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The Role of Feeding Specialization on Post-Prandial Metabolic Rate in Snakes of the Genus *Bothrops*

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Feeding specialization is a recurrent issue in the evolution of snakes and is sometimes associated to morphological and/or behavioral adaptations that improve snake performance to exploit a particular food type. Despite its importance for animal fitness, the role of physiological traits has been much less studied than morphological and behavioral traits in the evolution of feeding specialization in snakes. In this context, the energetic cost of post-prandial period is an important physiological factor due to the remarkable effect on the snake energy budget. We collected data on post-prandial metabolic rate (SDA) in five species of pit vipers from the genus *Bothrops* with different degrees of mammal feeding specialization to test the hypothesis that feeding specialist species have lower energy costs during the digestion of their regular food item when compared to species with a more generalist diet. Our results support this hypothesis and suggest that ontogenetic changes in diet can be accompanied by changes in energy cost of the digestion process.

Key words: feeding specialist, feeding generalist, pit viper, digestion, metabolism, energetics

INTRODUCTION

Feeding specialization is a recurrent phenomenon in various animal taxa (Joern, 1979; Simon and Toft, 1991; Beissinger et al., 1994; Britt et al., 2006; Henry and Stoner, 2011). The current theory about feeding specialization predicts that specialist animals enjoy an ecological advantage by exploiting a particular type of food when compared to generalist animals using the same resource (Futuyma and Moreno, 1988; Holbrook and Schmitt, 1992; Robinson and Wilson, 1998; Terraube et al., 2011). This advantage is usually related to morphological, physiological, and/or behavioral traits that allow specialist species to explore more efficiently a particular food resource (Futuyma and Moreno, 1988; Holbrook and Schmitt, 1992). On the other hand, the fitness of feeding specialists is highly associated with the abundance of their usual food item, so the scarcity of the particular item could decrease individual body condition and impact the population size (Terraube et al., 2011). Conversely, feeding generalist species exploit a wide range of food resources and can buffer drops in the abundance of specific items of their diet (Futuyma and Moreno, 1988; Hayward and Kerley, 2008). For instance, Terraube et al. (2011), reported a higher foraging efficiency in a specialist harrier (*Circus macrourus*) when their preferred prey was abundant compared to a situation in which this type of prey was scarce.

As with any animal taxa, snakes may be classified as feeding generalists or specialists (Greene, 1997; Martins et al., 2002; Maritz and Alexander, 2014). Feeding specialization in snakes has been studied in ecological, morphological, and toxicological contexts (Pough and Groves, 1983; Dwyer and Kaiser, 1997; Mori and Vincent, 2008). Cranial features that facilitate the swallowing process, vertebral specializations for breaking eggshells, and differences in venom toxicity are examples of some of the evolutionary trends in snake feeding specialization (Coleman et al., 1993; Dwyer and Kaiser, 1997; Richards et al., 2012; Klaczko et al., 2016). For instance, Mori and Vincent (2008) studied biometrical cranial features and prey handling performances of two sympatric snake species (a frog-eating specialist and a diet-generalist). In terms of morphology, the anuran specialist has a relatively larger head and a larger gape, seemingly enhancing efficiency during handling and ingestion of amphibians (Mori and Vincent, 2008). Analogous cases of specialization may also occur with respect to physiological traits, although this is aspect of snake feeding specialization is less well studied (Britt et al., 2006; Britt and Bennett, 2008). In this context, digestion physiology would be a key aspect of feeding specialization because of its exclusive role in energy uptake, so that improving digestive performance would imply enhancement of net energy gain (Britt et al., 2006; Britt and Bennett, 2008).

The reduction of digestion cost may involve considerable energy conservation in some snake species (Secor and Nagy, 1994), so a link between dietary specialization and energetics of digestion might be expected. A key issue to

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establish this link is that the energy cost of post-prandial period (usually called Specific Dynamic Action, SDA) is especially high in ambush foraging snake species (e.g., Pit vipers, Boas, Pythons) (Andrade et al., 1997; Bedford and Christian, 1998; Secor and Diamond, 2000; Mccue, 2006; Secor, 2009). The high SDAs displayed by these taxa are related to a substantial investment in the reactivation of gastrointestinal organs, in turn mediated by intense protein synthesis and proton-pump activity during the feeding period in comparison to the minimum maintenance cost of fasting (Secor and Diamond, 2000; Ott and Secor, 2007). A fasting snake would typically reduce drastically the amount of energy spent in maintenance via atrophy of intestinal brush-borders, liver, and kidneys, maintenance of a neutral stomach pH, and minimum activity level of intestinal membrane transporters (Secor, 2003; Enok et al., 2013). Meal intake, especially a large one, therefore requires a rapid upregulation of body function so as to restore the digestive capacities (e.g., intestinal hypertrophy, increase in the stomach proton-pump activity, and increase in the intestine membrane transporters activity) (Helmstetter et al., 2005; Mccue, 2006; Lignot et al., 2008; Enok et al., 2013). Such upregulation is necessarily paralleled by enhanced energy cycling, evidenced by the increased metabolic rate during SDA. Indeed, SDA can last for more than a week in many species and represents an important part of the energetic budget of some snakes (Andrade et al., 1997; Zaidan and Beaupre, 2003; Ott and Secor, 2007).

