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Where are the turtles when they are not? Underwater refuges used by *Hydromedusa tectifera* COPE, 1869 in a suburban stream

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Abstract

Habitat requirements of *Hydromedusa tectifera* from an urban impacted stream from the Buenos Aires province, the Rodriguez stream, are here studied. We describe the type of underwater refuges, how they are used in different stream sections, stream margins, year season, size class and sex of the individuals, and analyze patterns of recurrence and aggregative use of refuges. Fieldwork was carried out from March 2017 to January 2018. Turtles were caught manually recording the following variables: site, transect and margin, sex, straight carapace length, and weight. Substrate types are differently used according to stream section, stream margin, year season and size class of individuals, but no difference was found between sexes. In the whole stream, most used refuges were the hollows on the margins (55.05%), followed by marginal aquatic vegetation (19.27%). Turtles of all class sizes and both sexes refuge into hollows on the stream margins. Despite different microhabitats provide refuge to turtles during all year seasons, hollows are the most important refuge during extreme cold and extreme hot seasons. In addition, turtle recaptures evidence a recurrence and aggregative pattern in the use of refuges. This information provides insight into the key aspects of the microhabitat requirements of the species and will be certainly useful in mitigating and preventing negative effects of the current stream management policies. It is worth highlighting the need to keep the stream margins unaltered to favor the presence of turtles in urban environments.

Keywords Habitat use · *Hydromedusa tectifera* · Snake-necked turtle · Substrate type · Suburban stream · Underwater refuges

Introduction

Urban expansion has caused drastic changes in natural landscapes across the world. The undesirable effects of urbanization involve habitat fragmentation and degradation, pollution, introduction of exotic species and road mortality. The combination of all these factors leads to declining populations and local extinction of certain species. Mitchell et al. (2008) summarized most of the work in urban amphibians and reptiles, and recently, French et al. (2018) published a review of reptilian responses to specific urban features.

The impact of urbanization on several turtle species has been reported by many authors (e.g., Ner and Burke 2008; Plummer and Mills 2008; De Lathouder et al. 2009; Rees et al. 2009; Eskew et al. 2010a, b; Roe et al. 2011; Sterrett et al. 2011; Hill and Vodopich 2013; Stokeld et al. 2014; Elston et al. 2016; Ferronato et al. 2016, 2017; Santoro 2017; Bowne et al. 2018; Dupuis-Desormeaux et al. 2018; Semeñiuk et al. 2019; Vanek and Glowacki 2019). Many of them have demonstrated that turtle populations have a neutral, and even positive, response to urban impacts according the degree of habitat modification (Plummer and Mills 2008; Eskew et al. 2010a, b; Roe et al. 2011; Stokeld et al. 2014; Ferronato et al. 2017; Dupuis-Desormeaux et al. 2018; Semeñiuk et al. 2019). However, Eskew et al. (2010b) emphasize that there may be a lag period between the beginning of human disturbance and its effect on populations.

Knowledge of the spatial ecology and habitat requirements is essential to understand how urbanization impacts on the species, in order to effectively manage urban habitats (Ryan et al. 2014). However, for most turtles this is poorly known. Freshwater turtles are particularly vulnerable to habitat fragmentation, as they not only depend on the aquatic environment, but also need the terrestrial environment for nesting,

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basking, dispersal and hibernation (Marchand and Litvaitis 2004; Ryan et al. 2008; Wieten et al. 2012; Hill and Vodopich 2013; Ryan et al. 2014; Hamer et al. 2018).

