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Protein catabolism in pregnant snakes (*Epicrates cenchria maurus* Boidae) compromises musculature and performance after reproduction

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Abstract In many species the high energetic demands of reproduction induce a negative energy balance, and thus females must rely on tissue catabolism to complete the reproductive process. Previous works have shown that both fat and protein are energy resources during prolonged fasting in vertebrates. While many ecological studies on energy costs of reproduction have focused on variations in fat stores, the impact of protein investment on the female has not been thoroughly investigated. Notably, as there is no specialized storage form for proteins, intense catabolism is likely to entail structural (musculature) loss that may compromise maternal physical performance after reproduction. Measurements on captive rainbow boas (*Epicrates cenchria maurus*) confirm that reproducing females undergo significant protein catabolism (as indicated by elevated plasma uric acid levels) and show considerable musculature loss during gestation (as detected by reduced width of the epaxial muscles). Protein mobilization entailed a significant functional loss that was illustrated by decrements in tests of strength and constriction after parturition. In wild situations, such effects are likely to decrease the snakes' ability to forage and apprehend prey. Hence, the time period needed to recover from reproduction can be extended not only because the female must compensate

losses of both fat stores and functional muscle, but also because the ability to do so may be compromised. Performance alteration is likely to be of equal or greater importance than reduced energy stores in the physiological mediation of elevated post-reproduction mortality rates and infrequent reproductive bouts (e.g. biannual or triannual), two common ecological traits of female snakes.

Keywords Costs of reproduction · Musculature · Protein catabolism · Snakes · Viviparity

Introduction

Reproduction generally requires substantial energy investment, especially in females where energy and nutrients must be allocated to the developing ova and/or offspring (Clutton Brock 1991). Along with direct energy and nutrient allocation, reproduction frequently involves behaviors associated with maternal care (e.g., incubation of eggs, provisioning of food to offspring, offspring guarding) that result in substantial changes in maternal activity budget and energy balance and thereby constitute a significant investment (Clutton Brock 1991; Somma 2003). Reproduction thus imposes an extremely high energy demand, which requires the breeding organism to acquire substantial resources from its environment. In some cases, these resources can be gathered relatively rapidly through feeding. However, for most animal species, resources must be partially or completely accumulated over some period prior to their expenditure. In some cases, females may spend years accumulating sufficient energy resources between reproductive efforts (Bonnet et al. 1998).

The accumulation and use of internal energy resources are important facets of an animal's life history, with strong implications for many aspects of its ecology. Therefore, it is not surprising that this topic has become a major theme in recent research (Jönsson 1997; Bonnet

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et al. 1998), and published studies clearly reveal the importance of lipids as energetically dense resources (Sheridan 1994). Many species store large amounts of lipids, which can be mobilized for reproductive purposes at a later time (Bonnet et al. 1998). However, reproduction involves a diversity of materials other than lipids. For example, embryonic development requires essential amino acids (White 1991; Houston et al. 1995; Henen 1997, 2002) that must come from food intake or protein catabolism. In species where females have limited or no food intake during reproductive activity, such amino acids must originate from maternal protein resources. Furthermore, if food deprivation is prolonged, both lipids and proteins are mobilized to meet energetic requirements (Cherel et al. 1988, 1992; Oftedal 1993; Crocker et al. 1998; Cherel and Grocolas 1999; Schwilch et al. 2002). Lipids provide an energy-rich resource, but proteins provide the major means by which the body meets its essential need for glucose during fasting (via gluconeogenesis).

Interestingly, while proteins are also important to consider (Meijer and Drent 1999), many ecological studies on energy costs of reproduction have predominantly concentrated on lipid store dynamics in terms of female mass change. Such work has clearly demonstrated a positive relationship between body stores and reproductive parameters (e.g., reproductive frequency, reproductive output: Bonnet and Naulleau 1994; Chastel et al. 1995; Doughty and Shine 1997; Madsen and Shine 1999a; Crocker et al. 2001; Bonnet et al. 2001, 2002a, 2002b; Lourdais et al. 2002b). While protein mobilization has been extensively studied from a physiological perspective (Goodman et al. 1984; Lowell et al. 1986; Lowell and Goodman 1987; Cherel et al. 1992) only a limited number of ecological studies have considered the functional impact of extensive protein mobilization. Since protein exists predominantly as structural tissue (e.g., muscle), substantial proteolysis likely affects skeletal musculature and thus performance capabilities (Bordel and Haase 2000; Schwilch et al. 2002). In this context, if protein catabolism represents a considerable resource for the reproductive female, then the potential costs of reproduction are likely to be different than if only a change in lipid stores would have occurred.

Many squamate reptiles are lecithotrophic (i.e., provide nutrients to their developing offspring via yolk deposition during follicle development; Packard and Packard 1988) and thus invest massive amounts of energy stores (body fat) into follicular growth during vitellogenesis (Bonnet 1996). After ovulation, therefore, only limited amounts of stores are available (Bonnet et al. 2002b). Despite this lack of energy stores, a number of species endure an extended period during which they provide care to the developing embryos, either through brooding (oviparous forms) or embryo retention (viviparous forms, Shine 1985; Somma 2003). While these activities generally do not entail substantial amounts of energy investment, they impose considerable eco-physiological changes on the mother, most notably prolonged

food deprivation (Shine 1980; Ross and Marzec 1990; Madsen and Shine 1993, 1999b; Gregory and Skebo 1998; Gregory et al. 1999; Rivas 1999; Lourdais et al. 2002a). As a result, protein catabolism is likely to occur and introduce constraints on the female. Reptiles rely upon muscle strength for many fitness-related activities including foraging and predator avoidance (Cundal 1987; Moon 2000). Hence, a post-reproductive female with atrophied musculature may experience significant impairment in some or all of these activities, and such impairments would likely affect the time necessary to recover from a reproductive bout and may even affect survival.

