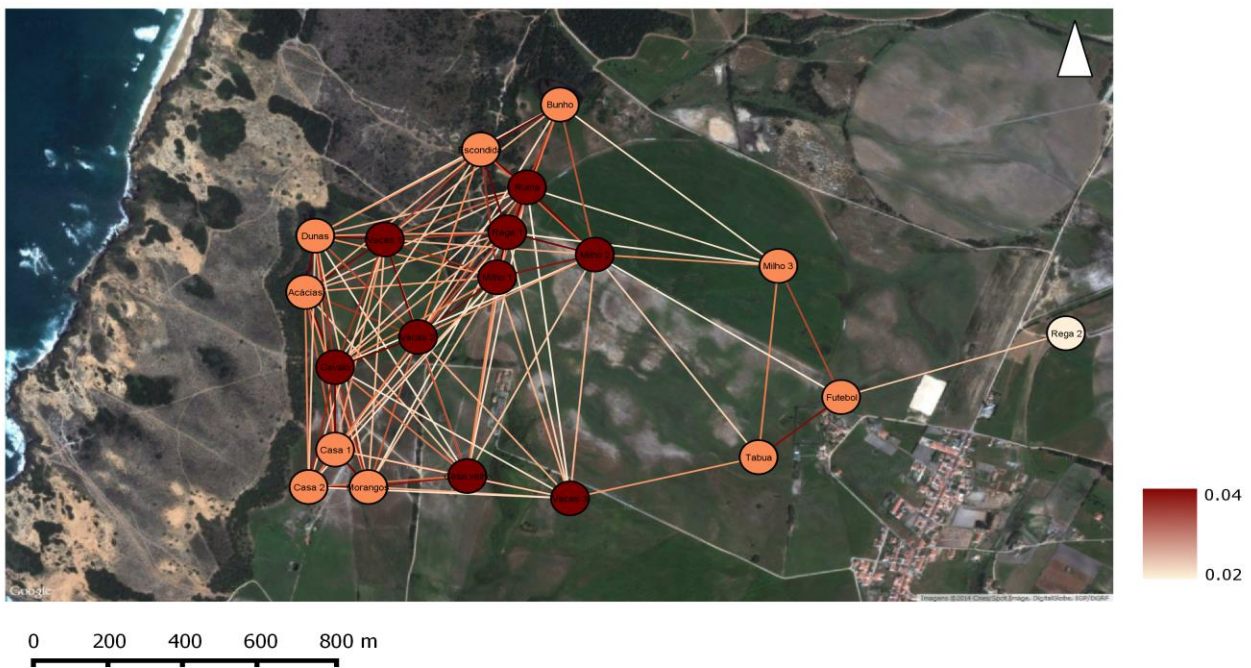

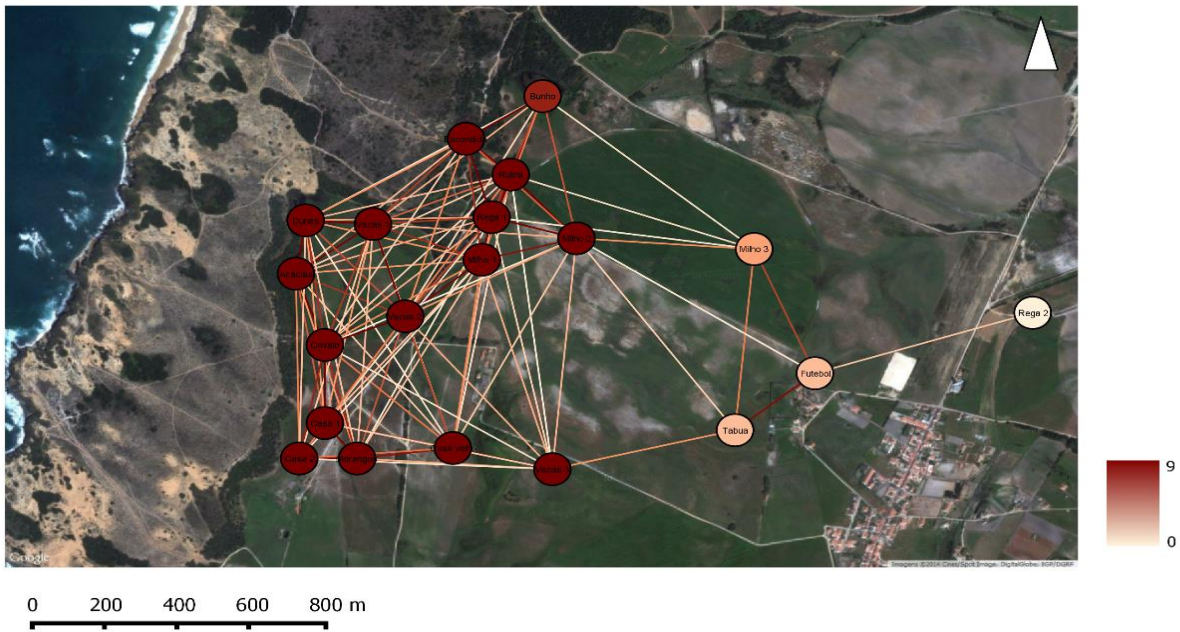Figure 7 – Closeness Centrality values for ponds in the study area

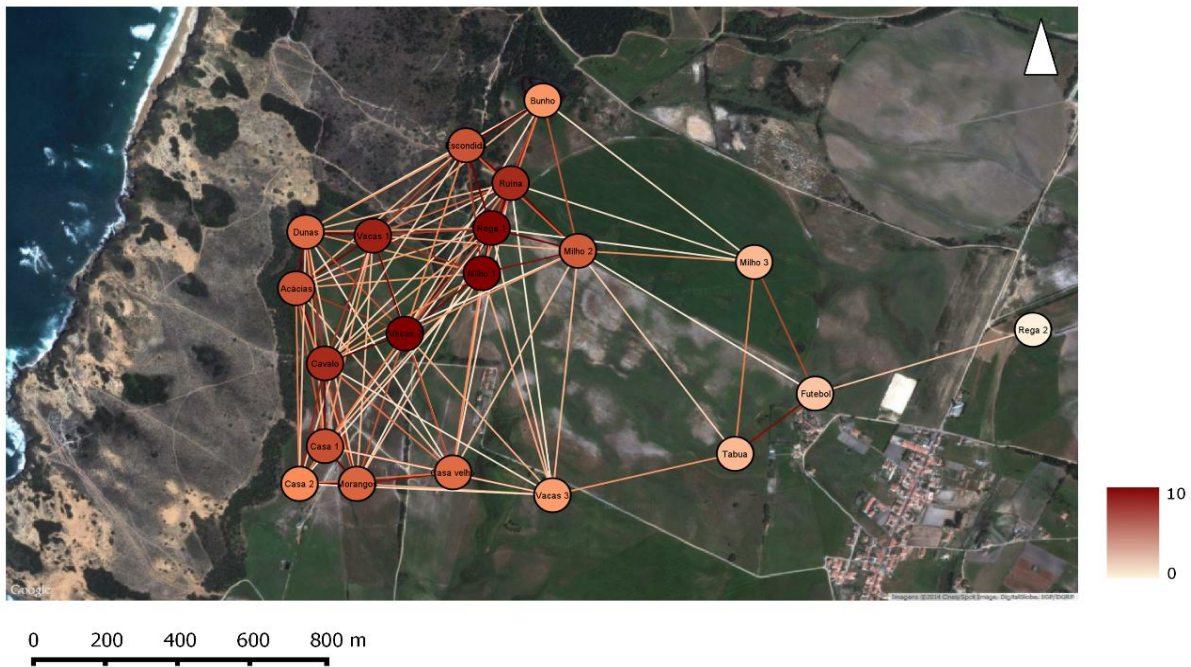Figure 8 – Maximum Cohesion values for ponds in the study area

Figure 9 – Probability of Connectivity values for ponds in the study area

Discussion

The purpose of this study was to assess the spatial ecology of the European pond turtle in a temporary pond complex, while analysing its empirical data movement through cost-distance modelling and the pond system's connectivity. We were able to demonstrate that a significant part of the population underwent terrestrial migrations and that the connectivity of the landscape plays a vital role when taking these movements into consideration. Moreover, landscape matrix composition is of importance for estimating the degree of connectivity and the favoured paths of dispersal. We also successfully used a graph-based approach to understand the importance of each pond to the overall connectivity (Bunn et al., 2000).

Terrestrial movements were frequent in both sexes and all age classes. Females tended to show higher movement rates and a larger average number of ponds visited. Analyses of movement rates between ponds in our study area suggest that individual ponds do not contain demographically independent populations but instead it is probably the core of a unique patchy population (Harrison, 1991), as reported for other vagile freshwater turtle species by different authors (Joyal et al., 2001; Bowne et al., 2006; Roe & Georges, 2008).

The fact that the average number of ponds visited by juveniles (slightly over 1.2 ponds) is lower than for both adult sexes, with close to 81% of their consecutive captures done in the same pond, might have to do with either a lack of perception of the individuals for the existence of more distant ponds (Bowne et al., 2006), or with risk avoidance, since younger (and thus smaller) individuals are more vulnerable to predation and desiccation (Finkler, 2001; Kolbe & Janzen, 2002; Delmas & Baudry, 2007). In fact, according to some studies, juvenile turtles tend to stay in the same pond for years (Rössler, 2000; House et al., 2010). The water bodies used by juveniles are most often shallower and warm up faster and so might offer food resources earlier than others, being usually densely vegetated with woody plants (Meeske & Mühlenberg, 2004; Ficetola et al., 2005; Cadi et al., 2008; Griffin, 2008). Even with the lower number of captured individuals, juveniles accounted for the highest captures per individual, which might be related to pond parameters (shallower and smaller) and/or disposition to be captured (due to higher feeding rate). Consequently, the movement rate was also lower for this age class (Table 2), as it happens with other species of semiaquatic freshwater turtles, such as *Chrysemys picta* (Griffin 2008; Bowne, Bowers, and Hines 2006).

