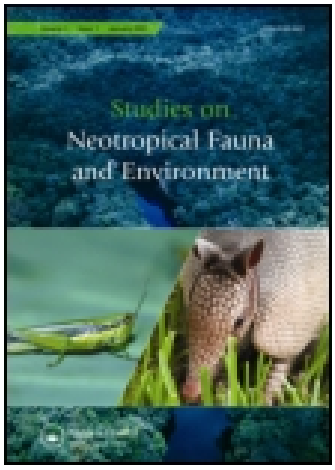

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Ana Filipa Palmeirim[ab], Melina de Souza Leite[b], Margarida Santos-Reis[a] & Fernando A. S. Fernandez[b]

[a] Universidade de Lisboa, Centro de Biologia Ambiental/Departamento de Biologia Animal; Faculdade de Ciências; Campo Grande, Lisboa, Portugal

[b] Laboratório de Ecologia e Conservação de Populações; Departamento de Ecologia; Universidade Federal do Rio de Janeiro; Rio de Janeiro, RJ, Brazil

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ORIGINAL ARTICLE

Habitat selection for resting sites by the water opossum (*Chironectes minimus*) in the Brazilian Atlantic Forest

Ana Filipa Palmeirim[a,b]*, Melina de Souza Leite[b], Margarida Santos-Reis[a] & Fernando A. S. Fernandez[b]

[a]*Universidade de Lisboa, Centro de Biologia Ambiental/Departamento de Biologia Animal; Faculdade de Ciências; Campo Grande, Lisboa, Portugal;* [b]*Laboratório de Ecologia e Conservação de Populações; Departamento de Ecologia; Universidade Federal do Rio de Janeiro; Rio de Janeiro, RJ, Brazil*

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We evaluated the selection of resting sites occupied by the water opossum *Chironectes minimus*, between 2004 and 2010, in streams of the Brazilian Atlantic Forest. Fourteen radio-tracked adult (males and females) opossums used natural cavities as resting sites. Opossums selected narrow river stretches and selected their resting sites mainly according to characteristics favoring protection against adverse weather conditions. Likewise, opossums avoided disturbed habitats and established their resting sites in well-preserved riparian forest sites, selecting river sections containing a high density of trees and a high proportion of forest cover between the river banks and 50 m from the river. Besides increasing our knowledge on this species habitat selection, such findings further highlight this species' sensitivity to human disturbance.

Nós avaliamos a seleção de abrigos ocupados pela cuíca-d'água *Chironectes minimus*, entre 2004 e 2010, em rios da Mata Atlântica Brasileira. Catorze cuícas (machos e fêmeas) radiomonitoradas utilizaram cavidades naturais como abrigos. As cuícas-d'água selecionaram secções mais estreitas de rios e selecionaram os abrigos principalmente de acordo com características que favorecem a sua proteção contra condições climáticas adversas. Adicionalmente, as cuícas-d'água evitaram habitas perturbados e estabeleceram os seus abrigos preferencialmente em locais com vegetação ripária preservada, selecionando seções do rio com elevadas densidade de árvores e proporção de cobertura florestal entre a margem e 50 m de distância do rio. Apesar do aumento do nosso conhecimento pela seleção de habitat da cuíca-d'água, os resultados aqui obtidos alertam para a sensibilidade desta espécie à perturbação humana.

Keywords: habitat resource; habitat selection; marsupial; Neotropics; semi-aquatic mammal

Introduction

Habitat selection is based upon an individual's choice of sites favoring survival and reproduction (Morin 2011). Therefore, individuals select a habitat according to its potential to provide food, shelter and encounters with potential sexual mates. Understanding species habitat requirements and selection is important to determine the potential ecological and physiological constraints on the species (Szor et al. 2008; Ross et al. 2010). Such knowledge can be used to focus on species conservation and management actions by mapping its most suitable areas (Ross et al. 2010).

During resting activity, individuals select sites that provide protection against adverse weather conditions and/or predators (Zalewski 1997a, 1997b; Bull & Heater 2000; Zabala et al. 2003). For species showing high fidelity to resting sites, these are an important habitat resource (Sepúlveda et al. 2007). The availability of suitable resting sites can affect species ecology, behavior and conservation and may further limit species populations (Beja 1996). Therefore, assessing habitat selection for resting sites is important, particularly for those species whose ecology and habitat requirements are still poorly known. This is the case for the water opossum (*Chironectes minimus*, Marshall 1978), a Neotropical marsupial that has seldom been recorded due to the ineffectiveness of traditional trapping methods for this species (Bressiani & Graipel 2008), and this constraint has resulted in the still scarce knowledge of this species ecology.