The South American snake-necked turtle *Hydromedusa tectifera* is widely distributed through Uruguay, southeastern Brazil, eastern Paraguay, and northeastern and central Argentina (Sánchez et al. 2019). In a recent paper, Semeñiuk et al. (2019), demonstrated that key population parameters of *H. tectifera* are not impacted by the moderate suburban settlement (La Plata city, eastern Argentina). In this work some aspects of habitat use in that population of the Rodriguez stream are further studied by (1) describing the type of underwater refuges use by turtles, (2) analyzing how these refuges are used in different stream sections, stream margins, year season, size class and sex of the individuals, and (3) the evaluation of recurrence patterns and aggregative use of refuges. Our interest was not to test any particular hypothesis, but to generate baseline information that could be useful to prevent the effects of urbanization on the species and, if necessary, to mitigate impacts by creating adequate habitats in streams already disturbed.

Materials and methods

Study area

Field work was made in a tributary of the Río de la Plata River, the Rodriguez stream, in the suburbs of La Plata city (Buenos Aires province, Argentina). We chose three stream sections with different degree of urbanization along the margins, in 10 ha that enclose each stream section: (S1) upstream, rural zone without housing on the margins; (S2) midsection, sparsely populated (urbanization density intermediate between S1 and S3: 3 houses/ha); and (S3) midsection downstream from S2, higher urbanization density with 5 houses/ha. Sites 1 and 2 are separated by 4.5 km of stream course (not in a straight line) and Sites 2 and 3 by 4.2 km. These sections are travesed by different street types and traffic intensities in the 10 ha that surround each site (Table 1). In sum, S1 lacks houses and is traversed by a single narrow foot path (not enabled for vehicles); S2 is moderately housed and traversed by a single paved street (very busy) and a net of low-traffic unpaved streets; and S3 highly housed and crossed by paved streets. Motor vehicle traffic also increases from S1 to S3, measured during 5 min in periods of intense traffic between 800 and 900 h (Table 1 quantifies these urban characteristics).

Table 1 Characteristics of the urbanization in the 10 ha that surround each stream section

Site	Degree of urbanization (houses/ha)	Paved street (m)	Unpaved street (m)	Motor vehicles/min
S1	0	0	0	0
S2	3	320	450	13
S3	5	700	0	22

Stream width is highest in S3 (range: 7.9–9.9 m, mean = 8.91 ± 0.55 m), followed by S2 (range: 4.2–7.3 m, mean = 6.27 ± 0.93 m) and S1 (range: 2.8–6 m, mean = 3.97 ± 0.92 m). Mean water depth is highest in S2 where it ranges from 18 to 43.67 cm (mean = 28.76 ± 6.74 cm), while in S3 it ranges from 6.67 to 23.67 cm (mean = 14.78 ± 5.15 cm) and in S1 it ranges from 8 to 22.17 cm (mean = 12.47 ± 4.19 cm, Table 2).

Sampling methods

Between March 2017 and January 2018 the stream was visited three times in autumn, one in winter, two in spring, and one in summer, totalizing seven work days (between 10:00 h and 16:00 h). Turtles were actively searched (visual and tactile encounters, hand collection) on both stream margins following five 30-m long transects by stream section, with 15 m separation between consecutive transects. Both stream margins of each transect were inspected simultaneously. The substrate types (Fig. 1) used by turtles as underwater refuges were grouped into four categories in order to facilitate analysis: (1) marginal aquatic vegetation; (2) hollows on the stream margins; (3) nude stream bottom, and (4) other substrate types such as garbage accumulations, logs and floating dams composed by rests of aquatic and terrestrial vegetation. The area (m^2) occupied by each substrate type in each of the three stream sections was estimated only once during winter (Table 3).

Each caught turtle was in-situ recorded for the following variables: (1) site (1–3), number of transect (1–5), and margin of stream (North-South); (2) capture location (position on transect); (3) substrate type (see above); (4) sex; (5) weight; and (6) straight carapace length (SCL: straight distance between anterior margin of nuchal scute and posterior margin of supracaudals). Turtles were sexed according to sexually dimorphic characteristics (Cabrera 1998) and, in the case of smaller specimens with no clear plastron concavity, by the presence of penis within the cloaca following Rodrigues et al. (2014). Finally, turtles were individually marked according to Cagle (1939) and released at the location of capture.

