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Aerobic metabolism during predation by a boid snake☆

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Abstract

We quantified the oxygen uptake rates ($\dot{V}O_2$) and time spent, during the constriction, inspection, and ingestion of prey of different relative sizes, by the prey-constricting boid snake *Boa constrictor amarali*. Time spent in prey constriction varied from 7.6 to 16.3 min, and $\dot{V}O_2$ during prey constriction increased 6.8-fold above resting values. This was the most energy expensive predation phase but neither time spent nor metabolic rate during this phase were correlated with prey size. Similarly, prey size did not affect the $\dot{V}O_2$ or duration of prey inspection. Prey ingestion time, on the other hand, increased linearly with prey size although $\dot{V}O_2$ during this phase, which increased 4.9-fold above resting levels, was not affected by prey size. The increase in mechanical difficulty of ingesting larger prey, therefore, was associated with longer ingestion times rather than proportional increases in the level of metabolic effort. The data indicate that prey constriction and ingestion are largely sustained by glycolysis and the intervening phase of prey inspection may allow recovery between these two predatory phases with high metabolic demands. The total amount of energy spent by *B. c. amarali* to constrict, inspect, and ingest prey of sizes varying from 5 to 40% of snake body mass varied inversely from 0.21 to 0.11% of the energy assimilated from the prey, respectively. Thus, prey size was not limited by the energetic cost of predation. On the contrary, snakes feeding on larger prey were rewarded with larger energetic returns, in accordance with explanations of the evolution of snake feeding specializations.

Keywords: Energetics; Feeding; Boidae; Snakes; Metabolism; Constriction; Predation; Prey ingestion

1. Introduction

Snakes have undergone an impressive adaptive radiation since their origin in the Cretaceous period (Greene, 1997; Pough et al., 1998). Beginning with fossorial ancestors, snakes diversified into an extraordinarily large number of species occupying almost all ecological niches in virtually all terrestrial environments except polar zones and high altitude regions (Greene, 1997). That notwithstanding, all living snakes share the same general body plan characterized by trunk elongation and limblessness features that influence many aspects of snake biology including feeding (Cundall, 1987; Pough et al., 1998). Elongation of the body results in a reduction of the cross-sectional area used for food acquisition, i.e. the gape of the mouth relative to body size (Gans, 1961, 1983; Pough et al., 1998). As a consequence, many species of snakes exhibit a number of morphological, physiological,

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and behavioral specializations for feeding (Cundall, 1983; Greene, 1983; Pough and Groves, 1983; Liem, 1990; Kardong et al., 1997; Young, 1999).

The feeding specializations in some snake groups were accompanied by shifts in foraging mode, from frequent ingestion of small prey to infrequent ingestion of relatively large prey (Greene, 1983). The adoption of these particular foraging modes required that the skull, particularly the jaw apparatus, be modified to increase the gape of the mouth since the prey is ingested whole (Cundall, 1987; Rodriguez-Robles et al., 1999). The skulls possess many distinctive osteological and muscular adaptations that facilitate the swallowing of prey with large cross-sectional areas (Gans, 1961; Cundall, 1987; Young, 1999). These same morphological specializations, however, made the skull a mobile, loose structure (Greene, 1997; Lee et al., 1999) unsuitable for the subjugation of large prey. It is thought, therefore, that snakes feeding on relatively large prey had to develop specialized strategies for prey subjugation (Gans, 1961; Kardong et al., 1997). Among the diverse techniques employed by snakes for capturing large prey, there are two extremes of a dichotomous division. (1) Envenomation, as seen in viperids, depending on a parenteral injection of venom by a rapid strike with a minimum of contact. The envenomated prey is released after the strike and relocated after death to be ingested (Sazima, 1992; Greene, 1992). (2) Constriction, typical of boid snakes, relying on the looping of the trunk around the prey and the exertion of pressure against it (Greene and Burghardt, 1978) causing circulatory arrest and death (Hardy, 1994). Unlike the situation with envenomation, prey constriction is associated with intense muscular activity that can last for extended periods of time (Greene and Burghardt, 1978; Moon, 2000).

The complex and unique suite of feeding specializations seen in snakes has been extensively examined from behavioral, ecological, and morphological perspectives (e.g. Arnold, 1983, 1993; Cundall, 1987; Mushinsky, 1987; Greene, 1992; Mori, 1996; Daltry et al., 1998; Marques and Puorto, 1998; Kardong and Berkhoudt, 1998; Andrade and Abe, 1999; Burghardt and Krause, 1999; Young, 1999; Rodriguez-Robles and Greene, 1999; Rodriguez-Robles et al., 1999). From a physiological perspective, however, most studies dealing with snake feeding largely focus on aspects of digestive physiology (Secor and Diamond, 1998; Andrade et al., 1997; Overgaard et al., 1999). In particular, the physiological and energetic aspects associated with the metabolic increase following meal ingestion (specific dynamic action, SDA) have attracted considerable attention in recent years (reviews in Wang et al., 2000; Secor, 2000). Nonetheless, SDA studies do not contemplate the physiological and energetic consequences associated with prey handling and ingestion and, therefore, such components of snakes feeding biology remain almost unstudied. To our knowledge, only two studies have addressed this subject. Feder and Arnold (1982) quantified anaerobic metabolism during predatory encounters by the snake, *Thamnophis elegans*, and Cruz-Neto et al. (1999) quantified aerobic metabolism during the ingestion of prey of different relative sizes by the viperid snake *Crotalus durissus*. As a consequence many aspects of the physiology and energetic of prey handling and ingestion in snakes are poorly understood, which hinder a better comprehension of the functional correlates of their feeding specializations, as well as of their energy budget.

