## Movement patterns and activity of the Brazilian snake-necked turtle Hydromedusa maximiliani (Testudines: Chelidae) in southeastern Brazil

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**Abstract.** To investigate the movement patterns and activity of *Hydromedusa maximiliani* in southeastern Brazil, sixteen adult freshwater turtles (eight males and eight females) were monitored with radio-transmitters from October 2009 to November 2010. An additional 22 turtles (11 males and 11 females) were monitored with thread-bobbins over a 3-day period. *Hydromedusa maximiliani* showed strong tendency to remain close to the river course. The distance moved each day varied from 0 to 179 m/day (radio-tracking) and 1.5 to 201 m/day (thread-bobbins). Males moved greater distances than females during the mating season (August to November), while females exhibited pronounced movements associated with egg-laying (December to February). Male and female movements did not differ significantly. Straight line distance measurements, usually obtained by radio-tracking, underestimate actual movement distances, since the nonlinearity of movements is not captured by radio-tracking as opposed to spool tracking, even when long distances are travelled. Results from this study are important for establishing conservation strategies for this vulnerable species.

Keywords: Atlantic Rainforest, daily movements, radio-tracking, thread-bobbins.

## Introduction

High human impact on the neotropical Atlantic Rainforest of Brazil raises serious concerns for the development of successful strategies for species conservation. Effectively protecting species habitat requires understanding of how individuals disperse through time and space in that habitat (Rasmussen and Litzgus, 2010). In turtles, movement patterns within and between terrestrial and aquatic habitats are strongly influenced by environmental attributes such as topography, climate, feeding resources, refuge availability, and reproductive requirements (Edge et al., 2010) with ultimate effects on demography and population struc-

\*Corresponding author; e-mail: shifacbio@yahoo.com.br ture (Gibbons, 1970). Differential movement patterns between individuals or demographic classes may reduce intraspecific competition, as well as influencing overall resource partitioning and interspecific competition (Bury and Germano, 2003; Tran, Moorhead and McKenna, 2007). Movements of freshwater turtles are also strongly associated with activity during the reproductive season (Mahmoud and Licht, 1997).

Recording the path of an animal using radiotracking or thread-bobbins yields a discrete representation of its habitat use, which is often complex (Roe and Georges, 2008). Data on animal movement reflects the continuous process of space use and habitat selection (Calenge, Dray and Royer-Carenzi, 2009), in response to resource availability. Movements affect survival in unique ways for freshwater turtles because they often engage in terrestrial activity which carries with it a risk of desiccation or starvation (Caldwell and Nams, 2006). The direction and pathway to water must be known, or strategies adopted to bring standing water within reach at regular intervals. Consequently, turtle movements will vary among seasons, coincident with changing availability of nesting and hibernation

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sites and availability of standing water. Their movements also will vary with age and sex. For example, females of Acanthochelys spixii monitored with thread-bobbins at Parque Nacional de Brasília, Central Brazil, were more active than males during the nesting season (Horta, 2008). In the Mamirauá Reserve, Amazonas state, northern Brazil, Podocnemis sextuberculata migrates in response to seasonal climate and water level changes (Fachín-Terán, Vogt and Thorbjarnarson, 2005). Females of P. sextuberculata move from the varzea to reach suitable nesting beaches, whereas males move primarily to seek potential mates (Fachín-Terán, Vogt and Thorbjarnarson, 2005). For instance, female turtles of most species often move greater distances than males during nesting season and males often move greater distances during mating season (Souza, 2004). Thus, understanding the extent and frequency of movements of these animals is needed to understand their distribution. abundance, gene flow and behavioral interactions (Carter, Haas and Mitchell, 2000).

Studies based only on radio-tracking may underestimate distances travelled, since linear measurements of the distances between capture/recapture sites (fixes) do not reveal the non-linear path taken between those two end points (Cunha and Vieira, 2002; Tozetti and Toledo, 2005). Alternative methods, such as direct observation or thread-bobbins (Boonstra and Craine, 1986), are required to access the non-linear movements between fixes (Tozetti, Vetorazzo and Martins, 2009) and to provide a more detailed picture of the path taken by the animal (Cunha and Vieira, 2002).

