Structural and Performance Costs of Reproduction in a Pure Capital Breeder, the Children's Python *Antaresia childreni*

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ABSTRACT

Females often manage the high energy demands associated with reproduction by accumulating and storing energy in the form of fat before initiating their reproductive effort. However, fat stores cannot satisfy all reproductive resource demands, which include considerable investment of amino acids (e.g., for the production of yolk proteins or gluconeogenesis). Because capital breeders generally do not eat during reproduction, these amino acids must come from internal resources, typically muscle proteins. Although the energetic costs of reproduction have been fairly well studied, there are limited data on structural and performance costs associated with the muscle degradation required to meet amino acid demands. Thus, we examined structural changes (epaxial muscle width) and performance costs (constriction and strength) over the course of reproduction in a pure capital breeder, the children's python (*Antaresia childreni*). We found that both egg production (i.e., direct resource allocation) and maternal care (egg brooding) induce muscle catabolism and affect performance of the female. Although epaxial muscle loss was minimal in nonreproductive females, it reached up to 22% (in females after oviposition) and 34% (in females after brooding) of initial muscle width. Interestingly, we found that individuals with higher initial muscular condition allocated more of their muscle into reproduction. The amount of muscle loss was significantly linked to clutch mass, underscoring the role of structural protein in egg production. Egg brooding significantly increased proteolysis and epaxial loss despite no direct allocation to the offspring. Muscle loss was linked to a significant reduction in performance in postreproductive females. Overall, these results demonstrate

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that capital-breeding females experience dramatic costs that consume structural resources and jeopardize performance.

Introduction

Reproduction is one of the most highly demanding activities. This is particularly true for females that expend massive amounts of energy into offspring production (direct allocation) and invest time and additional energy into behavioral modifications associated with care of the progeny (Bull and Shine 1979; Clutton-Brock 1991). Food availability can be highly heterogeneous in space and time, so food availability during the time of reproductive investment is often insufficient to cover the elevated energy requirements. Additionally, temporal and spatial demands associated with foraging and digestion may be incompatible with reproductive activities (Drent and Daan 1980; Jönsson 1997). Therefore, it can be advantageous to accumulate energy stores in advance of reproduction (i.e., capital breeding), because doing so allows reproductive investment independent of immediate resource availability and avoids physiological conflicts between the reproductive and digestive systems (Jönsson 1997; Bonnet et al. 1998; Varpe et al. 2009). Lipids constitute the most energetically dense nutrient and can be stored in specific tissues (e.g., fat bodies). Many vertebrates accumulate large amounts of fat before reproduction, and these stores can later be mobilized to support specific needs (e.g., incubation, lactation, and gestation; Oftedal 1993; Crocker et al. 1998; Senechal et al. 2011) or cover the entire energy requirements of reproduction (Bonnet et al. 2002; Crossin et al. 2009). Patterns of energy acquisition and allocation have attracted considerable scientific interest and stimulated discussion on the origin of energy used (capital vs. income; Jönsson 1997; Bonnet et al. 1998). Recent work suggests that, among vertebrates, there is a continuum rather than a dichotomy between capital and income strategies, and many species can combine the two (Meijer and Drent 1999; Wheatley et al. 2008; Warner et al. 2008; Telemeco and Baird 2011).

Importantly, reproduction requires an investment by the female of a myriad of components in addition to lipids, including proteins and essential mineral elements (White 1991). Proteins are a critical resource in several respects. First, amino acids provide raw materials necessary for subsequent protein synthesis, which is associated with somatic growth and development of the offspring. Considerable amounts of proteins are allocated to the developing embryos via egg yolk (White 1991; Santos et al. 2007; Senechal et al. 2011; Van Dyke et al. 2012)

or through postnatal provisioning (e.g., lactation). Also, during prolonged fasting (Lowell et al. 1986; Lowell and Goodman 1987; Cherel et al. 1992), the conversion of amino acids to glucose via gluconeogenesis is critical. Contrary to lipids that can be stored in specific cells (such as adipocytes), no equivalent storage tissues exist for proteins. Instead, proteins are principally located in muscle tissue and are tightly linked to muscle fiber function. Thus, in the absence of dietary protein intake, substantial protein mobilization will eventually induce muscle atrophy and subsequently decrease functional performance (Schwilch et al. 2002). This allocation trade-off is likely amplified in those species that undergo prolonged fasting during reproductive activity, most notably extended attendance of the eggs or neonates (Bull and Shine 1979). Hence, reproduction likely imposes contrasted constraints on female protein balance in relation to (i) reproductive investment (direct allocation) and (ii) an activity shift that is associated with parental care and may induce a reduction or complete cessation of food intake.