Constricting snakes, particularly those belonging to the boid family, are well known for the use of intense muscular activity during prey handling (constriction) (Moon, 2000), and for being able to ingest a broad range of prey size. However, the metabolic consequences of handling and ingesting prey of different sizes have never been determined in a constricting snake. Therefore, the goal of the present study was to quantify the aerobic metabolism during prey handling and ingestion of prey of different relative sizes in a prey-constricting snake, *Boa constrictor amarali*. The primary objectives of our study were to: (1) address whether the energetic expenditure of predation could constrain the size of ingested prey, i.e. could ingestion of larger prey cost disproportionately more and result in a reduced energy reward?; and (2) uncover the possible physiological constraints, particularly in terms of gas exchange, that could arise from the constriction and ingestion of prey of different sizes.

2. Material and methods

2.1. Animals

We used 14 juvenile *Boa constrictor amarali* (mean mass = 658 ± 39 g) born in captivity from a gravid female collected in São Paulo state, southeastern Brazil. *Boa constrictor* has the widest

range of the Neotropical boines with a distribution extending from northern Mexico south to Argentina. It is a large ground-dwelling species growing up to 4.5 m (SVL) and preying upon a large variety of vertebrates including fishes, lizards, birds, and mammals (Henderson et al., 1995; Stafford and Henderson, 1996). We maintained animals individually in wood cages ($27\times41\times35$ cm) lined with cardboard and provided with lateral holes for ventilation. The snakes were fed mice every other week from birth and had free access to water. By the time of the experiments, animals were 3 years old and all appeared healthy. We fasted snakes for at least 20 days prior to experiments and did not use snakes that were molting.

2.2. Experimental protocol

We quantified the aerobic cost of predation in boas by measuring the rates of oxygen consumption during rest [resting metabolic rate (RMR); see Andrews and Pough, 1985] and during the capture and ingestion of rats weighing 5, 10, 20 or 40% of snake body mass (±1% in all cases). Each of the 14 snakes used in this study were tested once for each category of prey size, with an interval of 20 days between measurements. We randomized the order of prey size presentation to avoid possible effects of training on predation ability and to balance, as closely as possible, snake masses among experimental groups. Hereafter, we refer to groups that consumed prey equaling 5%, 10%, 20% or 40% of snake body mass, as *G*5%, *G*10%, *G*20% and *G*40%, respectively. All experiments were carried out in a constant temperature room at 30 ± 2 °C.

For metabolic measurements, we placed each snake in a metabolic chamber (4.8–5.7 l) where it was left undisturbed overnight, during this time room air was pumped through the chamber. The next morning, the respirometer was hermetically sealed and for the next 3 h we took an air sample every 40 min. Following this period we opened the chamber and introduced a rat of appropriate body mass that had been freshly killed by cervical dislocation. To entice the snakes to attack and constrict the prey it was necessary to move the dead rat inside the respirometer. To do this, the tail of the rat was tightly tied to a piece of plastic tubing (internal diameter = 0.5 mm) and this was led out of the metabolic chamber through a sealed hole in the lid. Both ends of the tubing were also sealed and by pulling the tubing manually, it was possible to move the dead rat inside the sealed chamber while preventing any air exchange between the chamber and the outside.

Initially, the dead rat was moved gently until it attracted the snake's attention, Once this occurred the snake would immediately strike the prey and start to constrict around it. At this point, we took an air sample from the respirometer. Constriction was further stimulated by pulling on the plastic tube (a 1 cm pull every 10 s for five times) following the beginning of constriction to simulate struggling by the prey. We assumed that the constriction phase had finished once the snake released its grasp on the prey and started to loosen the coils around it. At this moment, we took another air sample from the respirometer. After this, we waited for the beginning of the prey ingestion phase. We identified this phase as starting at the moment the snake first positioned its open jaws on the prey, and at this point we took another air sample. The final air sample was taken when the prey had just disappeared into the mouth of the snake and the tongue was first protruded. Thus, our measurements of prey ingestion include only the transportation of the meal into the digestive system; we do not consider meal transportation within the digestive system, for example, from the esophagus down to the stomach.

After prey ingestion, the chamber was quickly opened and the plastic tube tied to the tail of the prey was cut as close as possible of the mouth of the snake. All the events occurring inside the respirometer chamber were followed visually through a transparent window built into the lid of the chamber. The duration of constriction (T_{CT}), prey inspection (T_{IP}) (defined as the period from the end of constriction until the beginning of ingestion), and ingestion (T_{IG}) were recorded with a stopwatch. Only snakes that swallowed prey headfirst and that did not display signs of disturbance during the experiment were included in the analysis.

2.3. Respirometry

Air sample (10 ml) were taken with an airtight syringe attached to the respirometer by a three-way stopcock. To ensure that the samples were representative of the composition of the air inside the respirometer, we flushed air back and forth several times before taking the definitive sample.

Immediately after the samples had been taken, we injected it through a tube containing CO_2 and water vapor absorbers, into an oxygen analyzer (AMETEK S3A) at a flow rate of 150 ml/min. Oxygen fractional concentration of the air samples was read directly from the digital output of the O_2 analyzer.

