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How Far from Water? Terrestrial Dispersal and Nesting Sites of the Freshwater Turtle *Phrynops hilarii* in the Floodplain of the Paraná River (Argentina)

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Terrestrial environments surrounding aquatic resources are important and intensively used by semi-aquatic species. In the present work, terrestrial dispersal and nesting sites of the freshwater turtle *Phrynops hilarii* were analyzed in the floodplain of the Paraná River, using field data and variables obtained from remote sensing. A total of 112 turtles and 44 nests were recorded during road sampling for one year (covered a total of 786 km in 30 surveys). Individuals were at a mean distance of 171.45 m from water, with a negative correlation between number of turtles and distance from water bodies. No significant differences in distance of turtles from water were observed among seasons. *Phrynops hilarii* nested at a mean distance of 136.51 m from water, showing a negative correlation between number of nests and distance from water bodies. Mean elevation of nests relative to maximum level of water body nearest each record was 1.13 m. The correlation between number of nests and elevation of the nearest water body was positive and significant. The landscape surrounding wetlands is important for *P. hilarii* to complete the life cycle, as nesting is done in this environment. Our results show that the habitat selected for nesting and terrestrial dispersal was proportionally different from that available in the entire study area, with a higher proportion of wetlands, grasslands and forests.

Key words: dispersal, freshwater turtle, habitat, nesting, *Phrynops hilarii*

INTRODUCTION

Land-use practices surrounding a wetland may be as important for maintaining wildlife populations as the wetland itself (Attum et al., 2007). Terrestrial environments surrounding aquatic resources are important and intensively used by semi-aquatic species, such as amphibians and reptiles (Bager and Rosado, 2010). For example, aquatic habitats are used by freshwater turtles for migration, feeding and courtship (Richard, 1999; Moll and Moll, 2004), whereas the terrestrial habitat surrounding wetlands is used for nesting, refuge, hibernation, estivation, and travel (Moll and Moll, 2004; Bager et al., 2007; Giraudo et al., 2007). For this reason, in recent years there has been growing interest in delineating terrestrial buffers surrounding aquatic habitats (Ficetola et al., 2008).

The movements of a given species are strongly influenced by the features and quality of the surrounding landscape (Attum et al., 2007; Ficetola et al., 2008; Olden, 2007; Olson et al., 2007; Steen et al., 2007). Forest loss may affect connectivity quality, as wetland species use forests as dispersal corridors to other wetlands (Taylor et al., 1993; Lees and Peres, 2008). For example, roads can disrupt connectivity because they fragment upland forest, acting as barriers to movement and causing mortality (Gibbs and Shriver, 2002; Mazerolle, 2004; Steen et al., 2006). The landscape surrounding wetlands is also important for the dynamics of metapopulations. For example, the presence of amphibians was found to be related to roads and the hydrographic network at large spatial scales, suggesting that these land-

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scape elements require wider buffers (Ficetola et al., 2008). One method to define the buffer zones required by a semiaquatics species is to determine the dispersal of individuals from the nearest water body. Thus, by knowing the position of an individual, we can estimate the distance they have moved, and then determine the types of environments in which the turtles disperse farther from the water.

Phrynops hilarii is a South American turtle with a wide geographical distribution, from southern Brazil to Paraguay, Uruguay and northern and central Argentina (Cei, 1993). This species is usually seen in ponds and lakes in the floodplain of the Paraná River and lotic channels (Cabrera, 1998). While it is often seen basking on the coast and sometimes searching nesting sites on land, no studies have dealt with the terrestrial movements of the species. Courtship and mating occur in water (Richard, 1999). In *P. hilarii*, reproduction starts in November-December (courtship, copulation and posture), and ends in February-March in northeastern Argentina (Cei, 1993). The scarce studies that have been reported on the reproductive biology of the species have mostly been conducted in southern Brazil (Bager, 1997; Bujes, 1998; Bujes and Verrastro, 2009; Bager and Rosado, 2010); however, the results of these works have not been mutually consistent, nor are they consistent with observations in Argentina.