Protein mobilization has been widely studied from a physiological perspective (Cherel et al. 1988, 1992; Challet et al. 1995; Bordel and Hase 2000). Even when extensive lipid stores are available, a prolonged fast may have dramatic consequences on critical protein-rich tissues (e.g., muscle), and various protein-sparing strategies have emerged (Cherel et al. 1988). Because it compromises short-term survival through reduced performance, protein depletion can drive behavioral choices, such as withdrawing from reproductive activities (Robin et al. 2001). To date, the impact of reproductive demands (resulting from direct allocation and parental care) on protein mobilization and, notably, the associated functional costs have not been explored in depth. Nevertheless, this facet of reproductive costs is particularly relevant in term of life history strategies. Because it affects functional performances and thus individual phenotypic quality, protein depletion may mediate substantial costs of reproduction. Protein depletion is likely to be most relevant in natural systems characterized by low rates of nutrient acquisition combined with prolonged fasting episodes.

Despite the high resource demands of reproduction, some species support reproductive investments through the use of stored resources (i.e., capital) rather than through increased food intake during reproduction (Drent and Daan 1980; Jönsson 1997). Because energy requirements for maintenance are quite low for ectotherms, pure capital breeding (i.e., complete reliance on resource stores during reproduction) is a particularly widespread strategy in ectotherms (Bonnet et al. 1998). Most ectotherms are lecithotrophic, and thus yolk lipoprotein synthesis and deposition into the developing follicles require considerable investment by the female (Sheridan 1994). After ovulation, some species provide nondepreciable care (Somma 2003) to the developing embryos (e.g., guarding and brooding) that will extend the duration of fasting and thus require additional investment of body lipids and proteins. Because proteins are paramount to reproduction and cannot be readily stored in the body, protein depletion is likely to be a significant reproductive constraint in ectotherms, but this aspect has attracted only limited interest (Santos et al. 2007).

Boas and pythons are renowned among squamates for their low metabolic requirements (Bedford 1998; Zaidan 2003), very large lipid storage, and extreme fasting abilities. Reproduction can be sustained without any food intake (Rivas 1999), which results in pure capital-breeding systems. Maternal activities shift after ovulation with attendance of the developing embryos (either through gestation or egg brooding) for prolonged periods (several months). Thus, these snakes are valuable models for addressing the impact of reproductive constraints on protein use, considering the relevance of direct allocation and activity shifts induced by prenatal care behaviors. We used the children's python *Antaresia childreni* (Gray 1842), a small python species that broods its eggs, to test the following predictions: (i) vitellogenesis and associated follicular growth should induce significant mobilization of structural proteins, (ii) maternal care of the eggs should extend the protein depletion induced by vitellogenesis and significantly amplify muscle alteration, and (iii) significant protein depletion and resultant muscle atrophy induced by reproductive constraints (allocation and prenatal care) alter physical performances after reproduction.

Material and Methods

Study Species and Maintenance

Antaresia childreni is a medium-sized (up to 1,200 mm snout-vent length [SVL], 600 g body mass [BM]), nonvenomous, constricting snake that inhabits rocky areas in northern Australia from Kimberley, Western Australia, to the eastern Gulf of Carpentaria, Queensland (Wilson and Swan 2003). Oviposition occurs toward the end of the dry season (July–August, austral winter), when ambient temperatures are relatively cool and dry. After oviposition, females brood their eggs but are not facultatively endothermic (Stahlschmidt and DeNardo 2009).