The respirometer remained hermetically sealed during the experiment and, therefore, O_2 uptake rates could be calculated from the depletion of the oxygen content occurring between the collection of two consecutive air samples (see Vleck, 1987). We assumed that the average of the two lowest $\dot{V}O_2$ values measured before feeding trials was the RMR (see Andrews and Pough, 1985). The O_2 uptake rates during constriction ($\dot{V}O_{2CT}$), prey inspection ($\dot{V}O_{2IP}$), and ingestion ($\dot{V}O_{2IG}$) were calculated from the depletion of oxygen content in the air of the respirometer during T_{CT}, T_{IP} and T_{IG}, respectively.

2.4. Data analysis and statistics

We transformed $\dot{V}O_2$ values and prey mass to energetic equivalents by calculating the following variables: (1) energy content of the prey, assuming that each g of a rat wet mass yields 8.95 kJ (Smith, 1976); (2) net energy assimilated from the prey (EAP), assuming that boas have a capacity to assimilate approximately 80% of the total energy content of the prey (Greenwald and Kanter, 1974); (3) net energetic cost of constriction (E_{CT}), prey inspection (E_{IP}), and ingestion (E_{IG}), assuming that each milliliter of O_2 used in aerobic metabolism, after subtracting the cost of maintenance during this period (calculated from the RMR) was equivalent to 19.8 J (Gessman and Nagy, 1988). The relative cost of constriction (%E_{CT}), prey inspection (%E_{IP}), and ingestion (%E_{IG}), expressed as a percentage of the energy assimilated from the prey, were calculated as (the net cost of each predation phase/net energy assimilated from the prey)$\times$100. The relative cost of the whole predatory event (%E_{TOT}) was calculated as the sum of %E_{CT}, %E_{IP}, and %E_{IG}.

Since the same individual snakes were tested for each prey size category, we employed a one way repeated-measures ANOVA (Potvin et al., 1990) to test for the occurrence of significant differences among the four prey mass/snake mass groups. In cases in which data variation compromised the premise of normality and homoscedasticity of this test, we log-transformed the data prior to analysis. If log-transformation did not effectively achieve normality and homogeneity, we used a Friedman Repeated Measures ANOVA on Ranks on the raw data. Whenever a statistically significant difference was detected by the ANOVA, we employed a Student–Newman–Keuls (SNK) post-hoc test to reveal which pairs of groups differed from each other. Statistical procedures follow Potvin et al. (1990) and all values are presented as mean$\pm$S.E.M. Differences were considered statistically significant at the level of $P \leq 0.05$.

3. Results

3.1. General

There were no significant differences in snake body mass ($P=0.705$; see Table 1) or RMR ($P=0.920$; see Table 2) among the four experimental groups. All snakes promptly attacked the rats upon their introduction into the respirometer. The number of coils applied by *B. c. amarali* during constriction increased with prey size ($P=0.009$; Table 1) with snakes using less coils to constrict prey with masses equaling 5% of their own body mass than snakes in all other experimental groups ($P<0.05$ in all cases). The stimulus of pulling the rat during constriction appeared effective in simulating the struggle of a live prey during subjugation since we clearly noted that boas pressed body loops tighter around the prey in response to pulling.

3.2. Effects of prey size on time spent in constriction, inspection and ingestion

Times spent for prey constriction, inspection, and ingestion are given in Table 1. Prey size affected T_{CT} ($P=0.0005$), but *G*20% vs. *G*40% and *G*10% vs. *G*5% did not differ significantly from each other ($P>0.05$ in both cases). There was a linear increase in T_{CT} for prey with masses ranging from 5 to 20% of snake body mass, however, the increase in prey size from 20 to 40% did not cause constriction time to increase (see Fig. 1d).

Time spent for prey inspection differed among experimental groups ($P=0.00091$) and the groups that presented a significant difference between each other were *G*20% vs. *G*5%, *G*20% vs. *G*10%,

Table 1
Meal mass, snake body mass, number of coils applied during constriction (no. of coils), and time needed for prey constriction (T_{CT}), inspection (T_{IP}), and ingestion (T_{IG}) by *Boa constrictor amarali* feeding on rats equaling 5%, 10%, 20% and 40% of its own body mass (experimental groups) at 30 °C

	Experimental groups			
	5%	10%	20%	40%
Meal mass (g)	34.6±1.7 (23.3−48.8)	66±4.3 (40−94)	128.5±6.8 (82−172)	254.7±11 (168−314)
Snake body mass (g)	692±34 (465−975)	660±43 (400−940)	641±34 (410−860)	637±28 (420−785)
T_{CT} (min)	7.6±0.7 (4.3−11.3)	10.4±0.8 (4.8−16.1)	16.3±2.1 (6.5−30.1)	15±2.4 (4.6−35.7)
T_{IP} (min)	3.6±0.7 (1.2−8.4)	5.3±0.7 (1.1−9.3)	10.3±1.7 (2.8−21.5)	7.8±1.3 (1.4−16.9)
T_{IG} (min)	3.9±0.3 (1.6−5.6)	9.6±1.4 (4.5−20.2)	9.8±0.9 (2.1−15.8)	20±1 (15.5−26.3)
No. of coils	1.3±0.2 (0−2)	1.9±0.2 (1−3)	1.9±0.2 (1−3)	2.1±0.2 (1−3)

Values presented as mean±1 S.E.M. Values in parentheses denote minimum−maximum values. $N=14$ for all experimental groups.

and *G*40% vs. *G*5% ($P>0.05$ for all pairwise comparisons). T_{IP} increased almost linearly with prey size from 5 to 20%, however, prey size did not affect this variable between prey sizes equaling 20% and 40% of snake body mass (Fig. 1e).