Nesting site selection is one of the most important reproductive parameters for turtles, because nests can be subjected to predation of eggs and hatchlings, and because embryo development is influenced by temperature and humidity (Flitz and Mullin, 2006). Bager (1997) considered that nest distribution of *P. hilarii* in southern Brazil, was determined by local topography, as this species is highly selective of the substrate in terms of elevation above water level. Distance of nests from water bodies is also an important parameter in several freshwater turtles, mainly in wetlands shaped by cycles of water level rises and falls (Moll and Moll, 2004; Ferreira Júnior and Castro, 2010). One of the principal causes for the loss of freshwater turtle nests is associated with changing river levels over the years (Ferreira Júnior and Castro, 2010). For example, flooding is common in the case of *P. expansa* and *P. unifilis* nests (Ferreira-Júnior and Castro, 2003, 2006); however, floods are also the main cause of nest loss in *Trionyx muticus* (Plummer, 1976) and *Emydoidea blandingii* (Standing et al., 1999). Therefore, distance between nests and water bodies, as well as nest elevation with respect to water level, can vary among species, geographical regions and river hydrodynamics. The existence of regional information may help to determine the appropriate width of land strips to protect semiaquatic species (Roe and Georges, 2007; Bager and Rosado, 2010).

In the present work we analyze terrestrial dispersal and nesting sites of the freshwater turtle *P. hilarii* in the floodplain of the Paraná River, using field data and variables obtained from remote sensing. We delimited the buffer area considering distance of individuals from water, and we propose that environmental factors, such as characteristics of habitats surrounding water bodies, and nest height influence the dispersal of individuals and nesting.

MATERIALS AND METHODS

Study area

Fieldwork was conducted in northeastern Argentina (Entre Ríos and Santa Fe provinces), in a lowland area on the alluvial floodplains of the Paraná River (Fig. 1). The vegetation is mosaic, ranging from wet savannahs and grasslands to subtropical dry forests, gallery forests, shrublands, and a wide variety of wetlands (e.g., rivers, streams, marshes, swamps). The dynamics of the floodplain of the Paraná River are strongly shaped by cycles of water level rises and falls (Neiff, 1990; Burkart et al., 1999; Casco et al., 2005). The climate is seasonal, with a principal rainy season in autumn and a secondary rainy season in spring. During the study period: from January to December 2010, mean temperature was 25.5°C in summer, 15.5°C in autumn, 14.06°C in winter, and 21.9°C in spring. Maximum values of river level and precipitations were recorded in February and March, whereas the minimum values were recorded in September (Fig. 2). Meteorological data were provided by the

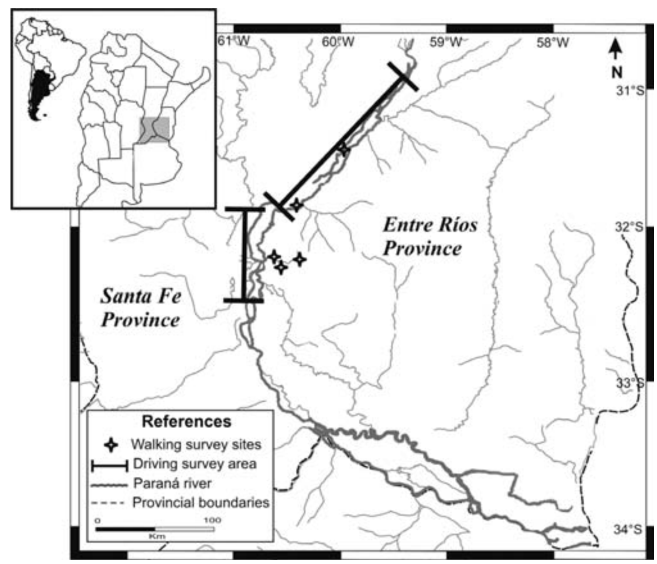

Fig. 1. Location of the study area in the Paraná River floodplain, Argentina.