*Hydromedusa maximiliani* (Mikan, 1825) is a small, semiaquatic chelid turtle endemic to eastern, mid-western and south-eastern Brazil. It occupies small streams of mountainous regions throughout its range in southern Bahia, Goiás, Minas Gerais, Espírito Santo, Rio de Janeiro and São Paulo states (ICMBIO-RAN, 2011). *H. maximiliani* is considered Vulnerable by the IUCN (2010), Critically Endangered in the Brazilian state of Minas Gerais, and Vulnerable in the states of São Paulo and Espírito Santo (Martins and Molina, 2008). Like many other turtles, H. maximiliani is long-lived, probably reaching more than 90 yr of age in their natural setting and with a delayed sexual maturity strategy (Martins and Souza, 2009; Famelli et al., 2012). It is able to reproduce only after reaching 13 yr for males and 15 yr for females (Martins and Souza, 2008; Famelli et al., 2014). In the last decade, a population of H. maximiliani has been intensively studied at the Parque Estadual Carlos Botelho, southeastern Brazil (Famelli et al., 2012), where it shows a peak in activity in the wet season, between September and December (Famelli et al., 2014) and a mean dispersal rate of 2 m/day (reviewed in Souza and Martins, 2009). However, no information is currently available on its movement patterns.

Here we report results of a study of the movement patterns and activity of adult *Hydromedusa maximiliani*. We compare the application of radio-tracking with thread-bobbins to ascertain the degree to which data collected with straight-line point estimates (radio-tracking) underestimate actual distance moved. Our results include preliminary data on nesting and estivation, two critical activities in the life cycle of these turtles.

## Material and methods

#### Study area

The study was conducted in the Parque Estadual Carlos Botelho (PECB, 24°00'00"-24°15'00"S; 47°45'00"-48°10'00"W), southern São Paulo State, in south-eastern Brazil. The park comprises only 37.6 ha, but is part of a vast ecological corridor of more than 120 000 ha prescribed as a Natural Site on the World Heritage List by UNESCO (Ferraz and Varjabedian, 1999). This corridor supports one of the largest continuous remnants of Atlantic Rainforest in Brazil, important for the persistence of many species and the maintenance of ecological processes (Leitão-Filho, 1982; Guix, 2002). The PECB has a network of hundreds of ephemeral and perennial rivers and streams occupied by H. maximiliani. These water bodies are 5 to 100 cm depth with clear, cold water, and sand and rocky substrata (Souza and Martins, 2009). The vegetation comprises complex forest with a large, dense canopy; little direct sunlight reaches the water surface.



Figure 1. Annual rainfall and mean air temperature at Parque Estadual Carlos Botelho, state of São Paulo, southeastern Brazil, between January 2008 and December 2010. Owing to technical problems, meteorological data were not collected between July and September 2008.

Monthly data collected during the study period at PECB (2008 to 2010) indicated precipitation varying from 6 mm (July 2008) to 391 mm (January 2008). The temperature varied between 13.1°C (July 2010) and 24.6°C (November 2008) (fig. 1).

#### Methods

Turtles were located visually during diurnal walks along the streams and hand-collected. All turtles not already marked in earlier studies were individually marked by notching marginal scutes (Cagle, 1939). Straight-line maximum carapace length (CL) was measured to the nearest 0.1 mm with callipers. The sex of individuals was identified using the combination of degree of plastron concavity and relative tail length (Souza, 1995a, 1995b; Souza and Abe, 1997). Males are also larger than females (Souza, 1995a; Souza and Abe, 1997).

#### Thread-bobbins

Field surveys were conducted in October 2007 and August 2008. Monthly from October 2008 to March 2009, and from March 2010 to April 2011 (table 1). During each sampling visit, two animals were captured (one male and one female) to monitor continuous movements with spooland-line tracking devices (thread-bobbins) (Wilson, 1994). This was not always achieved, and capture was irregular in some months (table 1). A total of twenty-two turtles (11 males and 11 females) were monitored with the thread-bobbins. Each thread-bobbin was constructed with spools

of nylon thread, 300 m long. The spools themselves were cylindrical, 37 mm long, 14 mm in diameter, and 6 g in weight. Two spools linked by their ends (600 m of thread in total) were wrapped in transparent plastic film and then attached to the carapace with adhesive tape (Tozetti and Toledo, 2005; Tozetti, Vettorazzo and Martins, 2009). Final weight of the device was less than 7% of body mass.

Turtles fitted with thread-bobbins were released at the site of capture with the thread anchored to branches or roots. As the water bodies are shallow (5-100 cm) and clear, trails could be easily seen in the water. The thread trail remains stationary in the environment, attached to the rocks and/or vegetation, revealing the animal's trajectory as it moves through the environment (Tozetti and Martins, 2007). We followed each animal trail once a day for three consecutive days in an interval of 24 hours to minimize auto-correlation among fixes (White and Garrott, 1990). Every day we collected the line abandoned between fixes, cut and reattached it to branches or roots near the turtle's current location. However, owing to the difficulties in tracking and collecting the abandoned line, it was difficult to monitor more than two animals per day, though in December 2010, three animals were monitored (table 1).