Thus, investigating whether reproducing females sacrifice muscle strength to fuel their reproductive expenditure has direct implications on assessment of "costs" of reproduction in animals. Studies based solely on energy stores per se, although useful in their own right, fail to capture this aspect of reproductive costs. To clarify this issue, we studied captive viviparous snakes to (1) assess whether or not pregnant females exhibited significant protein catabolism, (2) examine potential effects of catabolism on muscle dimensions, and (3) explore the consequences of such muscular atrophy on fitness-relevant tasks related to predator escape and prey constriction.

Materials and methods

Study species and maintenance

Rainbow boas (*Epicrates cenchria maurus* Gray 1849) are medium-sized (up to 150 cm snout-vent length) non-venomous constricting snakes from South and Central America (Ross and Marzec 1990; Matz 2001). The snakes in our study (22 females, plus 20 males used for breeding) were obtained in 2001 from a long-term captive snake colony. Snakes were maintained in the laboratory in separate cages (50×50×20 cm) that provided free access to a heat source (temperature range in the cage: 28–33°C). Water was available ad libitum in a bowl, and snakes were fed rats (mean mass 150 g) once per month. Breeding occurred after a 1.5-month wintering period during which temperature was allowed to drop overnight (24°C, Ross and Marzec 1990). Ultrasonography was used to assess reproductive status. Ten females commenced reproductive activity in early spring and ovulated in late April 2002. Thus, there were two groups of females: non-pregnant ($n = 12$) and pregnant ($n = 10$). Although no specific data are available for wild populations of *E. c. maurus*, females of many boid species cease feeding after ovulation and do not resume feeding until after they have given birth (Ross and Marzec 1990; Rivas 1999). Thus, we did not provide food to any of the snakes during the period when reproductive snakes were pregnant (3.5 months: from late April to July). Litters were removed from maternal cages immediately after parturition in July, because post-parturient females often ingest their unfertile eggs

and stillborns. In the present study, one female ate infertile ova after parturition. Data from this snake were thus deleted from the analysis of post-parturient condition because such ingestion of ova can significantly replenish part of the lost energy reserves (O. Lourdais et al., unpublished observations).

Variables measured

For each snake, we measured (± 0.5 cm) total body length (BL) and snout-vent length (SVL) in early May (early gestation for pregnant snakes) and late July (after parturition of pregnant snakes). Body mass (± 1 g) was recorded once every 2 weeks. Initial BL, SVL, and body mass did not differ significantly between pregnant and non-pregnant snakes (all $P > 0.1$). We measured a series of variables to assess (1) energy costs of pregnancy, and (2) responses of females to pregnancy. Pregnant female snakes are likely to expend more energy than non-pregnant females if (1) they maintain higher body temperatures (Ladyman et al. 2003), and (2) their metabolic rates (and thus, energy expenditure) increase at higher temperatures (Huey 1982). To assess the validity of these assumptions, we measured body temperatures and metabolic rates in our snakes. To assess the occurrence and possible consequences of protein catabolism, we measured plasma uric acid levels, width of the main epaxial musculature, and effects of reproduction on muscular performance.

Thermal preferences

When cages were being cleaned, we recorded the position of the snake in its cage. Snakes were classified as “basking” if they were found coiled above the heat source.

We placed all snakes in a thermal gradient in late May (mid-gestation for pregnant snakes) and then again in late July (after parturition of pregnant snakes). The thermal gradient was a 3×3 m enclosure that had a heat source (radiator) on one end and a cooling source (continuously sprayed water) on the other, allowing animals to select temperatures from 20 to 40°C. The enclosure floor was a concrete slab allowing the snakes to move and thermoregulate without disturbance. Cloacal temperatures were measured 24 h after introducing the snakes to the enclosure in the late afternoon.

Metabolic rates

We used a flow-through respirometry system in which dry incurrent air was drawn through a small, clear Perspex metabolic chamber (internal volume: 15×20×28 cm), at a rate of 250.2 ± 6 ml min^{-1} by a Bioblock Scientific 2-l air pump. Flow was controlled using a Platon mass flow controller. The metabolic chamber was located within a sealed Cryosystem

temperature-controlled room and snakes could be observed during the trial through a small viewing port. The metabolic chamber was calibrated to the outside atmosphere (Pressure Indicator Druck DPI 260) and set at zero oxygen consumption by running an empty chamber for 1 h prior to each snake being tested (Ladyman et al. 2003). Snakes were run for as long as necessary to obtain stable oxygen consumption for a period greater than half an hour. Animals were placed into a temperature-controlled room the night prior to testing to acclimate them to the trial temperature. After being placed in the metabolic chamber, the snake was covered with a small piece of cloth, which provided shelter. Using this method, snakes showed limited exploratory movements, rapidly coiled, and stay motionless during the entire trial. Data were obtained on eight pregnant and eight non-pregnant females of similar body sizes ($P > 0.20$). For each snake, we measured rates of oxygen consumption (ml h^{-1}) at two temperatures corresponding to mean thermal preferences of pregnant and non-pregnant snakes (33°C and 29°C, respectively, as determined from the body temperature measurements obtained in the thermal gradient). Snakes were tested randomly throughout the day to negate any effect of diel cycle.