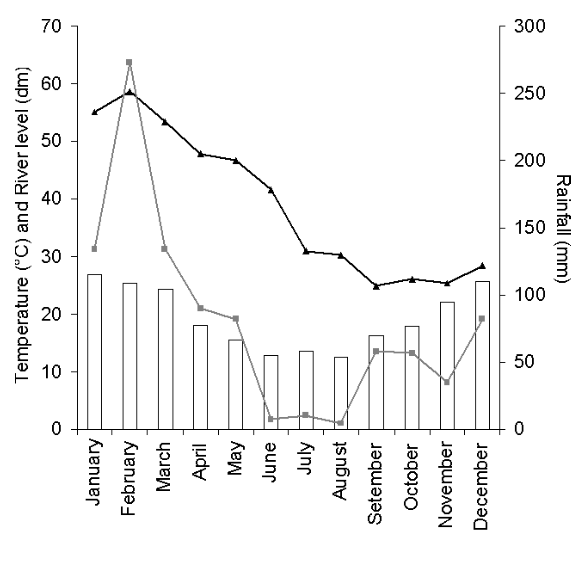

Fig. 2. Monthly variations in temperature, river level, and precipitation in the study area between January 2010 and December 2010. Temperature and river level values are monthly averages; rainfall values are monthly totals.

Centro de Informaciones Meteorológicas, Centro de Investigaciones Científicas y Transferencia de Tecnología a la Producción (CICyTTP-CONICET) and the Centro de Informaciones Meteorológicas, Facultad de Ingeniería y Ciencias Hídricas, Universidad Nacional del Litoral, Prefectura Naval Argentina and the Dirección de Construcciones Portuarias y Vías Navegables.

Turtle and nest sampling

We performed 30 surveys from January to December 2010, evenly distributed over the four seasons (summer, autumn, winter, and spring). Road sampling covered 786 km and consisted of 20 walking surveys (32 km in total) along different coastal and island areas in the Pre-Delta National Park (Entre Ríos province), and coastal areas along national road 168 and provincial road 1 (Santa Fe province); and 10 driving surveys (754 km in total) in coastal areas along national route 168 and provincial route 1. Walking surveys enabled us to detect active turtles and nests; during driving surveys we recorded road-killed turtles (Fitch, 1987). In most cases the nests were identified by the presence of females laying eggs; in other cases, nests were predated and contained some eggshells on the substrate surface and near the nest chamber. We conducted both walking and driving surveys during daylight hours and recorded site location (with GPS Garmin® eTrex Summit) and date of each record. Road-killed turtles were preserved and deposited in the collection of CICyTTP-CONICET.

Image acquisition and preprocessing

We used Landsat TM5 images, which were acquired from INPE (National Institute for Space Research, Brazil). The study area is covered by two Landsat TM5 satellite scenes (WRS-2 paths 226 and 227, rows 081 and 082). We used one satellite image per season because river level changes throughout the year. The criterion for image selection was proximity of cloud-free scenes to date of data records (turtles and nests). Eight Landsat TM5 images were used with the following dates: 13 Jan 2010, 20 Jan 2010, 10 Apr 2010, 5 May 2010, 16 Aug 2010, 26 Sep 2010, 19 Oct 2010, and 13 Nov 2010. Elevation data were taken from the Shuttle Radar Topography Mission (SRTM). The SRTM is an international project spearheaded by the National Imagery and Mapping Agency (NIMA) and NASA (Van Zyl, 2001). The objective of the mission was to obtain the most complete high-resolution digital topographic database of the Earth. SRTM consisted of a specially modified radar system that flew onboard the Space Shuttle Endeavour during an 11-day mission in February 2000. SRTM data provides the most complete global topographic map ever made, from 60° north to 56° south latitude. The SRTM data have a spatial resolution of 30 m. Landsat satellite images were georeferenced using IDRISI SELVA (Eastman, 2012).

Obtaining variables from remote sensing

We calculated the distance (m) between each record (turtle and nesting site) and the shore of the water bodies by measuring the shortest straight line between them, using Quantum Gis software. Given the spatial resolution of the images used to determine distance from water bodies (Landsat TM5), we used an approximate error of 30 m (one pixel).

