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SHORT COMMUNICATION

The trophic niche of *Mesoclemmys vanderhaegei* (Testudines: Chelidae): evidence from stable isotopes

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ABSTRACT. Ecological niche is the multidimensional space comprising the resources used by an organism. Intraspecific variation in resource exploitation is common in reptile populations to maximize coexistence of individuals. The use of stable isotope analysis is an effective tool when there are variations in resource exploitation, since it can provide quantitative information about food consumption and habitat use. *Mesoclemmys vanderhaegei* (Bour, 1973) is a medium-sized turtle with a limited distribution in south central Brazil and Paraguay. In spite of that, little is known about its ecology. In this study we used stable isotope analysis to understand the intraspecific trophic niche variation in *M. vanderhaegei* at Serra das Araras Ecological Station, state of Mato Grosso, Brazil. The isotopic ratios of $\delta^{15}N$ and $\delta^{13}C$ were determined in claw samples collected from 14 males and 14 females. Isotopic niche width values were not statistically different between the sexes, there was a high degree of overlap between sexual niches and there were no relationships between isotopic compositions and body size. These results suggest that individuals of both sexes and throughout their ontogenetic development exploit food resources with the same isotopic baseline.

KEY WORDS. Anthropic environment, freshwater turtle, isotopic niche, sexual niche

INTRODUCTION

Ecological niche is defined as the multidimensional space comprising the resources used by an organism (Hutchinson 1957). Resource partitioning within species is a strategy used by reptiles to maximize coexistence among individuals (e.g. Marques et al. 2013a, Richards-Dimitrie et al. 2013). Species exhibiting sexual size dimorphism may have sexual differences in nutritional requirements, which can result in the use of different resources (Wearmouth and Sims 2008). In addition, the increase in body size throughout the ontogenetic development is also an important factor that influences the exploitation of food resources (Werner and Gilliam 1984).

Stable isotope methodology has been used to identify overlaps or partitioning of resources within species, since it provides quantitative information about food resource consumption and habitat use (Newsome et al. 2007, Layman et al. 2012). This methodology has advantages over stomach or feces content analysis: it is less traumatic to the animals, it provides a time-integrated measure of diet, and it is not biased toward hard or less digestible food items (Bulté et al. 2008). These characteristics have made it possible to apply isotopic niche approaches toward a better understanding of the interactions among organisms (e.g., Jackson et al. 2011). Isotopic niche is defined as the hypervolume of the isotopic space (n-dimensional), where the axes are the isotopic compositions that represent the bionomic and scenopoetic components of the niche (Newsome et al. 2007). This approach has already been successfully used both to understand the variations in resource exploitation by *Chelodina longicollis* in a natural-urban gradient in southeastern Australia (Ferronato et al. 2016), and to identify the impact of the invasive species *Trachemys scripta elegans* (Wied-Neuwied, 1839) on *Emys orbicularis* (Linnaeus, 1758) in Italy (Balzani et al. 2016).

Mesoclemmys vanderhaegei (Bour, 1973) is a medium-sized chelid turtle with limited geographic distribution in South America: Amazonas, Tocantins, Paraguay, Parana, and Uruguay river basins (Marques et al. 2014). The species has been documented utilizing several different habitat types, including lagoons, small streams and urban/anthropogenic watercourses (Brandão et al. 2002, Brito et al. 2012, Marques et al. 2013b); however, little is known about its biology and ecology (Brito et al. 2009, Marques et al. 2014).

Here we applied stable isotope methodology to ascertain the intraspecific trophic niche variation in *M. vanderhaegei* at Serra das Araras Ecological Station, state of Mato Grosso, Brazil. First, we compared the isotopic niche width and the degree of overlap between males and females. Additionally, we tested the relationship between isotopic compositions ($\delta^{15}N$ and $\delta^{13}C$) and body size.