Females visited a higher number of ponds, hence, in average, were captured less in the same pond. The multiple comparison test after Kruskall-Wallis confirms the difference in number of ponds used between

adult females and juveniles. This could be related with different requirements and with sex-specific movements, namely nesting activity, since other studies report long-distance movements for this purpose: 143 ± 217 m in France (Cadi et al., 2008), 150-1000 m in Lithuania (Meeske, 1997), 150-600 m in Italy (highlighting the importance of small temporary ponds in nesting migrations) (Rovero & Chelazzi, 1996), 69-83 m in Poland (Najbar & Szuszkiewicz, 2007), 188-530 m in Slovakia (Bona et al., 2012), 500-2000 m in Ukraine (Kotenko, 2000) and 400-600 to 1500 m combining terrestrial and aquatic dispersal in Belarus (Drobenkov, 2000), although Mitrus (2010) states that movements over 1000 meters cannot be considered as usual.

Movement rates were significantly different across every sex and age class, possibly indicating that each has different requirements and behaviours that influence the way individuals occupy and move across the landscape, as it happens with the closely related *Emydoidea blandingii* (Congdon et al., 2011). One evidence of this comes from Table 2, showing that the movement rate is particularly high for females. Their higher movement rates might be related with nesting behaviour, as stated above.

These results contrast or agree with other studies focused on freshwater turtles. Females of the European pond turtle were found to have larger home range sizes in a study performed by Cadi et al. (2008). For *Actinemys marmorata* Sloan (2012) shows that females do have noticeable higher movement rates but only during the nesting season, whereas males presented higher movement rates throughout the rest of the year. The same trend was found with *Clemmys guttata* mean daily movements. For *Chrysemys picta*, Bowne, Bowers, and Hines (2006) report a female interpond movement rate of 0.46, contrasting with the value of 0.13 reported by Griffin (2008). Both authors suggest that habitat quality (namely pond drying) is the possible movement-inducing factor, with distance also playing an important role when it comes to move between water bodies. Roe and Georges (2008) also found that for *Chelodina longicollis*, the percentages of terrestrial locations and terrestrial activities duration increased linearly with decreasing hydroperiod, justifying it with the maximization of resource acquisition. This issue remains poorly studied and would be an interesting topic for further studies in spatial ecology and movements of the European pond turtle, since turtle movements between aggregation sites are probably also dependent on multiple biotic and abiotic factors that might alter the cost-benefit ratio, such as changes in the suitability of the wetlands (Bowne et al., 2006; Enneson & Litzgus, 2009; House et al., 2010).

Regarding the several tested interpond distance types (linear paths vs. least cost paths) and scenarios of landscape resistance to movement, the results seem to support one of our hypotheses: movement probabilities decrease as distance between ponds increase. This is most probably related to cost-reducing

strategies (the longer the migration the higher its energetic cost) and to the fact that other ponds in the way to more distant ponds might offer enough resources to annul the need to travel to the more distant pond in the first place.

The ability of turtles to move through landscape depends, among other factors, of the characteristics of the land in which the dispersal takes place. Namely landscape composition is certainly significant in affecting dispersal costs and benefits (Baguette & Dyck, 2007). The cost value attributed to each land use class represents the degree to which that specific component enables or impedes terrestrial movements for the individual in focus. It is also a particular individual's trait rather than a species-specific assigned cost since it shows differences both within and between populations (Joyal et al., 2001; Van Dyck & Baguette, 2005).

The interpond movement probabilities were more strongly related with the accumulated cost distances (that incorporate both length and underlying ecological cost to travel them) than with simple Euclidean distances or least cost distances. In fact, accumulated cost distances are considered to represent a good analytic metric because they can provide a more appropriate and complete measure of connectivity and, thus, a stronger explanatory power of movements (Etherington & Holland, 2013). However, the relationship of interpond movements with Euclidian distances was also very evident and statistically significant (especially when considering the total population), which has been also found in other studies (Etherington & Holland, 2013). Therefore, since the Euclidean distances are much simpler to compute and to extrapolate to new landscapes, we ended up using these distances to fit the calibration curve describing the decrease of interpond movements with interpond distances.

Among the several scenarios of landscape resistance to movement, the scenarios 1 (equidistant) and 3 (center-concentrated intervals) showed a higher relation with the observed movements and this seems to show that scenarios with more abrupt interclass difference of resistance to movement do not relate as good with the observed turtle movements (e.g. scenario 2 where there is a high increase of resistance to movement from 5 in dune to 16 in croplands). Similar scenarios were found to have a significant impact on the pond network structure, although showing little impact on the pond connectivity measures (Pereira et al., 2011). Rayfield et al., (2010) using simulated landscapes showed that setups with less extreme differences in the cost values show a less harsh spatial deviation to least-cost paths. The same study also found a significant interaction between habitat fragmentation (as are temporary ponds complexes), the amount of hospitable matrix of landscape use classes and the relative costs of the latter. Our results can

serve as a basis to study and analyse landscape connectivity through use of different wetlands (Fall et al., 2007).

The spatial graph approach to interpond connectivity allowed for a better understanding of each pond's significance for the overall connectivity of the system via results of Betweenness Centrality (BC), Closeness Centrality (CC), Maximum Cohesion (Maxco) and Probability of Connectivity (dPC). The pond with the highest BC, CC and Maxco values was "Milho 2". This is apparently the most relevant water body for the structural connectivity, since it serves as one of the main connectors, it is among the most central habitat patches and ranks highest in maximum cohesion. However, considering the functional connectivity, as measured by the dPC, this pond is only ranked in the 10th position, the ponds with higher dPC outcome being "Rega 1", "Milho 1" and "Vacas 2". All these ponds had also the maximum score for both CC and Maxco but their BC was not as noticeably high as it was for "Milho 2". Therefore, these 3 ponds ("Rega 1", "Milho 1" and "Vacas 2") contribute the most for the overall connectivity of this species, while "Milho 2" should not be overlooked in a more structural approach. This supports the idea that structural connectivity metrics may become meaningless unless when compared with reliable data movements and actual space usage by the studied species (Calabrese & Fagan, 2004). As Kindlmann & Burel (2008) described, landscape does not have a single fixed connectivity value but is instead composed of two components: landscape and species. Therefore different landscapes can have different connectivity results while the same landscape can have a difference in connectivity values for different species or even intraspecific variation (be it age class or sex), with the same degree of connectivity also depending on the used metrics. That is why one of the proposed solutions by many authors (Sutherland et al., 2006; Fall et al., 2007; Rayfield et al., 2010) is to utilize a combination of least-cost paths (inferred through data movements) with graph-theoretic techniques (considering habitat specificities and landscape matrix characteristics) so that we attain a better understanding of the species' spatial attributes and its relation with the landscape configuration.

Despite the important results we achieved, there were some limitations of the study that we consider important to deal with in further studies. The movement rate variation model is still too simple to fully explain the interpond movements, since it is based on mark-release-recapture techniques, which are often prone to suffer from behavioural biases, as some individuals might be easier to catch (Van Dyck & Baguette, 2005). Another limitation was that wetland quality was not incorporated as an explaining factor, especially since adult turtles are likely to choose better quality water bodies (Ficetola et al., 2005; Bowne et al., 2006). Unfortunately, no hydrological data is available for the wetlands in our study area and the

large inter annual variation in its regime made it especially difficult to properly assess. Additionally, a proper assessment of the habitat quality would require data on the presence and abundance of turtles in a much larger number of ponds and a wider gradient of conditions. Finally, some ponds have not been as systematically surveyed as others, while some were not sampled at all, and therefore it is difficult to infer the habitat quality of these ponds.

This study has contributed with important data to improve planning and management of this endangered habitat system and, therefore, also for an effective conservation of *Emys orbicularis*. The results obtained with this study, namely regarding the empirical estimation of interpond movement probabilities, will be essential to develop a pond connectivity model at the wider scale of the whole coastal plateau of the Sudoeste Alentejano e Costa Vicentina Natural Park. This wider approach would allow to evaluate the decrease of connectivity due to the agricultural intensification that became noticeable in the beginning of the 1990's, which destroyed about of 56% of the existing ponds (Ferreira & Beja, 2013).The results of this study also highlight the contribution of a graph-based approach to the best comprehension of how a scattered population occupies habitat patches in a fragmented landscape, making it especially useful when adding empirical data movement and its spatial analysis. As previous studies concluded (Joyal et al., 2001; Bowne et al., 2006; Roe & Georges, 2007; Fall et al., 2007; Cadi et al., 2008), both terrestrial and aquatic environments should be targeted when designing effective management actions, meaning that, even with some ponds being more highlighted and possibly having higher priorities, the focus of the studies and measures should be directed to the landscape. Complexes of wetlands should be considered as a distinct unit in order to attain a more effective conservation plan, since patchy populations use a multitude of this ephemeral water bodies to satisfy their life cycle requirements.

References

Aresco, M. J. (2005). The effect of sex-specific terrestrial movements and roads on the sex ratio of freshwater turtles. *Biological Conservation*, *123*(1), 37–44.

Baguette, M., & Dyck, H. (2007). Landscape connectivity and animal behavior: functional grain as a key determinant for dispersal. *Landscape Ecology*, *22*(8), 1117–1129.

Baranyi, G., Saura, S., Podani, J., & Jordán, F. (2011). Contribution of habitat patches to network connectivity: Redundancy and uniqueness of topological indices. *Ecological Indicators*, *11*(5), 1301–1310.