To analyze the characteristics of the upland, a 1000-m buffer zone around each record was defined (1000-m radius). The buffer polygon was then subdivided into 100×100 m cells (341 cells). A 1000-m buffer zone was set, as it was the maximum distance from the water at which an individual of *P. hilarii* was observed in this study (see results). Visual interpretation was then performed, and the proportion of cells occupied by each one of four habitat categories was calculated: wetlands, natural terrestrial environment (grassland, forest and savanna), agricultural fields (crops and livestock), and anthropogenic environment (cities, towns, streets, roads). Using these data we characterized the area of influence of each record (Fig. 3). When the presence of clouds in sectors of the scene hindered characterization, we excluded the record from the analysis. To determine whether habitats with presence of turtles and nests were random or not, random control points (that represented all available habitats in the entire study area) were assigned within the study area and subjected to the same analysis procedure as that of presence points (records), with the aim of determining if habitat selected was proportionally different from available habitat (see Statistical analyses). To select those random points, we used the roads where turtles and nests where sampled (National Route 168, provincial route 1 in Santa Fe, secondary roads and trails inside Pre-Delta National Park), which were downloaded from Open Street Maps (http://www.openstreetmap.org). A 50-m buffer on each side of the roads sampled was defined, and 120 points were randomly assigned to that area to be used as control points. A 50-m buffer zone was defined, as it is the maximum distance at which turtles and nests were observed during driving and walking surveys. Because habitat was analyzed in 120 records (of nests and turtles, see results), we have taken the same number of random points, where the habitat is also analyzed.

To estimate the elevation (m) of nests relative to maximum level of water body nearest each record throughout the year, we

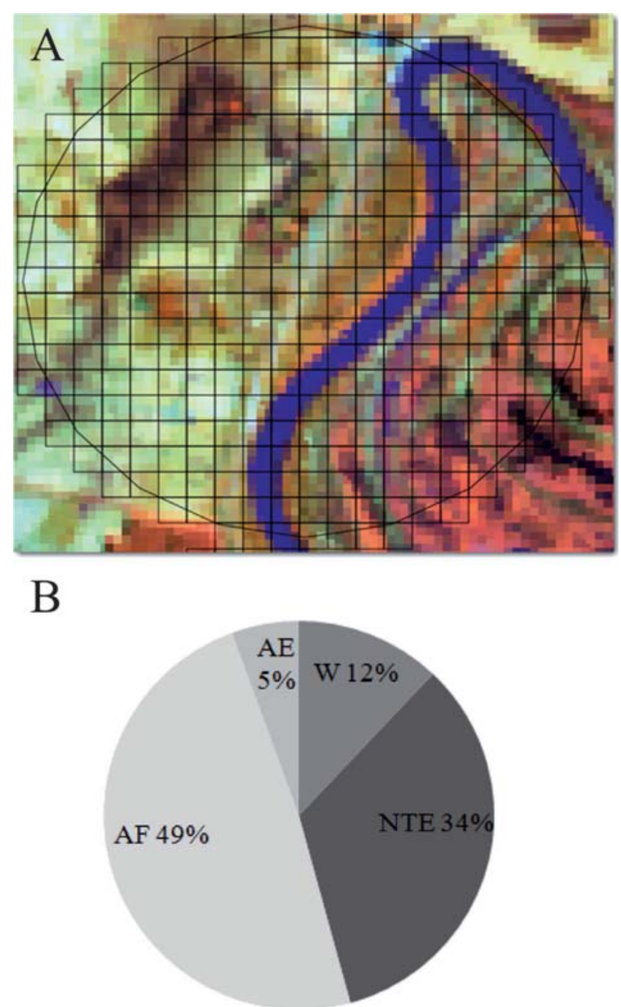

Fig. 3. Area of influence of each record: **(A)** 1000-m buffer zone around a record subdivided into 100 x 100 m cells; **(B)** graph showing the percentages of each category of habitat. W: wetlands, NTE: natural terrestrial environment, AF: agricultural fields and AE: anthropogenic environment.

first calculated the elevation of each nest, then calculated the maximum elevation of the water body nearest each nest, and finally we calculated the difference between the two values to obtain the relative elevation. With maximum water elevation we refer to the maximum level reached for each water body nearest the nests recorded throughout the study period. Here elevation is measured with respect to sea level. Elevation data were taken from the Shuttle Radar Topography Mission (SRTM). The SRTM data have a spatial resolution of 30 m and continental relative height error of 5.5 m (Rodríguez, 2006). Since the random error is dependent on the topography, with more errors in mountainous areas than in the plains areas (Rodríguez, 2006), it is likely that the error in the study area is less than 5.5 m.

