

Morphological and Physiological Changes during Reproduction and Their Relationships to Reproductive Performance in a Capital Breeder

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ABSTRACT

Current reproductive effort typically comes at a cost to future reproductive value by altering somatic function (e.g., growth or self-maintenance). Furthermore, effects of reproduction often depend on both fecundity and stage of reproduction, wherein allocation of resources into additional offspring and/or stages of reproduction results in increased costs. Despite these widely accepted generalities, interindividual variation in the effects of reproduction is common—yet the proximate basis that allows some individuals to mitigate these detrimental ef-

fects is unclear. We serially measured several variables of morphology (e.g., musculature) and physiology (e.g., antioxidant defenses) in female Children's pythons (*Antaresia childreni*) throughout reproduction to examine how these traits change over the course of reproduction and whether certain physiological traits are associated with reduced effects of reproduction in some individuals. Reproduction in this capital breeder was associated with changes in both morphology and physiology, but only morphological changes varied with fecundity and among specific reproductive stages. During reproduction, we detected negative relationships between morphology and self-maintenance (e.g., increased muscle allocation to reproduction was related to reduced immune function). Additionally, females that allocated resources more heavily into current reproduction also did so during future reproduction, and these females assimilated resources more efficiently, experienced reduced detriments to self-maintenance (e.g., lower levels of oxidative damage and glucocorticoids) during reproduction, and produced clutches with greater hatching success. Our results suggest that interindividual variation in specific aspects of physiology (assimilation efficiency and oxidative status) may drive variation in reproductive performance.

Introduction

Reproductive effort is an integral component of fitness, and life-history theory posits that individuals or species investing heavily into a reproductive event often incur effects that may reduce survival, reduce future reproduction, or both (Williams 1966; Stearns 1992; Roff 2001). This tenet has been empirically supported by both observational and manipulative studies (e.g., Madsen and Shine 1999 and Cox and Calsbeek 2010, respectively). The effects of current reproductive effort on future reproduction can be manifested through somatic function, such as altered morphology (e.g., body mass or musculature) or self-maintenance (e.g., immune function or oxidative status; e.g., Zera and Harshman 2001). Thus, aspects of morphology and self-maintenance reflect proximate physiological constraints that can be related to future reproduction and survival (Landwer 1994; Kalmbach et al. 2004; Cox et al. 2010). Under laboratory conditions, trade-offs between reproductive and somatic investment are particularly evident when individual variation in resource allocation is expected to exceed that of resource acquisition (Glazier 1999). Therefore, laboratory stud-

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ies of these relationships provide an effective complementary approach to the more traditional fitness assessments of survival and future reproductive success performed in the field, which can be extremely challenging for long-lived or secretive taxa.

Allocation into reproduction occurs through several avenues, each of which may impact the individual to different degrees. Furthermore, the extent of parental allocation—and thus the energetic costs associated with reproduction—can vary among stages within reproduction (e.g., French and Moore 2008; but see De Coster et al. 2010). The effects of reproduction may be fecundity dependent, wherein individuals that produce more or larger offspring experience greater costs in terms of survival, future reproduction, and/or body condition (Gustafsson and Sutherland 1988; Dijkstra et al. 1990; reviewed in Parejo and Danchin 2006). Conversely, some facets of reproduction may be largely fecundity independent (e.g., nest defense; reviewed in Clutton-Brock 1991). As a result of this variation, a thorough assessment of somatic changes during reproduction requires examining how specific reproductive stages and fecundity relate to both self-maintenance (e.g., oxidative damage, a source of senescence) and morphology (e.g., loss of musculature).

Despite the detrimental effects of reproduction, some studies have failed to detect a trade-off between important life-history traits (e.g., current and future reproduction; Bell 1984; reviewed in Sand 1998; Glazier 1999). Such cases may be due to inter-individual variation in quality wherein individuals that acquire more resources (or use resources more efficiently) can allocate more resources to multiple life-history traits (e.g., Clutton-Brock 1984; van Noordwijk and de Jong 1986; Sand 1998; Glazier 1999; Beauplet and Guinet 2007). Yet the proximate basis that allows individuals to mitigate the effects of reproduction is unclear, particularly if individuals do not acquire resources during reproduction (e.g., capital breeders [animals that fuel reproduction using only stored resources]). Pythons are a compelling model for examining physiological traits (e.g., antioxidant defenses or innate immunity) that could be associated with reduced reproductive trade-offs. On one hand, pythons are long-lived and can reproduce annually (Madsen and Shine 1999), suggesting relatively limited effects of each reproductive event. On the other hand, pythons are capital breeders that heavily allocate their body reserves into the production of large clutches (one-third or more of maternal body mass) and care to the eggs (brooding), and more than one-quarter of their adult life can be spent in an aphagic reproductive state (reviewed in Stahlschmidt and DeNardo 2011). Such traits could result in considerable physiological costs.

To disentangle this apparent contradiction, we used a correlative approach to measure a suite of morphological and physiological variables throughout reproduction in a pure capital breeder, the Children's python (*Antaresia childreni* Gray 1842), under captive conditions. Adequate energy reserves are required for reproduction in capital breeders, meaning that a significant allocation of body reserves to reproduction is a substantial component of reproduction. Thus, we assessed several morphological traits, including body mass, body size (snout-vent length [SVL]), body condition, and musculature (epaxial,

or dorsal, muscle width). We measured complementary physiological variables associated with animal well-being, such as oxidative status (antioxidant defenses and oxidative damage), corticosterone (CORT; a glucocorticoid hormone involved in energy mobilization and the stress response; Sapolsky et al. 2000), leukocyte profile (a ratio of heterophils to lymphocytes, which is indicative of a chronic stress response; Davis et al. 2008), innate immune function, and assimilation efficiency of energy, crude protein, and crude fat.