Data analysis

Multiple Correspondence Analysis was applied to elucidate the relationship among the number of turtles with the variables stream margin, stream section, substrate type, year season, size class and sex. In order to compare with previous works on the species we used five size classes (SC) based on SCL (see Semeñiuk et al. 2019).

Table 2 Detail of the stream width and water depth (means) of each transect along the three sections

	S1		S2		S3	
Transect	Mean Width	Mean Depth	Mean Width	Mean Depth	Mean Width	Mean Depth
1	3.57	10.33	7	34.39	9.3	19.36
2	5.2	14.71	6.47	27.67	9.03	16.97
3	4.27	15.36	5.77	25.2	8.57	12.97
4	3.13	9.78	5.23	27.11	9.3	11.91
5	3.7	12	6.87	29.45	8.33	12.71
Mean	3.97	12.44	6.27	28.76	8.91	14.78
Sd	0.80	2.52	0.75	3.49	0.44	3.22

A factorial analysis of variance (ANOVA) (Zar 1999) was run to evaluate effects of stream sections, stream margins and substrate types (factors) in the number of turtles caught (dependent variable). Transects were the sampling units, and data were fourth-root transformed to approach the assumptions of the test.

The aggregative or not aggregative use of refuges was evaluated taking into account the locations of turtle capture within each transect and margin, and measuring the distance (m) between adjacent individuals. Recaptures were used to assess patterns of recurrence in the use of refuges.

All analyses were performed with the software Statistica 7.0 under a significant p value of 0.05. Recaptured turtles were not included in the analyses (except to evaluate the recurrence of refuge use) to avoid data pseudoreplication.

Results

We caught 109 individuals of *H. tectifera* (mean ± SD = 15.71 ± 3.68 individuals/survey; $n = 7$): 56 males, 46 females and 7 hatchling turtles for which sex could not be determined.

Fig. 1 Substrate types used by turtles as underwater refuges: (1) marginal aquatic vegetation; (2) hollows on the stream margins; (3) nude stream bottom, and (4) other substrate types (garbage accumulations, logs and dams composed by rests of aquatic and terrestrial vegetation that floats freely)

Table 3 Area occupied (m^2) by each substrate type in the three studied stream sections of Rodriguez stream

Site	Margin	Marginal aquatic vegetation	Hollows on margins	Nude stream bottom	Other substrate types
S3	S	33.68	0.21	150	1.50
	N	9.49	1.94	150	1.70
S2	S	0	11.54	150	0.09
	N	0.24	0.31	150	1.46
S1	S	2.49	3.86	150	0
	N	0.88	8.54	150	0

Most turtles were caught in hollows in the stream banks (55.05%), being the remaining ones under marginal aquatic vegetation (19.27%), resting still on the nude stream bottom (17.43%), and under other substrate types such as garbage accumulations, logs and floating dams of aquatic and terrestrial vegetation; (8.26%).

Table 3 summarizes the area occupied by each substrate type on both margins of the three stream sections. In the least urbanized sites (S1 and S2) hollows prevailed over marginal aquatic vegetation and other substrate types. This fact explains the high use of hollows by turtles in these sections (see below), while site 3 has higher availability of marginal aquatic vegetation. Coincidentally, turtles of this site used this type of refuge more frequently than the other types (see below; Table 4).

The Multiple Correspondence Analyses (MCA) revealed the relationships among the studied variables and the different habitats used by *H. tectifera* in the Rodriguez stream (Fig. 2). In general, according to the MCA, turtles from the most urbanized site (S3) were predominantly found on the north margin (71.74%) and using the four types of substrate. The most used refuge by both sexes on site 3 was the marginal aquatic vegetation (males: 45.83%, females: 41.18%), whilst at the other sites the analysis reflected a high use of hollows at S1 (males: 83.33%, females: 85.71%), especially on the north margin, and S2 (males: 73.68%, females: 75.00%), mainly on the south margin (Table 4, Fig. 2a,b). Percentage of turtles caught on the nude stream bottom was highest at S2 followed by S3 (Table 4). On the other hand, individuals on other substrate types (garbage accumulations, logs and floating rests of aquatic and terrestrial vegetation) were found only in S3, being similar the percentage of males and females (20.83% and 23.53%, respectively) (Table 4, Fig. 2b).