The trail was mapped using a compass and a tape measure. Measurements taken from the spool trails are shown in table 2. The distance between points and the angle of movements were transformed to Cartesian coordinates and schematized using graph paper. The length of dispensed line gave us the distance effectively moved which was recorded as a per day estimate (EDM). The sum of EDM after 3 d monitoring gave us the total effective distance moved

Table 1. List of snake-necked turtles, *Hydromedusa maximiliani*, monitored by thread-bobbins and radio-transmitters at Parque Estadual Carlos Botelho, state of São Paulo, southeastern Brazil. CL, carapace length; N, number of fixes of animals radio-tracked.

Turtle	Sex	Thread-bobbins period	Radio-tracking period	Method	CL (mm)	N
#156	F	Oct 2007		Thread	144.6	
#242	М	Oct 2007	Oct 2009 to Jul 2010	Thread/radio	156.5	28
#216	Μ	Aug 2008	Nov 2009 to Oct 2010	Thread/radio	177.8	34
#159	Μ	Oct 2008	Oct 2009 to Oct 2010	Thread/radio	164.3	37
#88	F	Oct 2008		Thread	155.5	
#229	F	Nov 2008		Thread	135.6	
#30	F	Nov 2008	Oct 2009 to Dec 2010	Thread/radio	142.5	33
#150	Μ	Dec 2008	Nov 2009 to Oct 2010	Thread/radio	176.6	31
#158	F	Jan 2009	Oct 2009 to Dec 2010	Thread/radio	135.6	40
#206	Μ	Feb 2009	Nov 2009 to Mar 2010	Thread/radio	169.4	22
#162	F	Feb 2009		Thread	146.0	
#246	F	Mar 2009		Thread	135.0	
#153	Μ	Mar 2010		Thread	176.5	
#165	F	Jul 2010		Thread	146.6	
#167	F	Aug 2010		Thread	146.1	
#168	F	Oct 2010		Thread	145.0	
#298	М	Oct 2010		Thread	152.0	
#234	F	Dec 2010		Thread	140.2	
#75	Μ	Dec 2010	Oct 2009 to Nov 2010	Thread/radio	181.3	37
#222	Μ	Dec 2010		Thread	116.1	
#219	Μ	Apr 2011		Thread	203.0	
#238	Μ	Apr 2011		Thread	143.0	
#41	F	_	Oct 2009 to Dec 2010	Radio	153.0	39
#74	F	_	Nov 2009 to Oct 2010	Radio	155.5	37
#121	F	_	Oct 2009 to Jan 2010	Radio	145.4	12
#157	F	_	Sep 2009 to Jan 2010	Radio	144.6	25
#221	F	_	Nov 2009 to Jul 2010	Radio	140.0	30
#262	F	_	Nov 2009 to May 2010	Radio	132.7	23
#232	М	_	Nov 2009 to Jun 2010	Radio	124.3	17
#283	М	-	Nov 2009 to Apr 2010	Radio	173.0	23

(TEDM). The line abandoned on land was measured separately (e.g. terrestrial movements). Using a tape measure we obtained the straight-line distance between initial and final sites daily (SLD). The sum of straight-line distances between sequential fixes gave us the total distance moved (TDM). The distance from last (3rd day) to first location (1st day) was also obtained using a tape measure (DFIS: distance from final to initial site) (adapted from Tozetti, Vettorazzo and Martins, 2009). The animal's tendency to move around the initial site (e.g. zigzag or circular movements) was obtained by TDM/DFIS and called site fidelity (SF). Higher SF values reflect a tendency to remain near the initial site (adapted from Tozetti, Vettorazzo and Martins, 2009). In the 2007, 2010 and 2011 campaigns, the daily straight-line distance (SLD) was not measured, thus neither TDM nor SF were obtained for those periods. We also counted the number of burrows used along the trajectories.

# Radiographs and selection of turtles equipped with radio-transmitters

Between September and December 2009, female turtles were X-rayed with a portable 80 KV FNX X-ray machine to

verify the presence of shelled eggs using recommended dose rates (Famelli et al., 2014). These animals were kept in captivity and transported to a medical clinic for the radiograph procedure. Ten gravid females were selected and equipped with radio-transmitters. They were released at their initial site of capture. An additional three gravid females were fitted with thread-bobbins. Owing to difficulties in following their trail (even only for one day), data from the gravid females tracked with thread-bobbins were not included in the movement analyses, but relevant comments on their behaviour are included.

Another 10 males also received the radio-transmitters. Four males and four females equipped with radio-transmitters were also monitored with thread-bobbins method in previous years (table 1). Very High Frequency (VHF) transmitters TELENAX (Wildlife Telemetry) were affixed to the carapace of turtles (10 males and 10 females) using epoxy resin. The final weight of the transmitter was less than 7% of the individual's body mass.