Time spent for prey ingestion varied among the four experimental groups ($P<0.0001$), and the only groups that did not differ from each other were *G*10% vs. *G*20% ($P>0.05$, for both comparisons). Therefore, prey size produced a general increase in T_{IG}, but prey with masses ranging from 10 to 20% of snake body mass were ingested with the same time effort (Fig. 1f).

3.3. Effects of prey size on metabolism during constriction, inspection and ingestion

The effects of prey size on oxygen consumption rates during the different phases of predation by *B. c. amarali* are given in Table 2. Oxygen consumption rates increased only slightly with prey size, and this general pattern was similar for $\dot{V}O_{2CT}$ (Fig. 1a), $\dot{V}O_{2IP}$ (Fig. 1b), and $\dot{V}O_{2IG}$ (Fig. 1c). Accordingly, in all pairwise comparisons a significant difference was found only for $\dot{V}O_{2IP}$ between *G*5% and *G*40% and for $\dot{V}O_{2IG}$ between

Table 2
Rates of oxygen consumption during rest (RMR; $mlO_2\ g^{-1}\ h^{-1}$), during prey constriction ($\dot{V}O_{2CT}$; $mlO_2\ g^{-1}\ h^{-1}$), inspection ($\dot{V}O_{2IP}$; $mlO_2\ g^{-1}\ h^{-1}$), and ingestion ($\dot{V}O_{2IG}$; $mlO_2\ g^{-1}\ h^{-1}$) by *Boa constrictor amarali* preying upon rats equaling 5%, 10%, 20% and 40% of its own body mass (experimental groups) at 30 °C

	Experimental groups			
	5%	10%	20%	40%
RMR	0.035±0.004 (0.006−0.07)	0.039±0.003 (0.02−0.07)	0.043±0.007 (0.006−0.1)	0.039±0.004 (0.02−0.08)
$\dot{V}O_{2CT}$	0.234±0.02 (0.14−0.41)	0.23±0.02 (0.09−0.42)	0.276±0.05 (0.01−0.7)	0.325±0.04 (0.08−0.6)
$\dot{V}O_{2IP}$	0.14±0.02 (0.03−0.25)	0.15±0.01 (0.08−0.23)	0.19±0.03 (0.006−0.47)	0.23±0.03 (0.06−0.41)
$\dot{V}O_{2IG}$	0.15±0.02 (0.02−0.4)	0.15±0.01 (0.09−0.24)	0.212±0.03 (0.005−0.47)	0.251±0.02 (0.11−0.45)

Values presented as mean±1 S.E.M. Values in parentheses denote range of observations. $N=14$ for all experimental groups.