Given the importance of SDA in the energy budget of ambush-hunting snakes, evolution may be expressed in coadaptation between feeding ecology and digestive physiology, so that the digestion of preferred prey items is optimized relatively to the digestion of alternative prey items. This coadaptation hypothesis is testable under the expectation that feeding specialists present lower digestive cost (i.e., lower SDA) when digesting their typical food item than do generalist species consuming analogous prey items (regarding type and relative size). A study of natricine snakes of the genus *Thamnophis* suggests that this may be the case. Britt and colleagues (2006, 2008) showed that *Thamnophis e. elegans*, a mollusk-eating specialist, presents lower SDA than the generalist sub-species *Thamnophis e. terrestris* when both were fed on mollusks. However, different energetic patterns were detected for fish specialist snakes, so that the debate remains (Britt et al., 2006; Britt and Bennett, 2008). Here we investigate the relationship between the mammalian feeding specialization and the energy expenditure during post-prandial period (SDA) of this kind of prey in Neotropical pit vipers of the genus *Bothrops*. Specifically, our primarily assumption is that physiological variables related to SDA, such as metabolic scope (MS), time to SDA peak (Tpeak), SDA duration (SDAd) or overall SDA, may be lower for snakes more specialized in mammalian prey. We also tested the hypothesis that a higher degree of specialization is coupled to a lower SDA value in a phylogenetic independent comparison among species.

MATERIAL AND METHODS

Snakes of the genus *Bothrops* as experimental models

The genus *Bothrops* represents the most significant radiation of the subfamily Crotalinae in South America, widely distributed in most habitats of the continent (Campbell and Lamar, 2004). Regarding feeding habits, species in this genus vary in their degree of specialization (Egler et al., 1996; Martins et al., 2002; Hartmann et al., 2005; Monteiro et al., 2006; Marques and Sazima, 2009). The most prominent dietary specialization occurs in *Bothrops alternatus* group, in which three of the six species feed exclusively on mammals throughout their lives (Martins et al., 2002). Other species present an ontogenetic shift in diet changing from ectothermic prey (feeding mostly lizards and amphibians) to mammalian prey over the course development; in other words, these species become mammal specialists during ontogeny (Martins et al., 2002). Unlike mammal specialists, other species feed on small ectotherms as juveniles, adding mammals along ontogeny, but never abandon ectothermic prey (Martins et al., 2002). In this work we measured the post-prandial metabolic increment (SDA) on five *Bothrops* species with different diet specialization. *Bothrops alternatus* (Ba) is a highly mammal-specialist species that feeds exclusively on mammalian prey throughout its life; *B. jararaca* (Bj), *B. jararacussu* (Bjs), *B. atrox* (Bax), and *B. erythromelas* (Be) usually eat ectotherms at early ages and begin to incorporate mammalian prey during the ontogeny. Of these four species, *B. jararaca* consumes a high percentage of mammalian prey earlier with small SVL (snout-vent length) than *B. jararacussu*; *B. jararacussu* earlier than *B .atrox*; and *B. atrox* earlier than *B. erythromelas* (Martins, unpublished data).

Experimental animals

We used a total of 79 snakes of the five species of *Bothrops* (45 juveniles and 34 adults, table 1.0) from the Herpetology Laboratory at Instituto Butantan, São Paulo, Brazil. We considered as juveniles snakes with less than one year old and as adults snakes presenting at least three years old and/or with SVL compatible with sexual mature adults. Prior to the metabolic measurements, snakes were kept in containers (56.4 cm L × 38.5 cm W × 20.1 cm H) with cardboard as substrate and water *ad libitum*. The temperature was maintained between 20–28°C and the photoperiod was 12L: 12D. All procedures were allowed by the Ethics Committee on Animal Use of Instituto Butantan, São Paulo, Brazil.

Specific dynamic action

Specific dynamic action (SDA) was assessed by changes in oxygen consumption rates (VO_2) during the digestion process. The measurement started immediately before feeding (corresponding to the standard metabolic rates – SMR) and after meal ingestion, lasted until the VO_2 returned to pre-feeding fasting levels. We used a closed respirometric system for juveniles due to the low oxygen consumption by the young snakes; while for adults we used an open respirometric system. In both cases we used a PA oxygen-1 oxygen analyzer (Sable Systems) coupled to a suction pump and a flow meter (Omega Engineering Inc.). The air sample sent to the analyzer was dried by desiccant (Drierite®) resin and had its CO_2 content absorbed by Ascarite®. The signals emitted by the analyzer were computed and analyzed using the Expedata software (Sable Systems). The flow rate varied between 100 and 700 $ml.min^{-1}$ and VO_2 values were converted to standard temperature and pressure (Dejours, 1981), under the conditions of barometric pressure and temperature of the experiment. Results are expressed in $mlO_2.h^{-1}$ STPD.