#### Radio-tracking

Turtles were followed using a handheld radio receiver and Yagi antenna TELENAX (Wildlife Telemetry). When the

Acronym	Variable	Description	Way of measurement	Observations
Thread-bobb	ins			
EDM	Effectively distance moved per day	Length of dispensed line among fixes	Obtained daily	
TEDM	Total effective distance moved	Sum of EDM in 3-days of monitoring		
SLD	Straight-line distance between initial and final sites	Distance between fixes	Obtained daily with a tape measure	Not measured in 2007, 2010 and 2011 campaigns
TDM	Total distance moved (straight-line)	Sum of SLD	Obtained with a tape measure	Not measured in 2007, 2010 and 2011 campaigns
DFIS	Distance from final to initial site	Distance from last (3rd day) to first location (1st day)		
SF	Site fidelity	Ratio between TDM and DFIS TDM/DFIS		Not available for 2007, 2010 and 2011 campaigns
Radio-trackir	ıg			
TDMr	Total distance moved – radio-tracking	Sum of all the movements performed		
DFISr	Straight-line distance from first to last location – radio-tracking	Distance from last to first location (DFISr)	Measured on GPS TrackMaker	
SFr	Site fidelity radio-tracking	TDMr/DFISr	Ratio between TDMr and DFISr	SF was also measure per season (wet and dry)

Table 2. List of variables used as movement measurements and the methods used to obtain each one of them.

signal was strong, another antenna (directional antenna) was used to locate the animals. We were able to locate the tracked animals visually, eliminating triangulation errors. When animals were underground we used a digital photographic camera to locate them inside the burrows. After the initial location, subsequent fixes were based on visual contact only. We ensured turtles were not disturbed when approached for visual confirmation. Between September and November 2009, radio-tracking fixes were obtained once per day over a three-day period each fortnight, then once per day over a three-day period each month from October 2009 to October 2010. In November 2009, two transmitters detached from the turtle's carapace (one was reinstalled, the other one was lost) and in April 2010 another three transmitters were lost when the turtles shed their scutes. Only animals with more than 10 fixes were included in the analyses (eight males and eight females).

Locations were recorded using a GPS Garmin 60 CSX and later plotted using GPS TrackMaker to calculate daily distances movements (e.g. distances between the location of the first and second day and between the second and third day). Distances between monthly fixes (DMR) were collected on the first day of each fieldtrip. The sum of the distances between sequential fixes monitored with radio-tracking gave us the total distance moved (TDMr). The distance from last to first location (DFISr) was measured on GPS TrackMaker (adapted from Tozetti, Vettorazzo and Martins, 2009). The ratio between TDMr and DFISr (TDMr/DFISr) was used to obtain site fidelity for radio-tracking data (SFr). Higher SF values reflect a tendency of the animals to remain near the initial site (adapted from Tozetti, Vettorazzo and Martins, 2009). We measured the distance between the final to the initial location per season (wet and dry) and calculated site fidelity for each period. A summary of the variables used can be found in table 2.

#### Data analysis

Data were subjected to Analysis of Variance with repeated measures and was used to compare EDM as the response variable, across days for each day of thread-bobbins sampling (i.e.: 1st day  $\times$  2nd day  $\times$  3rd day). The same analysis was used to compare SLD for the three days of monitoring (except 2007, 2010 and 2011, insufficient data). Post-hoc Tukey-Kramer tests were performed where ANOVA showed significant differences, to determine which classes differed from each other. Prior to analysis, normality and homogeneity tests of variance were performed (Sokal and Rohlf, 1969).

SLD and EDM were compared by paired *t*-test. The same test was used to compare DFIS and TEDM. Normality and homogeneity tests of variance were performed prior to analysis. To compare TEDM and TDM we used the Wilcoxon matched pairs test (only data collected in 2008 and 2009). Mann-Whitney U tests were used to evaluate significant differences between sexes and seasons for radio-tracking and thread-bobbin data.

Differences in radio-tracking daily movements (DDM) and DMR were tested intragroup (males vs males and females vs females) and extra-group (females vs males) using ANOVA (with Welch's correction for the combination of unequal samples sizes and unequal variances), followed by Tukey-Kramer pairwise comparisons when significant differences were found. Differences between seasons (wet vs dry) were tested on a daily and monthly basis (DMR) using Mann-Whitney U tests (only for radio-tracking data). Linear regression was performed to examine correlation between daily movements and body size (CL). We used Wilcoxon test to compare TDMr, DFISr and SFr between sex and seasons for radio-tracking data. Differences were considered significant when p < 0.05. Statistical analyses were performed with PAST (Hammer, Harper and Ryan, 2001) and R (R Development Core Team, 2014). Means are presented with their standard errors unless otherwise specified.