The data for this study was collected on a reservoir at Serra das Araras Ecological Station (SAES; 15°49’31”S, 57°17’14”W, altitude: 800 m), a protected area in the state of Mato Grosso, Brazil. SAES is located between the cities of Porto Estrela and Cáceres and is dominated by savanna (Total area: 28,700 ha; Fig. 1). The small stream (first order) that originates the dam is oligotrophic, with sandy bottom, aquatic vegetation, periphyton and grass, which are partially submerged in the rainy season.

Mesoclemmys vanderhaegei individuals were captured with funnel traps (1.2 m long × 0.60 m external diameter × 0.30 m entrance diameter; plastic mesh 5.0 × 1.0 mm), between November 2010 and November 2011. The animals were attracted into the funnel traps by a mixture of bovine meat and commercial fish-flavored pellets. Trapped individuals were marked by notching the marginal scutes of their the carapaces (Cagle 1939) and were sexed based on the external examination of secondary sexual characteristics (Brito et al. 2009). All individuals had their straight line carapace length (Stainless Hardenedde caliper, millimeters) and body mass (Pesola, grams) taken. Claw samples (terminal 5 mm) were collected from individuals for the isotopic analyses. The animals were released where they had been captured.

Different types of tissue reflect distinct scales of exploitation of food resources (Dalerum and Angerbjörn 2005). Tissues with high turnover rates reflect the animal’s recent diet, however, inert tissues (e.g., claw) reflect the animal’s diet at the time those tissues were synthetized (Ethier et al. 2010). Therefore, inert tissues are considered good indicators of the general diet of animals (Bowen et al. 2005). Claw tissue can reflect changes in the diet of the freshwater ‘turtle after six months for $d^{15}N$ and greater than six months $\delta^{13}C$ (Aresco et al. 2015).

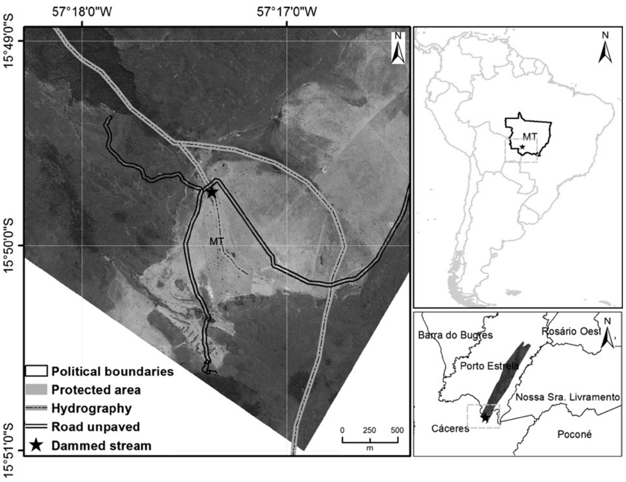

Figure 1. Maps illustrating study site at Serra das Araras Ecological Station, Mato Grosso state, Brazil (Projected Cordinate Sistem; Datum: Sirgas 2000).

The claw samples were washed with distilled water, dried at 60°C, fragmented to the smallest possible size and placed (0.8–1.0 mg) in small tin capsules, where they were heated in a Carlo Erba elemental analyzer (CHN-1110) coupled to a Delta Plus mass spectrometer in the Laboratório de Ecologia Isotópica, Centro de Energia Nuclear na Agricultura, Universidade de São Paulo, Brazil. The isotopic composition of carbon and nitrogen was expressed in delta (δ) per mil (‰) as folows: $\delta X = (R_{sample}/R_{standard} - 1) \times 1000$; where R are ratios of heavy to light isotopes ($^{15}N/^{14}N$ or $^{13}C/^{12}C$) in the sample and standard. The analytical error estimated by repeated measures of internal standard (sugarcane) was 0.5‰ for $\delta^{15}N$ and 0.3‰ for $\delta^{13}C$. The international standards used for nitrogen and carbon were atmospheric air (AIR) and Pee Dee Belemnite (PDB), respectively.

Normality data and homoscedasticity were tested prior to statistical analyses by Anderson Darling’s test and Levene’s test, respectively. The t-test was used to investigate sexual differences in biometric measurements (carapace length and body mass) and in stable isotope compositions ($\delta^{15}N$ and $\delta^{13}C$).