The water opossum is the only semiaquatic marsupial in the World (Marshall 1978) and has a wide distribution ranging from southern Mexico to northeastern Argentina (Nowak 1991). So far, the species has been reported using river channels characterized by stony substrate, clear and fast-running waters,

*Corresponding author. Email: anafilipapalmeirim@gmail.com.

and preserved riparian vegetation (Galliez et al. 2009; Galliez & Fernandez 2012). Similarly to other Neotropical marsupials, the water opossum is solitary and nocturnal, spending daytime in resting sites close to water bodies (Galliez et al. 2009; Galliez & Fernandez 2012; Leite et al. 2013). In the IUCN Red List, the water opossum is listed as a least concern species despite having a decreasing population trend (Cuarón et al. 2008). In Brazil, however, the species is considered as threatened in five states (Bergallo et al. 2000; Mikich & Bérnis 2004; Passamani & Mendes 2007), mainly due to habitat loss and degradation. Recently, the species' ecology and activity patterns were studied in the fast-flowing mountain rivers of the Brazilian Atlantic Forest, where the increasing altitude gradient often coincides with a decreasing habitat disturbance gradient (e.g. Galliez et al. 2009; Leite et al. 2013). Here we aim to evaluate the habitat selection of the water opossum during its inactivity period by determining the factors that affect resting site selection in streams of the Brazilian Atlantic Forest. For this, we considered factors related to both the habitat structure and the degree of human disturbance. We further describe in detail the structure and use patterns of the resting sites used by radio-tracked water opossums.

Materials and methods

Study area

This study was carried out at the Águas Claras River basin (22°30′ S, 42°30′ W), in Rio de Janeiro state, southeastern Brazil (Figure 1). The climate is tropical wet and warm, with monthly precipitation (mean ± SD) varying between 104.2 ± 50.5 mm in the dry season (April–September) and 272.6 ± 74.1 mm in the rainy season (October–March). Mean monthly temperatures range from 19°C to 25°C. Vegetation is characterized by submontane rainforest with a mixture of secondary forest. The study area is comprised by three sections of Águas Claras River – upper, middle, and lower course – and some of its tributaries. Habitat conditions vary along the river and its tributaries; there is a well-preserved riparian forest and rocky substrate in the upper and middle courses, whereas in the lower course the habitat is more degraded, being often surrounded by agricultural fields and pasturelands.

Trapping and radio-tracking methods

Water opossums were captured in five night-trapping sessions carried out monthly, from October 2004 to April 2010, using double-door wire-mesh live traps