#### Results

#### Thread-bobbins

Turtles showed intensive use of refuges along the trajectories (1 to 4 times), with strong fidelity to the river course. After release, animals tended to climb the riverbanks and move for a few metres on land (7.10  $\pm$  3.17 m, 0-34 m, except for gravid females), returning to the water and burying themselves (75% of the animals monitored) in the riverbank burrows. These burrows were used by the animals as tunnels and/or shortcuts along the stream. Sixty-four percent of the monitored animals (14 animals) showed terrestrial movements, mostly on the first day after being released. Nine animals (41%) showed movements only on the first day. Only two animals showed terrestrial movements on the second day (a male, 5.3 m; and a female, 7.9 m). Movements increased on the third day; only 27% of the monitored animals did not move.

Significant differences were observed when SLD was compared among the three days of monitoring ( $F_{(2,27)} = 4.214$ , p < 0.05); on the second day the distance moved was lower (2nd day:  $9.77 \pm 4.27$  m) than that observed on the other two days (1st day:  $57.68 \pm 14.47$  m; 3rd day:  $37.01 \pm 13.53$  m) (fig. 2A). The differences were also significant when EDM was compared among the three days of monitoring ( $F_{(2,27)} = 1.67$ , p < 0.05) (fig. 2B), though no significant difference was observed with the inclusion of data collected in 2010 and 2011 ( $F_{2.54} = 1.72$ , p = 0.21) (fig. 2C) (table 3).

For males, the mean SLD was 47.47  $\pm$  19.56 m and for females it was 8.0  $\pm$  2.44 m, a difference that was significant (U = 67.5, z = -1.75, p < 0.05) (table 3). Males EDM (57.18  $\pm$  14.19 m) was significantly lower than that for females (19.92  $\pm$  5.93 m) (U = 60, z = -2.038, p < 0.05). This significance remained when data collected in 2007, 2010 and 2011 was included in the analysis (U = 241, z = -2.642, p < 0.05) (males: 43.21  $\pm$  7.68 m; females: 18.10  $\pm$  4.27 m) (table 3).

The TEDM varied from 5.6 to 225.8 m (90.88  $\pm$  14.22 m) and DFIS from 1.5 to 158.0 m (38.47  $\pm$  7.93 m). TEDM and DFIS were significantly different (t = 5.86, p < 0.05, n = 22), even when females (t = 4.021, p < 0.05, n = 11) or males (t = 5.611, p < 0.05, n = 11) were compared separately (fig. 3; table 3). The mean TDM was 71.38  $\pm$  95.47 m (8.69-298.42 m). TEDM and TDM were not significantly different (t = 0.79, p = 0.43, n = 10). However, there was significant difference in TDM between males (142.41  $\pm$  126.53 m) and females (24.0  $\pm$  8.5 m) (U = 6, z = -1.552, p < 0.05) (table 3).

Although DFIS did not differ significantly between males (49.96  $\pm$  14.40 m) and females (26.9  $\pm$  5.5 m) (U = 67.5, z = -1.75, p = 0.08, n = 22), males moved significantly greater distances than females in term of TEDM (U = 60, z = -2.03, p < 0.05, n = 22). Males had mean TEDM of 126.88  $\pm$  21.42 m compared to 54.8  $\pm$  11.4 m for females (excluding



Figure 2. Comparison among three days of monitoring of *Hydromedusa maximiliani* tracked with thread-bobbins. (A) Straight line distance; (B) effective distance moved (2008 and 2009 data); (C) effective distance moved (all animals).

**Table 3.** Movement of *Hydromedusa maximiliani* monitored with thread-bobbins at Parque Estadual Carlos Botelho, State of São Paulo, southeastern Brazil. SLD, straight line distance; EDM, effective distance moved; TEDM, effective distance moved in three days of monitoring; DFIS, distance from final to initial site; TDM, total distance moved; SF, site fidelity (see methods section for more details). For EDM, TDM and SF data collected only in 2008 and 2009 (see methods session): n = 10 (six females and four males) for that period. \* indicates when females and males movements differed significantly (p < 0.05).