Snakes were fasted during 30 days before SDA measuring, weighed, fed (as described below) and then placed individually in acrylic metabolic chambers whose volume varied from 450 to 3300 ml, depending on the size of the animal. These chambers were kept in a climatic chamber (Eletrolab®) at 25 ± 1°C. The VO_2 measurements were carried out for 10–12 days, at 0 (SMR), 12, 18, 24, 30, 36, 42, 48, 54, 60, 66, 72, 78, 84, 90, 108, 120, 144, 168, 192, 240, 252, 264 hours after food intake.

To ensure that all snakes would eat the exact proportion of food in relation to body mass, we force-fed the animals. First, we saturated a chamber with carbon dioxide and then kept the snake inside for a period no longer than 2 min, to reach a minor narcosis state. Then, we manually introduced a dead mouse into the esophagus and conducted it to the stomach with a gentle massage. The recovery of the snakes (apparent by recovery of typical enclosure behavior like coiling, tong flicking and normal posture) took less than 3 minand, after that, we placed each animal back into the metabolic chamber. The amount of food used was 20 ± 1% of the equivalent of the body weight for juveniles (newborn mice 2–5 g) and 15 ± 1% of body weight for adults (1–3 adult mice).

We quantified total SDA for each animal by calculating the area under the curve obtained for oxygen consumption (VO_2 in mlO_2h^{-1}) versus time (hours), minus the standard metabolic rate (SMR). We also calculated the metabolic scope (MS) as the factorial increase in VO_2 from SMR to the postprandial metabolic peak, as well as the time to achieve the metabolic peak (Tpeak) and SDA duration in hours (SDAd).

We transformed all values of oxygen consumption to kilojoules (kJ) considering a 19.8 KJ ratio for each liter of consumed O_2 (Secor and Diamond, 2000; Zaidan and Beaupre, 2003). We use two different approaches in our analysis: 1) comparison among different species; and 2) comparison based on degree of specialization (considering species as random factor).

Analysis

All analyses were performed in R platform (version 3.3.3), using RStudio software (version 1.0.136). The post-hoc comparisons were performed in ‘emmeans’ package using *Tukey* method (Lenth, 2017) when no interaction was detected between variables, and in ‘phia’ package (Rosario-Martinez, 2015) when occurred interaction. The significance level adopted was 0.05.

First, we investigated possible differences among *Bothrops* species relative to the four post-prandial physiological parameters (i.e., MS, Tpeak, SDAd and SDA). As the species are not independent in their evolutionary history, we calculated the intraspecific phylogenetic independent contrasts (iPICs) for each trait and body mass using the method described by Felsenstein (2008), by function *pic.ortho* in ‘ape’ package (Paradis et al., 2004). This function computes orthonormal contrasts based on a list containing the individual observations for each species. In this case, individuals belonging to the same species are considered to be separated from each other by branches of length 0 and, by consequence, connected by external branches of equal length. The phylogenetic information used in this approach was gathered from a time-calibrated phylogeny for viper snakes based on 11 genes and 263 taxa proposed by Alencar et al. (2016). See Fig. 1 for the relationship among the five *Bothrops* species used in this study. After extracted the iPICs, we analyzed MS, Tpeak, SDAd and SDA through ANCOVA using ‘body mass’ as a covariate and ‘species’ as categorical factor, testing first for interaction between terms (slope differences), and passing the regression through the origin as recommended by Garland et al. (1992). We did not perform comparison between adults and juveniles because both groups received different amount and quality of food in terms of their energy content (newborn mice for juveniles and adult mice to adults). Thus, we chose to perform an ANCOVA test for each age class.

In our second approach, we categorized each tested individual based on size and according to the degree of mammal feeding spe-