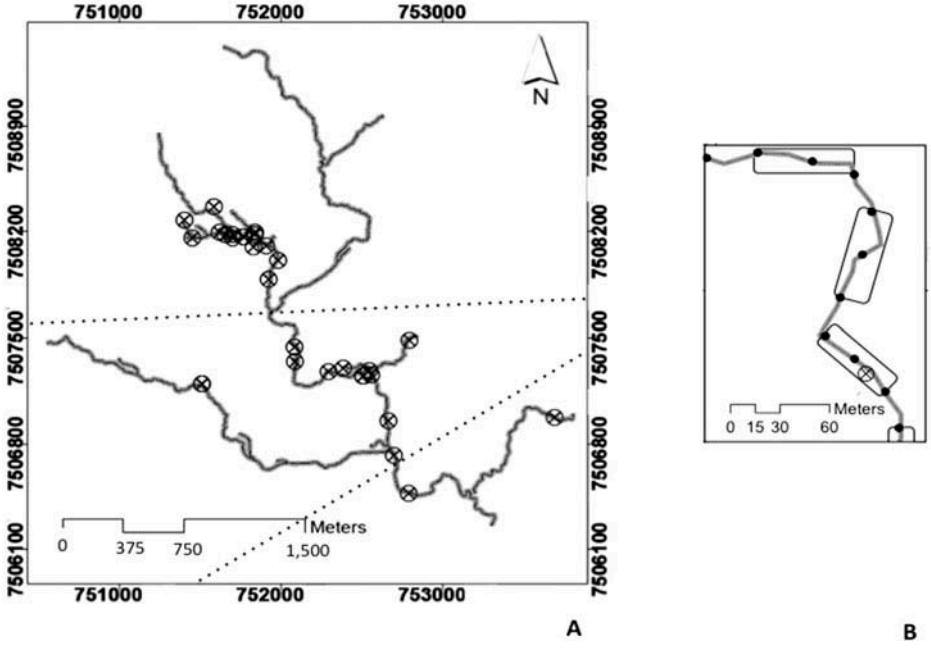

Figure 1. Location of the study area. A, Águas Claras river basin. The black line indicates the Águas Claras River. The circles with crosses indicate the locations of resting sites used by the radio-tracked water opossums. Towards the south, the dashed lines separate the upper, middle and lower courses, respectively. B, Representation of the sampling sites (solid dots) where the habitat variables were measured and the 100 m river bank sections (rectangles).

located in the channel of the rivers (Bressiani & Graipel 2008). Fourteen adult water opossums (four females and 10 males) were fitted with radio-collars (SOM-2380A, Wildlife Materials, Murphysboro, IL, USA; or TXE-207C, Telenax, Playa del Carmen, Mexico), weighing approximately 15 g (a maximum of 5% of the animal's body mass). No adverse effects of the radio-collars were observed for any of the animals. Individuals were monitored by radio-tracking using the homing-in technique (White & Garrot 1990), during the three months of battery life on the radio-collars. Three of the radio-tracked opossum were recaptured and refitted with new radio-collar. The detailed trapping and radio-tracking methods are described in Galliez et al. (2009) and Leite et al. (2013).

Resting site description and use patterns

During the period of inactivity, we located 14 radio-tracked opossums on 89 occasions (from one to 22 occasions per individual). Thirty-six different resting sites were georeferenced with a handheld GPS (Garmin 76cx) using the Universal Transverse Mercator (UTM) coordinates. From those resting sites, 25 (see the circles with crosses in Figure 1A) were described regarding their own characteristics such as entrance numbers and sizes (i.e. ellipsoidal area = $\pi \times$ largest entrance diameter $\times$ perpendicular diameter to the largest) and characteristics of both the habitat where it was located (substrate type and shortest distance to the river) and the surrounding habitat (river width and deepest part near the resting site).

From the total of 14 radio-tracked individual opossums, 10 had their linear home range estimated (Leite 2009; Palmeirim unpubl. data) and from those 10, six were located on more than five occasions during inactivity. We analyzed the resting site use patterns for these last six opossums (three males and three females), located during inactivity between six and 22 times (median = 9), by calculating the median, minimum and maximum numbers of resting sites used by each opossum. We compared the number of resting sites used by males and females using a Mann–Whitney U-test at a significance level of 0.05 (Zar 1999). We considered the cumulative number of different resting sites used by each individual to see how the number of different resting sites used by individual opossums increased with the number of locations at inactivity.

Resting site selection

In August 2010, we measured a set of habitat variables related to both habitat structure and degree of human disturbance in sampling sites 50 m apart from each other, within the home range of radio-tracked opossums (Figure 1). We considered 100 m river bank sections, by averaging the variables values measured at three contiguous sampling sites (see Figure 1). To minimize spatial autocorrelation, each 100 m section was 50 m apart from the border of the previous section. We then assigned each of those 100 m sections to either presence or absence of water opossum resting sites using a geographic information system (ArcMap 10.1; ESRI 2012). We did not take into account the presence of resting sites in the 50 m river sections separating the contiguous 100 m river sections ($n <$ 5 cases). The habitat variables measured included the river channel area, particle size of the river substrate, vegetation type, tree density at river banks and proportion of forest at river banks and its surroundings (see Table 1 for a description of variables).

Table 1. Variables used to assess the resting site selection of the water opossum at Águas Claras river basin, southeastern Brazil.