Beja, P., & Alcazar, R. (2003). Conservation of Mediterranean temporary ponds under agricultural intensification: an evaluation using amphibians. *Biological Conservation*, *114*(3), 317–326.

Berry, J., & Shine, R. (1980). Sexual size dimorphism and sexual selection in turtles (Order Testudines). *Oecologia*, *191*(2), 185–191.

Bona, M., Danko, S., Novotný, M., & Burešová, A. (2012). Nest site fidelity in the Slovakian population of the European pond turtle *Emys orbicularis*. *Amphibia-Reptilia*, *33*(2), 207–213.

Bowers, M., & Matter, S. (1997). Landscape ecology of mammals: relationships between density and patch size. *Journal of Mammalogy*, *78*(4), 999–1013.

Bowne, D., & Bowers, M. (2004). Interpatch movements in spatially structured populations: a literature review. *Landscape Ecology*, *19*(1), 1–20.

Bowne, D. R., Bowers, M., & Hines, J. (2006). Connectivity in an Agricultural Landscape as Reflected by Interpond Movements of a Freshwater Turtle. *Conservation Biology*, *20*(3), 780–791.

Bunn, A. G., Urban, D. L., & Keitt, T. H. (2000). Landscape connectivity: A conservation application of graph theory. *Journal of Environmental Management*, *59*(4), 265–278.

Cabral, M. J., Almeida, J., Almeida, P. R., Dellinger, T., Ferrand de Almeida, N., Oliveira, M. E., Palmeirim, J. M., Queiroz, A. I., Rogado, L., & Santos-Reis, M. (2005). *Livro Vermelho dos Vertebrados de Portugal*. Lisboa: Instituto da Conservação da Natureza.

Cadi, A., Nemoz, M., Thienpont, S., & Joly, P. (2008). Annual home range and movement in freshwater turtles: management of the endangered European pond turtle (*Emys orbicularis*). *Revista Española de Herpetología*, *22*, 71–86.

Cadi, A., Nemoz, M., Thienpont, S., Joly, P. (2004). Home range , movements , and habitat use of the European pond turtle (*Emys orbicularis*) in the Rhône-Alpes region , France. *Biologia, Bratislava*, *59*(14), 89–94.

Calabrese, J., & Fagan, W. (2004). A comparison-shopper's guide to connectivity metrics. *Frontiers in Ecology and the environment*, *2*(10), 529–536.

Cancela, L., Cristo, M., Machado, M., Sala, J., Reis, J., Alcazar, R., & Beja, P. (2008). Mediterranean temporary ponds in Southern Portugal : key faunal groups as management tools ? *Pan-American Journal of Aquatic Sciences*, *3*(3), 304–320.

Congdon, J. D., Kinney, O. M., & Nagle, R. D. (2011). Spatial ecology and core-area protection of Blanding's Turtle (*Emydoidea blandingii*). *Canadian Journal of Zoology*, *89*(11), 1098–1106.

Congdon, J., Dunham, A. E., & van Loben Sels, R. C. (1993). Delayed sexual maturity and demographics of Blanding's turtles (*Emydoidea blandingii*): implications for conservation and management of long-lived organisms. *Conservation Biology*, *7*(4), 826–833.

Crockett, T. (2008). Home range, movements, and habitat use of Blanding's Turtle (*Emydoidea blandingil*) in St. Lawrence County, *New York.* State University of New York College.

Curado, N., Hartel, T., & Arntzen, J. W. (2011). Amphibian pond loss as a function of landscape change – A case study over three decades in an agricultural area of northern France. *Biological Conservation*, *144*(5), 1610–1618.

Decout, S., Manel, S., Miaud, C., & Luque, S. (2012). Integrative approach for landscape-based graph connectivity analysis: a case study with the common frog (*Rana temporaria*) in human-dominated landscapes. *Landscape Ecology*, *27*(2), 267–279.

Delmas, V., & Baudry, E. (2007). The righting response as a fitness index in freshwater turtles. *Biological Journal of the Linnean Society*, *91*(1), 99–109.

Drobenkov, S. M. (2000). Reproductive ecology of the pond turtle (*Emys orbicularis L.*) in the Northeastern part of the species range. *Russian Journal of Ecology*, *31*(1), 49–54.

Enneson, J. J., & Litzgus, J. D. (2009). Stochastic and spatially explicit population viability analyses for an endangered freshwater turtle, *Clemmys guttata*. *Canadian Journal of Zoology*, *87*(12), 1241–1254.

Etherington, T. R., & Holland, E. P. (2013). Least-cost path length versus accumulated-cost as connectivity measures. *Landscape Ecology*, *28*(7), 1223–1229.

Fall, A., Fortin, M.-J., Manseau, M., & O'Brien, D. (2007). Spatial Graphs: Principles and Applications for Habitat Connectivity. *Ecosystems*, *10*(3), 448–461.

FAOSTAT. (2014). FAOSTAT data. Retrieved September 27, 2014, from http://faostat.fao.org

Ferreira, M. (2012). "Multi-species occupancy modeling of natural and anthropogenic habitats by Mediterranean amphibians: grim prospects for conservation in irrigated farmland." Universidade de Évora.

Ferreira, M., & Beja, P. (2013). Mediterranean amphibians and the loss of temporary ponds: Are there alternative breeding habitats? *Biological Conservation*, *165*(5), 179–186.

Ficetola, G. F., Monti, A., Massa, R., Bernardi, F. De, & Bottoni, L. (2005). The importance of aquatic and terrestrial habitat for the European pond turtle (*Emys orbicularis*): implications for conservation planning and management. *Canadian Journal of Zoology*, *1712*(2004), 1704–1712.

Finkler, M. (2001). Rates of water loss and estimates of survival time under varying humidity in juvenile snapping turtles (*Chelydra serpentina*). *Copeia*, *2001*(2), 521–525.

Firbank, L. G. (2005). Striking a new balance between agricultural production and biodiversity. *Annals of Applied Biology*, *146*(2), 163–175.

Fortin, G. (2012). Can landscape composition predict movement patterns and site occupancy by Blanding's turtles?: A multiple scale study in Québec, Canada. University of Ottawa.

Fortin, G., Blouin-Demers, G., & Dubois, Y. (2012). Landscape composition weakly affects home range size in Blanding's turtles (*Emydoidea blandingii*). *Ecoscience*, *19*(3), 191–197.

Gibbons, J. (1986). Movement patterns among turtle populations: applicability to management of the desert tortoise. *Herpetologica*, *42*(1), 104–113.

Gibbons, J. W. (2003). Terrestrial habitat: A vital component for herpetofauna of isolated wetlands. *Wetlands*, *23*(3), 630–635.

Griffin, K. (2008). Spatial population dynamics of western painted turtles in a wetland ecosystem in northwestern Montana. University of Montana.

Gustafson, E., & Gardner, R. (1996). The effect of landscape heterogeneity on the probability of patch colonization. *Ecology*, *77*(1), 94–107.

Harrison, S. (1991). Local extinction in a metapopulation context: an empirical evaluation. *Biological Journal of the Linnean Society*, *42*(1-2), 73–88.

House, W., Nall, I., & Thomas, R. (2010). Interpond movements of western painted turtles (*Chrysemys picta*) in east-central Kansas. *The Southwestern Naturalist*, *55*(3), 403–410.

Howeth, J. G., Mcgaugh, S. E., & Hendrickson, D. a. (2008). Contrasting demographic and genetic estimates of dispersal in the endangered Coahuilan box turtle: a contemporary approach to conservation. *Molecular Ecology*, *17*(19), 4209–4221.

Johnson, M., & Gaines, M. (1990). Evolution of dispersal: theoretical models and empirical tests using birds and mammals. *Annual Review of Ecology and Systematics*, *21*(1990), 449–480.

Joyal, L., McCollough, M., & Hunter, M. (2001). Landscape ecology approaches to wetland species conservation: a case study of two turtle species in southern Maine. *Conservation Biology*, *15*(6), 1755–1762.

Keller, C. (1997). Ecología de poblaciones de *Mauremys leprosa* e *Emys orbicularis* en el Parque Nacional de Doñana. Universidad de Sevilla, Sevilla.

Kindlmann, P., Aviron, S., & Burel, F. (2005). When is landscape matrix important for determining animal fluxes between resource patches? *Ecological Complexity*, *2*(2), 150–158.

Kindlmann, P., & Burel, F. (2008). Connectivity measures: a review. *Landscape Ecology*, *23*(8), 879–890.

Kolbe, J., & Janzen, F. (2002). Experimental analysis of an early life-history stage: water loss and migrating hatchling turtles. *Copeia*, *2002*(1), 220–226.

Kotenko, T. (2000). The European pond turtle (*Emys orbicularis*) in the steppe zone of the Ukraine. *Stapfia*, *149*, 87–106.

Kuzmin, S. L. (2002). The turtles of Russia and other ex-Soviet republics (former Soviet Union) (Chimaira.). Frankfurt am Main.

Litzgus, J. D., & Mousseau, T. A. (2004). Home range and seasonal activity of southern spotted turtles (*Clemmys guttata*): Implications for Management. *Copeia*, *2004*(4), 804–817.

Lue, K.-Y., & Chen, T.-H. (2008). Home ranges and movements of the Chinese stripe-necked turtle (*Ocadia sinensis*) in the Keelung River, northern Taiwan. *Amphibia-Reptilia*, *29*(3), 383–392.

Mantel, N. (1967). The detection of disease clustering and a generalized regression approach. *Cancer Research*, *27*(2), 209–20.

Meeske, A. C. M., & Mühlenberg, M. (2004). Space use strategies by a northern population of the European pond turtle, Emys orbicularis. *Biologia*, *Suppl. 14*(59), 95–101.