The snakes in our study (35 females, plus 11 males used for breeding) were part of a long-term captive snake colony. Snakes were housed individually in 91 × 71 × 46-cm cages. The room was maintained at 25°C with a 12L : 12D cycle. Permanent access to supplemental heat was provided using a subsurface heating element (Flexwatt, West Wareham, MA) below one end of each cage. The resulting thermal gradient was 26°–42°C. Water was available ad lib. in bowls, and snakes were fed mice (mean mass = 20 g) once every 2–4 wk.

Reproduction

The adult snakes were cooled for 2 mo (December and January) in a temperature-controlled room with a 6 : 18 h daily temperature cycle of 25° : 15°C. During this period, room light and supplemental heat were provided only during the 6 h when the room temperature was 25°C. Mating occurred from mid-February to mid-March, and ultrasonography (Concept MCV, Dynamic Imaging, Livingston, Scotland) was used periodically to assess reproductive status.

Reproductive females typically refuse to feed after the cooling

period. Thus, for consistency, we did not provide food to any of the snakes during the period between wintering and oviposition. Twenty-one of 35 females initiated a reproductive cycle, and the presence of both reproductive and nonreproductive females was favorable to assess the impact of reproduction on protein use. Individual cages were inspected daily for oviposition. Laying occurred between early April and mid-June. In each case, females rapidly adopted a typical coiled position around their eggs. After oviposition, the female and her eggs were removed from the cage, the clutch was weighed (± 0.1 g), and the eggs were counted. However, six females were minimally disturbed at oviposition (see group 2 in the experimental design below) and instead simply placed with their eggs in individual polypropylene boxes housed in a thermally controlled room (constant temperature of 30°–31°C, which is the preferred incubation temperature for children's pythons). We did not measure clutch characteristics for these females to avoid clutch abandonment by the female. Water was provided ad lib. in two water bowls at one side of the box. As is the case for other pythons, the eggs of children's pythons mostly stick together and generally form a compact conglomerate shortly after oviposition (Ross and Marzec 1990).

Experimental Design

The primary goal of the study was to determine the impact of egg production and egg brooding on protein balance in the female. Because repeated disturbances after oviposition can result in clutch desertion, it was not possible to sequentially study vitellogenesis and brooding for each individual. Thus, we examined structural and functional impacts of reproduction using three groups.

Group 1.   Fifteen females (postoviposition) were removed from the clutch shortly after oviposition to examine the impact of egg production. The period of fasting for these females depended on oviposition date and had a mean ($\pm$SD) of 85.4 ± 11.8 d.

Group 2.   Six females (postbrooding) were left undisturbed to brood their eggs until hatching to measure the combined impact of egg production and brooding. The period of fasting for these females depended on hatching date and had a mean ($\pm$SD) of 129.5 ± 24.0 d (including vitellogenesis and egg brooding).

Group 3.   The 14 females that did not initiate a reproductive cycle were considered a group that did not make any reproductive investment. It is critical to note that these females were similar in size, initial mass, and body condition (BC) to those females that did reproduce. That is, the metrics of interest for this study were not the cause of the failure to reproduce, which, instead, was likely attributable to differential responses to the nonnatural stimulation of reproduction in captivity and perhaps inappropriate timing of pairings with males. Additionally, reproducing and nonreproducing females were all exposed to the same environmental conditions throughout the experiment. Because there was no reproductive end point (e.g., oviposition or hatching) for this group, the final measurements for this group were collected toward the end of oviposition for the two other groups (mean fasting time, 95.7 ± 0.5 d). Fasting duration in nonreproductive females (group 3) was similar to that in group 1 to quantify the absolute cost of folliculogenesis, which is a primary step of protein allocation into the eggs (Santos et al. 2007; Van Dyke et al. 2012). Using nonreproducing females rather than true controls (i.e., females that were not allowed to reproduce) potentially confounds results, but we took this approach because all females were of similar body and muscle condition, and randomly allocating females to the three groups would have led to significantly reduced sample sizes for the two reproductive groups (because some females allocated to those groups would have failed to reproduce).