Isotopic niche can be defined as the polygon area formed by the isotopic compositions in δ space (Newsome et al. 2007). Sexual niche widths were evaluated using Bayesian standard ellipse methodology (SEA_B – Jackson et al. 2011). Differences between male and female in niche widths were tested by estimating the number of simulations where one group had a larger SEA than the other. Niche overlap was measured by comparing the extent of male and female overlap of corrected standard ellipses (SEA_c – Jackson et al. 2011). These analyses were performed in the package Stable Isotope Analysis (SIAR – Parnell et al. 2010) of the software R (R Core Team 2013). Linear regression was used to test the relationship to isotopic compositions and biometric

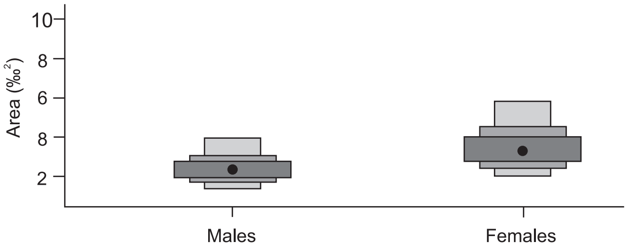

Figure 2. Niche widths for males and females of *Mesoclemmys vanderhaegei* estimated by Bayesian standard ellipse areas (SEA_B). The black points correspond to the mean SEA_B for each group, while shaded boxes representing the 50%, 75% and 95% credible intervals from dark to light grey.

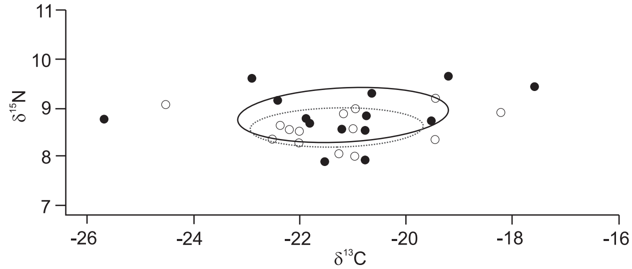

Figure 3. Corrected standard ellipses (SEA_c) overlaping of standard ellipse between males (white circles: dashed line) and females (black circles: black line) of *Mesoclemmys vanderhaegei* in $\delta^{15}N$-$\delta^{13}C$ space.

measurements (carapace length and body mass). These analyses were performed using Minitab 16 (Minitab Inc., State College, Pennsylvania, USA).

We captured 28 individuals during the study period, 14 males and 14 females. Females had significantly longer carapaces (female = 165.9 ± 12.2 mm; male = 142.1 ± 16.4 mm; $t = 4.29$; $df = 27$; $p < 0.001$) and greater body mass than males (female = 392.9 ± 99.7 g; male = 259.3 ± 73.5 g; $t = 4.03$; $df = 27$; $p < 0.001$).

Niche widths (SEA_B) were not statistically different between the sexes (SEA_B female = 3.76 ‰^{2}, 95% Cr.I. = 2.02–5.79 ‰^{2}; SEA_B male = 2.56 ‰^{2}, 95% Cr.I. = 1.36-3.93 ‰^{2}; $p = 0.145$; Fig. 2) and there was a high degree of overlap between the corrected standard ellipses of males and females (M/F: 88.2%; F/M: 50.5%; Fig. 3). The mean values of $\delta^{15}N$ were 8.87 ± 0.54 ‰ (7.89-9.65 ‰) for females and 8.60 ± 0.36 ‰ (8.01–9.19 ‰) for males. The mean values of $\delta^{13}C$ were -21.19 ± 1.88 ‰ (-25.68–17.58 ‰) for females and -21.30 ± 1.56 ‰ (-24.54–18.22 ‰) for males. There was no difference for both $\delta^{15}N$ ($t = 1.45$; $df = 27$; $p = 0.162$) and $\delta^{13}C$ ($t = 0.15$; $df = 27$; $p = 0.879$) between sexes.