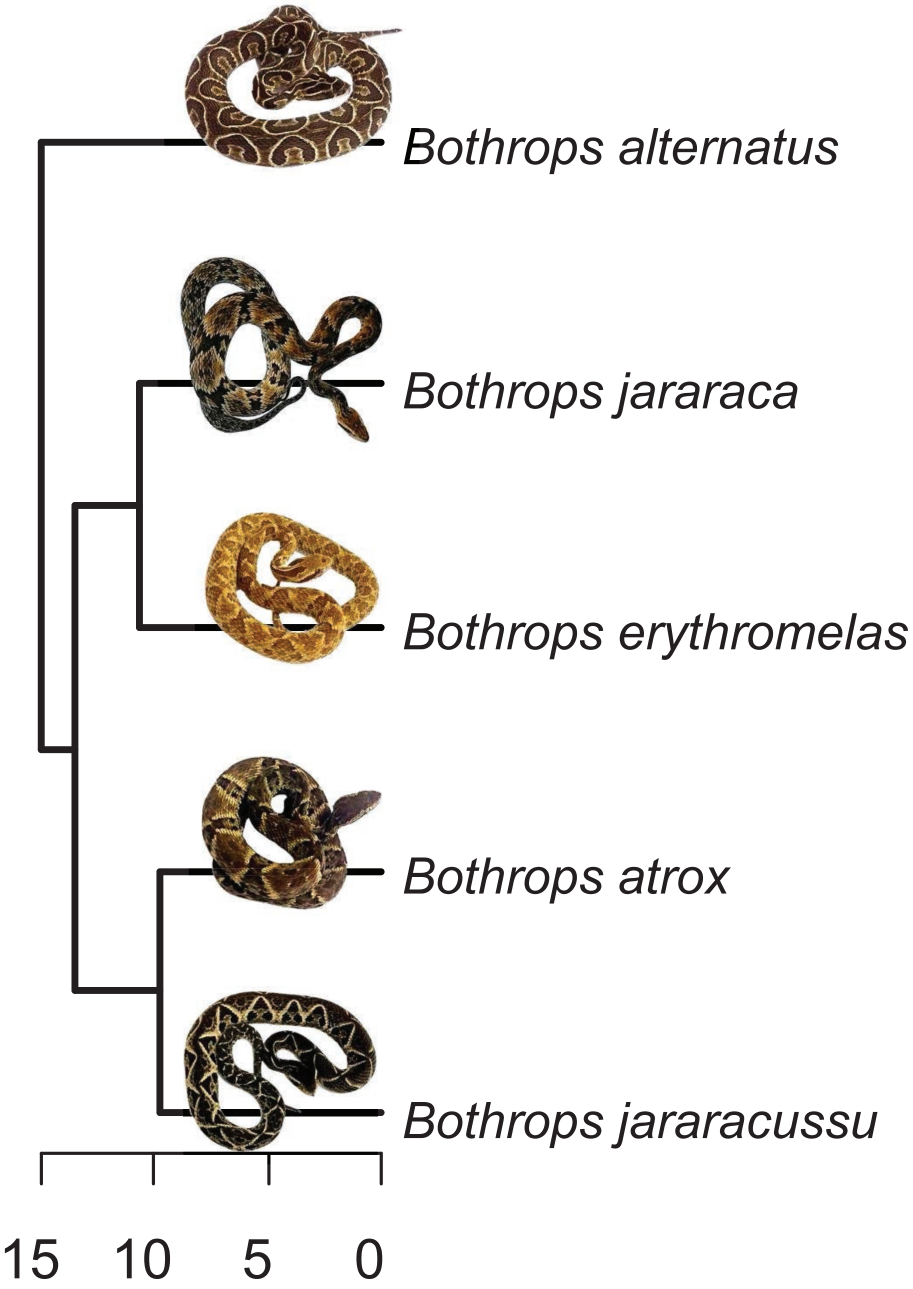

Fig. 1. Cladogram representing phylogenetic relationship between *Bothrops* species used in this study following hypothesis proposed by Alencar et al. (2016). Y-axis represents evolutionary time (in millions of years).

Table 1. Average, standard deviation and range values for specific dynamic action (SDA), metabolic scope (MS), time to VO_2 peak (Tpeak), and SDA duration (SDAd) for juveniles and adults of five species of *Bothrops* with varying degrees of specialization in mammals.