Variables Collected

Body size and BM.   We measured (± 0.5 cm) maternal total body length (BL), SVL, and BM at the end of the wintering period. At that time, ultrasonography indicated that no follicular growth had occurred. The BC (the residual of the linear regression of BM against SVL) was calculated just after the wintering period (initial BC).

Musculature.   In snakes, epaxial muscles lying beside the vertebral column are of primary importance for locomotion (Cundall 1987) and constriction (Moon 2000). Magnetic resonance imaging of epaxial musculature has verified that external measurements of epaxial muscle width with callipers provide accurate estimates of actual muscle size (Lourdais et al. 2005). We measured the width of the epaxial muscles at four equally spaced points between the head and the vent. For each location, the mean value was derived from four consecutive measures. Measurements were made shortly after wintering for all individuals after oviposition (group 1), after egg incubation (group 2), and after the imposed fasting on nonreproductive females (group 3). We averaged values collected from the four body locations to derive mean epaxial muscle width. We report mean epaxial muscle change (mm) as well as the rate of mean epaxial muscle change (mm/d), because the duration between measurements varied among the groups (see above). We independently considered the four different locations to address variation in muscle change along the body.

Reproductive effort.   Maternal allocation into the eggs occurs during vitellogenesis and follicular growth. We expected that female muscle mobilization would be linked to reproductive effort. Because reproductive effort is usually linked to body size in snakes (Bonnet et al. 2003), we calculated SVL-adjusted clutch mass (residuals of the regression of clutch mass on mother SVL). The relationship between muscle loss and reproductive effort was restricted to group 1, for which we had clutch characteristics at the time of oviposition.

Performance measures.   Pythons rely on 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 postpartum snakes (nonpregnant females were tested at the same time) in two different contexts designed to mimic the following biologically impor-
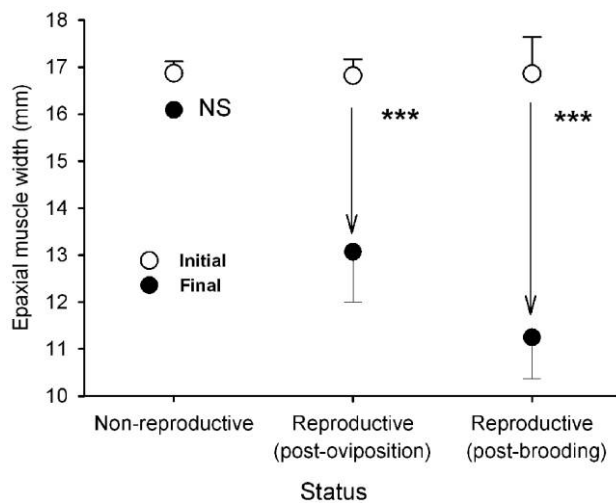
Figure 1. Initial and final epaxial muscle width (mm) measured in nonreproductive and reproductive female children's python (*Antaresia childreni*). Reproductive females were measured after oviposition or egg brooding. Error bars represent standard error. NS = nonsignificant. Three asterisks indicate $P < 0.001$.

tant situations. The validity of those estimators is described in a related article (Lourdais et al. 2005). (1) 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 over a 5-min period of repeated stretching and contraction. (2) Prey handling: We measured the intensity of muscular contraction during prey constriction using a compressible lure (a water-filled rubber balloon, 10 cm long and 2 cm in diameter, surrounded by mouse-scented cloth; Lourdais et al. 2005). 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 video recorded so that maximal displacement (cm) could be subsequently measured.

Statistics

All statistics were performed with Statistica 6.0. The BC was compared among groups using a simple ANOVA procedure. To examine the influence of treatment on musculature, we first used a repeated-measures ANOVA with initial and final epaxial muscle width as repeated dependent variables and treatment group as a factor. Second, we used an ANOVA procedure that used either absolute muscle change or daily rate in muscle change as the dependent variable, treatment group as the factor, and independent linear covariates (SVL and initial muscle

width). Changes in musculature across body locations were examined in a mixed-model procedure using muscle change as a dependent variable, body location and treatment group as factors, and individual identity as a random factor. Changes in force were examined with a repeated-measures ANOVA procedure using initial and final traction forces as repeated dependent variables, treatment group as a factor, and initial epaxial muscle width as a linear covariate. Finally, differences in constriction forces were analyzed using an ANOVA procedure. Tukey post hoc tests were conducted for 2 × 2 comparisons. Unless otherwise stated, values are reported as means ± standard deviations.