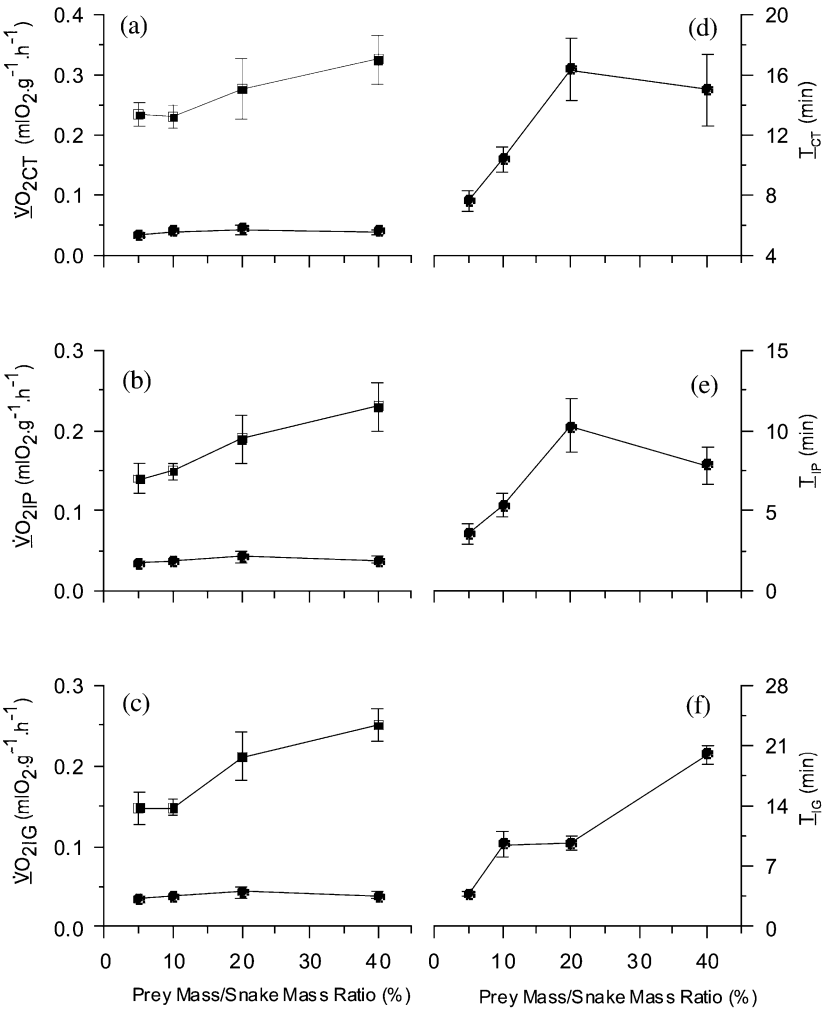

Fig. 1. Effects of prey size on the oxygen consumption rates and the duration of the different predation phases in *B. c. amarali* at 30 °C. $\dot{V}O_2$ variation during prey constriction (a), inspection (b), and ingestion (c) are shown on the left panel (■). The graphs a–c include the boas RMR values (●). Prey size effects on the duration of prey constriction (d), inspection (e), and ingestion (f) are showed on the right panel. Dots and transversal bars represent mean values and ±1 S.E.M, respectively. $N=14$ for all experimental groups.

*G*40% compared with *G*5% and *G*10% ($P>0.05$, in all cases). The average oxygen consumption rate, regardless of prey size, varied significantly among the different phases of predation ($P<0.0001$) with $\dot{V}O_{2IP}<\dot{V}O_{2IG}<\dot{V}O_{2CT}$ ($P<0.05$, in all cases). On average, $\dot{V}O_{2IP}$, $\dot{V}O_{2IG}$, and $\dot{V}O_{2CT}$ were 4.5, 4.9, and 6.8 times higher than RMR values, respectively.

3.4. Effects of prey size on the energetics of constriction, inspection and ingestion

The effects of prey size on the energetic expenditure during the different phases of predation by *B. c. amarali* are presented in Table 3. The amount of energy that could be assimilated from the prey increased with prey size ($P<0.05$ for all pairwise comparisons). Prey size did not affect the net energetic cost of prey constriction ($P=0.19$), however, this parameter tended to increase with prey size (Fig. 2a). However, expressed as % of the EAP, cost of constriction tended to decrease with prey size (Fig. 2d) being higher in G5% than all other experimental groups ($P<0.05$ for all pairwise comparisons).

No significant differences were found in the cost of prey inspection among the different prey

Table 3
Energetic profiles for *Boa constrictor amarali* feeding on rats with masses equaling to 5%, 10%, 20% and 40% of its own body mass (experimental groups) at 30 °C

	Experimental groups			
	5%	10%	20%	40%
EAP (kJ)	248 ± 12 (166 − 349)	472 ± 30 (286 − 673)	920 ± 49 (587 − 1231)	1824 ± 79 (1203 − 2249)
E_{CT} (kJ)	0.37 ± 0.07 (0.08 − 1.1)	0.43 ± 0.06 (0.11 − 0.8)	0.72 ± 0.14 (0.03 − 1.61)	0.93 ± 0.19 (0.1 − 2.64)
E_{IP} (kJ)	0.08 ± 0.02 (−0.01 − 0.24)	0.16 ± 0.03 (0.01 − 0.36)	0.29 ± 0.06 (0.001 − 0.67)	0.33 ± 0.08 (0.01 − 1.05)
E_{IG} (kJ)	0.1 ± 0.02 (−0.0014 − 0.27)	0.24 ± 0.06 (0.04 − 0.7)	0.3 ± 0.05 (−0.004 − 0.6)	0.85 ± 0.08 (0.4 − 1.45)
$\%E_{CT}$	0.14 ± 0.02 (0.05 − 0.32)	0.09 ± 0.012 (0.025 − 0.18)	0.078 ± 0.014 (0.003 − 0.16)	0.051 ± 0.01 (0.005 − 0.13)
$\%E_{IP}$	0.032 ± 0.006 (−0.005 − 0.076)	0.028 ± 0.006 (0.004 − 0.07)	0.032 ± 0.007 (0.0001 − 0.08)	0.019 ± 0.005 (0.001 − 0.05)
$\%E_{IG}$	0.039 ± 0.008 (−0.0006 − 0.11)	0.048 ± 0.008 (0.01 − 0.11)	0.034 ± 0.006 (−0.0003 − 0.08)	0.047 ± 0.004 (0.02 − 0.07)
$\%E_{TOT}$	0.211 ± 0.03 (0.071 − 0.42)	0.167 ± 0.019 (0.06 − 0.27)	0.143 ± 0.024 (0.003 − 0.27)	0.116 ± 0.014 (0.047 − 0.21)

EAP, net energy assimilated from the prey; E_{IP}, energy cost of constriction; E_{IP}, energy cost of prey inspection; E_{IG}, energy cost of ingestion; $\%E_{CT}$, percentage of EAP used for constriction; $\%E_{IP}$, percentage of EAP used for inspection; $\%E_{IG}$, percentage of EAP used for ingestion; $\%E_{TOT} = \%E_{CT} + \%E_{IP} + \%E_{IG}$. Values presented as mean ± 1 S.E.M. Values in parentheses denote range of observations. $N = 14$ for all experimental groups. *Note*: calculations were based on the values of the parameters presented in Tables 1 and 2, see text for details.

size groups, either as net values ($P = 0.246$) or as % of the EAP ($P = 0.23$). Nonetheless the lack of statistical significance, the net cost of prey inspection showed a trend to increase linearly for prey with masses ranging from 5 to 20% of snake body mass, with little increase occurring for prey larger than 20% (Fig. 2b). No particular pattern was recognizable for the variation in the cost for prey inspection (as % of EAP) as a function of prey size (Fig. 2E).