Explanatory variables	Type	Description
River channel area (Riv)	Continuous	River width and deepest part directly measured (m^2)
Index of particles size of the river substrate (Subs)	Continuous	Index of particle size classes visually estimated following Somers and Nel (2004): [(%clay × 1) + (%gravel × 4) + (%stones × 7) + (%boulders × 10)]/10
Index of riparian vegetation (Veg)	Continuous	Index of the proportion of each class of riparian vegetation visually estimated: [(absent or herbaceous × 3) + (shrub × 6) + (arboreal × 10)]/10
Tree density (Tree)	Continuous	Number of trees with diameter at breast height > 10 cm recorded in two plots of 2 × 5 m, each one located at one of the river banks
Forest proportion at river banks (F5)	Quantitative	Proportion of forest at 5 m distance from the river estimated by direct observation
Forest proportion at river vicinities (F50)	Quantitative	Proportion of forest at 50 m distance from the river estimated by direct observation
Response variable	Type	Description
Resting site occurrence	Binary	Presence (1) or absence (0) of water opossum resting sites

To evaluate the water opossum resting sites selection, we modeled the occurrence of resting sites used by the 10 radio-tracked opossums whose home ranges were previously estimated, corresponding to a total of 106 river sections of 100 m each. For this modeling approach, because the opossums' home ranges overlapped in 55 of the 106 river sections (ranging from two to seven individuals), we considered all the sampled 106 river sections nested within the seven levels of opossums' home range overlap observed (random term). One level of opossums' home range overlap means that two opossums' home ranges are overlapping, two levels are corresponding to three opossums' home ranges overlapping and so on. Then, we performed binomial generalized linear mixed models (GLMMs) with a logit-link function. To construct the candidate model set, we used all additive combinations of the entire set of habitat variables (fixed factors), as no significant correlation was observed between the pairs of those variables ($r < 0.65$, $p > 0.05$). We then ranked the 51 alternative models, plus the complete and the null models, based on their maximum likelihood using the Akaike information criterion corrected for small sample size (AICc: Burnham & Anderson 2002). Habitat variables were standardized ($x = 0$, $\sigma = 1$) before fitting each model to the data in order to both improve convergence of the fitting algorithm and place coefficient estimates on the same scale. These analyses were performed using the "glmmML" package within R version 2.1.11 (R Core Team 2013). Following the approach of Rhodes et al. (2009), we performed a model averaging using only the most plausible models (i.e. models having ΔAICc values between 0 and 2, considering $\Delta AIC = AIC_i - AIC_{min}$ in which $i = i$th model). By using this procedure, more robust predictions can be obtained when there is no single model that clearly performs better (Burnham & Anderson 2002). The relative importance (RI) of each variable contained in the set of more plausible models was obtained by the sum of the Akaike weights of the models where that variable was included (Rhodes et al. 2009).

Results

Resting site description and use patterns

All of the 25 resting sites used by the radio-tracked opossums were natural cavities. Those sites were evenly located in rocky substrate ($n = 8$; Figure 2A), root systems within bare soil ($n = 8$) or in a mixture of the three different types of substrate (i.e. rocks, roots and bare soil; $n = 9$). Most resting sites presented only one entrance ($n = 22$), but multiple entrances were observed in three sites (two with two and one with four entrances). The area of the entrance varied, with a range of 35.33–1004.80 cm^2 (median = 254.34 cm^2; $n = 15$), as well as its shortest distance to the river, 0–183 cm (median = 32.5 cm; $n = 20$). Almost all habitat cover surrounding resting sites was arboreal vegetation ($n = 24$), except in one site located under shrubby vegetation. The width of the rivers near resting sites varied between 0.56 and 12.20 m (median = 3.80 m, $n = 20$) and depth was 2–52.70 cm (median = 18.30 cm, $n = 20$).

Water opossums used three resting sites more frequently (from two to eight sites, $n = 6$ individuals). Both sexes used a similar number of resting sites although males more frequently used four (2–5 sites) and females three (3–8 sites; $U = 4$, $n_{males} = 3$, $n_{females} = 3$, $p = 0.83$). In general, the number of resting sites used did not tend to stabilize with increasing number of individual locations during the resting activity. However, individuals re-used the same range of resting sites during frames from 2–9 locations (Figure 3). Along the monitoring period, five resting sites were used by two opossums at different times (two males on three occasions and one male and one female on two occasions).