Statistical analyses

We analyzed the normality of the data using Shapiro-Wilk tests. We used Spearman's correlation coefficient to analyze the relationship between number of turtles/nests and distances from water bodies (the distance data were categorized every 100), and number of nests and relative nest elevation with respect to the nearest water body (categories: < 0, 0–1, 1–2, 2–3, 3–4, 4–5, and 5.1–6 m). We used Kruskal-Wallis tests to determine possible differences in distances between individuals and water bodies in the four seasons. To determine whether the buffer zones of the records (nests and turtles) were different from those of the randomly assigned control points (representing all available habitats in the entire study area), we performed a Chi-square test considering the types of environments as strata (wetlands, natural terrestrial environment, agricultural fields and anthropogenic environment). All analyses used a significance level of 0.05. Statistical analyses were performed using the program Infostat version 5.1 (Di Rienzo et al., 2005).

RESULTS

Distance of turtles and nests from water bodies

A total of 112 turtles and 44 nests were recorded throughout the study period. Individuals were at a mean distance from water of 171.45 m (n = 112, $S.D.$ = 162.45 m, range = 0–1085 m); 75% of individuals were detected within the first 200 m (Fig. 4A). A negative and significant correlation between number of turtles and distance from water bodies was observed (r = −0.93, n = 11). There were no significant differences in the distance of turtles from water among seasons (P = 0.28, H = 3.78, n = 112, df = 3). The turtles nested at a mean distance from water of 136.51 m (n = 44, $S.D.$ = 124.20 m, range = 0.50–344 m); 52.71% of the nests were recorded within the first 100 m (Fig. 4B). There was a negative and significant correlation between the number of nests and distance from water bodies (r = −0.80, n = 5).

Nest elevation above water level

Mean elevation of nests relative to maximum level of water body nearest each record was 1.13 m (n = 44, $S.D.$ = 1.14, range = −1–3.17 m). The correlation between number of nests and elevation of the nearest water body was significant (r = 0.63, n = 44). Ninety-four of nests not flooded, 47% of the nests is the same height from water and another 47% of nests located above the water level (Fig. 5). Distribution of nest height above the sea level is shown in Fig. 6.

Characteristics of the upland

The buffer zones of the records (nests and turtles) were statistically different from those of the random control points (P < 0.0001, χ^2 = 174.38, df = 1). Habitat composition of the buffer zones of the records included 76% of wetlands and natural terrestrial environments (Fig. 7A); instead habitat composition of control points included 68% of agricultural fields and anthropogenic environment (Fig. 7B).

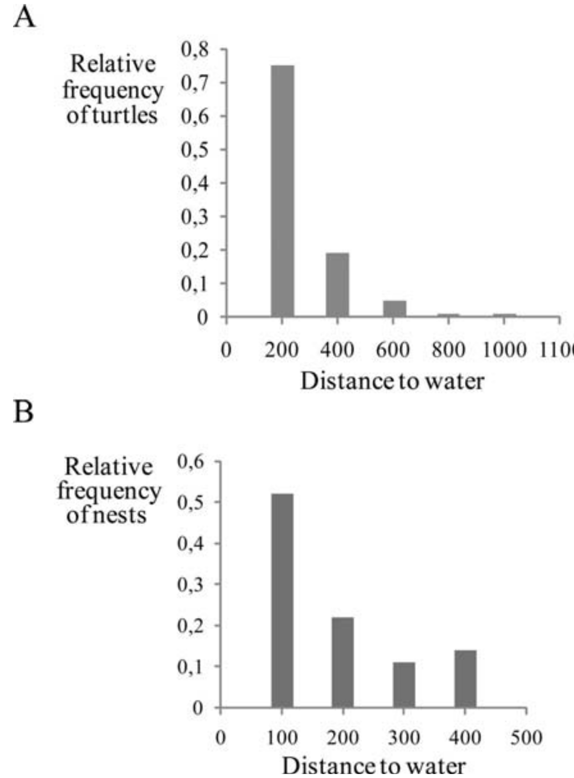

Fig. 4. **(A)** Histogram of relative frequency of turtles and distances from water bodies, **(B)** Histogram of relative frequency of nests and distances from water bodies.

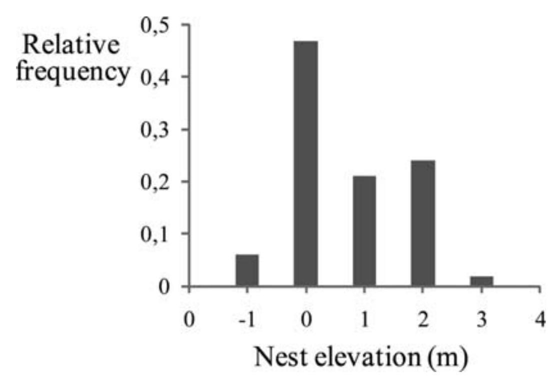

Fig. 5. Histogram of relative frequency of nests and nest elevation.