	SLD (m/day) (2008 and 2009)	EDM (m/day) (2008 and 2009)	EDM (m/day) (+2007, 2010 and 2011)	TEDM (m)	DFIS (m)	TDM (m)	SF
Females	$8.0 \pm 2.44$	$19.92\pm5.93$	$18.10\pm4.27$	$54.8 \pm 11.4$	$26.9\pm5.5$	$24.0 \pm 8.5$	$1.2 \pm 0.66$
(n = 11)	(0-28.72)*	(0-76.42)*	(0-76.42)*	(5.6-118.2)*	(1.5-54.0)	(9.5-34.61)*	(0.5-2.4)
Males	$47.47 \pm 19.56$	$57.18 \pm 14.49$	$43.21\pm7.68$	$126.88 \pm 71.05$	$49.96 \pm 14.40$	$142.41 \pm 126.53$	$1.6\pm0.60$
(n = 11)	(0-201)	(0-136.45)	(0-136.45)	(28.5-225.8)	(6.5-158.02)	(8.69-298.42)	(1.1-2.5)
Both sexes	$23.79\pm8.54$	$34.82\pm7.46$	$29.99\pm34.40$	$90.88\pm60.70$	$38.47\pm37.19$	$71.38\pm95.47$	$1.3\pm0.64$
	(0-201)	(0-136.45)	(0-136.45)	(5.6-225.8)	(1.5-158.02)	(8.69-298.42)	(0.5-2.5)



Figure 3. Movement patterns of females (A) and males (B) of *Hydromedusa maximiliani* tracked by thread-bobbins (DFIS, distance from final to initial site, and TEDM, total effective distance moved).

gravid females) (fig. 3; table 3). Three gravid females monitored during the nesting period (November and December 2008) moved more extensively than the other animals monitored, including excursions of more than 70 m from the resident stream. Unfortunately, we were unable to follow the trail of the gravid females for more than 1 day, because the thread was broken or missed in the terrestrial habitat.

TEDM of males seemed to increase progressively from October 2008 until the end of the wet season (February) then decreased during the dry season (March to August 2010) (fig. 3B). The biggest distances recorded by males were observed in October 2008 and December 2010, corresponding to the mating season (fig. 3B). However, males and females had different peaks in their movements, with females showing a maximum in January 2009 and December 2010 (fig. 3A; table 3). There was no significant difference in site fidelity (SF ratio all turtles =  $1.3 \pm 0.64$ ; 0.5-2.5) between females ( $1.2 \pm 0.66$ ; 0.5-2.4) and males ( $1.6 \pm 0.60$ ; 1.1-2.5) (p = 0.22) (table 3).

## Radio-tracking

No terrestrial movement was observed for animals monitored with radio-tracking, except for the use of burrows along the riverbank. In November 2009, we found a male equipped with a radio-transmitter sharing a refuge with a female, as evidenced by a photograph taken from inside the burrow (fig. 4).

The mean daily movement for both sexes combined was  $30.23 \pm 6.68$  m/day (0-179 m/day) and mean DMR was  $111.01 \pm$ 15.06 m (0-757 m) (table 4). Females had mean daily movements estimated with radio-tracking of  $27.79 \pm 5.70$  m/day (0-109 m/day), with no significant added variance attributable to



Figure 4. Male *Hydromedusa maximiliani* tracked with a radio-transmitter sharing a refuge with a female at the Parque Estadual Carlos Botelho, state of São Paulo, southeastern Brazil. This figure is published in colour in the online version.

**Table 4.** Average movements of *Hydromedusa maximiliani* radio-tracked at Parque Estadual Carlos Botelho, state of São Paulo, southeastern Brazil. DDM, daily distance moved (DDM), distance moved from final to initial site (DFISr) and site fidelity (SFr) (see methods section for more details). Results were not significantly different (p < 0.05).

	DM (m/day)	DMR (m)	TDMr (m)	DFISr (m)	SFr
Females $(n = 8)$	$27.79 \pm 5.7$ (0-109)	$91.4 \pm 20.7$ (0-680)	$\begin{array}{c} 1078.52 \pm 558.81 \\ (5031824) \end{array}$	$50.62 \pm 35.30$ (8-123)	$31.19 \pm 21.37$ (4.89-62.87)
Males $(n = 8)$	$32.66 \pm 7.0$ (0-179)	$\begin{array}{c} 123.2 \pm 20.9 \\ (0\text{-}757) \end{array}$	$\begin{array}{c} 1512.81 \pm 701.26 \\ (593\text{-}2282) \end{array}$	99.37 ± 124.36 (10-382)	$52.88 \pm 75.09 \\ (4.89-228.2)$
Both sexes	$30.23 \pm 6.68$ (0-179)	$\begin{array}{c} 111.01 \pm 15.06 \\ (0-757) \end{array}$	$\begin{array}{c} 1295.66 \pm 652.31 \\ (503\text{-}2282) \end{array}$	$75.0 \pm 91.83$ (8-382)	$\begin{array}{c} 42.03 \pm 54.50 \\ (4.89\text{-}228.2) \end{array}$

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differences among individuals (Welch's  $F_{7,19} = 0.7958$ , p = 0.61). Significant differences were found when comparing DMR travelled (Welch's  $F_{7,13} = 1.551$ , p < 0.05), attributed to one female that showed larger movements than average between November and December 2009, using adjacent streams and temporary pools. Mean DMR for females was 91.4  $\pm$  20.7 m (0-680 m).