Meeske, M. (1997). Nesting Ecology of European Pond Turtle (*Emys Orbicularis*) in South Lithuania. *Acta Zoologica Lituanica*, *7*(1), 138–142.

Millar, C., & Blouin-Demers, G. (2011). Spatial ecology and seasonal activity of Blanding's turtles (*Emydoidea blandingii*) in Ontario, Canada. *Journal of Herpetology*, *45*(3), 370–378.

Millar, C. S. (2010). The spatial ecology of Blanding's turtles (*Emydoidea blandingii*): from local movement patterns, home ranges and microhabitat selection to Ontario-wide habitat suitability modelling. Ottawa-Carleton Institute of Biology.

Mitrus, S. (2010). Is the European pond turtle *Emys orbicularis* strictly aquatic?–Habitats where the turtle lives in central Europe. *Acta Herpetologica*, *5*(1), 31–35.

Mönkkönen, M., & Reunanen, P. (1999). On critical thresholds in landscape connectivity: a management perspective. *Oikos*, *84*(2), 302–305.

Morreale, S., Gibbons, J., & Congdon, J. (1984). Significance of activity and movement in the yellow-bellied slider turtle (*Pseudemys scripta*). *Canadian Journal of Zoology*, *62*(6), 1038–1042.

Najbar, B., & Szuszkiewicz, E. (2007). Nest-site fidelity of the European pond turtle *Emys orbicularis* (LINNAEUS, 1758)(Testudines: Emydidae) in western Poland. *Acta Zoologica Cracoviensia*, *50A*(1-2), 1–8.

Nikolakaki, P. (2004). A GIS site-selection process for habitat creation: estimating connectivity of habitat patches. *Landscape and Urban Planning*, *68*(1), 77–94.

Pardini, R., de Souza, S. M., Braga-Neto, R., & Metzger, J. P. (2005). The role of forest structure, fragment size and corridors in maintaining small mammal abundance and diversity in an Atlantic forest landscape. *Biological Conservation*, *124*(2), 253–266.

Pascual-Hortal, L., & Saura, S. (2007). Integrating landscape connectivity in broad-scale forest planning through a new graph-based habitat availability methodology: application to capercaillie (Tetrao urogallus) in Catalonia (NE Spain). *European Journal of Forest Research*, *127*(1), 23–31.

Pereira, M., Segurado, P., & Neves, N. (2011). Using spatial network structure in landscape management and planning: A case study with pond turtles. *Landscape and Urban Planning*, *100*(1-2), 67–76.

Pimentel, D., Stachow, U., Takacs, D. A. ., & Brubaker, H. W. . (1992). Conserving biological diversity in agricultural/Forestry Sistems. *BioScience*, *42*(5), 354–362.

Pinto-Cruz, C., Barbosa, a. M., Molina, J. a., & Espírito-Santo, M. D. (2011). Biotic and abiotic parameters that distinguish types of temporary ponds in a Portuguese Mediterranean ecosystem. *Ecological Indicators*, *11*(6), 1658–1663.

Pittman, S. E., & Dorcas, M. E. (2009). Movements, habitat use, and thermal ecology of an isolated population of bog turtles (*Glyptemys muhlenbergii*). *Copeia*, *2009*(4), 781–790.

Project Development Team. (2012). Quantum, G. I. S. Quantum GIS Geographic Information System. Open Source Geospatial Foundation.

Proulx, C. L., Fortin, G., & Blouin-Demers, G. (2014). Blanding's turtles (*Emydoidea blandingii*) avoid crossing unpaved and paved roads. *Journal of Herpetology*, *48*(2), 267–271.

R Core Team. (2013). R: A language and environment for statistical computing. Vienna, Austria: R Foundation for Statistical Computing.

Rasmussen, M. L., & Litzgus, J. D. (2010). Habitat Selection and Movement Patterns of Spotted Turtles (Clemmys guttata): Effects of Spatial and Temporal Scales of Analyses. *Copeia*, *2010*(1), 86–96.

Ray, N. (2005). Pathmatrix: a geographical information system tool to compute effective distances among samples. *Molecular Ecology Notes*, *5*(1), 177–180.

Rayfield, B., Fortin, M.-J., & Fall, A. (2010). The sensitivity of least-cost habitat graphs to relative cost surface values. *Landscape Ecology*, *25*(4), 519–532.

Roe, J., & Georges, A. (2007). Heterogeneous wetland complexes, buffer zones, and travel corridors: Landscape management for freshwater reptiles. *Biological Conservation*, *135*(1), 67–76.

Roe, J., & Georges, A. (2008). Maintenance of variable responses for coping with wetland drying in freshwater turtles. *Ecology*, *89*(2), 485–494.

Rössler, M. (2000). der Lebensraum der Europäischen Sumpfschildkröte *Emys orbicularis* (L.) in den niederösterreichischen donau-Auen (Reptilia: Testudines: Emydidae). *Stapfia* (Vol. 149).

Rovero, F., & Chelazzi, G. (1996). Nesting migrations in a population of the European pond turtle *Emys orbicularis* (L.) (Chelonia Emydidae) from central Italy. *Ethology Ecology & Evolution*, *8*(3), 297–304.

Russell, R. W. (1999). Comparative demography and life history tactics of seabirds: implications for conservation and marine monitoring. In *American Fisheries Society Symposium* (pp. 51–76).

Ryan, T. J., Peterman, W. E., Stephens, J. D., & Sterrett, S. C. (2013). Movement and habitat use of the snapping turtle in an urban landscape. *Urban Ecosystems*, *17*(2), 613–623.

Saura, S., & Pascual-Hortal, L. (2007). A new habitat availability index to integrate connectivity in landscape conservation planning: Comparison with existing indices and application to a case study. *Landscape and Urban Planning*, *83*(2-3), 91–103.

Saura, S., & Torné, J. (2009). Conefor Sensinode 2.2: A software package for quantifying the importance of habitat patches for landscape connectivity. *Environmental Modelling & Software*, *24*(1), 135–139.

Sexton, O. J. (1959). Spatial and temporal movements of a population of the painted turtle, *Chrysemys picta marginata* (Agassiz). *Ecological Monographs*, *29*(2), 113–140.

Sheperd, B., & Swihart, R. (1995). Spatial dynamics of fox squirrels (*Sciurus niger*) in fragmented landscapes. *Canadian Journal of Zoology*, *105*(73), 11.

Sloan, L. M., & Marks, S. (2012). Population structure, life history, and terrestrial movements of western pond turtles (*Actinemys marmorata*) in lentic habitats along the Trinity River, California. Humboldt State University.

Spinks, P. Q., Pauly, G. B., Crayon, J. J., & Bradley Shaffer, H. (2003). Survival of the western pond turtle (*Emys marmorata*) in an urban California environment. *Biological Conservation*, *113*(2), 257–267.

Stevens, C. J., Dise, N. B., Mountford, J. O., & Gowing, D. J. (2004). Impact of nitrogen deposition on the species richness of grasslands. *Science (New York, N.Y.)*, *303*(5665), 1876–9.

Stoate, C., Báldi, a, Beja, P., Boatman, N. D., Herzon, I., van Doorn, a, de Snoo, G. R., Rakosy, L., & Ramwell, C. (2009). Ecological impacts of early 21st century agricultural change in Europe--a review. *Journal of Environmental Management*, *91*(1), 22–46.

Stoate, C., Boatman, N., Borralho, R., Carvalho, C. R., Snoo, G. R. d., & Eden, P. (2001). Ecological impacts of arable intensification in Europe. *Journal of Environmental Management*, *63*(4), 337–365.

Sutherland, G., Waterhouse, F., Fall, A., O' Brien, D., & Harestad, A. (2006). A framework for landscape analysis of habitat supply and effects on populations of the northern spotted owl in British Columbia. Victoria BC.

Taylor, P., Fahrig, L., Henein, K., & Merriam, G. (1993). Connectivity is a vital element of landscape structure. *Oikos*, *68*(3), 571–573.

Tortoise & Freshwater Turtle Specialist Group. (1996). The IUCN Red List of Threatened Species. *Version 2014.2*. Retrieved September 27, 2014, from http://www.iucnredlist.org/

Tscharntke, T., Klein, A. M., Kruess, A., Steffan-Dewenter, I., & Thies, C. (2005). Landscape perspectives on agricultural intensification and biodiversity-ecosystem service management. *Ecology Letters*, *8*(8), 857–874.

Turtle Conservation Fund. (2002). A Global Action Plan for Conservation of Tortoises and Freshwater Turtles: Strategy and Funding Prospectus 2002–2007. Washington, D.C.: Conservation International and Chelonian Research Foundation.

Urban, D., & Keitt, T. (2001). Landscape connectivity: a graph-theoretic perspective. *Ecology*, *82*(5), 1205–1218.

Urban, D. L., Minor, E. S., Treml, E. a, & Schick, R. S. (2009). Graph models of habitat mosaics. *Ecology Letters*, *12*(3), 260–73.

Van Dyck, H., & Baguette, M. (2005). Dispersal behaviour in fragmented landscapes: Routine or special movements? *Basic and Applied Ecology*, *6*(6), 535–545.

Zacharias, I., Dimitriou, E., Dekker, A., & Dorsman, E. (2007). Overview of temporary ponds in the Mediterranean region: threats, management and conservation issues. *Journal of Environmental Biology*, *28*(1), 1–9.

Zacharias, I., & Zamparas, M. (2010). Mediterranean temporary ponds. A disappearing ecosystem. *Biodiversity and Conservation*, *19*(14), 3827–3834.

Zeller, K. a., McGarigal, K., & Whiteley, A. R. (2012). Estimating landscape resistance to movement: a review. *Landscape Ecology*, *27*(6), 777–797.