DISCUSSION

Habitat selection

The movement of aquatic or semi-aquatic turtles in a landscape is usually affected by various biological and environmental factors (Moll and Moll, 2004; Bowne et al., 2006). Land use change not only results in a decrease in the amount of original habitat but also alters the ability of an organism to move among remaining patches of natural habitat (Bowne et al., 2006). The persistence and metapopula-

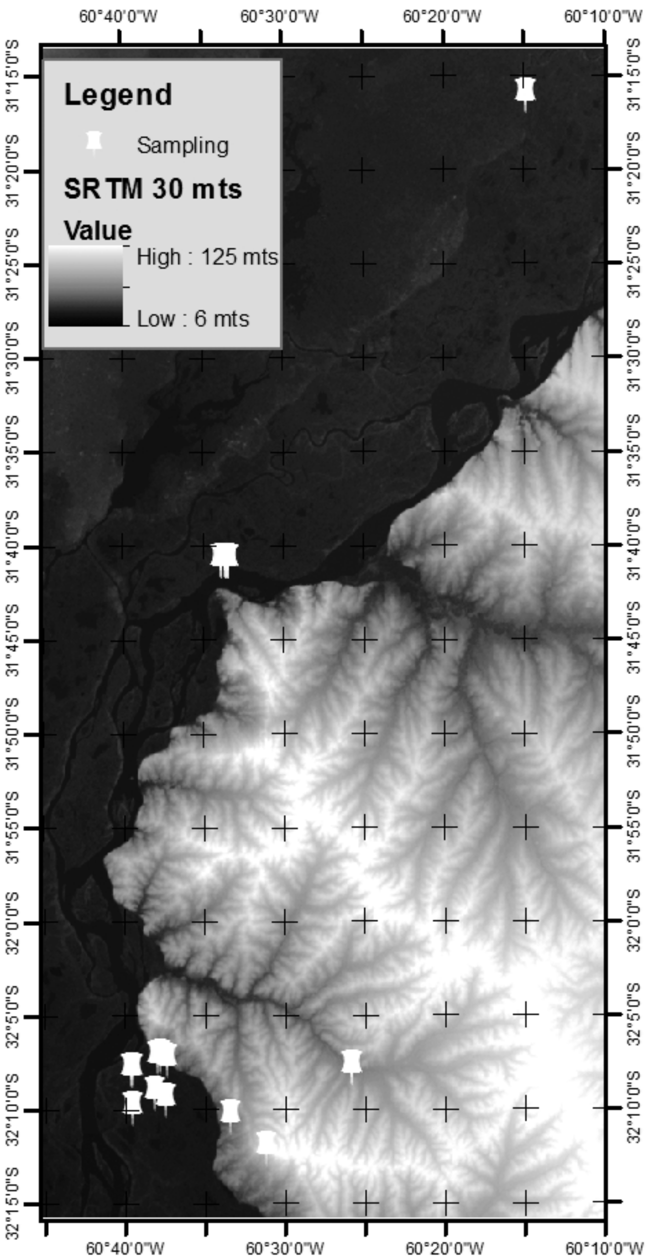

Fig. 6. Distribution of nest elevation; the graph shows the main sites where nests were recorded and elevation ranges correspond to the height above the sea level.