Results

Changes in Musculature

Initial body mass was closely related to body size ($F_{1,33} = 26.29$, $P < 0.001$, $r^2 = 0.74$). No differences in initial BC were found among the three experimental groups (ANOVA, $F_{2,32} = 0.004$, $P = 0.94$). Initial muscle width was not linked to SVL ($F_{1,33} = 1.59$, $P = 0.21$) but significantly related to initial body mass ($F_{1,33} = 7.95$, $P < 0.008$, $r^2 = 0.19$) and BC ($F_{1,33} = 11.19$, $P < 0.002$, $r^2 = 0.24$). Initial muscle width was not different in the three groups ($F_{2,32} = 0.95$, $P = 0.39$).

Fasting was associated with a significant decrease in musculature (repeated-measures ANOVA, specific time effect, $F_{1,32} = 254.51$, $P < 0.001$). We also found an influence of treatment ($F_{2,32} = 14.32$, $P < 0.001$) as well as an interaction term between time and treatment ($F_{2,33} = 44.46$, $P < 0.001$; fig. 1). Mean change in epaxial muscle width was -5.60 ± 1.30 mm (34%), -3.75 ± 1.4 mm (22%), and -1.04 ± 1.23 mm (4%) for postbrooding, postoviposition, and nonreproductive females, respectively (Tukey post hoc test, all P values < 0.001). Postbrooding females had lower final epaxial muscle width than did postlaying females, and postlaying females had lower values than did nonreproductive females (Tukey post hoc test, all P values < 0.001). Final epaxial muscle width was closely related to SVL ($F_{1,33} = 9.05$, $P = 0.004$, $r^2 = 0.24$).

Determinants of Muscle Change

In addition to treatment, SVL and initial muscle width also explained change in muscle width (table 1). SVL had a positive influence on the change in muscle width ($\beta = 0.22 \pm 0.06$).

Table 1: Influences of snout-vent length (SVL), initial epaxial muscle width, and reproductive status on muscle change in female children's pythons

Source	df	SS	F ratio	P
SVL	1	6.10	11.18	.002
Initial epaxial muscle width	1	24.26	44.46	<.0001
Reproductive status	2	98.56	90.31	<.0001
Error	30	16.36		

Note. $r = 0.95$; $r^2 = 0.88$; $n = 35$; $F_{4,30} = 65.91$; $P < 0.0001$. See text for statistics. SS = sum of squares.
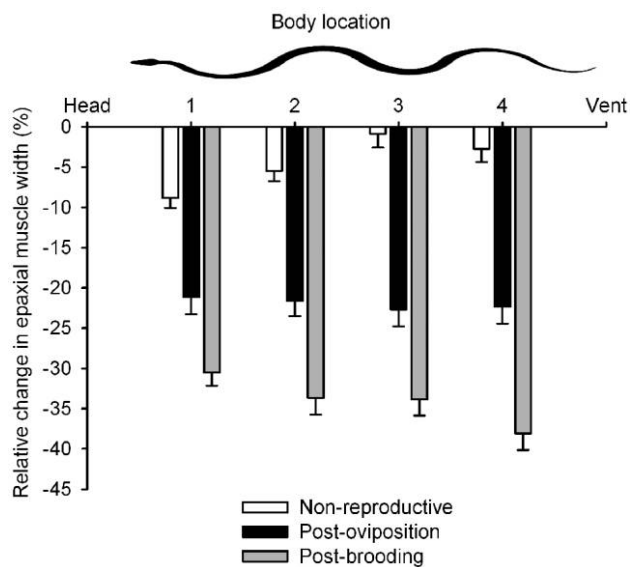
Figure 2. Relative changes in epaxial muscle width (%) at four locations along the body in nonreproductive females, reproductive females after oviposition, and reproductive females after brooding. Error bars represent standard error. See text for detailed statistics.