Nesting activity and movements of the European pond turtle (*Emys orbicularis* L. 1758)

Abstract

Since 2009, the National Association for Nature Conservation, Quercus, has been promoting a management action for the conservation of the European pond turtle, *Emys orbicularis*, in a system of ponds located in SW Alentejo, Portugal. This system is located within a private property mainly dedicated to livestock farming. This action involves an agreement with the land owner to ensure good agriculture and livestock practices, namely from the point of view of turtle conservation. An important aspect that should be taken into account in this conservation effort is the protection of the turtle main nesting sites. For this purpose, in 2013, a radio telemetry program was conducted to study the movements and nesting activity of female turtles. The main objective of this study was to identify and characterize the main nesting sites. Ten females were fitted with transmitters and all individual's frequencies were searched twice a day from early June to August. A total of nine nesting behaviour events were identified, but only six were confirmed as effective nesting sites. The distance of nesting sites to the nearest pond varied between 20 to 100 m. Total movements of tagged females ranged from 551 to 2709 meters (4 ponds used and several ditches). The maximum movement exclusively through land was 367 m. No aggregating behaviour of females seems to occur in the study area. Most nesting sites were found in open areas dominated by pastures used by the cattle. This study provides preliminary data on individual female turtle's movements along the reproductive season as well as the first details of nesting site characteristics for this species in Portugal. Further data on turtle movement activity along the whole activity cycle and extended to adult males are needed to support effective management actions for the conservation of this important population.

Postura e movimentos do cágado-de-carapaça-estriada (*Emys orbicularis* L. 1758)

Resumo

Desde 2009, a Associação Nacional de Conservação da Natureza, Quercus, tem vindo a promover uma ação de gestão de um sistema de charcos de carácter temporário no Sudoeste Alentejano para a conservação do cágado-de-carapaça-estriada (*Emys orbicularis*). Este sistema de charcos encontra-se em grande parte incluído numa propriedade privada dedicada predominantemente à criação de gado e envolve um acordo com o proprietário que garante boas práticas agropecuárias, nomeadamente sob o ponto de vista da conservação dos cágados. Um aspeto importante que deveria ser considerado neste esforço de conservação refere-se à proteção dos locais de postura dos cágados. Para este propósito, em 2003, foi realizado um programa de radio telemetria para estudar os movimentos e atividades de postura de tartarugas fêmeas. O principal objetivo deste estudo foi o de identificar e caracterizar os principais locais de postura. Foram colocados transmissores de rádio em 10 fêmeas adultas e todas as frequências individuais foram controladas duas vezes por dia de Junho a Agosto. Um total de nove eventos de comportamento de postura foram detetados, 6 dos quais foram confirmados como locais de postura. A distância dos locais de postura ao corpo de água mais próximo variou entre os 20 e os 100 metros. Os movimentos totais de fêmeas variaram entre 551 e 2709 metros (4 charcos utilizados e diversas valas). O movimento mais longo detetado exclusivamente em terra foi de 367 metros. Não foi observado nenhum comportamento de agregação de fêmeas na área de estudo. A maior parte dos locais de postura foram encontrados em zonas abertas, predominantemente de pastagem usadas por gado. Este estudo forneceu dados preliminares sobre os movimentos individuais de fêmeas ao longo do período reprodutor. De futuro, é necessário recolher mais dados sobre o movimento das tartarugas, nomeadamente ao longo de todo o ciclo de atividade e estendido aos machos adultos, para apoiar medidas de gestão efetivas para a conservação desta importante população.

Palavras-chave: Conservação, *Emys orbicularis*, movimentos, locais de postura, telemetria.

Introduction

Turtles are long-lived organisms with delayed sexual maturity and a long reproductive life span. Hence, their demographic strategy implies high adult survivorship and high reproductive output with low egg and hatchling survivorship as to maintain viable populations (Congdon et al., 1993). Semi-aquatic turtles require terrestrial habitat in which to deposit eggs and so they undergo terrestrial migrations to nest in suitable habitat (Najbar & Szuszkiewicz, 2007). This is an extremely important activity between egg production and reproductive success (Zuffi & Rovina, 2006). Reproductive output of turtles is linked to habitat productivity (Kennett, 1999), hence anthropogenically altered landscapes present various challenges to semi-aquatic turtle reproduction. Nest-site selection is essential to ensure progeny' survival (Lindeman, 1992), relying on parameters such as solar exposure, inclination, soil type, distance to nearest body water and vegetation cover (tree, shrub or grass) (Andreas, 2000; Zuffi & Rovina, 2006). Nest-site selection has as impact on the hatchling's survival via probability of nest predation (Burke et al., 1998) or behaviour and survival of recently-emerged turtles (Kolbe & Janzen, 2001) and may even affect the offspring' sex ratio (Ferreira Junior, 2009).

The European pond turtle (*Emys orbicularis*) is a semi-aquatic freshwater turtle with a distribution that ranges from Portugal in the west to the area of the Aral Sea (Kazakhstan) in the east, from Denmark and northern Germany to southern Italy, Sicily and northern Africa and south-eastwards from Turkey to the eastern Caucasus Republics and the southern shoreline of the Caspian Sea. Despite being considered as "Near Threatened", its populations tend to be extremely localized and in many countries (*e.g.* Portugal) the species is classify as "Endangered" (Cabral et al., 2005). Although having an extensive distribution, information regarding the European pond turtle's nesting migrations and egg-laying parameters is scarce (Rovero & Chelazzi, 1996; Najbar & Szuszkiewicz, 2007), especially in the most western part of its distribution (i.e. Iberian Peninsula) (Alarcos, 2008). Zuffi & Rovina (2006) suggested that nesting characteristics data could be easily gathered by following a few selected number of females during nesting season (from May to late July), captured prior to their nesting occasion and Steen et al., (2012) refers that a synopsis of covered distances by nesting females could provide useful information when conservation planning conservation measures.

This study, the first to be undertaken in Portugal, aims to answer questions regarding this specie's nesting migration and selection of nesting habitat, as well as to assess if temporal synchronization and spatial aggregation of egg-laying occur.

Materials and methods

Study area

The study was carried out within an area of the Natural Park of Southwest Alentejo and Vicentina Coast, located on the coastal plain of southwest Portugal (37º 30' N, 8º57' W) (Beja & Alcazar, 2003). Climate is typical Mediterranean with oceanic influence. Aridity increases southwards, with annual mean temperature increasing from 15 to 16º C, and annual precipitation going from 650 to 400 mm, of which >80% falls in the October– March period (Ferreira, 2012). The landscape is flat throughout most of its extension with some tree cover composed of small woods, windbreaks and stream valleys. Agriculture and livestock farming are the most significant land uses, with extensive cultivation of winter cereals on a cereal–fallow rotation basis, and beef cattle, respectively. Since the early 90's there has been an increase of irrigation systems and, as a consequence, an increase in the use of fertilizers and chemical-based products such as pesticides.

Temporary ponds are scattered in this landscape, occupying shallow depressions and varying greatly in terms of depth, water volume, permanence and the associated biotic factors. These ponds are intimately associated with changes in the hydrological regime, filling in the winter and drying in the summer. Some of these ponds were deepened by farmers and used as reservoirs transforming them in permanent water bodies (Beja & Alcazar, 2003). This affects the functioning of the pond's biotic components, becoming more susceptible to exotic species, such as the Louisiana crayfish (*Proclambarus clarki*), the pumpkinseed sunfish (*Lepomis gibbosus*) and the small-mouth bass (*Micropterus salmoides*). During the extent of this study nine ponds were considered in the capture-mark-recapture overview, all of them included in the surrounding agricultural fields of Longueira-Almograve.

Capture

From early June to mid-July turtles were captured either by hand or by traps baited with sardine placed perpendicularly along the pond shoreline. Traps were checked and baited on a daily basis and captured turtles were individually marked by notching the marginal scutes (Keller, 1997).

Biometric data was recorded for each individual at each capture event, including carapace length (CL), measured with a Vernier calliper to the nearest millimetre, and weight (W), measured with a Pesola scale.

The age class of the individuals was classified according to the carapace length, following the criteria used by (Keller, 1997): juveniles CL < 115mm; subadult males CL < 120 mm, adult males CL > 120 mm; subadult

females CL < 130mm, adult females CL > 130mm. Adult and subadult individuals were sexed based on the differentiation of secondary sexual characters. Males attain smaller sizes (Berry & Shine, 1980) and have smaller and more concave plastrons with a longer precloacal tail.

Telemetry

During the 2013 sampling season ten female individuals were monitored by radio telemetry. The females were fitted with transmitters (W-31/2AA tags transmitters programmed with a 30ppm pulse rate) in the posterior left marginal scute (being careful not to get epoxy glue on the scute sutures or neighbouring scutes) using 5-minute epoxy glue and released in the following 1 - 24 h in the exact same location. All transmitters weighed less than 5% of the female's body mass and the whip antenna was allowed to trail behind the turtle (Martini-Lamb, 2004).

After their release the individual's frequencies were searched twice a day from early June to mid-July, as this period is referenced as the nesting season for most freshwater turtles, including *E. orbicularis*, by several studies (Cadi et al., 2004; Litzgus & Mousseau, 2004; Cadi et al., 2008; Crockett, 2008; Refsnider & Linck, 2012). Female detection consisted of either direct observation or triangulation with a three-element Yagi antenna with a bandwidth of 147-150 Mhz and a R-1000 receiver and was carried out during the morning and the late afternoon/soon after dusk. The late afternoon detection aimed at confirm laying and to identify nesting sites (Kotenko, 2000; Mitrus & Zemanek, 2000) while the morning detection aimed at finding if the individuals had returned to the water body. For each radio location, the date, hour, weather conditions, habitat type and coordinates, using a handheld GPS unit, were recorded.

