

SHORT COMMUNICATION

Isotopic signatures ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of muscle, carapace and claw in  
*Phrynops geoffroanus* (Testudines: Chelidae)

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**ABSTRACT.** The isotopic composition ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of muscle, carapace and claw was determined from six wild individuals of *Phrynops geoffroanus* (Schweigger, 1812) in order to verify the variation between those tissues. The mean values of  $\delta^{13}\text{C}$  e  $\delta^{15}\text{N}$  were, respectively,  $-19.48 \pm 0.81\text{‰}$  ( $-20.8 - -18.64\text{‰}$ ) and  $7.23 \pm 0.67\text{‰}$  ( $6.49 - 8.3\text{‰}$ ) for muscle,  $-16.52 \pm 0.98\text{‰}$  ( $-17.88 - -15.43\text{‰}$ ) and  $7.29 \pm 0.54\text{‰}$  ( $6.74 - 7.97\text{‰}$ ) for carapace and  $-18.57 \pm 0.97\text{‰}$  ( $-19.97 - -17.26\text{‰}$ ) and  $4.36 \pm 0.33\text{‰}$  ( $3.93 - 4.79\text{‰}$ ) for claw. We found a significant difference for the tissues evaluated for both  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . Muscle and claw were statistically similar and more depleted in  $^{13}\text{C}$  than the carapace. Nonetheless, claw was more depleted in  $^{15}\text{N}$  than muscle and carapace tissues. These results are likely related to differences in metabolic processes involved on each tissue formation. The description of the isotopic signatures variation in *P. geoffroanus* tissues provides a baseline for future investigations on the diet reconstruction of this species when more than one tissue is involved.

**KEY WORDS.** Diet; freshwater turtles; Geoffroy's side-necked-turtle; metabolic processes; stable isotope.

Stable isotope analyses present a great potential to be explored in animal ecology studies (GANNES *et al.* 1997). This technique can provide information on animals' movement patterns, diet reconstruction, trophic level, protein balance, turnover of nutrients, and nutrient allocation (GANNES *et al.* 1997, 1998, HOBSON 1999). However, investigations based on this technique for Neotropical chelonians are scarce.

The use of stable isotopic technique on chelonians is generally concentrated in marine turtles (e.g., GODLEY *et al.* 1998, HATASE *et al.* 2002, BIASATTI 2004, WALLACE *et al.* 2006, REICH *et al.* 2007). However, some information on freshwater turtle's species is also available (ARESCO & JAMES 2005, SEMINOFF *et al.* 2007, BULTÉ *et al.* 2008). The Geoffroy's side-necked turtle, *Phrynops geoffroanus* (Schweigger, 1812), is widely distributed in South America, ranging from Venezuela and Colombia to southern Brazil, Paraguay, northern Argentina and Uruguay (PRITCHARD & TREBBAU 1984, ERNST & BARBOUR 1989). It is a common inhabitant of urban polluted rivers (SOUZA & ABE 2000, MARQUES *et al.* 2008, FERRONATO *et al.* 2009a, c, PIÑA *et al.* 2009), where it can coexist with the invasive exotic species *Trachemys scripta elegans* (Wied, 1838) (FERRONATO *et al.* 2009b).

The goal of this study was to describe the variation in isotopic signatures ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) found in muscle, carapace

and claw tissues of Geoffroy's side-necked turtle and to provide a better understanding of the isotopic relationships of its different tissues.

In this investigation, we used turtles from two polluted watercourses in southeastern Brazil, the Piracicaba river and its tributary, the Piracicamirim stream, Piracicaba Municipality, state of São Paulo ( $22^{\circ}42'52.18''\text{S}$ ,  $47^{\circ}37'38.95''\text{W}$ ). The Piracicaba river basin has suffered an intense change in land use, which altered the original landscape and water quality (DEL GRANDE *et al.* 2003). For further information on the study sites see MARQUES *et al.* (2008), FERRONATO *et al.* (2009a), and PIÑA *et al.* (2009). Muscle samples from different body regions (abdomen, arm, leg and neck), as well as carapace and claw samples were collected from six Geoffroy's side-necked turtles (three from each watercourse). Claw samples represented the terminal 8 mm of the claw. Despite the relatively small sample-size, this study generates valuable data considering the ethical difficulty in sacrificing a larger number of animals.