No significant relationship was found between the values of $\delta^{15}N$ for both carapace length ($F = 1.15$; $df = 27$; $p = 0.294$; $r^2 = 0.04$) and body mass ($F = 1.83$; $df = 27$; $p = 0.188$; $r^2 = 0.06$). Similar pattern occurred for values of $\delta^{13}C$ (CL: $F = 3.94$; $df = 27$; $P = 0.058$; $r^2 = 0.13$; BM: $F = 4.13$; $df = 27$; $p = 0.053$; $r^2 = 0.13$; Fig. 4).

Mesoclemmys vanderhaegei showed consistent sexual dimorphism in body size, corroborating previous studies on the species in others localities (Brito et al. 2012, Marques et al. 2013b), which also found that females are larger than males. However, despite the marked morphological difference between the sexes, their isotopic niche widths did not differ.

Isotopic niche of wildlife species can be influenced by what they consume as well as their habitat (Newsome et al. 2007). The knowledge about the diet of *M. vanderhaegei* is extremely limited since there have been few studies in natural conditions. This turtle is omnivorous with affinity to carnivory (Cabrera 1998, Rueda-Almonacid et al. 2007). Brito et al. (2016) showed that aquatic insects are the main food item consumed by this species in the Cerrado (frequency of occurrence: Odonata (25%), Diptera (25%), Hemiptera (16%) followed by fish (39%), aquatic plants (9%), fruits (9%), and leaves (6%)). The lack of differences in isotopic niche widths suggests that both sexes can exploit a similar range of food resources available in the study area.

Habitat use can also influence the isotopic niche because animals can move between areas with different isotopic baselines (Hobson 1999, Hobson and Wassenaar 2008). This is the case of crocodilians in the coastal region, which have access to marine and estuarine/freshwater environments (Rosenblatt and Heithaus 2011, Hanson et al. 2015) or felids in agricultural landscapes (forest remnants – C3 *x* monocultures of sugarcane – C4; Magioli et al. 2014). Males and females of *M. vanderhaegei* in this study were captured in the same water bodies and have similar $\delta^{13}C$ values, which suggests that both sexes have the same isotopic baseline. However, future studies should determine isotopic compositions from prey representing the different potential baselines (e.g., benthic, pelagic, terrestrial) in the system, in order to verify their importance in the intersexual niche of *M. vanderhaegei*.

Several studies indicate ontogenetic differences in the diet of turtles (e.g., Clark and Gibbons 1969, Souza and Abe 1995). Dietary partitioning is a strategy adopted by many species and has the advantage of reducing the intraspecific competition and increasing fitness (Riklefs 2008). However, there was no relationship between isotopic composition and body size in *M. vanderhaegei*. Nitrogen isotopic composition ($\delta^{15}N$) indicates the trophic level of an organism, whilecarbon isotopic composition ($\delta^{13}C$) indicates the different carbon sources of diet exploited by individuals (Minawaga and Wada 1984, Fry 2006). Future long-term studies should understand how the trophic niche of *M. vanderhaegei* vary in space (e.g., natural and man-made habitats) and time (e.g., seasonaly).