Seven land use classes were identified in the study area: ponds, meadows, grazing fields, dunes, croplands, forests and urban areas along with three linear structures (barriers) – sand roads (no traffic but human use and presence), dirt roads (low traffic) and national roads (high traffic). The perimeter of each land class use was then digitised into the GIS software (Project Development Team, 2012), using aerial images from Google Earth (version 7.1.2.2041) as a template. All turtles were classified as being detected in either aquatic or terrestrial environments. The outer limits of the ponds were used as borders between aquatic and terrestrial environments and each telemetry location was then assigned to a specific land class. For each female, distances between locations of each detection were calculated and thus nesting migrations and nesting sites were determined.

Analysed nesting parameters

To assess the nesting requirements and microhabitat features we evaluated 12 parameters, 7 quantitative and 5 qualitative. The quantitative parameters were: Distance to nearest water body (DNWB) in meters, inclination (I) in degrees, bare soil cover (BSC) % in a 1 meter radius), tree cover (TC, % in a 1 meter radius), shrub cover (SC, % in a 1 meter radius), grass cover (GC, % in a 1 meter radius) and number of predated eggs (PE). The qualitative parameters were: Land use class (LUC), Orientation (O), Soil type (ST) and of date (D) and time (H). Soil type classes were: Sand, Silt and Clay.

Results

We caught 10 females of which 9 were positively identified as gravid (Table 1). Weight ranged from 540 (for the non-gravid female) to 810 g and the carapace length values varied from 141.6 to 161 g.

Table 1 – Morphometric parameters of radiotagged females

Turtle #	Status	Weight (g)	Carapace lenght (mm)
16	G	680	151.1
27	G	600	143.5
45	G	620	145.3
56	G	810	161.0
64	G	550	141.6
93	G	640	144.2
95	G	700	149.3
144	NG	540	147.2
151	G	620	145.4
228	G	660	146.0

Nesting sites

We found a total amount of 13 nesting sites (Fig. 1) (3 of which were either prospection or abandoned because of human disturbance and 9 actual nesting sites of which 6 were detected via Radiotelemetry and 3 detected through vestigial nesting activity).

Table 2 – Spatial characteristics of the detected nesting sites

Turtle #	DNWB (m)	LUC	O	I (º)	ST	BSC (%)	TC (%)	SC (%)	GC (%)
151	50	Grazing field	-	0	Sand	5	0	0	100
16	50	Grazing field	-	0	Sand	10	0	0	100
16	55	Grazing field	-	0	Sand	10	0	0	100
64	40	Grazing field	-	0	Sand	0	5	0	90
45	40	Grazing field	W	5	Sand	20	0	10	90
16	100	Grazing field	-	0	Sand	10	5	5	90
93	30	Grazing field	-	0	Sand	10	0	0	100
-	30	Dune	SE	5	Sand	80	0	70	30
-	25	Dune	SE	5	Sand	50	0	0	100
151	50	Grazing field	-	0	Sand	5	0	0	100
151	30	Grazing field	-	0	Sand	5	0	0	100
27	40	Grazing field	-	0	Sand	5	0	0	100
-	30	Dune	SE	5	Sand	50	0	80	20

Concerning nesting parameters (Table 2): distance to nearest water body ranged from 25 to 100 meters with an average of 43.8 meters. Inclination was only present for 4 nesting occasions and Southeast-oriented sites attained the biggest proportions (3 of them) with the remaining nest facing West. As for land-use classes used for laying eggs they were mostly Grazing fields (76.9%) with the remaining carried out in Dunes (23.1%) and the soil type being sand for all detected occasions. In spite of the xerothermic characteristics of the habitat, nesting was only observed in microhabitats with bare soil of 50% or more 3 out of 13 times. Regarding vegetation cover, the predominant class was grass for most nesting sites (mean of 86%), followed by shrubby (most represented in 2 occasions) with a mean of 13% of cover. Tree cover was only present twice, with both representing only 5% of the land's total vegetated cover.No nests were predated in spite of sightings and evidence of presence of several potential predator species like common crow (*Corvus corone*), wild boar (*Sus scrofa*), genet (*Genetta genetta*) and red fox (*Vulpes vulpes*). One nest (f64) however was destroyed by tractor plowing.
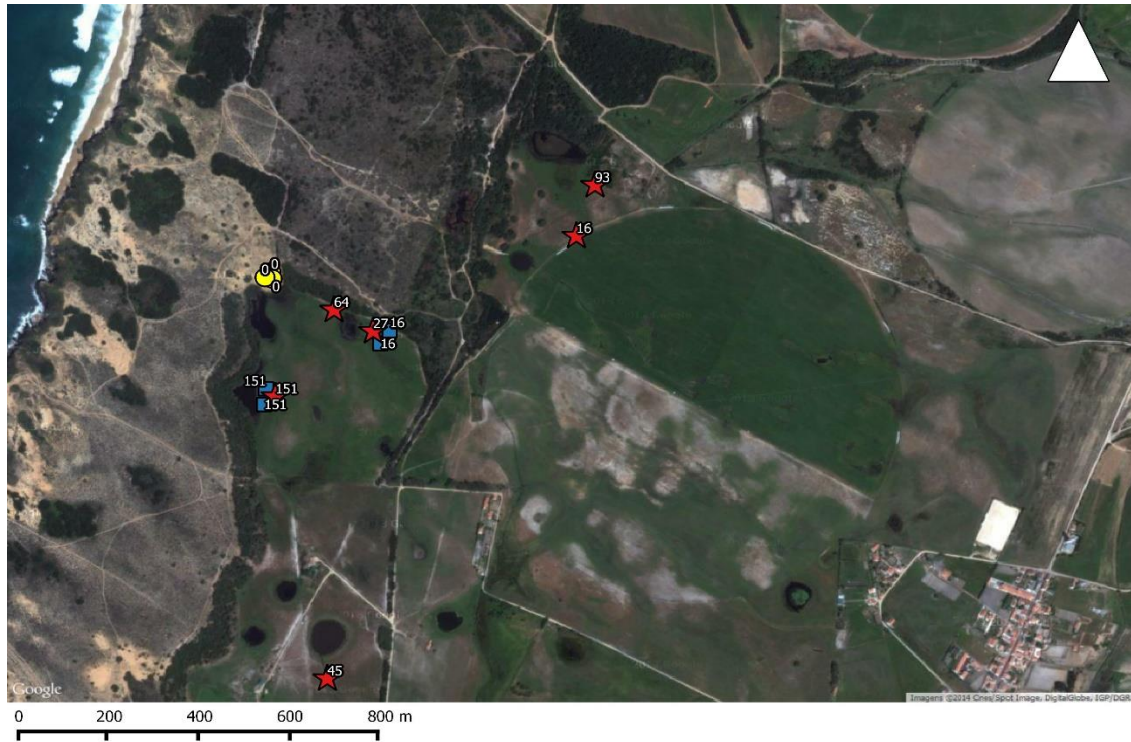
Fig. 1 – Map of nests in the study area (★ - detected through radiotelemetry, ■ - abandoned/disturbed, ○ - detected through vestigial activity)

Table 3 – Temporal parameters of nesting activity (* was used if the nest was abandoned or prospection)

Turtle #	Date (dd/mm/yyyy)	Hour	Detection method
16	16/06/2013	20:27	Radiotelemetry*
16	17/06/2013	20:33	Radiotelemetry*
64	19/06/2013	20:20	Radiotelemetry
45	20/06/2013	21:50	Radiotelemetry
16	20/06/2013	20:33	Radiotelemetry
93	30/06/2013	20:19	Radiotelemetry
-	30/06/2013	-	Vestigial activity
-	03/07/2013	-	Vestigial activity
151	04/07/2013	20:45	Radiotelemetry*
151	05/07/2013	22:00	Radiotelemetry*
151	06/07/2013	21:31	Radiotelemetry
27	09/07/2013	20:33	Radiotelemetry
-	11/07/2013	-	Vestigial activity

The earliest date of egg-laying was on the 16th of June and the latest one was detected on the 11th of July. All oviposition behaviour took place at dusk, from 20:19 to 22:00 and we only found more than one female laying eggs in the same night once (Table 3). No second clutches were detected.

Covered distances

We observed nesting behaviour from mid-June to beginning of July.

Table 4 – distances covered (in meters) by radiotracked females (G – Gravid; NG – non-gravid).

Turtle #	Status	Nesting migration distance	Total covered distance	Average daily distance	Maximum daily distance
f16	G	648.5	2708.9	45.9	367.3
f27	G	58	551.3	19.0	84.9
f45	G	95.5	1627.8	30.1	127.1
f56	G	-	1757.1	27.9	102.9
f64	G	60.3	1989.9	32.1	158.5
f93	G	164.5	1068.2	24.3	227.8
f95	G	-	1816.1	29.8	80.5
f144	NG	-	806.1	20.2	86.7
f151	G	66.3	838.0	23.3	75.8
f228	G	-	1325.6	22.9	306.2
Mean	-	181.7	1448.9	27.5	161.8

While moving towards the nesting site, females underwent migrations with distances varying from 58 to 648.5 m (mean = 181.7) and total covered distances (Table 4) ranged from 551.3 to 2708.9 m (mean = 1448.9 m). Female 16 attained the highest of all the analysed parameters. For maps of females' nesting migration see Appendix I.

Discussion

In the mid nineties, data of nesting behaviour of freshwater turtles, especially on their nesting movement patterns and site choices, were scant (Rovero & Chelazzi, 1996), but since then many studies have been carried out, with a few focused on the nesting behaviour of European pond turtles (Meeske, 1997; Andreas, 2000; Kotenko, 2000; Drobenkov, 2000; Zuffi et al., 2007; Bona et al., 2012). However, to date none has been conducted on Portuguese territory and so our study yields the first results for this species in Portugal.