		B. alternatus	*B. atrox*	*B. erythromelas*	*B. jararaca*	*B. jararacussu*
Juveniles	Mass (g)	239.11 ± 130.11 (97.00–440.00)	449.00 ± 193.59 (69.00–600.00)	152.31 ± 122.79 (46.00–340.00)	222.60 ± 87.09 (155.00–356.00)	204.17 ± 65.14 (140.00–319.00)
	SDA (KJ)	39.46 ± 22.16 (16.63–72.52)	59.25 ± 24.64 (12.09–83.10)	29.92 ± 24.32 (9.64–70.37)	36.73 ± 13.52 (22.71–54.33)	33.21 ± 10.64 (24.65–52.45)
	MS	7.99 ± 3.61 (4.84–17.29)	6.28 ± 2.02 (2.75–8.77)	8.28 ± 2.08 (5.39–11.40)	8.39 ± 0.66 (7.32–8.98)	6.50 ± 2.12 (4.11–8.90)
	Tpeak (days)	50.00 ± 8.49 (30.00–60.00)	48.00 ± 9.30 (30.00–54.00)	51.00 ± 7.17 (36.00–60.00)	44.40 ± 8.05 (30.00–48.00)	37.00 ± 8.83 (30.00–48.00)
	SDAd (days)	231.00 ± 30.37 (196.00–264.00)	234.00 ± 21.13 (192.00–252.00)	252.00 ± 12.00 (240.00–264.00)	240.00 ± 14.70 (216.00–252.00)	230.00 ± 15.95 (216.00–252.00)
Adults	Mass (g)	18.49 ± 2.15 (13.80–20.90)	15.09 ± 15.19 (4.00–39.60)	9.32 ± 2.16 (7.41–12.33)	15.09 ± 7.33 (8.60–26.40)	13.27 ± 1.58 (9.60–14.83)
	SDA (KJ)	2.07 ± 0.47 (1.23–2.61)	2.04 ± 0.70 (1.21–2.71)	3.06 ± 0.74 (2.08–3.90)	2.79 ± 2.29 (0.89–7.56)	4.14 ± 1.14 (2.81–6.49)
	MS	3.00 ± 0.47 (2.28–4.05)	6.61 ± 2.72 (3.73–12.14)	4.72 ± 0.98 (3.72–5.99)	2.76 ± 0.92 (1.91–4.17)	4.64 ± 1.05 (2.64–6.02)
	Tpeak (days)	38.57 ± 5.63 (30.00–48.00)	41.14 ± 9.44 (24.00–48.00)	31.50 ± 9.00 (24.00–42.00)	28.27 ± 4.55 (23.94–36.00)	32.00 ± 11.54 (24.00–60.00)
	SDAd (days)	154.29 ± 51.74 (96.00–240.00)	241.71 ± 33.53 (168.00–264.00)	231.00 ± 43.13 (168.00–264.00)	236.00 ± 37.24 (172.00–264.00)	253.00 ± 10.80 (240.00–264.00)

The values are expressed in KJ for SDA, number of the times that SMR increases during SDA peak for MS data, and hours after the meal for Tpeak and SDAd data.

Table 2. Results of ANCOVA for intraspecific phylogenetic independent contrasts (iPICs) of metabolic scope (MS), time to SDA peak (Tpeak), SDA duration (SDAd) and SDA value against body mass in relation to adult and juvenile snakes. Significant values are in bold. First models were tested for equal slope and the interaction term was retired in case of no-significant results. Df = degrees of freedom; bm = body mass; sp = species-type.

Variable	Age	H_0 = equal slopes (body mass*species)				H_0 = equal elevation (body mass+species)			
		Factors	Df; residual Df	F-value	p-value	Factors	Df; residual Df	F-value	p-value
MS	Adult	bm	1; 19	0.027	0.87	bm	1; 23	1.358	0.256
		sp	5; 19	3.496	**0.021**	sp	5; 23	4.141	**0.008**
		bm:sp	4; 19	0.312	0.867				
	Juvenile	bm	1; 29	0.09	0.767	bm	1; 33	11.898	**0.002**
		sp	5; 29	6.894	**< 0.001**	sp	5; 33	7.143	**< 0.001**
		bm:sp	4; 29	0.914	0.469				
Tpeak	Adult	bm	1; 19	0.977	0.335	bm	1; 23	0.061	0.807
		sp	5; 19	0.461	0.8	sp	5; 23	0.398	0.845
		bm:sp	4; 19	0.627	0.649				
	Juvenile	bm	1; 29	2.848	0.102	bm	1; 33	0.001	0.98
		sp	5; 29	2.359	0.065	sp	5; 33	1.723	0.157
		bm:sp	4; 29	1.795	0.157				
SDAd	Adult	bm	1; 17	2.102	0.165	bm	1; 21	0.01	0.922
		sp	5; 17	0.984	0.456	sp	5; 21	0.456	0.804
		bm:sp	4; 17	2.494	0.082				
	Juvenile	bm	1; 29	2.434	0.13	bm	1; 33	1.79	0.19
		sp	5; 29	0.527	0.754	sp	5; 33	1.497	0.218
		bm:sp	4; 29	1.199	0.332				
SDA	Adult	bm	1; 17	99.739	**< 0.001**	bm	1; 21	223.517	**< 0.001**
		sp	5; 17	0.864	0.525	sp	5; 21	1.552	0.217
		bm:sp	4; 17	2.278	0.103				
	Juvenile	bm	1; 27	1.971	0.172	bm	1; 31	38.785	**< 0.001**
		sp	5; 27	1.546	0.209	sp	5; 31	0.742	0.598
		bm:sp	4; 27	1.431	0.251				