The net cost for prey ingestion increased with prey size ($P < 0.0001$; Fig. 2c), with significant differences occurring between snakes in *G*40% compared to all other experimental groups and between snakes in *G*20% compared to those in G5% ($P < 0.05$, for all pairwise comparisons). Nevertheless, when expressed as % of the EAP, ingestion cost did not vary significantly among the different experimental groups ($P = 0.389$), and no particular pattern was apparent for the variation in this variable as a function of prey size (Fig. 2f). The relative cost of the whole predatory event, expressed as a % of the EAP, varied among experimental groups ($P = 0.024$), being greater for snakes in *G*5% than those in *G*40% ($P < 0.05$). The general trend was for a decrease in the relative cost of the whole predatory event, expressed as % of the EAP, as prey size increased (Fig. 3a,b).

4. Discussion

4.1. General

The RMR of *B. c. amarali* measured in this study was 30% above the value predicted from the allometric equation derived for this species (Chappell and Ellis, 1987), but within the limit of accuracy of such equations (see Andrews and Pough, 1985).

The method we used to incite the snakes to attack prey was effective as was the method used to simulate prey struggle and induce constriction. It is possible, however, since we standardized the number of simulated prey movements during constriction, regardless of prey size, that our procedure may have eliminated some differences that would have existed between experimental groups arising from the fact that larger prey may have struggled more. Moon (2000) found that colubrid snakes responded to simulated prey heartbeats, lung ventilation, and muscular movement by increasing the applied muscular force during constriction of

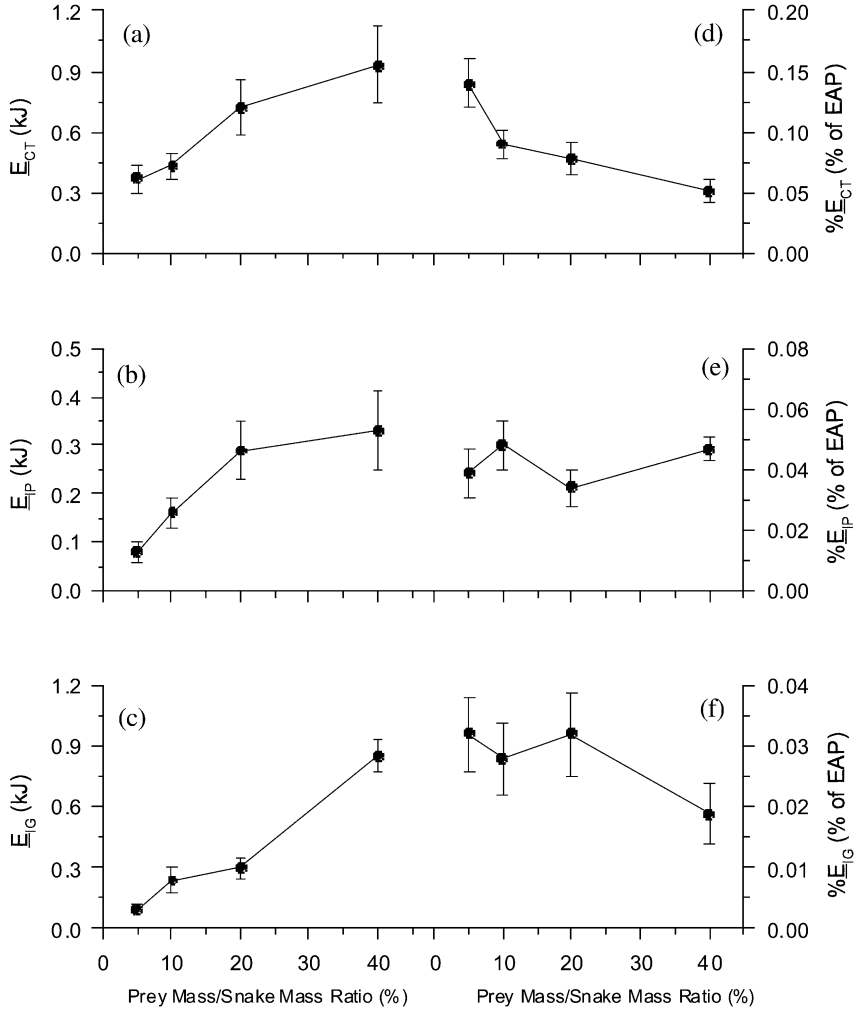

Fig. 2. Prey size effects on the energetics of the different predation phases in *B. c. amarali* at 30 °C. The left panel shows the variation in the net energy cost of prey constriction (a), inspection (b), and ingestion (c) as a function of relative prey size. The right panel presents the variation in the relative cost of prey constriction (d), inspection (e), and ingestion (f), expressed as % of the energy assimilated from the prey (% of EAP), as a function of relative prey size. Dots and transversal bars represent mean values and ± 1 S.E.M., respectively. $N = 14$ for all experimental groups.

rodent prey. Specifically, he found that the responses of the snakes were transitory and occurred only intermittently in response to each stimulus. Thus, it seems plausible that prey size, under natural conditions, could have a greater effect on prey constriction than was seen in our study. However, both we (this study) and others (Mendes, personal communication) have noted that prey struggle has limited, if any, effect on the number of coils used during prey constriction; the number of coils applied during prey constriction are set up immediately after the prey have been struck and usually do not change during the constriction process regardless of the amount of struggle exhibited by the prey.