Mean daily movements for males were estimated using radio-tracking was  $32.66 \pm 7.04 \text{ m/day} (0-179 \text{ m})$ , with no added variance component attributable to significant differences among individuals (Welch's  $F_{7,21} = 0.8236$ , p = 0.57). Distances between monthly fixes (DMR) did not differ significantly among males monitored with radio-transmitters (Welch's  $F_{7,13} = 2.07$ , p = 0.13). Males mean DMR was  $123.2 \pm 20.9 \text{ m} (0-757 \text{ m})$ .

The difference in DMR of males and females was not significant (U = 17, z = -1.114, p = 0.27), nor was there a significant difference between the sexes in daily movements estimated with radio-tracking (t = 1.267, p = 0.25, n =16). However, there was a significant correlation between distance travelled and body size (CL) ( $r^2 = 0.57$ ,  $F_{1,14} = 6.805$ , p < 0.05) (fig. 5). Males and females subjected to radio-tracking differed significantly in carapace length (t =-3.627, p < 0.05) (table 1).

Monthly DMR was significantly different between wet and dry seasons (U = 15, z = 1.972, p < 0.05), as well as on daily basis (U = 58, z = 1.92, p < 0.05). However, there was no significant trend in movement of females with season, neither on a daily basis (U = 26, z = 1.623, p = 0.10) nor on a monthly basis (DMR) (U = 44, z = 0.09617, p = 0.92). Female movement peaked in November (one female moved 151 m), with no movements detected at all in June, when females were probably inside burrows or moved less than the error of measurement ( $\pm$  3.5 m). For males, a significant trend was observed only on DMR (U = 32, z = 2.804, p < 0.05), but this was not signifi-



**Figure 5.** Relationship between body size (carapace length) and dispersal (daily movements) of *Hydromedusa maximiliani* at the Parque Estadual Carlos Botelho, state of São Paulo, southeastern Brazil. Circles, females; triangles, males; the line represents a positive correlation between CL and DMR.

cant when measured on a daily basis (U = 26, z = 1.408, p = 0.10).

The mean TDMr was  $1295 \pm 652$  m (range: 503-2282 m). No significant difference was found on TDMr when comparing females (1078  $\pm$  558 m; 503-1824 m) and males (1512  $\pm$  701; 593-2282) (t = 1.762, p = 0.12, n = 16). The mean DFISr was 75  $\pm$  92 m and varied from 8 to 383 m. There was no difference between sexes (t = 1.082, p = 0.33, n = 16). Females showed mean DFISr of  $50 \pm 35$  m (8-123 m) and males 99  $\pm$  124 (10-382 m). The SF for males was 53  $\pm$  75 (4.89-228.2) and females  $31.2 \pm 21.4$  (4.9-62.9), with no significant difference between them (t = 0.9161, p = 0.89, n = 16).

The distance from final to initial site was significantly different between wet and dry seasons (t = 1.757, p < 0.05). The mean DFISr on dry season was  $70 \pm 59$  m (12-219 m) and on the wet season was  $143 \pm 196$  m (20-780 m). The tendency to move around the same place or site fidelity was higher on dry season, which varied from 3.6 to 50.3 (15.2  $\pm$  14.2) on the dry season and 1.4 to 32.4 (12.5  $\pm$  7.6) on wet season, however, there was no significant difference between seasons (t = 0.899, p = 0.38).

#### Discussion

#### **Biological findings**

The daily distances moved by Hydromedusa maximiliani (thread: 1.5 to 201 m/day; radiotracking 0 to 179 m/day) are comparable to those reported for another Brazilian chelid species with a similar body size, such as Acanthochelys spixii (5.3 to 237.8 m/day; Horta, 2008). Some Chelidae may have the ability to move greater distances, such as Chelodina expansa in Australia (with mean movements of 0.65 km for females and 1.29 km for males), which may be an advantage for species in dynamic river systems to access temporary ponds or streams (Bower, Hutchinson and Georges, 2012). The association of river turtles with temporary resources was observed in another Australian chelid, Chelodina longicollis, which travels 427 m (40-1470 m) overland between wetlands (Roe and Georges, 2007). Often, major activity and movements are associated with the wetter months and when the demands of reproduction are to be met (Souza, 2004), or during the transition from wet to dry season, as observed in Mesoclemmys dahli (Forero-Medina, Cárdenas-Arevalo and Castaño-Mora, 2011) and Platemys platycephala, with a combination of long time resting with far movements (Böhm, 2013). Though males and females show different peaks of activity during the year (nesting season for females and the presumed mating season for males), the overall distances moved were not different when the animals were engaged in activities that demand movements over long distances.