Uric acid levels

Plasma uric acid levels provide a reliable indication of protein breakdown in uritelic vertebrates. Blood samples were obtained by cardiocentesis in late May (mid-gestation) and late July (1 week after parturition for pregnant females). Plasma uric acid values were determined by an automatic spectrophotometer (Technicon RA1000) and Biomérieux enzymatic reagents. Total uric acid ($\mu\text{mol l}^{-1}$) was assayed using a coupled enzymatic system involving uricase and peroxidase.

Changes in epaxial musculature

In snakes, complex epaxial muscles lying beside the vertebral column are of primary importance for locomotion (Cundall 1987) and constriction (Moon 2000). Magnetic resonance imaging (MRI) of epaxial musculature in *E. c. maurus* has verified that external measurements of muscle width with callipers provide accurate estimates of actual muscle size (O. Lourdais et al., unpublished observations). We thus measured the width of the epaxial muscles at a single point (located at mid-SVL) in late April (early gestation) and mid July (late gestation). Shortly after parturition (late July for non-pregnant snakes), we measured the width of the epaxial muscles at four-equally spaced points between the head and the vent.

Performance measures

Boid snakes rely upon muscular contractions to generate the force needed to subdue and kill prey by

constriction, as well as to escape from predators (Cundall 1987).

We measured muscle strength of post-partum snakes (non-pregnant females were tested at the same time) in three different contexts designed to mimic the following biologically important situations.

Escape from a predator

These snakes react to handling, especially attempts to stretch out their bodies, by vigorous and extended body contraction. We quantified the strength of these contractions using a dynamometer connected to the snake's tail (Pesola, Baar, Switzerland). Snakes were stretched and then stimulated to provoke body contractions. A drag pointer on the dynamometer recorded the maximum tension during a five-minute period of repeated stretching and contraction.

Prey handling

We measured the intensity of muscular contraction during prey constriction using a compressible lure (10 cm long×2 cm diameter water-filled rubber balloon surrounded by mouse-scented cloth; O. Lourdais et al., unpublished observations). The lure was connected to an open water column via a rigid plastic tube (5 mm in diameter). When presented with the lure, snakes readily bit and coiled around the lure, which was then wiggled for 10 s to simulate normal prey reaction. Displacements of the water column were videotaped so that maximal displacement could be subsequently measured (cm).

Climbing ability

When held by the tail, these snakes climb up their own body towards the hand of the operator. We measured the length of time required for the snake's head to reach the operator's hand on three consecutive trials.

Statistics

All statistics were performed with Statistica 6.0. Data on thermal preferences and uric acid levels were not normally distributed and were therefore examined using non-parametric tests. To evaluate changes in musculature width during gestation we performed *T*-tests on paired samples. Metabolic rates were examined using repeated-measures analysis of variance (ANOVA) with oxygen consumption as the dependent variable and both reproductive status and temperature as the factors. Differences in musculature profile after parturition were examined using ANCOVA analysis using muscle width as the dependent variable, reproductive status as the factor, and SVL as the covariate. Finally, differences in performances were assessed using *T*-tests for independent samples. Unless otherwise stated, values are reported as mean ± SD.

Table 1 Variation in the number of individuals found coiled on the basking source during six random observations during gestation (*P* pregnant, *NP* non-pregnant)

Observation no.	<i>P</i> (<i>n</i> = 10)	<i>NP</i> (<i>n</i> = 12)	χ^2	<i>df</i>	<i>P</i>
1	9	5	5.50	1	0.018
2	7	1	8.96	1	0.002
3	6	5	0.73	1	0.39
4	9	2	7.82	1	0.002
5	9	4	7.24	1	0.007
6	9	6	4.02	1	0.044

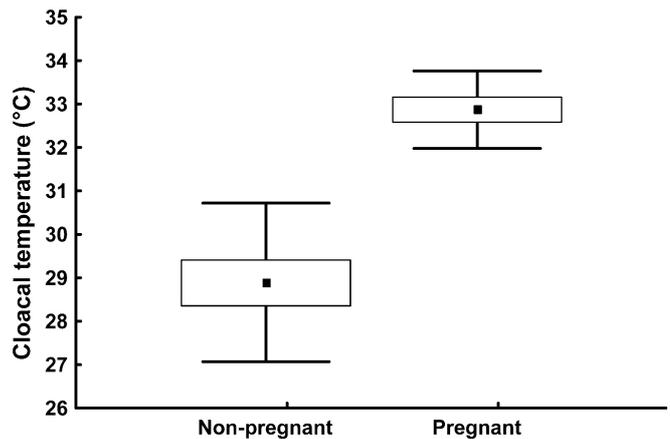


Fig. 1 Mean selected temperatures (°C) measured in non-pregnant and pregnant female rainbow boas. *Black squares* represent mean values, *open squares* represent standard error, and error bars represent standard deviation

Results

Thermal and metabolic changes

Thermal preferences: pregnant females were found coiled above the heat source significantly more often than were non-pregnant snakes (Table 1). Similarly, pregnant snakes selected significantly higher body temperatures in a thermal gradient (Kolmogorov-Smirnov test, $P < 0.001$; Fig. 1). Interestingly, body temperatures were less variable among pregnant than non-pregnant females (Kolmogorov-Smirnov test, $P < 0.05$, using standard deviation as the dependent variable; Fig. 1). Selected temperature differences disappeared when we tested the snakes in the thermal gradient after parturition (mean values 26.5 ± 2.4 and 26.7 ± 2.1 °C, respectively, for post-pregnant and non-pregnant females).