In turn, initial muscle width had a negative impact ($\beta = -0.40 \pm 0.06$). When considering daily rate of change in muscle width as the response variable (i.e., dividing absolute loss by fasting duration), the influence of treatment was maintained (ANOVA, $F_{2,31} = 35, 49$, $P = 0.001$) with changes of -0.008 ± 0.007, -0.043 ± 0.015, and -0.044 ± 0.011 mm/d for nonreproductive, postoviposition, and postbrooding females, respectively. However, postovipositing and postbrooding females did not diverge in their daily rate of muscle loss (Tukey post hoc test, $P = 0.99$).

Muscle change was not homogenous along the body (generalized linear model treating individual identity as a random factor, specific location effect, $F_{3,96} = 4.45$, $P = 0.005$) with significant variation among groups (interaction term between groups and location, $F_{6,96} = 7.05$, $P < 0.001$). Although absolute muscle loss was minimal in nonreproductive females, it was mainly concentrated in the cranial locations (1 and 2). In contrast, absolute muscle loss was greater and homogeneous in postlaying females. Finally, for postbrooding females, muscle loss was more pronounced in the caudal locations (fig. 2).

Reproductive females showed significant variation in clutch size (mean no. eggs, 9.7 ± 2.4; range, 7–15 eggs) and clutch mass (mean mass, 96.61 ± 29.77 g; range, 53.33–163.81 g). Clutch mass was significantly related to female SVL ($F_{1,13} = 8.22$, $r^2 = 0.38$, $P = 0.013$). We found that mean muscle change was negatively related to reproductive effort (size-adjusted clutch mass; $r^2 = 0.36$, $F_{1,13} = 6.85$, $P = 0.02$).

Functional Impact of Reproduction

The three groups did not differ in their initial traction force (ANOVA, $F_{2,32} = 0.33$, $P = 0.71$). Traction force was closely related to epaxial muscle width ($F_{1,33} = 15.49$, $r^2 = 0.32$, $P < 0.001$). A significant decrease in traction force was observed over the fasting period (ANOVA, specific time effect, $F_{1,32} = 135.26$, $P < 0.001$), and the three groups significantly differed in the magnitude of strength reduction (interaction between time and group, ANOVA, $F_{2,32} = 17.71$, $P = 0.002$). Strength reduction was more pronounced in postbrooding females and minimal in nonreproductive ones. Postlaying females were in intermediate position $F_{2,32} = 19.81$, $P = 0.001$ by Tukey post hoc tests, all P values <0.001; fig 3). Traction force change was proximately linked to the change in mean epaxial muscle width ($F_{1,33} = 42.10$, $r^2 = 0.57$, $P < 0.0001$).

Constriction performance was measured in nonreproductive and postlaying females at the end of their fasting periods. Postlaying females showed significantly lower maximal constriction values (3.75 ± 1.72 vs. 5.23 ± 1.05 cm; $F_{1,20} = 5.63$, $P = 0.027$), but there was no difference in constriction duration between the two groups ($F_{1,20} = 0.65$, $P = 0.42$).

Discussion

Capital breeding provides significant benefits, notably when energy requirements are high, resources are scarce, or foraging time is limited during the reproductive season (Jönsson 1997; Bonnet et al. 1998; Varpe et al. 2009). Nevertheless, current theories predict that reliance on energy storage can be costly as a result of either (i) locomotor impairment (due to storage) or (ii) structural and performance costs induced by acute resource mobilization (Senechal et al. 2011). To date, structural costs of capital breeding remain largely understudied, yet such costs are suspected to be significant in pure capital breeders (i.e., species that can successfully reproduce without food intake). Here, we provide evidence that both egg production (i.e., direct allocation) and maternal care (egg brooding) induce
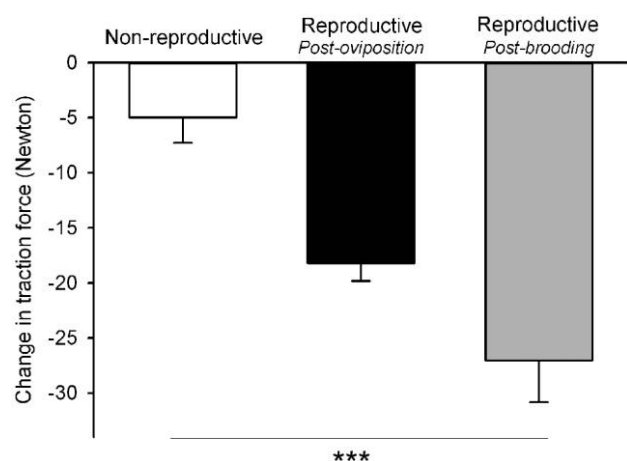