Thus, according this analysis, the refuges used by turtles varied among sites but not between sexes. As well, turtles used several patterns of refuge according to size class and year season. Although all size classes used hollows, the small (SC I) and medium-sized (SC III) turtles were mostly found on nude stream bottom, whereas large turtles (SC IV, 200–250 mm) predominated under marginal aquatic vegetation (Fig. 2c). Throughout the year, marginal hollows were the most used substrate (Table 5), especially in autumn and spring; in winter, in addition to hollows, most turtles were associated with marginal aquatic vegetation; and in summer, with nude stream bottom (Fig. 2d, Table 5). Although turtles were caught in the three sites across all year seasons (Table 6), during autumn turtles predominated at S2, while in winter and summer they appeared mainly in S3, and in spring, they were mostly found in S1 (Fig. 2e).

In sum, the MCA analysis showed that turtles display a different use of refuges according to size class, stream section, stream margin, and year season, but not according to sex (see Discussion).

The ANOVA results were significant for the number of turtles found under different substrate types among stream margins and sections (interaction: $F_{4,612} = 7.48$, $p < 0.0001$), and confirmed the relationship reflected by the MCA (Fig. 2a).

Each of the 14 turtles recaptured during this work was found in the same stream section, and many of them were even in the same stream margin and transect (ten cases) indicating a high site fidelity during the one-year period of the study. Regarding the substrate types, turtles from the most urbanized site (S3) were mainly recaptured in a different

Table 4 Percentage (%) of turtles caught associated to each type of refuge in the three sections of the Rodriguez stream

Site	Sex	Hollows on margins	Nude stream bottom	Marginal aquatic vegetation	Other substrate types
S3	F	17.65	17.65	41.18	23.53
	M	25.00	8.33	45.83	20.83
S2	F	75.00	18.75	6.25	0.00
	M	73.68	26.32	0.00	0.00
S1	F	85.71	7.14	7.14	0.00
	M	83.33	8.33	8.33	0.00