When tracking the females during their nesting season, 13 actual or potential sites were identified. Among these, 9 were actual nesting sites (6 detected by following the tagged females and the other 3 found by vestigial nesting activity). The remaining 4 were either prospections or abandoned due to human disturbance. The latter has been reported for freshwater turtles by other studies (Kotenko, 2000; Novotný et al., 2004; Moore & Seigel, 2006). Although being a detrimental side of studies, they still can give an insight to the type of nesting habitat chosen by the female prior to its abandonment and so we chose to incorporate its parameters in the analysis.

The mean distance of nesting sites to the nearest water body found in this study (46 meters, varying from 25 to 100 m) is among the lowest distance recorded in similar studies with this species: 2 to 5 m in Ukraine (Kotenko, 2000); 30 to 200 m in South Lithuania (Meeske, 1997, A. C. M. Meeske & Mühlenberg, 2004); 188 to 530 (average distance of 349 m) in Slovakia (Bona et al., 2012); 200 to 800 m (Novotný et al., 2004) also in Slovakia; 30 to 250 m in Poland (Najbar & Szuszkiewicz, 2005), with Mitrus (2006) finding most nests within 150m or less to the closest water bodies. Steen et al., (2012) states that, for semi-aquatic freshwater turtle species, 95% of the nests are located 210 m or less from the nearest water body, with a recorded maximum of 810 m, though most of the analysed species were American emydids. For *Emydoidea blandingii* (Millar, 2010), it is reported that nesting areas within 500 m from water bodies of origin will result in emerging turtles with higher probabilities of ending up in the original wetland of the nesting female than do more distant nests sites.

Nest-site ecological characteristics were very similar to the ones reported in other studies: xerothermic conditions (Andreas, 2000; Novotný et al., 2004; Zinenko, 2004; Mitrus, 2006), where the most predominant vegetation cover is the herbaceous type (Rovero & Chelazzi, 1996; Kotenko, 2000; Novotný et al., 2004; Zinenko, 2004; Najbar & Szuszkiewicz, 2005). The actual cover of herbaceous shows some variation across the studies, ranging from 5-20% (Kotenko, 2000), 25% (Andreas, 2000) or 15-65% (Najbar & Szuszkiewicz, 2005). None of the studies found arboreous cover to be significant. The reason why turtles seem to prefer open grassy areas relates to the fact that, besides being important in terms of insolation, it helps the nests to have better evaporation rates (caused by the wind) and also because temporary shading (provided by grass and nearby bushes) have influence on the hatchling's sex ration (Ferreira Junior, 2009). As predicted, South-exposed nests were preferred (Rovero & Chelazzi, 1996; Andreas, 2000; Novotný et al., 2004; Zinenko, 2004; Najbar & Szuszkiewicz, 2005) since it favours insolation (hence incubation rates). Another parameter that differed from previous studies was the almost absolute lack of inclination in nest-sites. Most authors found inclination in nesting sites to be an important factor (Andreas,

2000; Kotenko, 2000), arguing that steeper sites might prevent flooding and increase solar exposition. Sand was the only type of soil where nests were detected as it was reported in previous studies (Meeske, 1997; Kotenko, 2000; Drobenkov, 2000; Novotný et al., 2004; Zinenko, 2004; Najbar & Szuszkiewicz, 2005; Zuffi & Rovina, 2006).

Concerning a possible spatial nesting aggregation, the results do not show a noticeable pattern and, except in two studies in Ukraine (Kotenko, 2000) and in Hungary (Kotenko, 2000; Novotný et al., 2004), it is not common for *Emys orbicularis*.

Regarding temporal aspects of our study, the nesting season corresponded to the one mentioned by many authors (Farkas, 2000; Rössler, 2000; Bona et al., 2012), since reproductive activity in temperate latitudes is largely constrained by weather conditions, with nesting ocurring only in the warmer months (Kennett, 1999). All turtles left the water and started digging after sunset (Kotenko, 2000; Novotný et al., 2004). No evidences of temporal synchronicity of nesting activities among the tracked females was found in this study.

Nesting migration have been documented for the European pond turtle in several studies (Rovero & Chelazzi, 1996; Kotenko, 2000; Ficetola et al., 2005; Najbar & Szuszkiewicz, 2005; Zuffi & Rovina, 2006). Studies suggest that long-range migrations and extended adult longevity provide females with opportunities to develop navigation capability and topographic memory, thus becoming familiar with large portions of terrestrial habitat (Drobenkov, 2000; Millar, 2010). However,there are still some unanswered questions regarding how habitat characteristics and choice influences these movements (Steen et al., 2012).

Our results show similar nesting migration distances to some previous studies: 150 – 600 m in Italy (Rovero & Chelazzi, 1996); 150 - 800 m in Lithuania (Meeske, 1997), 10 - 300 m and 30 – 490 m in France (Cadi et al., 2008) whereas others report much higher results: up to 2000 m in Ukraine (Kotenko, 2000) and 400-1500 m (using both land and water habitats) and 1500-3000 m through streams in Belarus (Drobenkov, 2000). Mitrus (2010) states that movements exceeding 1000 meters can not be considered as usual.

In spite of perhaps being slightly underestimated, the reported distances might actually present some approximation to the real migration length since turtles tend to adopt straight and direct displacements in scattered-resource scenarios (Baguette & Dyck, 2007).

Assessing and understanding the species' life history traits are essential when designing management and conservation programmes, especially reproductive behaviour and spatial ecology (Rovero & Chelazzi,

1996; Meeske & Mühlenberg, 2004; Cadi et al., 2004; Najbar & Szuszkiewicz, 2005). One fundamental aspect of freshwater turtles conservation relates to the need to protect both aquatic and terrestrial habitats (Semlitsch & Bodie, 2003; Steen et al., 2012), which is often overlooked by land managers.

Since no spatial aggregation of nesting sites seems to occur in the study area, general management actions for nest protection should be implemented for the whole area. Nevertheless, most nests were found within an approximate 100 meter strip from the water bodies and therefore management actions should focus primarily on these areas. More data on the effects of human activities on nests, such as soil tillage and cattle livestock, are needed to help more effective protection of nesting sites. Regulating human activities within these areas will simultaneously protect nesting females, nest sites, and hatchlings dispersing to nearby wetlands.

While the results of this study are specifically focused in this particular species and population, they may have important applications on the design of other conservation programmes and contribute to the improvement of wetland management.

References

Alarcos, G. (2008). Preliminary data on the structure of freshwater turtle populations (*Emys orbicularis* and *Mauremys leprosa*) in a stream in the Natural Park of Los Arribes del Duero (Zamora, Spain). *Revista Española de Herpetologia*, *22*, 33–43.

Andreas, B. (2000). Reproductive ecology and conservation of *Emys orbicularis* in Brandeburg (NE Germany). *Chelonii*, *2*, 58–62.

Baguette, M., & Dyck, H. (2007). Landscape connectivity and animal behavior: functional grain as a key determinant for dispersal. *Landscape Ecology*, *22*(8), 1117–1129.

Beja, P., & Alcazar, R. (2003). Conservation of Mediterranean temporary ponds under agricultural intensification: an evaluation using amphibians. *Biological Conservation*, *114*(3), 317–326.

Berry, J., & Shine, R. (1980). Sexual size dimorphism and sexual selection in turtles (Order Testudines). *Oecologia*, *191*(2), 185–191.

Bona, M., Danko, S., Novotný, M., & Burešová, A. (2012). Nest site fidelity in the Slovakian population of the European pond turtle *Emys orbicularis*. *Amphibia-Reptilia*, *33*(2), 207–213.

Burke, V. J., Rathbun, S. L., Bodie, J. R., & Gibbons, J. W. (1998). Effect of density on predation rate for turtle nests in a complex landscape. *Oikos*, *83*(3), 3–11.

Cabral, M. J., Almeida, J., Almeida, P. R., Dellinger, T., Ferrand de Almeida, N., Oliveira, M. E., Palmeirim, J. M., Queiroz, A. I., Rogado, L., & Santos-Reis, M. (2005). *Livro Vermelho dos Vertebrados de Portugal*. Lisboa: Instituto da Conservação da Natureza.

Cadi, A., Nemoz, M., Thienpont, S., & Joly, P. (2008). Annual home range and movement in freshwater turtles: management of the endangered European pond turtle (*Emys orbicularis*). *Revista Española de Herpetología*, *22*, 71–86.

Cadi, A., Nemoz, M., Thienpont, S., Joly, P., Cnrs, U. M. R., Hydrosyst, E., Claude, U., Lyon, B., Cedex, V., Rhône-alpes, C., Naturels, E., & Vourles, F. (2004). Home range , movements , and habitat use of the European pond turtle (*Emys orbicularis*) in the Rhône-Alpes region , France. *Biologia, Bratislava*, *59*(14), 89–94.

Congdon, J., Dunham, A. E., & van Loben Sels, R. C. (1993). Delayed Sexual Maturity and Demographics of Blanding's Turtles (*Emydoidea blandingii*): Implications for Conservation and Management of Long-Lived Organisms. *Conservation Biology*, *7*(4), 826–833.

Crockett, T. (2008). Home Range, Movements, And Habitat Use Of Blanding's Turtle (*Emydoidea blandingii*) in St. Lawrence County, New York. Master's thesis, State University of New York College.

Drobenkov, S. M. (2000). Reproductive ecology of the pond turtle (*Emys orbicularis* L.) in the Northeastern part of the species range. *Russian Journal of Ecology*, *31*(1), 49–54.

Farkas, B. (2000). *The European pond turtle Emys orbicularis (L.) in Hungary*. *Stapfia* (Vol. 149, pp. 127–132).