All samples were dried at  $50^{\circ}\text{C}$  in an oven device until they reached a constant mass (MAGNUSSON *et al.* 1999). The carapace and claw samples were manually cleaned to remove contaminants from both the environment and the turtle itself, and fragmented into very small pieces, while the muscle samples

were ground to a fine powder. The resultant material was weighted (claw: 1.2-1.5 mg; carapace: 1-1.2 mg; muscle: 1-1.2 mg) and put inside small tin capsules. The isotopic composition of carbon and nitrogen were determined by “online” combustion of the samples by CF-IRMS (Continuous Flow – Isotope Ratio Mass Spectrometers), using an elemental analyzer Carlo Erba (CHN-1110) interfaced to an isotope ratio mass spectrometer Delta Plus, in the Isotopic Ecology Laboratory/CENA/University of São Paulo. Carbon and nitrogen isotopic composition were calculated according to JESPEN & WINEMILLER (2002). The results are defined in delta notation ( $\delta$ ) and reported in parts per thousand (‰) relative to international standards (see CRAIG 1957). The results are represented in delta ( $\delta$ ) notation in ‰. Routine measurements were precise to within 0.3‰ for  $\delta^{13}\text{C}$  and 0.5‰ for  $\delta^{15}\text{N}$ .

Data normality was tested using the Anderson-Darling test. To verify any difference in the isotopic signatures ( $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$ ) of muscle samples from the abdomen, arm, leg and neck, we used the Kruskal-Wallis test as the data were not normally distributed. In addition, we used the same test to check for any differences between muscle, carapace and claw. If a difference was detected, we used the Mann-Whitney Test between the treatments. We used Minitab 16 software for the statistical analyses.

The turtles used in the present study had a mean straight line carapace length of  $284 \pm 22$  mm (243-307 mm) and mean body mass of  $2038 \pm 434$  g (1300-2450 g). There was no significant difference in isotopic signatures among muscle samples collected from different body regions (Fig. 1;  $\delta^{13}\text{C}$ :  $df = 3$ ,  $h = 0.8$ ,  $p = 0.84$ ;  $\delta^{15}\text{N}$ :  $df = 3$ ,  $h = 2.65$ ,  $p = 0.44$ ); consequently we could use a general mean of muscle from each individual, when comparing the data with other tissues (carapace and claw). The isotope ratios of muscle, claw, and carapace are shown in figure 2 and table I.

Table I. Mean isotopic signatures ( $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ ) of muscle, carapace and claw tissues of Geoffroy's side-necked turtle. Differences between treatments are marked by different letters.

|          | $\delta^{13}\text{C}$                        | $\delta^{15}\text{N}$                  |
|----------|--|--|
| Muscle   | $-19.5 \pm 0.8$ (-20.8 – -18.6) <sup>B</sup> | $7.2 \pm 0.7$ (6.5 – 8.3) <sup>A</sup> |
| Carapace | $-16.5 \pm 0.9$ (-17.9 – -15.4) <sup>A</sup> | $7.3 \pm 0.5$ (6.7 – 7.9) <sup>A</sup> |
| Claw     | $-18.6 \pm 0.9$ (-19.9 – -17.2) <sup>B</sup> | $4.3 \pm 0.3$ (3.9 – 4.8) <sup>B</sup> |

There was a significant difference between the tissues sampled (muscle, carapace, and claw) for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  ( $\delta^{13}\text{C}$ :  $df = 2$ ,  $h = 11.56$ ,  $p = 0.003$ ;  $\delta^{15}\text{N}$ :  $df = 2$ ,  $h = 11.47$ ,  $p = 0.003$ ). There was no difference between claw and muscle samples for  $\delta^{13}\text{C}$  ( $w = 29$ ,  $p = 0.12$ ). However, there was a significant difference between carapace and muscle/claw (carapace/muscle:  $w = 57$ ,  $p = 0.005$ ; carapace/claw:  $w = 55$ ,  $p = 0.01$ ). In addition, in the case of  $\delta^{15}\text{N}$  there was a significant difference for

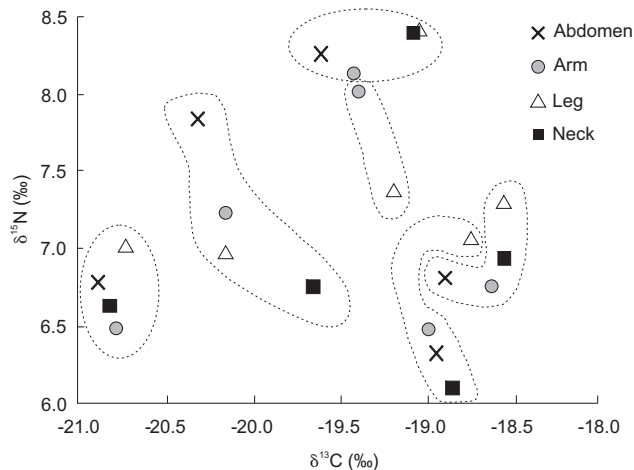


Figure 1. Relationships of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  among muscle tissues from different body regions (abdomen, arm, leg and neck) in Geoffroy's side-necked turtle. The dash line circles represent the same individual samples.