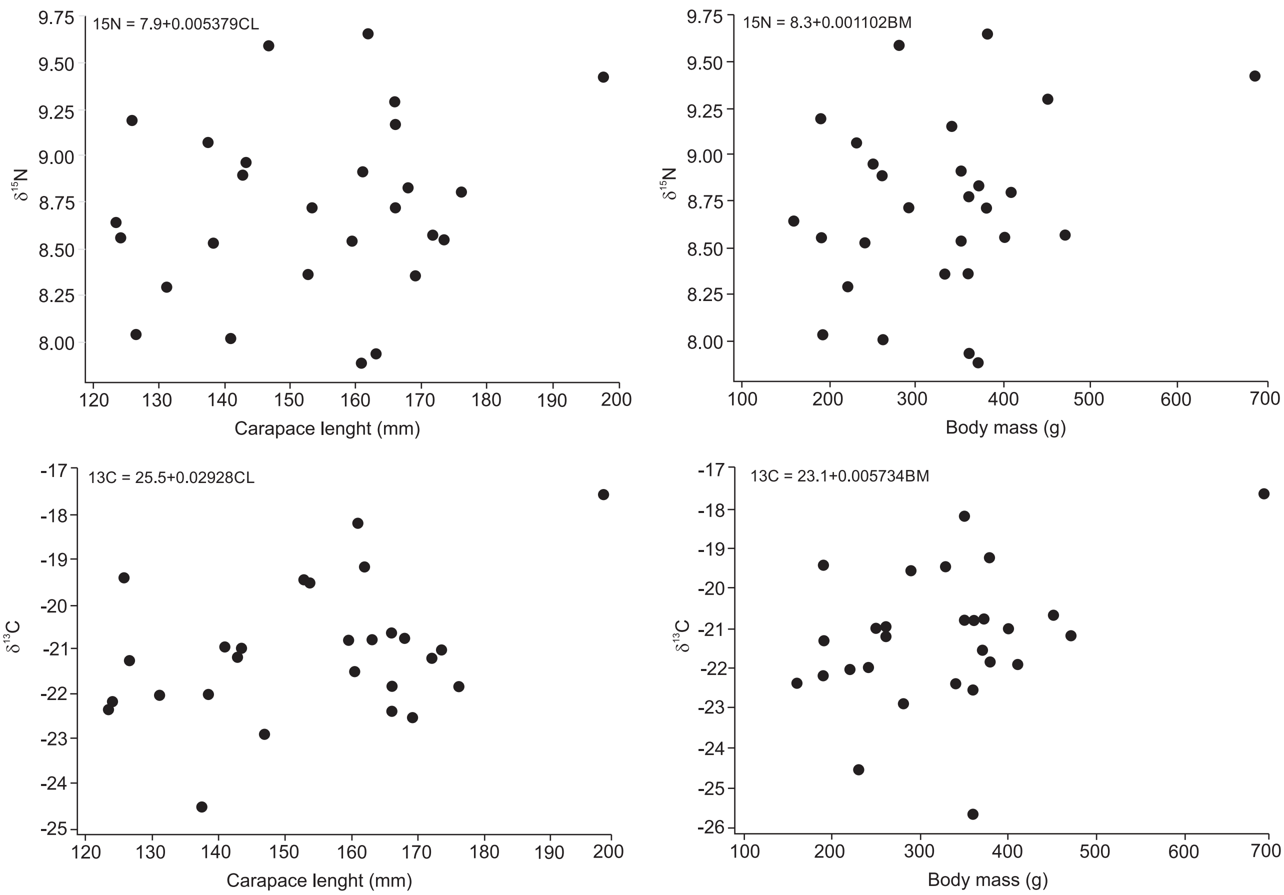

Figure 4. Relationship between the stable isotope compositions ($d^{15}N$-$d^{13}C$) and body size (carapace length and body mass) of *Mesoclemmys vanderhaegei* at Serra das Araras Ecological Station, Mato Grosso state, Brazil.

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LITERATURE CITED

Aresco MJ, Trans J, Macrae PSD (2015) Trophic interactions of turtle in a North Florid Lake food web: Prevalence of Omnivory. Copeia 103: 343–356. https://doi.org/10.1643/CE-13-130

Balzani P, Vizzini S, Santini G, Masoni A, Ciofi C, Ricevuto E, Chelazzi G (2016) Stable isotope analysis of trophic niche in two co-occurring native and invasive terrapins, *Emys orbicularis* and *Trachemys scripta elegans*. Biological Invasions 18: 3611–3621. https://doi.org/10.1007/s10530-016-1251-x

Bowen GJ, Wassenaar LI, Hobson KA (2005) Global application of stable hydrogen and oxygen isotopes to wildlife forensics. Oecologia 143: 337–348. https://doi.org/10.1007/s00442-004-1813-y

Brandão RA, Zerbini GJ, Sebben A, Molina FB (2002) Notes on distribution and habitats of *Acanthochelys spixii* and *Phrynops vanderhaegei* (Testudines, Chelidae) in central Brazil. Boletín de la Asociación Herpetológica Española 13: 11–15.