Metabolic rates

A repeated measure ANOVA showed that oxygen consumption was significantly higher at 33°C than at 29°C [$F_{(1,14)} = 66.3$, $P < 0.00001$; Fig. 2], whereas no significant differences were detected between pregnant and non-pregnant animals [$F_{(1,14)} = 3.04$, $P = 0.14$; Fig. 2].

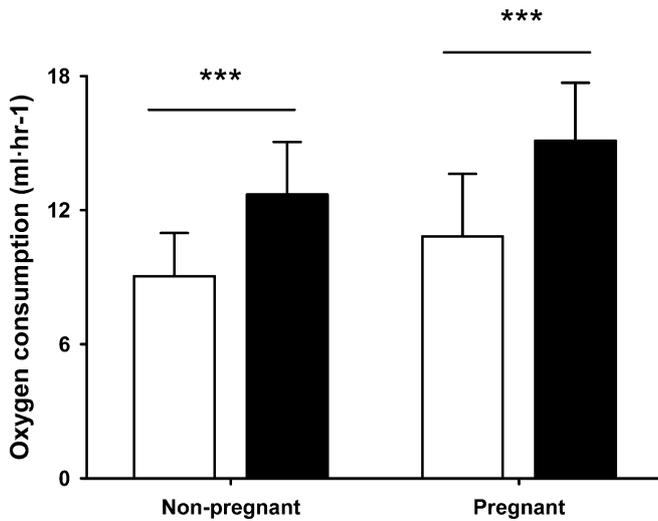


Fig. 2 Mean oxygen consumption ($\text{ml O}_2 \text{ h}^{-1}$) measured at 29°C (open bars) and 33°C (black bars) in non-pregnant and pregnant female rainbow boas. Error bars represent standard deviation

Evidence of protein catabolism

Uric acid levels (Fig. 2): plasma uric acid levels were significantly higher in pregnant compared to non-pregnant females (mean values 318 ± 118 versus $178 \pm 37 \mu\text{mol l}^{-1}$, respectively; Kolmogorov-Smirnov Test, $P < 0.01$). No such difference was detected after parturition (mean values 193 ± 78 versus $172 \pm 49 \mu\text{mol l}^{-1}$, respectively, for post-pregnant and non-pregnant females; $P = 0.39$).

Changes in epaxial muscle dimensions: both non-pregnant and pregnant females lost significant mass over the 4-month period without food [T -test for paired samples: $t_{(1,20)} = 6.11$, $P < 0.001$]. However, daily rate of mass loss was higher in pregnant than in non-pregnant females [respective mean values -3.10 ± 0.81 versus $-1.12 \pm 0.59 \text{ g day}^{-1}$; T -test for paired samples: $t_{(1,20)} = -3.11$; $n = 22$, $P = 0.005$]. Repeated measurements of the width of the epaxial muscles indicated a significant decrease over time [T -test for paired samples: $t_{(1,20)} = 6.40$, $P < 0.001$], with pregnant females decreasing more than non-pregnant animals [T -test for independent samples: $t_{(1,20)} = 2.59$, $P < 0.02$].

Structural and functional impact

Musculature profile after parturition: the influence of reproductive status on post-partum musculature was examined using an ANCOVA procedure with muscle width as the dependent variable, reproductive status as factor, and SVL as the covariate. Musculature profiles diverged strongly between the two groups of snakes with post-partum females significantly less muscular than non-pregnant animals for three of the four measurement sites considered (Fig. 3).

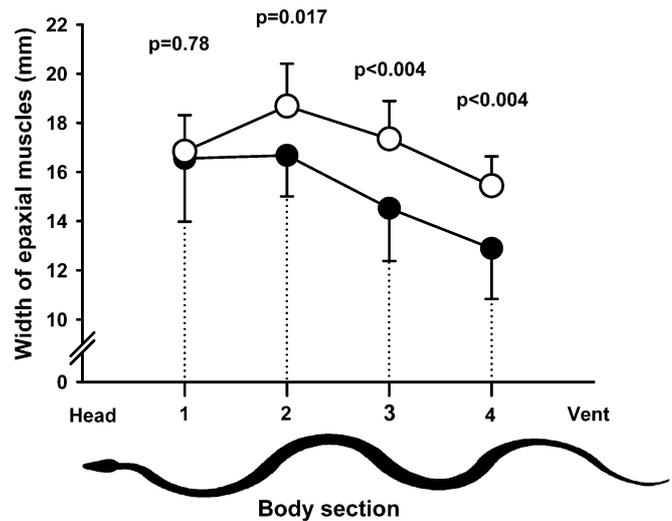


Fig. 3 Musculature profiles measured in non-pregnant (open circles) and post-pregnant (black circles) female rainbow boas. Width of epaxial muscles (mm) was measured at four equidistant points between the head and the vent (1–4 starting from the head). P values were obtained from ANCOVA analysis using muscle width as the dependent variable, reproductive status as the factor, and snout-vent length (SVL) as the co-factor. Error bars represent standard deviation