Habitat selection for resting sites

We recorded the presence of opossum resting sites in 25 of the 106 sections of 100 m of river banks included in the sampled river extension. That river extension was characterized by a variable river channel area (median: 2.64; range: 0.01–17.34 m^2), moderate size of river substrate particles [mean ± SD: 5.50 ± 1.60; index ranging from 1 (clay) to 10 (small stones)], predominant arboreal vegetation [0.93 ± 0.14; vegetation index ranging from 0 (herbaceous) to 1 (arboreal)], low but highly variable tree density (3.33, 0.33–11.00 trees/20 m^2 of river banks) and moderate to high proportion of forest both in the river channel banks (72.2 ± 40.0%) and in the surroundings (56.1 ± 47.7%).

The river channel area was the most explanatory variable in explaining the presence of water opossum resting sites, accumulating the higher relative importance (RI = 0.392) between the variables included in the set of the plausible GLMMs (ΔAICc ≤ 2; Table 2). According to this set of models, opossums selected river stretches with narrower river channels [β_{Riv} = negative relationship (–)]. Secondarily, opossums selected preserved river stretches, containing both a high density of trees [β_{Tree} = (+), RI = 0.129] and a high proportion of forest in the river banks surroundings [β_{F50} = (+), RI = 0.108]. A less-important variable of opossum resting site selection was the proportion of forest in the river bank [β_{F5} = (+), RI = 0.074]. Opossums did not necessarily select resting sites based on the particle size of the river substrate (Subs), nor on the

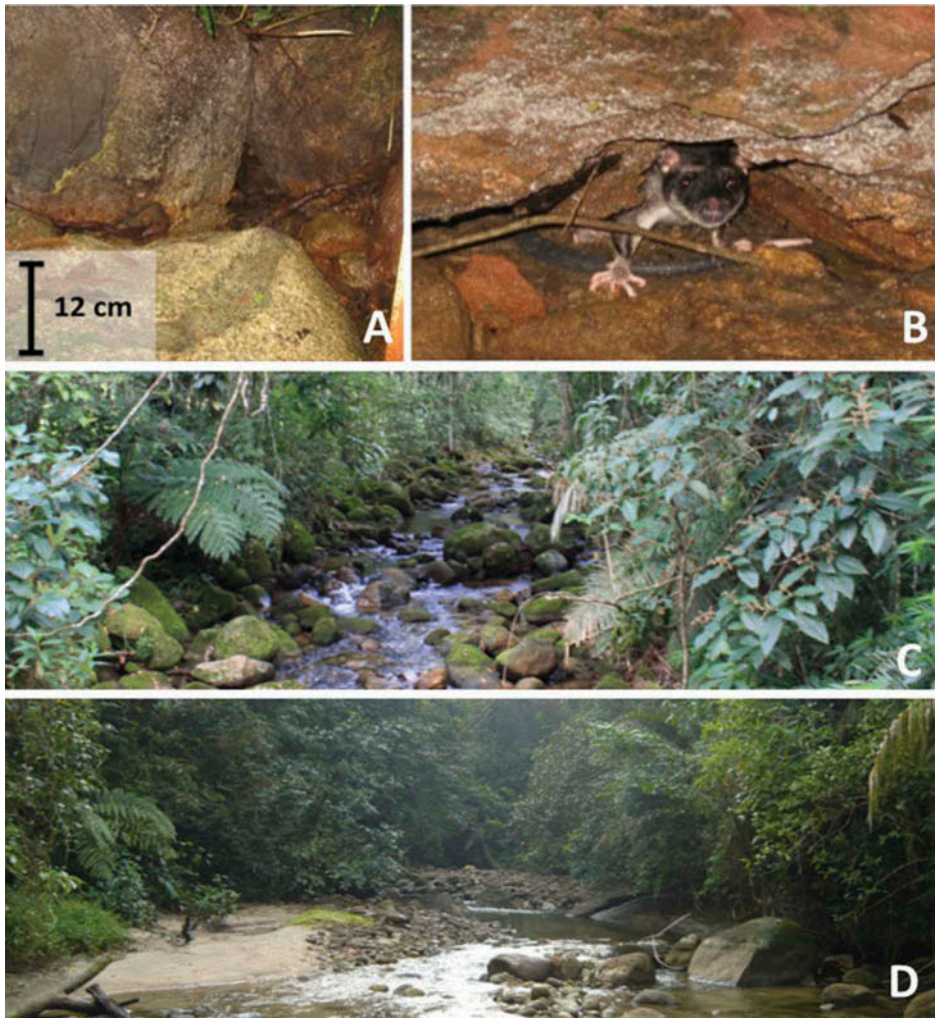

Figure 2. The Águas Claras River Basin, in southeastern Brazil. A, B, Examples of resting sites on rocky substrate used by the radio-tracked water opossums; C, D, typical stretches of the mountain rivers at the Águas Claras River Basin illustrating C, a narrow and D, a wide river channel section.