Brito ES, Souza FL, Strüssmann C (2016) Feeding habits of *Mesoclemmys vanderhaegei* (Testudines: Chelidae). Acta Herpetologica 11: 05–17.

Brito ES, Strussmann C, Penha JMF (2009) Population structure of *Mesoclemmys vanderhaegei* (Bour, 1973) (Testudines: Chelidae) in the Cerrado of Chapada dos Guimarães, Mato Grosso, Brazil.

Biota Neotropica 9: 245–248. https://doi.org/10.1590/S1676-06032009000400024

Brito ES, Strüssmann C, Kawashita-Ribeiro RA, Morais DH, Ávila RW, Campos VA (2012) New records and distribution extensions of three species of *Mesoclemmys* Gray, 1863 (Testudines: Chelidae) in Mato Grosso state, Brazil, with observations on terrestrial movements. Check List 8: 294–297. https://doi.org/10.15560/8.2.294

Bulté G, Gravel MA, Blouin-Demers G (2008) Intersexual niche divergence in northern map turtle (Graptemys geographica): the roles of diet and habitat. Canadian Journal of Zoology 86: 1235–1243. https://doi.org/10.1139/Z08-107

Cabrera MR (1998) Las Tortugas Continentales de Sudamérica Austral. Córdoba, Argentina, Privately printed.

Cagle FR (1939) A system of marking turtles for future identification. Copeia 1939: 170–173. https://doi.org/10.2307/1436818

Clark DB, Gibbons JW (1969) Dietary shift in the turtle *Pseudemys scripta* (Schoepff) from youth to maturity. Copeia 1969: 704–706. https://doi.org/10.2307/1441797

Dalerum F, Angerbjorn A (2005) Resolving temporal variation in vertebrate diets using naturally occurring stable isotopes. Oecologia 144: 647–658. https://doi.org/10.1007/s00442-005-0118-0

Ethier DM, Kyle CJ, Kyser TK, Nocera JJ (2010) Variability in the growth patterns of the cornified claw sheath among vertebrates: implications for using biogeochemistry to study animal movement. Canadian Journal of Zoology 88: 1043–1051. https://doi.org/10.1139/Z10-073

Ferronato BO, Marques TS, Lara NRF, Martinelli LA, Verdade LM, Camargo PB, Roe JH, Georges A (2016) Isotopic niche in the eastern long-necked turtle *Chelodina longicollis* (Testudines: Chelidae) along a natural-urban gradient in southeastern Australia. Herpetological Journal 26: 297–304.

Fry B (2006) Stable Isotope Ecology. New York, Springer Science, Business Media. https://doi.org/10.1007/0-387-33745-8

Hanson JO, Salisbury SW, Campbell HA, Dwyer RG, Jardine TD, Franklin CE (2015) Feeding across the food web: The interaction between diet, movement and body size in estuarine crocodiles (*Crocodylus porosus*). Australian Journal of Ecology 40: 275–286. https://doi.org/10.1111/aec.12212

Hobson KA (1999) Tracing origins and migration of wildlife using stable isotopes: a review. Oecologia 120: 314–326. https://doi.org/10.1007/s004420050865

Hobson KA, Wassenaar LI (2008) Tracking Animal Migration with Stable Isotopes. Academic Press, London.

Hutchinson GE (1957) Concluding remarks. Cold Spring Harbor Symposia on Quantitative Biology 22: 415–427. https://doi.org/10.1101/SQB.1957.022.01.039

Jackson AL, Inger RI, Parnell AC, Bearshop S (2011) Comparing isotopic niche widths among and within communities: SIBER – Stable Isotope Bayesian Ellipses in R. Journal of Animal Ecology 80: 595–602. https://doi.org/10.1111/j.1365-2656.2011.01806.x

Layman CA, Araujo MS, Boucek R, Hammerschlag-Peyer CM, Harrison E, Jud ZR, Matich P, Rosenblatt AE, Vaudo JJ, Yeager LA, Post DM, Bearhop S (2012) Applying stable isotopes to examine food-web structure: an overview of analytical tools. Proceedings of the Cambridge Philosophical Society 87: 545–562. https://doi.org/10.1111/j.1469-185X.2011.00208.x