Figure 3. Change in force (Newtons) measured in nonreproductive and reproductive (postoviposition or postbrooding) female children's python (*Antaresia childreni*). Error bars represent standard error. Tukey post hoc tests were conducted for 2 × 2 comparisons. Three asterisks indicate $P < 0.001$.

muscle catabolism and decrease performance in the pure capital breeding children's python.

We report an important impact of reproduction in inducing up to a 34% loss in epaxial muscle width compared with that in nonreproductive females. The majority of this loss (64%) occurred during the vitellogenic period, which constitutes the major time of resource allocation in lecithotrophic species. Vitellogenic females undergo major physiological changes and mobilize body stores to support the synthesis of vitellogenin and yolk deposition into the growing follicles (Speake et al. 2003). Protein breakdown is necessary to provide the essential amino acids required for yolk protein synthesis in the liver (Santos et al. 2007; Van Dyke et al. 2012). Additionally, vitellogenesis and gravidity impose a significant thermoregulatory shift and a metabolic increase (Lourdais et al. 2008). As a result, proteolysis is required to support elevated maintenance costs, notably through gluconeogenesis. Although our study does not allow us to fully separate direct allocation from maintenance requirements, the relationship between muscle loss and adjusted clutch mass underlines structural protein mobilization for follicular growth as the primary utilizer of maternal proteins. Interestingly, we found that individuals with higher initial epaxial muscle width allocated proportionally more of their muscles into reproduction. Such correlation suggests that body muscles, although they have a specific contractile function, could represent a form of protein "capital" that could be mobilized and allocated for reproduction (quite similarly to fat stores).

All python species show maternal care to their eggs in the form of egg brooding (Somma 2003). Although some medium to large species are capable of facultative endothermy through shivering thermogenesis (Hutchinson 1966; Slip and Shine 1988), small species, such as the one we studied, do not produce significant metabolic heat during brooding. Still, we found that muscle loss during brooding comprised 36% of total muscle loss, and maternal body condition was significantly lower after egg attendance than after laying. As a result, maintenance requirements during egg brooding further increased proteolysis and epaxial muscle loss. We found that muscle atrophy was not homogenous along the body, notably when considering postbrooding females. We detected a progressive increase in epaxial muscle loss from the cranial to the caudal locations, which is similar to results obtained with pregnant rainbow boas (*Epicrates cenchria maurus*), which is another constricting species (Lourdais et al. 2004). In contrast, muscle loss was spatially homogenous in postoviposition females, which suggests that selective muscle mobilization may be specific to situations of prolonged fasting.

The importance of muscle as a capital resource during extended fasting is further exemplified by the rate of muscle loss in reproductive females during brooding relative to the rate of muscle loss in nonreproductive females. Because *Antaresia childreni* females are not thermogenic and do not elevate metabolism during brooding (Stahlschmidt and DeNardo 2009), energetic expenditures between nonreproductive and brooding females held under identical conditions should be similar. The rate of muscle loss during brooding can be estimated from the difference in muscle loss between postbrooding and postlaying females (5.60 mm − 3.70 mm = 1.85 mm) and the difference in fasting duration (129.5 d − 85.4 d = 44.1 d). Despite the suspected similarity in metabolism, epaxial muscle loss was dramatically greater during brooding (0.042 mm/d) than it was for the nonreproductive snakes (0.008 mm/d). This difference is likely due to brooding occurring at the end of a long fasting period, when much of the lipid stores have been lost (i.e., maintenance costs during brooding are likely predominantly supported by muscle catabolism rather than lipolysis). Thus, despite brooding being energetically inexpensive, its impact on muscle morphology, and thus female performance, is considerable (fig. 3).