4.2. Effects of prey size on time spent on constriction, inspection and ingestion

Constriction time (T_{CT}) increased with relative prey size from 5 to 20% snake body mass, but remained a plateau thereafter (Fig. 1d). These observations suggest that the time taken to subdue prey is less for small prey and increases with size to a point beyond which the forces developed during constriction are equally effective regardless of prey size.

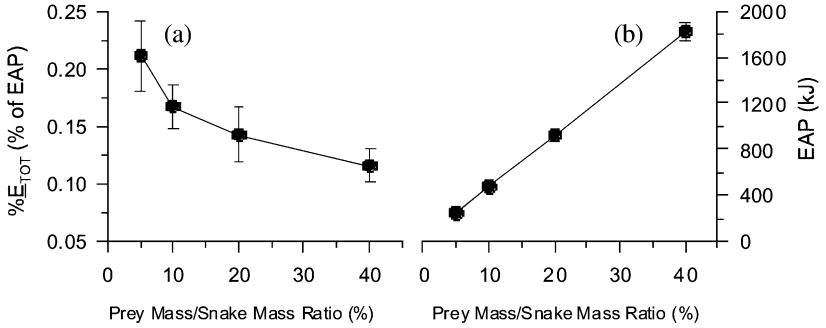

Fig. 3. Variation in the relative cost of the whole predatory event in *B. c. amarali* feeding on prey with different prey mass/snake mass ratios (a). This cost decreases with prey size, while the net energy assimilated from the prey increases linearly as a function of prey size (b). Dots and transversal bars represent mean values and ±1 S.E.M., respectively. $N=14$ for all experimental groups.

The time spent inspecting prey (T_{IP}) was shorter than the time spent in constriction and also increased with prey size in a similar fashion to the pattern observed for T_{CT} (Fig. 1e). During this time, it is thought that snakes assess whether the prey is dead, determine the direction in which the prey will be swallowed, and align their bodies in an optimal fashion for prey ingestion (see Sazima, 1992). None of these factors seem likely to be affected by prey size. The great similarity in the relationships between T_{CT} and T_{IP} and prey size, however, suggests that there is a causal link between the constriction and inspection phases. It is possible that the time spent inspecting the prey also serves to provide a recovery time following the metabolically active constriction phase and this is discussed further in the following section.

Ingestion time, T_{IP}, also increased with prey size (Fig. 1f). This suggests that as prey size increases, prey ingestion becomes more difficult. One can imagine at least two strategies that snakes could adopt to adjust ingestion to prey size: (1) maintain a constant level of metabolic effort but increase the duration of the process or; (2) increase the level of metabolic effort while keeping ingestion time to a minimum. Both strategies may be combined of course, but our data suggest that boas mainly adopt the first strategy. The second strategy (greater effort for a shorter period) may be precluded by functional constraints on ventilation and oxygen uptake during prey ingestion. Whatever the case, the strategy these snakes do employ means that they will remain in a vulnerable condition while ingesting larger meals. This increases their own risk of predation what may act as an ecological factor determining optimal prey size (see also Cruz-Neto et al., 1999).

4.3. Effects of prey size on metabolism during constriction, inspection and ingestion

During prey constriction the O_2 consumption rate of *B. c. amarali* increased by 6.8-fold above RMR. While there was a trend for the metabolic rate to increase with increasing prey size, this was not significant (Fig. 1a). The factorial aerobic scope for *B. c. amarali*, determined by submitting snakes to forced and strenuous locomotor muscular activity is 4.7 (Andrade et al., unpublished data) which is far below the values observed during prey constriction. This points out that the aerobic capacity of the muscles involved in prey constriction may be greater than those used for locomotion or, alternatively, that prey constriction can elicit greater levels of O_2 consumption than that attained during forced activity (argument also valid for prey ingestion, see below). Whatever the reason, it seems that during prey constriction *B. c. amarali* was operating near to their maximal level of aerobic metabolism since they were not able to increase metabolic rate further in response to relative prey sizes greater than 20%. Therefore, some of the energy required by the snakes to constrict prey may also be provided by anaerobic metabolism (see also Feder and Arnold, 1982; Cruz-Neto et al., 1999). This would lead to lactate accumulation during prey constriction and result in an oxygen debt. This in turn would lead to increased levels of metabolism during the inspection phase as the oxygen debt was repaid. Thus, it

seems plausible that prey inspection has the additional role of allowing recovery time between the energy demanding predation phases of constriction and ingestion and would explain why the relationship between $\dot{V}O_{2IP}$ and prey size paralleled that of $\dot{V}O_{2CT}$ and prey size (Fig. 1b). The high levels of O_2 consumption during prey constriction also indicate that gas exchange probably is not hindered during this predatory phase. Moon (2000) found that during prey constriction, snakes apply force only intermittently, thus it is possible that the relaxing intervals are important, not only to reduce the cost of the process but also to allow the snakes to breathe during the process.