Ferreira Junior, P. D. (2009). Aspectos ecológicos da determinação sexual em tartarugas. *Acta Amazonica*, *39*(27), 139–154.

Ferreira, M. (2012). "Multi-species occupancy modeling of natural and anthropogenic habitats by Mediterranean amphibians: grim prospects for conservation in irrigated farmland." Master's thesis, Universidade de Évora.

Ficetola, G. F., Monti, A., Massa, R., Bernardi, F. De, & Bottoni, L. (2005). The importance of aquatic and terrestrial habitat for the European pond turtle (*Emys orbicularis*): implications for conservation planning and management. *Canadian Journal of Zoology*, *1712*(2004), 1704–1712.

Keller, C. (1997). *Ecología de poblaciones de Mauremys leprosa e Emys orbicularis en el Parque Nacional de Doñana*. Universidad de Sevilla, Sevilla.

Kennett, R. (1999). Reproduction of two species of freshwater turtle, *Chelodina rugosa* and *Elseya dentata*, from the wet–dry tropics of northern Australia. *Journal of Zoology*, *247*(4), 457–473.

Kolbe, J., & Janzen, F. (2001). The influence of propagule size and maternal nest-site selection on survival and behaviour of neonate turtles. *Functional Ecology*, *15*(6), 772–781.

Kotenko, T. (2000). The European pond turtle (*Emys orbicularis*) in the steppe zone of the Ukraine. *Stapfia*, *149*, 87–106.

Lindeman, P. (1992). Nest-site fixity among painted turtles (*Chrysemys picta*) in northern Idaho. *Northwestern Naturalist*, *73*(1), 27–30.

Litzgus, J. D., & Mousseau, T. A. (2004). Home range and seasonal activity of southern spotted turtles *(Clemmys guttata)*: implications for management. *Copeia*, *2004*(4), 804–817.

Martini-Lamb, J. (2004). Distribution and habitat use of Pacific pond turtles in a summer impounded river. *Transactions of the Western Section of the Wildlife Society*, *40*, 84–89.

Meeske, A. C. M., & Mühlenberg, M. (2004). Space use strategies by a northern population of the European pond turtle, *Emys orbicularis*. *Biologia*, *Suppl. 14*(59), 95–101.

Meeske, M. (1997). Nesting ecology of European pond turtle (*Emys orbicularis*) in South Lithuania. *Acta Zoologica Lituanica*, *7*(1), 138–142.

Millar, C. S. (2010). The spatial ecology of Blanding's turtles (*Emydoidea blandingii*): from local movement patterns, home ranges and microhabitat selection to Ontario-wide habitat suitability modelling. Ottawa-Carleton Institute of Biology.

Mitrus, S. (2006). Fidelity to nesting area of the European pond turtle, *Emys orbicularis* (Linnaeus, 1758). *Belgian Journal of Zoology*, *136*(January), 25–30.

Mitrus, S. (2010). Is the European pond turtle *Emys orbicularis* strictly aquatic?–Habitats where the turtle lives in central Europe. *Acta Herpetologica*, *5*(1), 31–35.

Mitrus, S., & Zemanek, M. (2000). Distribution and biology of *Emys orbicularis (L.)* in Poland. *Stapfia* (Vol. 149, pp. 107–118).

Moore, M. J. C., & Seigel, R. a. (2006). No place to nest or bask: Effects of human disturbance on the nesting and basking habits of yellow-blotched map turtles (*Graptemys flavimaculata*). *Biological Conservation*, *130*(3), 386–393.

Najbar, B., & Szuszkiewicz, E. (2005). Reproductive ecology of the European pond turtle *Emys orbicularis* (LINNAEUS, 1758)(Testudines: Emydidae) in western Poland. *Acta Zoologica Cracoviensia*, *48A*(1-2), 11–19.

Najbar, B., & Szuszkiewicz, E. (2007). Nest-site fidelity of the European pond turtle *Emys orbicularis* (LINNAEUS, 1758)(Testudines: Emydidae) in western Poland. *Acta Zoologica Cracoviensia*, *50A*(1-2), 1–8.

Novotný, M., Danko, S., & Havaš, P. (2004). Activity cycle and reproductive characteristics of the European pond turtle (*Emys orbicularis*) in the Tajba National Nature Reserve, Slovakia. *Biologia, Bratislava*, *59*(Suppl. 14), 113–121.

Project Development Team. (2012). Quantum, G. I. S. Quantum GIS Geographic Information System. Open Source Geospatial Foundation.

Refsnider, J., & Linck, M. (2012). Habitat use and movement patterns of Blanding's turtles (*Emydoidea blandingii*) in Minnesota, USA: A landscape approach to species conservation. *Herpetological Conservation and Biology*, *7*(2), 185–195.

Rössler, M. (2000). der Lebensraum der Europäischen Sumpfschildkröte *Emys orbicularis* (L.) in den niederösterreichischen donau-Auen (Reptilia: Testudines: Emydidae). *Stapfia* (Vol. 149, pp. 157–168).

Rovero, F., & Chelazzi, G. (1996). Nesting migrations in a population of the European pond turtle *Emys orbicularis* (L.) (Chelonia Emydidae) from central Italy. *Ethology Ecology & Evolution*, *8*(3), 297–304.

Semlitsch, R. D., & Bodie, J. R. (2003). Biological criteria for buffer zones around wetlands and riparian habitats for amphibians and reptiles. *Conservation Biology*, *17*(5), 1219–1228.

Steen, D. A., Gibbs, J. P., Buhlmann, K. A., Carr, J. L., Compton, B. W., Congdon, J. D., & Doody, J. S. (2012). Terrestrial habitat requirements of nesting freshwater turtles. *Biological Conservation*, *150*, 121–128.

Zinenko, O. (2004). Notes on egg-laying, clutch size and hatchling feeding of *Emys orbicularis* in the Kharkiv region, Ukraine. *Biol Bratislava*, *59*(14), 149–151.

Zuffi, M. A. L., & Rovina, L. (2006). Habitat characteristics of nesting areas and of predated nests in a Mediterranean population of the European pond turtle , *Emys orbicularis* galloitalica. *Acta Herpetologica*, *1*(1), 37–51.

Zuffi, M., Celani, A., Foschi, E., & Tripepi, S. (2007). Reproductive strategies and body shape in the European pond turtle (*Emys orbicularis*) from contrasting habitats in Italy. *Journal of Zoology*, *271*(2), 218–224.

Appendix I – Maps of nesting season migration


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0        200        400        600        800 m
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Fig I.1 – Female 16's nesting season migration. ⭐ - detected through Radiotelemetry, 🟦 - abandoned/disturbed,


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0              100              200 m
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Fig I.2– Female 27's nesting season migration. ⭐ - detected through Radiotelemetry.
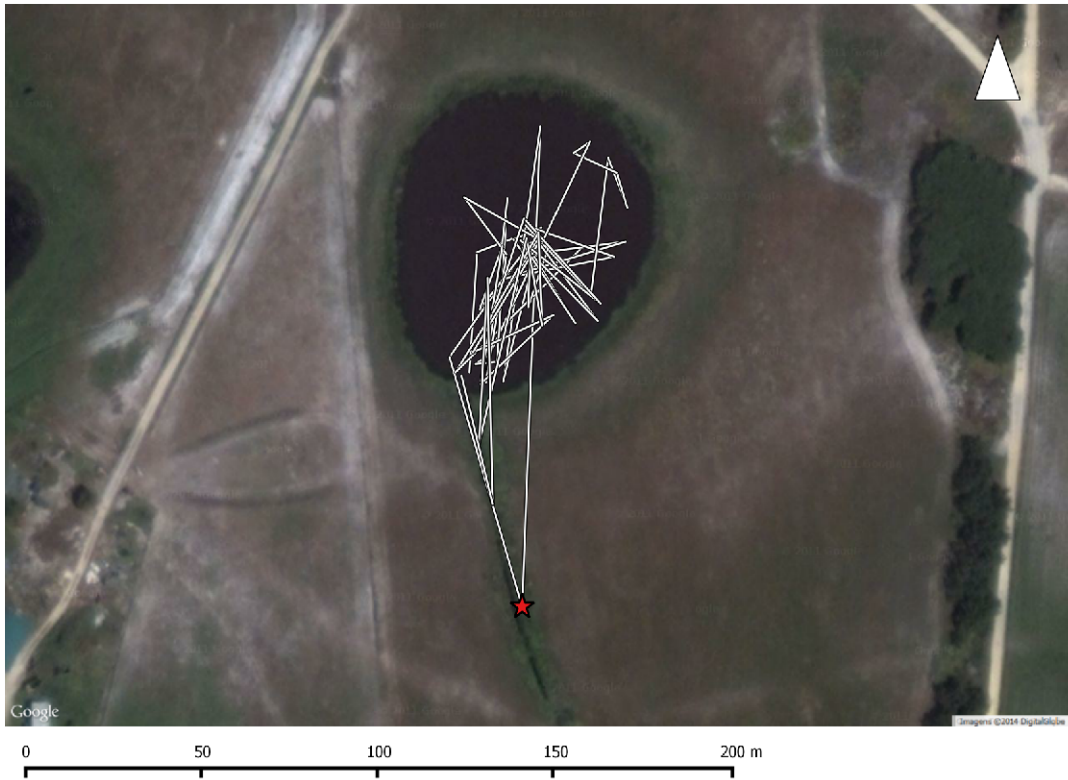
Fig I.3 – Female 45's nesting season migration. ★ - detected through Radiotelemetry.

Fig I.4 – Female 64's nesting season migration. ★ - detected through Radiotelemetry.

Fig. I.5 – Female 93's nesting season migration. ⭐- detected through Radiotelemetry.

Fig. I.6 – Female 151's nesting season migration. ⭐- detected through Radiotelemetry, ■- abandoned/disturbed.