Magioli M, Moreira MZ, Ferraz KMB, Miotto RA, de Camargo PB, Rodrigues MG, da Silva Canhoto MC, Setz EF (2014) Stable Isotope Evidence of *Puma concolor* (Felidae) Feeding Patterns in Agricultural Landscapes in Southeastern Brazil. Biotropica 46: 451–460. https://doi.org/10.1111/btp.12115

Marques TS, Lara NRF, Bassetti, LAB, Piña CI, Camargo PB, Verdade LM (2013a) Intraspecific isotopic niche variation in broad-snouted caiman (*Caiman latirostris*). Isotopes in Environmental and Health Studies 49: 325–335. https://doi.org/10.1080/10256016.2013.835309

Marques TS, Lara NRF, Bassetti LAB, Ferronato BO, Malvásio A, Verdade LM (2013b) Population structure of *Mesoclemmys vanderhaegei* (Testudines, Chelidae) in a silvicultural system in southeastern Brazil. Herpetology Notes 6: 179–182.

Marques TS, Böhm S, Brito ES, Cabrera MR, Verdade LM (2014) *Mesoclemmys vanderhaegei* (Bour 1973) – Vanderhaege's Toad-headed Turtle, Karumbé-hy. Chelonian Research Monographs 5: 083.1–8. https://doi.org/10.3854/crm.5.083.vanderhaegei.v1.2014

Minagawa M, Wada E (1984) Step-wise enrichment of ^{15}N along food chains: further evidence and the realtion betwenn $\Delta^{15}N$ and animal age. Geochimica et Cosmochimica Acta 48: 1135–1140. https://doi.org/10.1016/0016-7037(84)90204-7

Newsome SD, Martinez del Rio C, Bearhop S, Phillips DL (2007) A niche for isotopic ecology. Frontiers in Ecology and the Environment 5: 429–436. https://doi.org/10.1890/060150.1

Parnell AC, Inger R, Bearhop S, Jackson AL (2010) Source partitioning using stable isotopes: coping with too much variation. Plos One 5: e9672. https://doi.org/10.1371/journal.pone.0009672

Riklefs RE (2008) The Economy of Nature. W.H. Freeman, New York.

R Core Team (2013) R: A language and environment for statistical computing. R Foundation for Statistical Computing. Vienna, available online at: http://www.R-project.org [Accessed: 30/10/ 2013]

Richards-Dimitrie T, Gresens SE, Smith SA, Seigel RA (2013) Diet of Northern Map Turtles (*Graptemys geographica*): Sexual Differences and Potential Impacts of an Altered River System. Copeia 3: 477–484. https://doi.org/10.1643/CE-12-043

Rosenblatt AE, Heithaus MR (2011) Does variation in movement tactics and trophic interactions among American alligators create habitat linkages? Journal of Animal Ecology 80: 786–798. https://doi.org/10.1111/j.1365-2656.2011.01830.x

Rueda-Almonacid JV, Carr JL, Mittermeier RA, Rodriguez-Mahecha JV, Mast RB, Vogt RC, Rhodin AGJ, Ossa-Velaquez J, Rueda JN, Mittermeier CG (2007) Las tortugsd y los crocodilianos de los paises andinos del tropic. Conservation Internacional, Bogotá.

Souza FL, Abe AS (1995) Observations of feeding habitats of *Hydromedusa maximiliani* (Testudines: Chelidae) in southwestern Brazil. Chelonian Conservation and Biology 1: 320–322.

Wearmouth VJ, Sims DW (2008) Sexual segregation in marine fish, reptiles, birds and mammals: Behaviour patterns, mechanisms and conservation implications. Advances in Marine Biology 54: 107–170. https://doi.org/10.1016/S0065-2881(08)00002-3

Werner EE, Gilliam JF (1984) The ontogenetic niche and species interactions in size-structured populations. Anual Review of Ecology and Systematics 15: 393–425. https://doi.org/10.1146/annurev.es.15.110184.002141

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