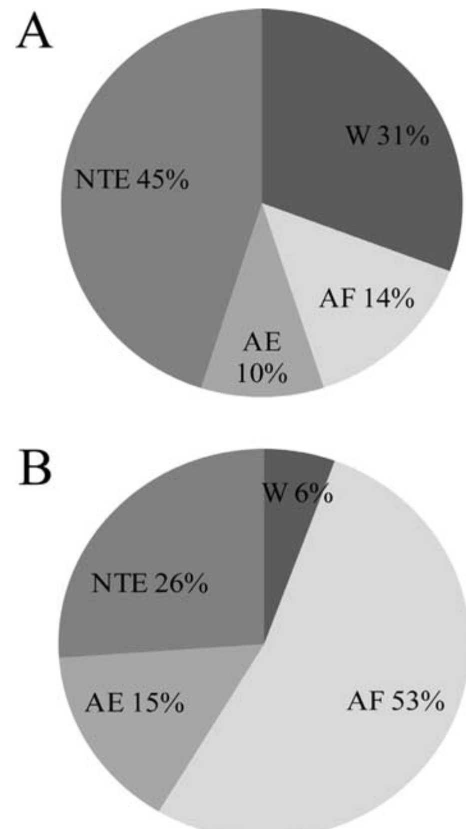

Fig. 7. **(A)** Percentage of habitat composition in 120 records of turtles and nests, **(B)** Percentage of habitat composition in 120 control points. W: wetlands, NTE: natural terrestrial environment, AF: agricultural fields and AE: anthropogenic environment.

tion structure of many amphibians and reptiles is dependent on both aquatic environments and terrestrial biotic corridors (Gibbons, 2003). Our results suggest that turtle dispersal and nesting is influenced by characteristics of the upland. Both the individuals and the nests were found at sites with a greater proportion of aquatic and natural terrestrial environments with respect to sites randomly assigned in the study area. These results differ from records reported for the congeneric species *Phrynops geofroanus*, which can occur at very high densities in urban rivers (Souza and Abe, 2000) and is well adapted to polluted habitats (Souza et al., 2008). In the Paraná River, specimens of *P. hilarii* tend to remain near water bodies; indeed, 75% of individuals were detected within the first 200 m; however, this dispersal is even lower in *P. geofroanus* (up to 115 m), probably due to food supplementation provided by a polluted habitat (Souza et al., 2008). These values suggest that *P. hilarii* may use terrestrial environments in good condition surrounding the wetlands for short-distance movements, possibly in search of food, nesting or refuge sites, and less frequently for long-distance movements, such as migrations or search of new habitats.

Nest site selection

Most of the nests were found within 100 m from the shore; these results agree with findings reported by Bager and Rosado (2010). However, they recorded a mean distance from shore to nest of 50.5 m, with a maximum of 222.6 m, whereas in our work, the mean distance from shore to nest was 136.51 m, with a maximum distance of 344 m. Differences between those findings and the present results may be due to the types of environments studied, since Bager and Rosado (2010) analyzed terrestrial environments surrounding a permanent pond. Availability of terrestrial environments may be different for each area, possibly influencing the distance that females can move away from water for nesting.

The elevation of the nest sites above water level is crucial both for embryo development and hatchling success

(Ferreira Júnior and Castro, 2003, 2006). Prolonged flooding periods may cause egg loss (Moll and Moll, 2004); however, flooding of nests at hatching can facilitate eclosion and hatchling emergence from the nest (Doody et al., 2001; Moll and Moll, 2004). Nest elevation has been found to vary among freshwater turtle species in relation to the dynamics of the water body they inhabit.

In the present work, *P. hilarii* females nested at a mean elevation of 1.13 m relative to the maximum river water level reached during the study period. Of the 44 nests recorded, 41 (93.18%) were found at the same level or above the maximum river level, indicating that most of the nests are not inundated during most of the incubation period. Thus, nest site selection may avoid loss of most of the eggs. Field observations (only three nests flooded) and Bager (1997) that consider that *P. hilarii* is likely to be selective of water level for nesting agree with above observations taken from satellite imagery. However, because SRTM has a relative height error of 5.5 and our results discussed differences within 3 m, we believe that our conclusions should be taken with caution, and future research reinforced.

Our work provides the first data on the knowledge of dispersal and nesting patterns of the freshwater turtle *P. hilarii*, and how they are influenced by habitat. The landscape surrounding wetlands is essential to complete the life cycle of the species. We calculated an average of 171 m of coastline needed by *P. hilarii* for activities such as nesting and dispersal. The terrestrial environments required by the species included a higher proportion of wetlands, grasslands and forests than control points. *Phrynops hilarii* is not considered a vulnerable or endangered species; however, there is a significant lack of knowledge about its life history that hinders appropirate categorization (Souza, 2004). Additionally, the loss of natural habitats and the use of agrochemicals are major risk factors for both adults and embryos (Muñoz et al., 2005; Saumure et al., 2006; Attademo et al., 2011). The results reported in this work provide basic knowledge that can contribute to management of the species.

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