cialization, referred to as feeding specialization index (FSI, Supplementary Table S1). To perform this categorization, we used a large dataset of stomach contents related to snake sizes previously collected (Stuginski et al. in preparation). Categories varied from low to high, where low (L) = almost do not eat mammal prey (0–24% of gut content represented by mammals); medium low (ML) = feed mostly on ectotherms but occasionally eat mammal prey (25–49% of gut content represented by mammals); medium high (MH) = feed mostly on mammal prey but occasionally eat ectothermic prey (50–74% of gut content represented by mammals); and high (H) = feed almost exclusively on mammal prey (75–100% of gut content represented by mammals). After categorization, we performed a general linear mixed-model testing the variation in SDA among the different feeding categories, in 'nlme' package (Pinheiro et al., 2016). We first pooled the dataset of juveniles and adult snakes together and considered the amount of food offered to individuals (food intake) as a covariate to avoid bias. The dependent variable was the residual of SDA, once body mass strongly influenced this parameter. We initiated the analysis testing for the interaction between SDA and food intake, with 'species' as a random factor. We used a backward elimination to choose a more parsimonious model with less parameters, based on Akaike information criterion with a correction for finite sample sizes ['AICc' function in 'AIccmodavg' package (Mazerolle, 2016)], following Burnham and Anderson (2002).

RESULTS

Differences among species

The means on SDA, MS, Tpeak, SDAd for adults and juveniles of each snake species are shown in Table 1. The iPICs of SDA, Tpeak and SDAd did not differ among the five *Bothrops* species, neither in adults nor in juveniles, although SDA scaled positively with body mass in both ontogenetic stages (Juveniles: estimate value = 0.303, t = 6.228, P < 0.001; Adults: estimate value = 0.147, t = 14.950, P < 0.001; Table 2 to access F-value). The independent contrast for MS, on other hand, presented significant differences among species in juveniles and adult snakes (Table 2; Fig. 2). In juveniles, *B. atrox* presented the lowest values of MS, differing from all other species (Bax – Ba dif. = −2.544; t = −4.637; P < 0.001; Bax – Be dif. = −2.706; t = −3.733; P = 0.006; Bax – Bj dif. = –3.095; t = −4.577; P < 0.001; Bax – Bjs dif. = −2.358; t = −4.335; P = 0.001), with no differences between other species. The MS in juveniles is also influenced by body mass, being that the heavier the juvenile the greater its metabolic scope (estimate value = 0.096, t = 3.449, P = 0.002; Table 2 to access F-value). In adult snakes, MS varies among *Bothrops* species independently of body mass, and in a different way from the pattern observed in juveniles. *Bothrops alternatus* presented the lower values of MS, differing from *B. jararacussu* (Ba – Bjs dif. = −4.565; t = −3.992; P < 0.005), and marginally from *B. atrox* and *B. erythromelas* (Ba – Bax dif. = −3.287; t = −2.875; P = 0.05; Ba – Be dif. = −3.201; t = −2.900; P = 0.056), with no differences between other species.

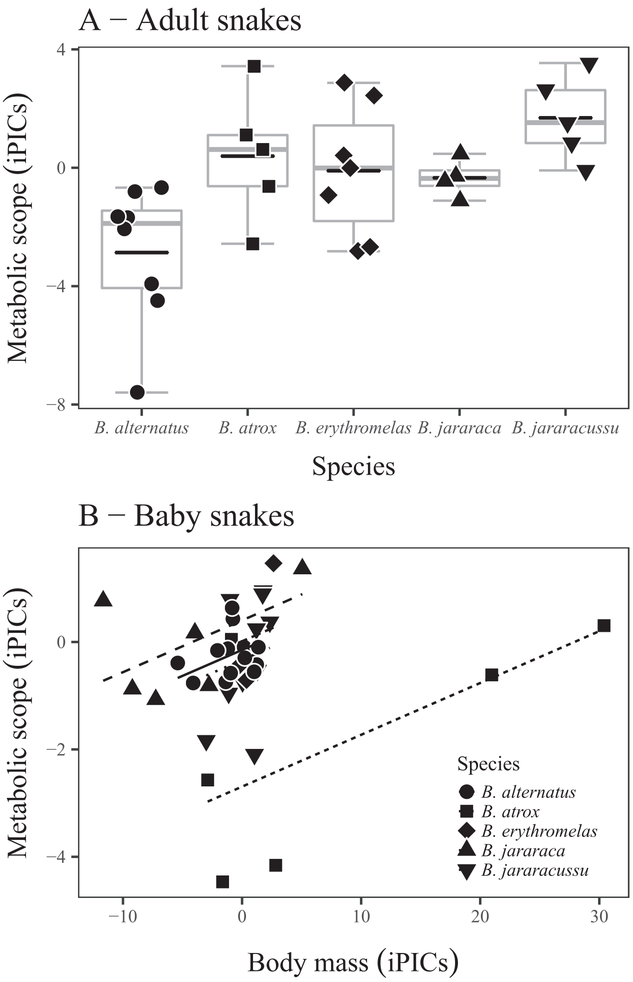

Fig. 2. Variation in metabolic scope of post-prandial energy expenditure between species of *Bothrops* snakes observed in adults **(A)**; and juveniles **(B)**. Note that in juveniles a positive relationship is also observed between MS and body mass.