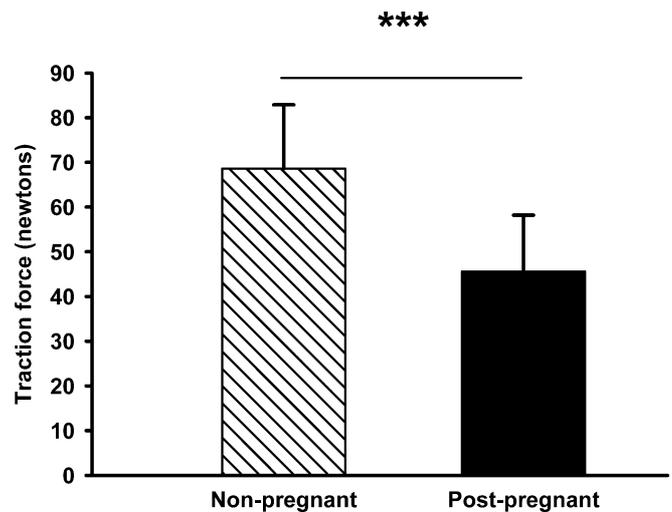


Fig. 4 Mean traction force (N) measured in non-pregnant and post-pregnant female rainbow boas. Error bars represent standard deviation

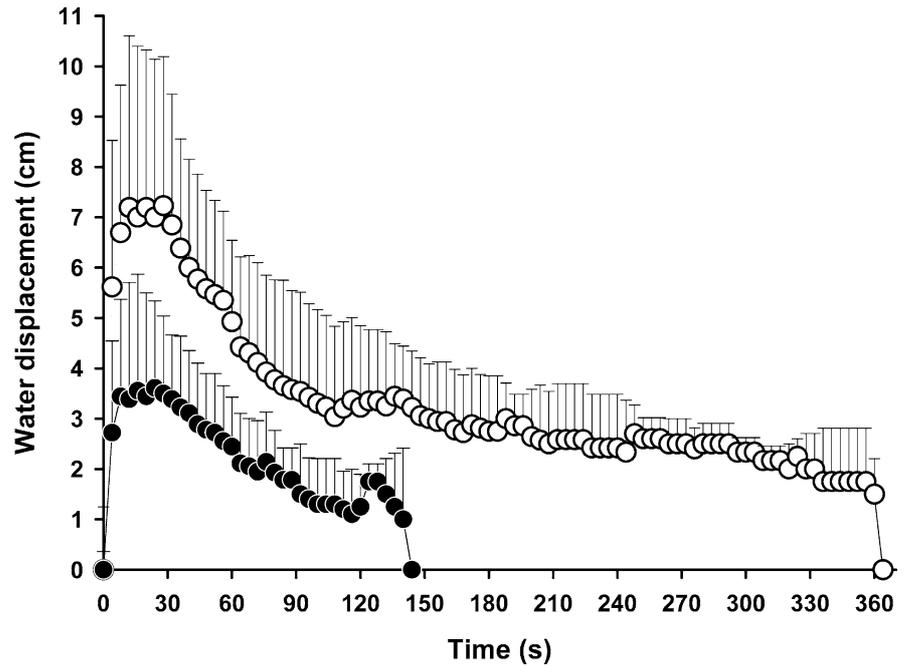
Physical performance

Post-partum snakes had reduced performance relative to non-reproducing ones in each of the three parameters measured.

Contraction strength

Contraction strength was consistently lower in post-partum females relative to non-pregnant females tested at the same time [T -test for independent samples: $t_{(1,19)} = -3.83$; $n = 21$; $P < 0.001$; Fig. 4].

Fig. 5 Constriction performance (cm of water displaced) measured in non-pregnant (*open circle*) and post-pregnant (*black circle*) female rainbow boas. The time interval between values is 4 s. Error bars represent standard deviation



Constriction strength

Constriction performance diverged between the two groups of snakes, with non-pregnant females displaying maximal constriction values twice that of post-partum females [*T*-test for independent samples: $t_{(1,19)} = -3.27$; $n = 21$; $P < 0.005$; Fig. 5]. Interestingly, the non-pregnant snakes also maintained their constriction for much longer than did the post-partum snakes [mean values 234 ± 100 versus 112 ± 36 s, respectively; *T*-test for independent samples: $t_{(1,19)} = -3.45$; $n = 21$; $P < 0.002$; Fig. 5].

Climbing ability

Finally, climbing ability was also affected by reproduction. When suspended by the tail, post-partum snakes took significantly more time to reach the hand of the operator than did the non-pregnant females [mean values 31 ± 17 s versus 13 ± 5 s, respectively; *T*-test for independent samples, $t_{(1,19)} = 3.50$; $n = 21$; $P < 0.002$].

Discussion

This work provides the first quantitative evidence for significant protein catabolism during pregnancy in squamate reptiles. As previously documented by experimental and field studies (Shine 1980, 1985; Guillette 1982; Charland and Gregory 1990; Ladyman et al. 2003), we found that gestation strongly impacted female energy balance. For instance, selection of higher body temperatures during gestation dramatically increased maternal rates of oxygen consumption. More importantly, we showed that in the absence of food intake