*Hydromedusa maximiliani* showed high degree of fidelity to the water and burrows along the river which, coupled with their relatively sedentary behavior (Souza et al., 2002; Souza and Martins, 2006), suggests that their needs for feeding and refuge are adequately provided within a small area of aquatic habitat (Souza and Abe, 2000; Bower, Hutchinson and Georges, 2012). Additional investigation is needed to explain their use of terrestrial habitats, especially since limitations of our dataset may have resulted in missing important movements associated with nesting and estivation, two critical stages of turtle life cycle (Ryan and Burgin, 2007). Hydromedusa maximiliani nesting season occurs in late spring and early summer (Famelli et al., 2014), which coincides with the increased distances moved by females. Terrestrial excursions conducted by gravid females of H. maximiliani in this period suggest they are searching for nest sites (Guix, Miranda and Nunes, 1992; Famelli et al., 2014). Our data suggest that nests are far from the water line, as observed in the critically endangered chelid Mesoclemmys dahlia (Forero-Medina, Cárdenas-Arevalo and Castaño-Mora, 2011), though we were unable to locate any nest. Nest site selection also requires further investigation, with gravid females being monitored with thread-bobbins for longer periods, to locate nesting areas and understand nest site selection (e.g., Kinosternon baurii in Florida; Wilson, 1998). Monitoring of hatchlings and juveniles may also bring essential insights to the movement patterns, since activity and resource partitioning are already stated among males, females and juveniles of H. maximiliani (Souza and Abe, 1998).

#### Radio-tracking and thread-bobbins comparison

As expected, the effective distances moved (EDM or TEDM) were significantly higher than comparable estimates from point-to-point data collected either by radio-tracking or threadbobbins (SDL or DFIS and DFISr). This indicates that thread-bobbins provide more information than radio-tracking, especially regarding distance travelled (Tozetti and Martins, 2007). As in our study, Souza et al. (2008) observed more intense movements of P. geoffroanus in the first hours after release and a progressive decrease after two days. Climbing the riverbank was a common behaviour of H. maximiliani released after capture and handling for biometrical procedures at Parque Estadual da Serra do Mar (PESM) (Famelli, 2005), suggesting that the terrestrial movements observed in animals tracked with thread-bobbins could be related to stress. According to Horta (2008), *Acanthochelys spixii* also seek for refuge after released, a behaviour also observed in *H. maximiliani* at PESM (Famelli, 2005). This suggests that a settling in period is required if the data gathered is to be a reflection of normal behaviour. In this sense, radio-tracking could be more effective, as it requires less handling of the animals.

The intense use of refuges coupled with the cryptic colouration of *H. maximiliani* make it difficult to detect these animals in the wild (Souza and Abe, 1997; Famelli et al., 2011). Radio-tracking is a feasible way to overcome this, as it is effective even when the turtles are sheltered in burrows (Tozetti and Martins, 2007). However, the simultaneous use of thread-bobbins and radio-tracking, as complementary methods, is strongly recommended when studying turtle movements.

Although tropical reptiles experience considerably higher temperatures during periods of low activity than overwintering ectotherms in temperate regions, estivation is a common behaviour in many tropical turtles, whereby they achieve substantial energy savings by reducing their metabolism and activity levels (Christian et al., 2007). In this study, we showed that turtles decrease their activity during late fall, as observed at the Parque Estadual da Serra do Mar, where H. maximiliani were recaptured after winter with mud around the shell and odour of organic matter in decomposition (Famelli et al., 2011). Using thread-bobbins and radiotransmitters it was possible to see that animals are sheltered in burrows during the period of low activity. However, intense use of burrows was observed all year, which may not be related to aestivation. Use of GPS data loggers should be considered to locate turtles' aestivation sites and to better document microhabitat use and habitat selection (Dubois et al., 2009), following trials to address if they function under the heavy forest canopy. GPS data loggers could also provide important information on the thermoconformity strategy of *H. maximiliani* (Souza and Martins, 2006).

#### Conclusions and conservation implications

This study represents the first detailed investigation of activity and movements of H. maximiliani, a turtle considered Vulnerable by IUCN (2010). Landscape structure and animalmovement behaviour, such as the high use of burrows and use of temporary pools, must be taken into consideration when determining future conservation strategies for H. maximiliani. Gravid females were observed using adjacent streams and temporary pools, indicating the importance of considering the influence of landscape connectivity as a vital component of suitable habitat for this species (Fordham et al., 2015). The assessment of the minimum requirements of suitable habitat for H. maximiliani reported here can be used as input to future population modelling and assist in decisionmaking and development of conservation strategies (Famelli et al., 2012).

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