Table 3. Random effect linear models and AICc test results for SDA, considering pooled dataset (juvenile and adult snakes). Symbols are related to each factor: FI = $\log_{10}$ of food intake (in grams), EC = ecological categories (i.e. high, medium-high, medium-low and low mammalian feeding specialization). Df = degrees of freedom. Values in bold are significative.

	Model Type	Dependent Variable	Factor (random fator)	Covariate	Associations	Df	χ^2	P-value	AICc	ΔAICc	Weight
1	Interaction model	residual SDA	ecological category (specie)	food intake	FI	1; 62	35.138	**< 0.001**	−86.681	4.843	0.082
					EC	3; 62	38.527	**< 0.001**			
					FI:EC	3; 62	5.826	0.120			
2	Reduced model	residual SDA	ecological category (specie)	food intake	FI	1; 65	39.014	**< 0.001**	−91.524	0.000	0.918
					EC	3; 65	94.639	**< 0.001**			
3	ANOVA model	residual SDA	ecologica category (specie)	–	EC	3; 66	7.607	0.055	−69.728	22.796	0.000

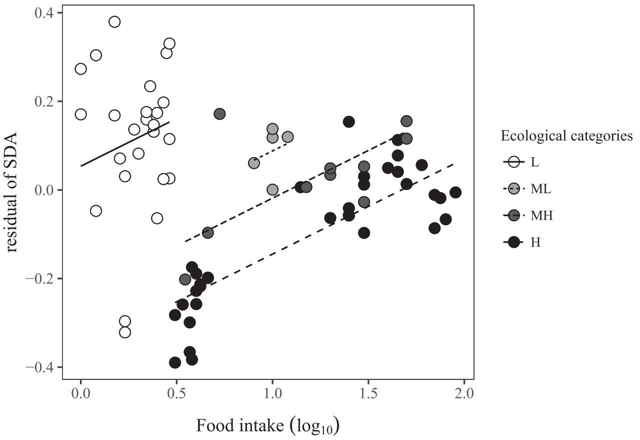

Fig. 3. Association between SDA (residual) and feeding specialization categories in *Bothrops* snakes, considering the food intake as a covariate, and specie-type as a random factor. Predicted values and points distribution showed that highly specialized snakes (H) spent less energy to digest a mammalian-prey, while generalists (L) presented the highest values to SDA.

Feeding specialization index

There is an evident decrease of residual of SDA as increases feeding specialization index in our pooled dataset (Table 3; Fig. 3). The residual of SDA was also positively correlated with food intake (Table 3; Fig. 3). Specifically, residual of SDA of the H category was lower than all other categories (H-L dif. = −0.414, $P < 0.001$; H-ML dif. = −0.233, $P < 0.001$; H-MH dif. = −0.126, $P = 0.014$), and low specialists (L) presented higher residual of SDA values than ML and MH (L-MH dif. = 0.287, $P < 0.001$; L-ML dif. = 0.181, $P = 0.018$), with no difference between ML and MH.

DISCUSSION

SDA and feeding specialization

Our results for five *Bothrops* species showed a considerable effect of feeding specialization on the energy cost of digestion, which largely agrees with the findings of Britt et al. (2006) that shows lower SDA values for snakes specialized in slugs when feeding its preferred food item. However, differently from previous work that studied two subspecies with divergent diet along their lifetime, our study employed species with distinctive specialization levels along the ontogenesis (Martins et al., 2002). The comparison among species differences, without taking mammalian feeding specialization into account, showed no SDA differences among species for all parameters measured except in MS (for both, juveniles and adults), independent of the phylogenetic relations among this particular group of *Bothrops*.

Differences in MS among *Bothrops* species however were not correlated to the decrease in energy amount employed in digestion (assessed by the SDA values), and the interpretation of these results remains elusive. Accepting that at least a part of the energy employed during SDA is actually obtained from the actual meal (Starck et al., 2004), lower MS but equal SDA values could represent different pre-prandial energetic reserves of the animals. Thus, a snake with low energetic reserves could, in theory, invest less energy in digestion before start using the energy provided directly from the meal to support the remaining digestion. However, if the energy necessary to promotes digestion must be mobilized from previously stocked energetic (according to the “pay before pumping theory” proposed by Secor and Diamond, 1995), such differences in MS should be related to other physiological issues instead the energetic investment. In the case of our study with *Bothrops* species, taking into account that we used only individuals in good health and body conditions, we strongly believe that such differences in MS are related to differences among species rather than differences in the amount of energetic reserves.

By testing for differences among snakes using their feeding specialization index (FSI) as a factor, we obtained a clear relationship between FSI and digestion cost (SDA) where the higher mammalian specialists individuals spend less energy to process their meals than those with a lesser degree of specialization. We were not able to perform experiments using other prey items (e.g., anurans or lizards) to discuss possible trade-offs associated with mammal feeding specialization related to the digestion of other kinds of food items (Britt et al., 2006; Britt and Bennett, 2008). However, our results support the hypothesis that feeding specialization has an impact on the energy balance during the post-prandial period.