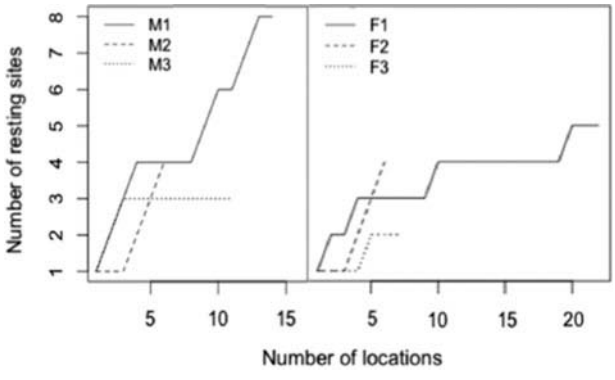

Figure 3. Number of different resting sites used by radio-tracked water opossums males (on the left) and females (on the right) in the Águas Claras River Basin, according to the number of locations during the animals' period of inactivity. We present only the number of resting sites used by the individuals located more than five times during their periods of inactivity.

type of riparian vegetation (Veg). These variables were not included in the set of 'best' models explaining the presence of water opossum resting sites.

Discussion

We found that water opossums use more than one natural cavity in the river bank as a resting site within its home range. Water opossums selected narrower river stretches to establish their resting sites, which might be related to characteristics favoring their protection against adverse weather conditions. Also, along the increasing degree of disturbance comprised within the study area, opossums preferred to establish their resting sites where well-preserved riparian forest was present, as indicated by the selection of river

Table 2. Set of the plausible GLMMs 'best' explaining the water opossum resting site selection, ordered by AICc [Akaike information criterion for small samples (i.e. ΔAICc > 2, considering $\Delta AICc = AICc_i - AICc_{min}$, i = ith model]. Random effects correspond to the number of individuals home range overlapping and fixed effects to the habitat variables [river channel area (Riv), index of particles size of the river substrate (Subs), index of riparian vegetation (Veg), tree density (Tree), forest proportion at river banks (F5) and forest proportion at river vicinities (F50)]. K corresponds to the number of parameters included in each model, W_i to the Akaike weights and RI to the relative importance of each habitat variable in the model averaging.

	Model							
Rank	Riv	Tree	F5	F50	K	AICc	ΔAICc	W_i
1	–0.918				2	116.317	0.000	0.137
2	–0.373	0.249			3	116.435	0.119	0.129
3	–0.289		1.241		3	117.535	1.219	0.074
4				1.206	2	118.117	1.800	0.056
5	–0.267			0.878	3	118.235	1.919	0.052
RI	0.392	0.129	0.074	0.108				

sections characterized by a high tree density and a high proportion of forest cover in the vicinity.

Habitat selection for resting sites

The water opossums' selection for narrower river stretches to establish their resting sites can be seen as a consequence of water dynamics in the study area. The Águas Claras river flow is highly influenced by local rainfall and the occurrence of torrential rains can drastically change the river flow within short time frames (Queiroz 2010). Wider river stretches are subject to higher water flows that further cause higher erosion of river banks (Tomasella et al. 2008). Narrower stretches of the river have higher bank stability, which is enhanced by the densely interwoven roots of the riparian vegetation, the protection from direct impact of rainfall by the forest canopy and the stable soil structure (Espírito-Santo et al. 2013). Therefore, by choosing narrower river sections (see Figure 2B) when resting, opossums might be decreasing the risk of destruction of their resting sites in such a dynamic habitat. With a similar purpose, the Neotropical otter *Lontra longicaudis*, which typically establishes its resting sites along the main river course, selects sites according to the distance to water (Pardini & Trajano 1999).