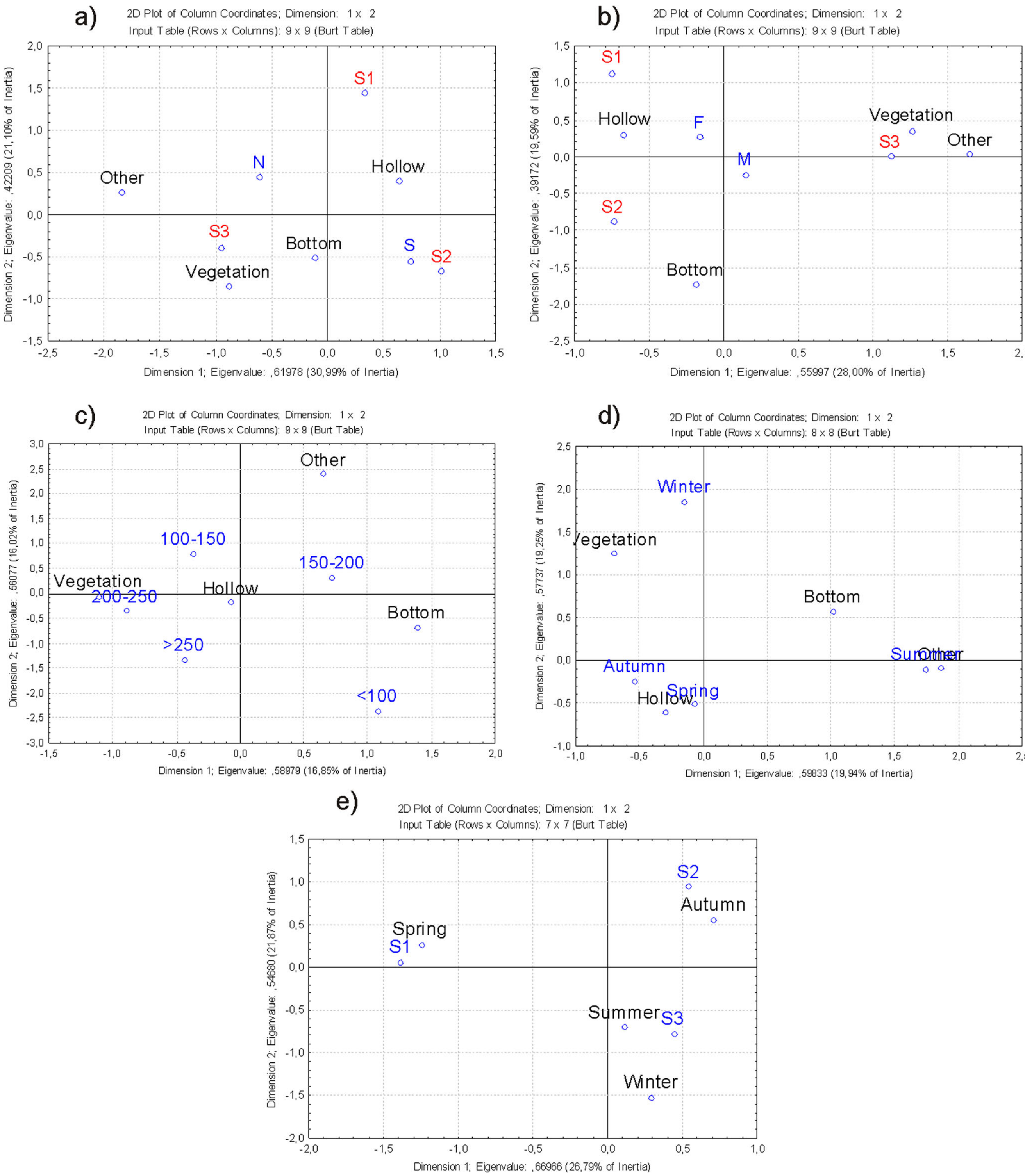

Fig. 2 Multiple correspondence analysis (MCA) plots for (a) substrate type in relation to stream section (Sites 1–3: S1, S2, S3) and stream margin (N: north, S: south); (b) sex of turtles (male: M, female: F) in relation to substrate type and stream section; (c) class size in relation to substrate type; (d) substrate type in relation to year season, and (e) year season in relation to stream section

substrate type respect to the first capture. Instead, S2 and S1 turtles that were first captured in hollows were always recaptured in hollows on the same margin but never in the same hollow than the first capture.

In relation to the aggregative patterns of *H. tectifera*, we obtained a mean distance between consecutive turtles of: 3.50 ± 3.46 m (S1), 5.35 ± 7.23 m (S2), and 4.79 ± 4.70 m (S3). We observed an aggregative use of hollows in 11 cases (3 in S3, 7

Table 5 Percentage (%) of turtles captured in each type of refuge during the year seasons

Year season	Marginal aquatic vegetation	Nude stream bottom	Hollows on margins	Other substrate types
Autumn	23.26	11.63	65.12	0.00
Winter	35.71	21.43	42.86	0.00
Spring	16.13	16.13	61.29	6.45
Summer	14.29	28.57	57.14	0.00

in S2, 1 in S1), being in some cases up to four turtles together in the same hollow (e.g., in S2).

Discussion

This is the first study of the habitat requirements of *Hydromedusa tectifera* in an urban-impacted stream of the suburbs of La Plata city. This study complements a previous paper on abundance and sex-size class structure of the same population (Semeñiuk et al. 2019). Turtles use different types of underwater refuges depending on stream section, stream margin, year season, and size class of individuals, but show no difference between sexes.