during pregnancy, this higher metabolism led to an elevated level of proteolysis, as indicated by higher plasma uric acid concentration and a significantly greater loss of epaxial musculature. It is important to note that withholding food throughout the 4-month gestation period (as in our study) offers a reasonable simulation of circumstances occurring in nature. Field studies have demonstrated that pregnancy is associated with a reduction or cessation in food intake in many snakes (Shine 1980; Madsen and Shine 1993; Gregory and Skebo 1998; Gregory et al. 1999; Lourdais et al. 2002a) including boids (Ross and Marzec 1990; Rivas 1999). Thus, significant levels of protein catabolism, and hence a reduction in maternal musculature, are likely to be observed in the wild as well as in the artificial conditions of captivity. In fact, the extent of protein catabolism required for reproduction is likely even greater than demonstrated here, since our study focused solely on post-ovulatory implications of pregnancy. Most reptiles are lecithotrophic and thus provide the vast majority of energy and nutrients to their young via the formation of yolk that contains a substantial amount of protein (White 1991; Henen 1997, 2002). Therefore, it is likely that vitellogenesis also puts a significant demand on maternal protein resources. If one considers the combined impact of vitellogenesis and pregnancy on energy demands as well as the length of anorexia, the extent of protein catabolism in natural situations would presumably be even greater than that detected in our study.

In addition to verifying the use of protein resources to support reproductive effort, our work provided an original assessment of the functional impact of protein loss on maternal performance after parturition. We found that the reduced epaxial muscle mass of post-

parturient females strongly impacted various estimators of physical performance. First, traction forces, which are closely related to epaxial musculature (O. Lourdais, F. Brischoux, L. Barentin, unpublished observations), were much lower in post-parturient females. Second, the ability to constrict prey was severely compromised in terms of force and duration of muscular contraction (Fig. 5). The fact that muscle atrophy was concentrated in the posterior rather than anterior part of the female's body (Fig. 3) is interesting in this respect. The anterior muscles are likely to be most important for prey constriction (Moon 2000) and, thus, the snakes may benefit from selective catabolism of the least critical muscles. Finally, the behavioral response to dangling was also modified, as climbing times were longer for post-pregnant females. Importantly, the performance traits examined are likely ecologically relevant. Traction forces measured here provide a good index of defense capability and our results suggest a possible delayed impact of gestation on female anti-predator responses. Similarly, constriction characteristics represent an indicator of the ability to hold and kill a prey item. Lowered constriction competency thus may reduce foraging success. Though very little is known about the biology of *E. c. maurus* in its natural habitat, major activities such as predator escape, prey constriction, and arboreal maneuverability likely affect maternal fitness.

Our results clearly demonstrate the importance of protein catabolism in supporting reproduction as well as the impact that such catabolism has on post-parturient performance. Since protein catabolism leads to impaired performance, one might expect females to minimize the duration of time in which they are in post-ovulatory negative energy balance (as a result of increased thermal preference and anorexia). However, viviparity has evolved independently in more than 100 lineages of lizards and snakes (Shine 1985), suggesting a considerable benefit to this mode of reproduction, at least in some ecological contexts. One major advantage of egg retention is probably improved regulation of developmental conditions (Shine 1985). Reptile eggs and embryos are very sensitive to ambient thermal conditions (Packard and Packard 1988; Shine 1991), and egg retention allows females to actively (typically through behavioral means) regulate the temperature experienced by the embryo. Conversely, egg-retention substantially lengthens the post-ovulatory state and thus exacerbates reproductive costs by (1) extending the increased metabolism associated with the maintenance of high body temperature, and (2) inducing dramatic changes in thermoregulatory behavior that may well be the proximal cause of reduced food intake. Thus, egg retention places greater demand on functional energy resources that may affect the female after reproduction (this study). Therefore, the evolution of viviparity is likely based on a trade-off between costs to the female (in terms of functional capacity as well as energy) versus benefits to the offspring.

Previous works on squamates clearly emphasize high reproductive costs imposed by the viviparous reproductive mode (Brown 1993; Madsen and Shine 1993; Bonnet et al. 1999, 2002a, 2002b). For instance, in European vipers post-partum females are often emaciated and suffer high rates of mortality (Madsen and Shine 1993). In addition, the degree of maternal emaciation after parturition predicts the probability of female survival in the year following reproduction (Bonnet et al. 1999). Along with an increased survival cost, viviparity classically is associated with low reproductive frequency with extensive delays (sometimes several years) between successive reproductive efforts (Bull and Shine 1979). Such attributes generally have been interpreted and discussed in terms of reduced body reserves. Our studies provide a novel perspective on this issue in showing that protein as well as fat stores may be important substrates that are utilized during reproduction. Reduced performance as a result of muscle catabolism would be likely to increase an animal's vulnerability to predation and to decrease its foraging effectiveness. This latter effect may extend the timescale over which females pay "costs" of pregnancy beyond that which might be expected based on simple energy budget estimates (Jönsson et al. 1995; Jönsson 1997; Bonnet et al. 1999). Hence, the high mortality rates and low reproductive frequencies displayed by many viviparous squamates may reflect slow rates of recovery in body condition (in turn due to compromised functional abilities) as well as depleted energy reserves.

In conclusion, we have shown that gestation is associated with intense protein mobilization and significant structural and functional decrements in a squamate reptile. Protein catabolism has been frequently reported in vertebrates, notably in endotherms, and the metabolic pathway has been studied extensively (Cherel et al. 1992 and references therein). The functional costs of intense protein use have attracted much less attention, and further studies are needed to more fully understand this topic. For instance, the degree to which protein catabolism is required may well depend upon post-ovulation fat reserves or feeding rates during pregnancy, which in turn depend upon local conditions of prey availability. Thus, the degree of reliance on catabolism may differ even among females within a single population, or between successive pregnancies of the same female. Additionally, comparative studies of protein catabolism would clarify the costs and benefits of maternal care in reptiles. For example, it would be interesting to examine whether similar performance costs exist in taxa showing alternative forms of parental care such as egg brooding. Many female pythons show prolonged periods of egg attendance (Madsen and Shine 1999b; Somma 2003) during which food intake does not occur and, in some cases, active metabolic heat production does (i.e., shivering thermogenesis: Slip and Shine 1988; Pearson et al. 2003). Such situations are likely to induce costs similar to those detected in this study of a viviparous species.