O_2 consumption during prey ingestion increased 4.9-fold above the RMR, but this increase was not affected by prey size (Fig. 1c). Thus, as noted above, prey size affected T_{IG} more markedly than $\dot{V}O_{2IG}$. This indicates that the extent to which metabolism can increase during prey ingestion may be limited by blockage of the upper airways during prey ingestion (personal observation) constraining lung ventilation and limiting the increase in aerobic metabolism. If large increases in gas exchange are precluded by ingestion of large prey in *B. c. amarali*, it is also possible that the process is fueled, to some extent, by glycolysis. Feder and Arnold (1982) found that the colubrid snake *Thamnophis elegans* eating the salamander *Plethodon jordani* (a prey mass of 14% of the snake's body weight) generated as much as 26% of its total energy during prey ingestion from the anaerobic pathway. Similarly, Cruz-Neto et al. (1999) suggested that the viperid snake *Crotalus durissus* may also use anaerobiosis during prey ingestion, particularly for prey over 30% of the snake's body weight. All these examples are in accord with the general view that reptiles and amphibians rely heavily on glycolysis to sustain short-term increases in metabolic demand (Pough et al., 1998).

In *B. c. amarali* the metabolic increase following meal ingestion, SDA, can reach values ranging from three- to six-fold times above the RMR for snakes digesting prey equaling 5–40% of their body mass at 30 °C (Toledo et al., unpublished data). This post-prandial increase in metabolism, however, lasts for many days and is entirely supported by the aerobic metabolism (see Wang et al., 2000). Thus, the aerobic factorial scope during prey handling and ingestion in *B. c. amarali* are comparable with that observed during digestion, while differing in their time basis and, probably, in the incidence of anaerobiosis. Meal digestion involves tissues and processes diverse from those involved in prey handling and ingestion and, accordingly, may present differences in their capacity to support increased levels of metabolism.

4.4. Effects of prey size on energetics during constriction, inspection and ingestion

Clearly the cost of the constriction phase is the most expensive phase of predation by *B. c. amarali* (Fig. 2). Moreover, if part of the cost of prey constriction is included in estimates of the cost of prey inspection (as we have suggested in the preceding section) then this cost may be even higher. The energy expended in all three phases increased with prey size.

Animals invest both time and energy in obtaining food, and this has ecological consequences (Pough et al., 1998; see also previous sections). For a snake, prey size may become limiting, in energetic terms, if the costs associated with predation increase faster than the net profit provided by the prey (Cruz-Neto et al., 1999). However, the total aerobic energy cost of *B. c. amarali* for prey constriction, inspection, and ingestion equaled approximately 0.2% of the total energy that could be assimilated from the prey making it improbable that the size of rodent prey that *B. c. amarali* can capture and eat is limited by a negative energy balance (see Arnold, 1993). Similarly, the energetic cost for meal digestion, that involves higher levels of metabolism during extend periods of time, also does not seem to limit the size of prey eaten by *B. c. amarali*. For this species, the cost of meal digestion varies from 10 to 15% of the caloric content of the prey, in snakes fed with rats of sizes varying from 5 to 40% of snake body mass at 30 °C (Toledo et al., unpublished data). This observation suggests that digestion probably is the most expensive phase of feeding in snakes (see also Andrade et al., 1997; Cruz-Neto et al., 1999), and that it is more likely that prey size limitation be set by morphological, physiological, and ecological factors, as discussed in the preceding sections, rather than energetic constraints (see also Cruz-Neto et al., 1999).

Our data show that the energy assimilated from prey increases rapidly with relative prey size (Fig. 3b), and since the net energy spent for predation did not increase proportionately (Fig. 2a–c) this

caused the relative cost of predation (%) to decrease with prey size (Fig. 3a). Therefore, feeding on larger prey rewarded snakes with more net energy adding experimental support to the scenario proposed by Greene (1983) to explain the selective forces involved in the change from frequent ingestion of small items to infrequent ingestion of relatively large prey.

5. Concluding remarks

Prey size markedly affected the time spent, and metabolic cost of predation by *B. c. amarali*. During prey constriction aerobic metabolism increased to levels greater than the maximum aerobic capacity measured during forced locomotor activity. Prey size affected ingestion time, perhaps because prey ingestion limited gas exchange forcing the process to be prolonged. These data suggest that both high levels of muscular activity during prey constriction, and oxygen limitation during prey ingestion, may lead to some anaerobic glycolysis. Such observations lead us to further suggest that the intermediate phase of prey inspection could serve to provide snakes with recovery time between these two strenuous and, partially anaerobic, activities.

The aerobic cost of prey ingestion by *B. c. amarali* was somewhat greater than the value previously measured for the viperid snake *Crotalus durissus* (see Cruz-Neto et al., 1999). For example, to ingest a prey item with a relative body mass of 40% *B. c. amarali* will expend 0.047% of the EAP whereas *C. durissus* would expend approximately 0.02% of the EAP (calculated from Cruz-Neto et al., 1999). Since viperid snakes possess the most derived modifications in cranial morphology related to increase in gape size (Cundall, 1983; Pough and Groves, 1983), our data suggest that these specializations could render prey ingestion less costly for *C. durissus* than for *B. c. amarali*. Such a relationship, however, is based only on two phylogenetically distant species and should be taken as tentative and clearly deserving further examination.

The total amount of energy spent by *B. c. amarali* to constrict, inspect, and ingest prey of sizes varying from 5 to 40% of snake body mass was estimated to vary from 0.21 to 0.11% of the energy assimilated from the prey, respectively. These figures were largely influenced by the cost of prey constriction, which is the most energy consuming phase of predation. Since the energy provided by prey of increasing size increased faster than the cost to capture and ingest it, snakes benefited more from feeding on larger prey.

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