Water opossums did not necessarily select sites according to the size of particles of the river substrate, or to the type of riparian vegetation. This was not surprising, since these two variables only presented a modest variation along the study area extent. In addition, the protection provided by resting sites may be achieved by intrinsic features of the resting site. For example, water opossums may choose resting sites characterized by modest entrance sizes to inhibit access by predators like raptors (e.g. the diurnal raptor *Rupornis magnirostris*: Tortato 2009). Likewise, the multiple entrances observed in three resting sites might facilitate an easy escape, a strategy also adopted by other mammals (e.g. *Caluromys philander*, Cobra 2010).

The selection of the habitat variables corresponding to a highly preserved riparian forest (i.e. high density of trees and high proportion of forest 5 and 50 m away from the river) is an indicator of the water opossums' sensitivity to human disturbance. The water opossums' selection for well-preserved habitats was previously reported (Mondolfi & Padilha 1958; Voss et al. 2001; Galliez et al. 2009) and, recently, this species has also been reported to be more frequently captured in areas containing a high density of trees (Galliez & Fernandez 2012). Similarly to other mammal species (e.g. black bears, *Ursus americanus*: Reynolds-Hogland et al. 2007; Pallas's cats, *Otocolobus manul*: Ross et al. 2010), water opossums also seek to avoid disturbances by humans when resting.

Resting site use patterns

The radio-tracked opossums used relatively few resting sites (three sites in ~7 months), compared to other mammals, including marsupials and semi-aquatic mammals that also do not construct their resting sites (Table 3). In general, individuals that do not invest in resting site construction are usually able to explore a larger area of available habitat (Garin et al. 2002) and, consequently, tend to use a higher number of resting sites, each of them being less intensively reused (Zabala et al. 2003; Rosalino et al. 2005). This scenario is expected unless the availability of resting

Table 3. Summary of resting sites used by mammal species, indicating the number of resting sites used per period of time (when mentioned in the reference).

Species	Common name	No. of resting sites[a]	Reference
Chironectes minimus	Water opossum	4.2/~7 months	This study
Glirulus japonicus	Japanese dormouse	4.1/week	Shibata et al. (2004)
Dasyurus maculatus	Tiger quoll	9	Glen and Dickman (2006)
Spilogale putorius	Eastern spotted skunks	10/summer	Lesmeister et al. (2008)
Martes foina	Stone marten	>20	Pereira (1999)
Genetta genetta	Common genet	>20	Pereira (1999)
Martes zibellina	Sable	44/year season	Cheng-Shui et al. (2000)
Didelphis virginiana	Virginia opossums	41/100 days	Hossler et al. (1994)
Mustela lutreola	European mink	No re-use	Zabala et al. (2003)

[a]This information is identical to that provided in the reference.

sites, or suitable habitat to establish them, is reduced. For example, the European mink *Mustela lutreola* does not re-use resting sites, further denoting a high availability of that habitat resource (Zabala et al. 2003). For the water opossum, the present findings suggest that sites with the necessary requirements to perform their main function of protection are at least not a normally abundant resource in the species' habitat. This is further supported by evidence of resting site sharing, a fact that, for solitary species, can be regarded as an indication of scarce resting resources in the habitat (Lesmeister et al. 2008). Also, Galliez et al. (2009), working at the same place, reported on one occasion the simultaneous use of the same resting site by a male and a lactating female opossum.

Conservation insights

Under the current scenario of increased deforestation in the Neotropics, which includes the riparian vegetation, one would expect a decrease in the suitable habitat area for the water opossum (Galliez & Fernandez 2012). According to the review of the Brazilian Forest Act, the forest protection law now states that riparian corridors with a minimum width of 15 m must be preserved, along both river banks, within private landholdings (Law nº 12.727, reviewed 17 October 2012). This measure will greatly reduce the previously required corridors with a width of 30 m (Metzger 2010); therefore a significant reduction in the availability of suitable resting sites for opossums can be anticipated, which will negatively affect this species' distribution and abundance, as observed in other mammal species (Beja 1996; Halliwell & Macdonald 1996; Ross et al. 2010). These threats to the long-term survival of this unique species stress the urgent need for studies that will improve the knowledge base on the factors determining its distribution and ecological requirements, allowing the revision of the species conservation status on the basis of scientifically sound arguments.

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