Most of the studies about habitat use of freshwater turtles are focused on species from the Northern Hemisphere (Hartwig and Kiviat 2007; Haramura et al. 2011; Millar and Blouin-Demers 2011; Forero-Medina et al. 2012; Refsnider and Linck 2012; Wieten et al. 2012; Hill and Vodopich 2013; Markle and Chow-Fraser 2014; Brown 2016; Pittfield and Burger 2017; Dupuis-Desormeaux et al. 2018). But, only a few researchers have focused on how urbanization impacts the habitat of turtles (Ryan et al. 2008; De Lathouder et al. 2009; Refsnider and Linck 2012; Hill and Vodopich 2013; Ryan et al. 2014; Brown 2016; Elston et al. 2016; Ferronato et al. 2016; Pittfield and Burger 2017).

Ryan et al. (2008) reported that human activities influence freshwater turtle habitat selection. Complex habitat types provide shelter and camouflage from possible disturbances, and increase food resources; some turtles may benefit from nutrients by taking advantage of the high productivity in these areas (Wieten et al. 2012).

In this study, we demonstrate that availability of turtle refuges differs among the three studied sections of the Rodriguez stream and that their use varies in relation to availability. The most used refuges considering the three sections as a whole were the hollows on the margins (55.05%) and marginal aquatic vegetation (19.27%), both explaining the 74.32% of the refuges occupied by turtles. Thus, it is esential to keep the stream margins unaltered as a conservation policy in order to favor the presence of turtles in urban environments. Margins of the studied stream, mainly in the most urbanized site (S3), are periodically altered by channelization works (held every 4–5 years) and vegetation clearing (monthly or bimonthly) tending to improve water drainage without considering the preservation of the fauna that inhabits the stream. Channelization works are carried out with heavy backhoe shovels that enter the stream causing a high impact not only by alteration of the habitat characteristics but also by direct damage to many species, as we personally verified how some turtles were crushed during works (see reports of similar damage in Plummer and Mills 2008).

Table 6 Percentage (%) of turtles captured in the three sections of the Rodriguez stream during all year seasons

Year season	S3	S2	S1
Autumn	41.86	44.19	13.95
Winter	50.00	28.57	21.43
Spring	29.03	22.58	48.39
Summer	35.71	35.71	28.57

Systematic weeding works made on marginal aquatic vegetation do not appear to be as invasive as channelization but introduce a periodic stressing factor that also reduces microhabitat availability. Turtles choose certain streams over others based on vegetation cover, since riparian vegetation modulates water temperature, improves water quality, provides refuge, and increases food availability (Cortelezzi et al., 2013; Forero-Medina et al., 2012), being the last two factors important habitat requirements for turtle populations (Ercolano, 2008).

In soft bottom streams, turtles are capable to bury themselves in the mud to avoid predators, in addition to the use of hollows, as reported by Famelli et al. (2016) for *H. maximiliani*; but the bottom of the Rodriguez stream is predominantly rough and with scarce soft areas where turtles could dig into. In fact, only a few turtles from the Rodriguez stream (17.43%) seem to choose the nude stream bottom to stay still and camouflage instead of taking refuge within hollows or under floating or rooted vegetation. The strategy of staying still and camouflaging on the nude bottom but without digging into was reported for the population of *H. tectifera* from the mountain streams of Córdoba province. The bottom of these streams is rich in fixed and free stones among which turtles can better camouflage themselves (Molina and Leynaud 2017). The frequency of use of nude bottom by the studied population of *H. tectifera* was low in the stream section characterized by low water depth (S1 with 7.14%) and increased in the deeper sections (S2 and S3 with 22.86% and

19.57% respectively). The greater depth in S2 and S3 sections is in part explained by water discharges constantly produced by the surrounding urbanization. The deepest section (S2) is located downstream of a closed neighborhood and occasionally receives water discharges, and S3 receives lots of pluvial drainages from the surrounding houses and streets. It seems evident that higher urbanization levels cause higher water levels in the middle and lower sections of the Rodriguez stream, providing additional refuge for the turtles during seasonal fluctuations and influencing the dynamics of microhabitat use. Thus, destruction of microhabitats on the stream margins (vegetation and hollows) should surely have a greater impact in the S1 section, since low water depth tends to avoid the use of nude bottom as refuge.