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References

- Birchard GF, Black CP, Schuett GW, Black V (1984) Influence of pregnancy on oxygen consumption, heart rate and haematology in the garter snake: implications for the cost of reproduction in live bearing reptiles. *Comp Biochem Physiol* 77:519–523
- Bonnet X (1996) Gestion des réserves corporelles et stratégie de reproduction chez *Vipera aspis*. Thèse de Doctorat, Université Claude Bernard, Lyon I
- Bonnet X, Naulleau G (1994) Utilisation d'un indice de condition corporelle (BCI) pour l'étude de la reproduction chez les serpents. *C R Acad Sci* 317:34–41
- Bonnet X, Bradshaw SD, Shine R (1998) Capital versus income breeding: an ectothermic perspective. *Oikos* 83:333–341
- Bonnet X, Naulleau G, Shine R, Lourdaïs O (1999) What is the appropriate time scale for measuring costs of reproduction in a capital breeder? *Evol Ecol* 13:485–497
- Bonnet X, Naulleau G, Shine R, Lourdaïs O (2001) Short-term versus long-term effects of food intake on reproductive output in a viviparous snake (*Vipera aspis*). *Oikos* 92:297–308
- Bonnet X, Lourdaïs O, Shine R, Naulleau G (2002a) Reproduction in snakes (*Vipera aspis*): costs, currencies and complications. *Ecology* 83:2124–2135
- Bonnet X, Naulleau G, Lourdaïs O (2002b) The benefits of complementary techniques: using capture-recapture and physiological approaches to understand costs of reproduction in the asp viper. In: Schuett GW, Hoggren M, Greene HW (eds) *Biology of the vipers*. Biological Science Press, Traverse City, MI
- Bordel R, Haase E (2000) Influence of flight on protein catabolism, especially myofibrillar breakdown, in homing pigeons. *J Comp Physiol B* 170:51–58
- Brown WS (1993) Biology, status and management of the timber rattlesnake (*Crotalus horridus*): a guide for conservation. *Herpetol Circ* 22:1–78
- Bull JJ, Shine R (1979) Iteroparous animals that skip opportunities for reproduction. *Am Nat* 114:296–316
- Calow P (1979) The cost of reproduction—a physiological approach. *Biol Rev* 54:23–40
- Charland MB, Gregory PT (1990) The influence of female reproductive status on thermoregulation in a viviparous snakes, *Crotalus viridis*. *Copeia* 1990:1089–1098
- Chastel O, Weimerskirch H, Jouventin P (1995) The influence of body condition on reproductive decisions and reproductive success in the blue petrel. *Ecology* 76:2240–2246
- Cherel Y, Grocolas R (1999) Relationship between nutrient storage and nutrient utilization in long-term fasting birds and mammals. In: Adams NJ, Slotow RH (eds) *Proceedings of the 22nd International Ornithology Congress*. Durban, Johannesburg, pp 17–34
- Cherel Y, Robin JP, Walch O, Karmann H, Netchitaïlo P, Le Maho Y (1988) Fasting in king penguin. I. Hormonal and metabolic changes during breeding. *Am J Physiol* 254:R170–R177
- Cherel Y, Robin JP, Heitz A, Calgari C, Le Maho Y (1992) Relationships between lipid availability and protein utilization during prolonged fasting. *J Comp Physiol B* 162:305–313
- Clutton-Brock TH (1991) *The evolution of parental care*. Princeton University Press, Princeton, New Jersey
- Crocker DE, Webb PM, Costa DP, Le Boeuf BJ (1998) Protein catabolism and renal function in lactating northern elephant seals. *Physiol Zool* 71:485–491
- Crocker DE, Williams JD, Costa DP and Le Boeuf BJ (2001) Maternal traits and reproductive effort in northern elephant seals *Ecology* 82:3541–3555
- Cundall (1987) Functional morphology. In: Seigel RA, Collins JT, Novak SS (eds) *Snakes: ecology and evolutionary biology*. Macmillan, New York, pp 210–252
- Doughty P, Shine R (1997) Detecting life history trade-offs: measuring energy stores in “capital” breeders reveals costs of reproduction. *Oecologia* 110:508–513
- Drent RH, Daan S (1980) The prudent parent: energetic adjustments in avian breeding. *Ardea* 68:225–252
- Goodman MN, Lowell B, Belur E, Ruderman NB (1984) Sites of protein conservation and loss during starvation— Influence of adiposity. *Am J Physiol* 246:E383–E390
- Gregory PT, Skebo KM (1998) Trade-offs between reproductive traits and the influence of food intake during pregnancy in the garter snake, *Thamnophis elegans*. *Am Nat* 151:477–486
- Gregory PT, Lisa HC, Skebo KM (1999) Conflicts and interactions among reproduction, thermoregulation and feeding in viviparous reptiles: are gravid snakes anorexic? *J Zool (London)* 248:231–241
- Guillette LJ (1982) Effects of gravidity on the metabolism of the reproductively bimodal lizard, *Sceloporus avenues*. *J Exp Zool* 223:33–36
- Henen BT (1997) Seasonal and annual energy budget of female desert tortoise (*Gopherus agassizii*). *Ecology* 78:283–296
- Henen BT (2002) Reproductive effort and reproductive nutrition of female desert tortoises: essential field methods. *Integr Comp Biol* 42:43–50
- Houston DC, Donnan D, Jones PJ (1995) Use of labeled methionine to investigate the contribution of muscle proteins to egg production in zebra finches. *J Comp Physiol B* 165:161–164
- Huey BR (1982) Temperature, physiology, and the ecology of reptiles. In: Gans C, Pough HF (eds) *Biology of the Reptilia*, vol 12. Physiological ecology. Academic Press, pp 25–95
- Jönsson KI (1997) Capital and income breeding as alternative tactics of resource use in reproduction. *Oikos* 78:57–66
- Jönsson KI, Tuomi J, Järemo J (1995) Reproductive effort tactics: balancing pre- and postbreeding costs of reproduction. *Oikos* 74:35–44
- Ladyman M, Bonnet X, Lourdaïs O, Bradshaw D, Naulleau G (2003) Gestation, thermoregulation and metabolism in a viviparous snake, *Vipera aspis*: evidence for fecundity-independent costs. *Physiol Biochem Zool* 76:497–510
- Lourdaïs O, Bonnet X, Doughty P (2002a) Costs of anorexia during pregnancy in a viviparous snake (*Vipera aspis*). *J Exp Zool* 292:487–493
- Lourdaïs O, Bonnet X, Shine R, DeNardo D, Naulleau G, Guillon M (2002b) Capital-breeding and reproductive effort in a variable environment: a longitudinal study of a viviparous snake. *J Anim Ecol* 71:470–479
- Lowell BB, Goodman MN (1987) Protein sparing in skeletal-muscle during prolonged starvation—dependence on lipid fuel availability. *Diabetes* 36:14–19
- Lowell BB, Ruderman NB, Goodman MN (1986) Regulation of myofibrillar protein degradation in rat skeletal muscle during brief and prolonged starvation. *Metabolism* 35:1121–1127
- Madsen T, Shine R (1993) Costs of reproduction in a population of European adders. *Oecologia* 94:488–495
- Madsen T, Shine R (1999a) The adjustment of reproductive threshold to prey abundance in a capital breeder. *J Anim Ecol* 68:571–580
- Madsen T, Shine R (1999b) Life history consequences of nest-site variation in tropical pythons (*Liasis fuscus*). *Ecology* 80:989–997
- Matz G (2001) Biologie de la reproduction de *Epicrates maurus* GRAY, 1849. *Situla* 3:10–15
- Meijer T, Drent R (1999) Re-examination of the capital and income dichotomy in breeding birds. *Ibis* 141:399–414
- Moon BR (2000) The mechanics and muscular control of constriction in gopher snakes (*Pituophis melanoleucus*) and a king snake (*Lampropeltis getula*). *J Zool (London)* 252:83–98
- Oftedal OT (1993) The adaptation of milk secretion to the constraints of fasting in bears, seals, and baleen whales. *J Dairy Sci* 76:3234–3246