The possible mechanism underlying coadaptation between digestive cost and feeding specialization in *Bothrops* remains unclear. However, it could be related to differences in: (1) the digestive cost *per se* and/or (2) the differential action of the venom. Assuming that most of the SDA is associated with the post-absorptive protein synthesis and gastrointestinal activity (Secor, 2003; Mccue, 2006; Enok et al., 2013), it is possible that this difference in SDA of more specialized snakes is related to a different cost during organ up regulation or membrane transporters activity (Lignot et al., 2005; Cox and Secor, 2006; Secor et al., 2010; Enok et al., 2013).

The role of venom in enhancing the digestion process has been extensively discussed and is a controversial matter in the literature (Nicholson et al., 2006; Mccue, 2007; Chu et al., 2009; Labonte et al., 2011; Alexander et al., 2012). In fact, venom composition has a strong ontogenetic component that could lead to different actions during juvenile and adult phases (Flachsenberger and Mirtschin, 1995; Andrade and Abe, 1999). However, studies involving crotalines with similar venom proprieties (*Trimeresurus stejnegeri*, *Trimeresus gracilis*, *Crotalus atrox*, and *Crotalus helleri*) did not detect any improvement in the energetics of snake digestive performance induced by the action of venom on experimental prey items (Mccue, 2007; Chu et al., 2009; Labonte et al., 2011). Furthermore, it should be noted that in our work we force-fed the snakes, thus it is unlikely that significant amounts of venom were inoculated.

Evolution of feeding specialization in snakes

Feeding specialization is sometimes associated with the use of a less exploited resource. For example, slug-eating snakes of the families Dipsadidae and Pareatidae are highly specialized in eating soft-body prey (i.e., molluscans) (Sazima, 1989; Salomao and Laportaferreira, 1994; Gotz, 2002; Hoso et al., 2007; Hoso and Hori, 2008; Ray et al., 2012; Zaher et al., 2014; Danaisawadi et al., 2015). Gastropods are resources poorly exploited by other snake groups so that, in the case of these snakes, feeding specialization (in terms of its ecological, morphological and toxicological aspects) might reduce competition with other snakes. Indeed, goo-eaters tend to feed frequently on small prey (i.e., prey with low prey-predator mass ratio; Otávio A. V. Marques, pers. comm.), a feeding strategy more similar to those of lizards than to those of most snakes (see Greene, 1983). The mammalian dietary specialization of some snakes could also potentially reduce competition with sympatric species that feed mainly on ectothermic prey (Martins et al., 2002). However, pit vipers are organisms with low maintenance costs, which are able to live under limited amounts of food (Beaupre, 1993; Cruzneto and Abe, 1994; Secor and Nagy, 1994; Beaupre and Duvall, 1998; Beaupre and Zaidan, 2001; Mccue and Lillywhite, 2002; Tsai et al., 2008). Suggestively, the extremely high density of *Gloydius shedaoensis* at Shedao Island, China, and of *Bothrops insularis* at Queimada Grande Island, Brazil (Shine et al., 2003; Marques et al., 2012) seems to be related mostly to the absence of predators and not to food abundance (Liu et al., 2010). Thus, competition for food resources seems not to be a plausible explanation for the appearance of feeding specialization in vipers.

Feeding specialization on mammals as a way to improve feeding performance seems to be a more reliable hypothesis for some snake taxa, especially those with reduced energy demands as the pit vipers. Mammals are particularly profitable food items in terms of energy content (KJ/g) when compared to other vertebrate prey animals of the same size (Bessler et al., 2010). This issue seems to be especially important for snakes that can achieve large size and rely on ambush foraging mode, as those animals usually exhibit lower feeding rates than active hunting snakes and are able to ingest larger prey than small ambush predators, and thus prey items highly profitable in terms of energy and with adequate size like rodents and other small mammals seem to be an excellent choice (Secor and Nagy, 1994; Zuffi et al., 2010). These factors may have favored dietary specialization in the vipers studied herein even when other food items were abundant. Further studies on other clades of vipers as well as other snake families would show how widespread the results described herein are shared by other snakes in general.

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COMPETING INTERESTS

None.

AUTHOR CONTRIBUTIONS

DS: Experimental design, conduct of experiments, interpretation of data and writing of the manuscript; CN: Conduct of experiments, interpretation of data and writing of the manuscript; FB: Statistical analysis, interpretation of data and writing of the manuscript; KG: Conduct of experiments and writing of the manuscript; MM: Interpretation of data and writing of the manuscript; JC: Experimental design, interpretation of data and writing of the manuscript.

SUPPLEMENTARY MATERIALS

Supplementary material for this article is available online (URL: http://www.bioone.org/doi/suppl/10.2108/zs170058).

Supplementary Table S1. Original dataset including all species and individuals.

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