In addition, our data show that turtles of all class sizes and of both sexes refuge into hollows on the stream margins. Although different microhabitats provide refuge for turtles during all year seasons, hollows are the most important refuge during extreme cold and extreme hot seasons. Similarly, Famelli et al. (2016) reported that the sister species *Hydromedusa maximiliani* displays intense use of burrows throughout the year.

Turtle recaptures evidence a recurrence and aggregative pattern in the use of refuges. This recurrence would be related, at least in 1-year period, to a high spatial fidelity of individuals of the studied population, since all recaptures have occurred in the same stream section as the first capture and most of them even in the same transect. Substrate availability modulates the recurrence in the use of refuges; our results also revealed that turtles from stream sites with high availability of refuge types (S3) were recaptured on a different substrate type than the first capture. Conversely, at S1 and S2 sites (lower availability of refuge types) individuals were always recaptured in hollows. A similar spatial fidelity was reported for *H. maximiliani*, a species that uses a small area of aquatic habitat to feed and refuge (Famelli et al. 2016).

The aggregation of two or more turtles into the same hollow is another characteristic of the microhabitat use of *H. tectifera*. This phenomenon was observed along the entire stream but was marked at site S2 which can be explained by the large number of hollows at this site.

Many authors have foccused attention on the worldwide decrease of several populations of freshwater turtles (Browne and Hecnar 2007; Enneson and Litzgus 2009; Lovich et al. 2018; Howell et al. 2019; Van Dyke et al. 2019). The factors causing such a decline seem to be habitat destruction and fragmentation as the most important for several species, but harvesting and pollution have also impact on others (Lovich et al., 2018; Van Dyke et al. 2019). With respect to habitat loss, important areas of the geographic distribution of certain species coincide almost totally with human settlements as is the case of *Hydromedusa tectifera*. Thus, urban development and the different ways of habitat modification that cities imply are one of the most crucial factors for the survival of the species in certain areas. Although it is currently recognized as not threatened for Argentina (Prado et al. 2012) and Uruguay (Carreira et al. 2007), as well as "Least Concern" for Brazil (Vogt et al. 2015) and also internationally (Rhodin et al. 2017, 2018), the southernmost core populations of *H. tectifera* coincide almost totally with the second largest human settlement in South America: the Area Metropolitana y Conurbano Bonaerense (AMBA, Argentina), where more than 10 million people live (INDEC 2016; Sánchez et al. 2019). In this context, the generation of knowledge and implementation of basin management policies for the streams of the area are crucial to avoid a severe decline and even local extinction of *H. tectifera*.

Finally, some general conclusions may be drawn: (1) *Hydromedusa tectifera* uses a diversity of underwater refuges; (2) the use of the different refuges varies according to the refuge availability in relation to the characteristics of the stream and to the urban impacts along the stream sections; (3) hollows on the stream margins are a key refuge for *H. tectifera* since they are the most used refuge by turtles of both sexes and all class sizes throughout the stream during all year seasons; (4) patterns of recurrence depend on the availability of substrate type; (5) aggregative use of refuges (hollows) occurs in response to a lower offer of this type of refuge. This information provides insight into key aspects of the microhabitat requirements of *Hydromedusa tectifera* that should be useful in mitigating and preventing the negative effects of the current stream management policies carried out by municipal authorities with no regard for effects on biodiversity of streams, particularly turtles.

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