- Packard GC, Packard MJ (1988) The physiological ecology of reptilian eggs and embryos. In: Gans C, Huey R (eds) *Biology of the Reptilia*. Liss, New York, pp 523–605
- Pearson D, Shine R, Williams A (2003) Thermal biology of large snakes in cool climates: a radio-telemetric study of carpet pythons (*Morelia spilota imbricata*) in south-western Australia. *J Therm Biol* 28:117–131
- Rivas JA (1999) Life history of the green anaconda (*Eumeces murinus*) with emphasis on its reproductive biology. Ph.D. thesis, University of Tennessee, Knoxville
- Ross RA, Marzec G (1990) The reproductive husbandry of pythons and boas. institute for herpetological research. Stanford, California
- Schwilch R, Grattarola A, Spina F, Jenni L (2002) Protein loss during long-distance migratory flight in passerine birds: adaptation and constraint. *J Exp Biol* 205:687–695
- Sheridan MA (1994) Regulation of lipid-metabolism in poikilothermic vertebrates. *C Biochem Physiol B* 107:495–508
- Shine R (1980) “Costs” of reproduction in reptiles. *Oecologia* 46:92–100
- Shine R (1985) The evolution of viviparity in reptiles: an ecological analysis. In: Gans C, Billet F (eds) *Biology of the Reptilia*, vol. 15. Wiley, New York, pp 605–694
- Shine R (1991) Influences of incubation requirements on the evolution of viviparity. In: Deeming DC, Ferguson MWJ (eds) *Egg incubation: its effects on embryonic development in birds and reptiles*. Cambridge University Press, Cambridge, pp 361–370
- Slip DJ, Shine R (1988) Reptilian endothermy: a field study of thermoregulation by brooding diamond pythons. *J Zool (London)* 216:367–378
- Somma LA (2003) Parental behavior in lepidosaurian and testudinian reptiles, a literature survey. Krieger, Malabar, Florida
- White HB (1991) Maternal diet, maternal proteins and egg quality. In: Deeming DC, Ferguson MWJ (eds) *Egg incubation: its effects on embryonic development in birds and reptiles*. Cambridge University Press, Cambridge, pp 1–15