Our performance results clearly underline the impact of structural alteration. Importantly, traction force and epaxial muscle reductions were closely correlated, which demonstrates the quality of our estimators. Traction force was reduced in postreproductive females compared with nonreproductive ones, and, furthermore, loss in traction force was 50% higher in postbrooding females than in postlaying females. Similarly, constriction performance was also affected by reproduction. Maximal constriction strength was lower in postreproductive females, which revealed that protein mobilization compromised constriction and, thus, foraging capabilities. The selective muscle mobilization observed in postbrooding females (i.e., greater muscle loss caudally) could be considered adaptive, because more cranial musculature is presumably critical for defense and prey constriction (Moon 2000).

Our results demonstrate the structural and performance costs induced by reproduction in a pure capital breeder. The effects we report are likely ecologically relevant and are consistent with the reduced postreproductive body condition observed in the field in other pythons (Madsen and Shine 1999a; Ujvari et al. 2011). Ectotherms are unique for their reduced energy requirements, and pythons can be considered low-energy specialists that can endure extended periods of fasting. Theoretical models predict that capital breeding can be costly because of significant locomotor impairments induced by storage. Such costs are likely minimal or absent in slow-moving and rather elusive predators, such as pythons, that do not rely on speed to subdue their prey or evade predators. Still, reproduction implies a massive energy allocation toward egg production and care to the developing embryos (egg brooding). Costs of reproduction are high, and reproductive frequency is low, because energy stores must be replenished up to a threshold level to reengage in reproduction (Madsen and Shine 1999a, 1999b). Low frequency of reproduction is a particular life-history trait that has attracted considerable interest and is usually observed in species showing "accessory" activities associated with reproduction (breeding migrations, egg brooding, or viviparity; see Bull and Shine 1979). Our data may provide proximal mechanisms to better understand delayed costs of reproduction. Indeed, protein alteration induced by reproduction likely alters performance in postreproductive females and induces long-term energy and ecological costs (Bonnet et al.

1999). For instance, altered performance will possibly reduce foraging efficiency and thereby reduce the ability to replenish body reserves or generate survival costs by altering the ability to escape from predators.

We provide clear evidence that brooding compromises epaxial muscles and critical performances in a species that does not allocate energy to heat production. Even when it is nonthermogenic, egg brooding can still provide alternate thermal benefits (e.g., from altering insulation of the clutch; Stahlschmidt and DeNardo 2009) as well as hydric benefits (Lourdais et al. 2007; Stahlschmidt et al. 2008). Our results are likely applicable to pythons in general, but with some expected variation. Notably, costs of brooding are expected to be higher in species capable of shivering thermogenesis that specifically involves muscle contraction and induces a dramatic increase in metabolic rate (Hutchinson et al. 1966). Structural and performance costs of brooding may explain maternal behavioral strategies reported in other species. For instance, in the water python, there is a dichotomy in nest attendance, in which some females stay with their clutches throughout incubation, whereas others desert their clutch shortly after oviposition (Madsen and Shine 1999b; Stahlschmidt et al. 2012a). Postreproductive females are particularly emaciated, and their body condition likely reflects altered musculature. Thus, desertion behavior could be related to critical levels of protein mobilization (phase III) associated with fasting, similar to egg abandonment in birds (Robin et al. 2001; Spee et al. 2010). In support of this conjecture, postreproductive female water pythons had similar epaxial muscle widths regardless of whether they were short- or long-duration brooders (Stahlschmidt et al. 2012b).

Our study provides original insight and points out the high demand on proteins for reproduction. In capital breeders, fat mobilization supports lipid and energy demands of reproduction and does not impact female function (in fact, reduced fat often increases performance). However, muscles are also a critical resource tissue for reproduction, but muscle tissue differs from adipocytes in that muscle cells have a specific contractile function that is compromised to support reproduction.

Acknowledgments

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