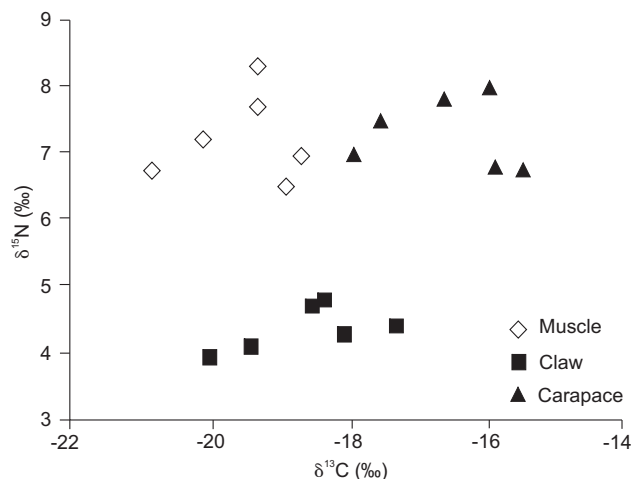


Figure 2. Relationships of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  among different muscle tissues, carapace and claw in Geoffroy's side-necked turtle.

claw and the other tissues (claw/muscle:  $w = 57$ ,  $p = 0.005$ ; claw/carapace:  $w = 21$ ,  $p = 0.005$ ), which did not differ from each other (Tab. I;  $w = 36$ ,  $p = 0.68$ ).

ARESCO & JAMES (2005) found no difference in the values of  $\delta^{15}\text{N}$  and  $\delta^{13}\text{C}$  when comparing muscle and claw tissues in two north American freshwater turtles, *Trachemys scripta scripta* (Schoepff, 1792) and *Pseudemys floridana* (LeConte, 1829). REVELLES *et al.* (2007) did not find differences in  $\delta^{13}\text{C}$  composition between muscle and carapace of loggerhead sea turtle, *Caretta caretta*, however they found differences for  $\delta^{15}\text{N}$  values. In the present study, we found the same pattern only for the  $\delta^{13}\text{C}$  in muscle and claw samples. Apparently, it is not clear if

there is a relation for chelonians muscle and carapace samples when analysing  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  composition.

The tissues of consumers are synthesised by nutrients present in the diet, so they usually reflect the isotopic composition of their food (DeNIRO & EPSTEIN 1978, HOBSON & CLARK 1992a, CRAWFORD *et al.* 2008). The differences found in the isotopic signatures of the tissues can result from different metabolic processes involved in the formation of different tissues. TIESZEN *et al.* (1983) suggested such a mechanism in their study of the rodent *Meriones unguiculatus* (Milne-Edwards, 1867) under controlled conditions.

When the animal's diet changes, the isotopic signature of the tissues does not change homogeneously in time (REICH *et al.* 2008). Tissues with high metabolic rate reflect the recent diet of the animal, while tissues with low metabolic rates reflect the diet from a longer period (HOBSON & CLARK 1992b). This is why using different tissues with distinct metabolic rates could help in reconstructing the animal's diet, as well as detecting seasonal differences and migratory trends (DALERUM & ANGERBJÖRN 2005). However, in the present study our claw samples probably represent a mix of old and new tissues due to the length of claw material that was sampled (see ETHIER 2010).

Investigations on tissues turnover patterns in freshwater turtles are scarce. SEMINOFF *et al.* (2007) estimated for pond sliders (*Trachemys scripta*) a turnover period for blood plasma, whole blood and liver of 142, 155 and 210 days, respectively. The isotopic signatures of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  of the claw of pond sliders took, respectively, twelve and six months to reflect changes in the turtle's diet (ARESCO & JAMES 2005). This information could also be relevant to our results because the turtles were captured in the wild and there is no information on possible temporal changes in their diet.

Most dietary studies on chelonians use stomach flushing techniques (LEGLER 1977) and faeces analysis (BURKE *et al.* 1993, WITZELL & SCHMID 2005, CAPUTO & VOGT 2008) These methods impose some limitations, such as the overestimation of the items with hard digestion structures, and the underestimation of the food items that are completely digested and absorbed (BULTÉ *et al.* 2008). In addition, they could include non-dietary items incidentally ingested. The isotopic analysis applied for dietary studies using different tissues is a powerful tool in the diet reconstruction, since they provide measurements of food assimilation for different periods of time (MARTINELLI *et al.* 2009).

In addition, if the proportionality of  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$  in claw and muscle of *Phrynops geoffroanus* demonstrated in the present study is confirmed, there will be no need to sacrifice or harm animals in order to get muscle samples in this species. This technique could also be applied in other freshwater turtles that show the same similarity for  $\delta^{13}\text{C}$  and  $\delta^{15}\text{N}$ . Besides helping investigating long-term dietary trends, stable isotopes also provide an alternative to traditional diet studies which allows for non-destructive sampling. This is the first step in order to carry out diet studies on *P. geoffroanus* by the use of isotopes.

Our results are original in describing the variation in the isotopic signatures of Geoffroy's side-necked turtle tissues, which can be used in future investigations of the species diet reconstruction. In complement, future studies of isotopic ecology under controlled conditions should be prioritized for the species. It could also help solving questions of temporal or spatial variation in the use of feeding resources.

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