Our first hypothesis states that reproduction entails correlated changes in morphology and self-maintenance (e.g., loss of body reserves and altered somatic function). We tested the following predictions: (1) morphology and self-maintenance (physiology) are associated with both reproductive stage (non-reproductive vs. gravid vs. egg brooding) and fecundity (clutch size and mass) and (2) morphological and physiological changes during reproduction are related to one another (e.g., allocation of body reserves into reproduction is related to reduced self-maintenance during reproduction). Our second hypothesis states that variation in interrelated physiological traits is associated with variation in reproductive performance. We predicted that interindividual variation in physiology (e.g., indices of immune function, antioxidant defenses, or assimilation efficiency of macronutrients) explains variation in future reproduction (whether a given female reproduced the following breeding season). Together, our results will provide insight into possible physiological traits associated with the mitigation of costs of reproduction.

Material and Methods

Subjects and Husbandry

Antaresia childreni is a medium-sized (up to 1.2 m SVL and body mass of 600 g) constricting snake of the Pythonidae family from northern Australia (Wilson and Swan 2008; Stahlschmidt and DeNardo 2009). *Antaresia childreni* has multiple attributes that make it an ideal model for investigating the effects of reproduction, including (1) natural variation in offspring number, (2) several stages of reproduction (vitellogenesis [yolk production], gravidity [egg bearing], and nonthermogenic egg brooding [females brood their clutch of eggs until hatchlings emerge, which is typically 50 d or more after oviposition]) over a prolonged reproductive period (100 d or more), (3) a body size that enables serial measurement of physiological variables throughout reproduction, and (4) adult growth (reviewed in Stahlschmidt and DeNardo 2011). This species is also a capital breeder, with energy reserves driving reproductive decisions and supporting reproductive effort. Thus, the potential confounding effect of energy intake on reproduction-related trade-offs (e.g., van Noordwijk and de Jong 1986; Zera and Harshman 2001; King et al. 2010) is eliminated because *A. childreni* does not eat during reproduction (Lourdais et al. 2013). However, individuals enter the breeding season in varying body condition (a proxy for energy reserves), allowing for examination of the effect of interindividual variation in resource acquisition on reproductive effort.

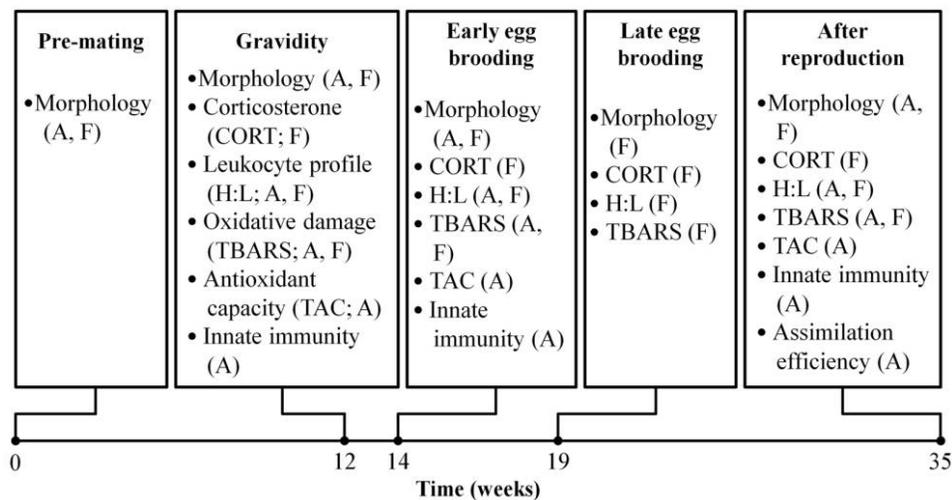


Figure 1. Summary and time line of sampling regimes for maternal variables in two colonies (A and F) of *Antaresia childreni*. Whether a colony was sampled for a variable at a given time point is denoted parenthetically. For example, morphological traits were sampled from colonies A and F before mating. See text for details about specific time points and measured variables. CORT = plasma corticosterone, H : L = ratio of heterophils to lymphocytes (leukocyte profile), TAC = total antioxidant capacity, TBARS = thiobarbituric acid–reactive substances.

We used two captive colonies of *A. childreni*, one maintained at Arizona State University (ASU), Tempe, Arizona, and the other at the Centre National de la Recherche Scientifique (CNRS), Chizé, France, for this study (colonies A and F, respectively). Colony A ($n = 16$ females) was maintained for >10 yr at ASU, and colony F ($n = 15$ females) was started >5 yr ago using individuals from colony A (mean age of individuals from both colonies: 7 yr). All procedures used in this study were similar between colonies (e.g., brooding females and eggs were kept at $31.5 \pm 0.3^\circ\text{C}$). Work at ASU and the CNRS was conducted under ASU Institutional Animal Care and Use Committee protocol 08-967R and CNRS permit 79-158, respectively. Despite the similarities, data from the two colonies were statistically analyzed separately due to some slight variations in methodologies and the techniques available at each institution (outlined below).

During the breeding season (January–April), we determined the reproductive status of fasting female pythons through weekly ultrasonograms using a portable ultrasound system (Concept/MCV; Dynamic Imaging, Livingston, Scotland). From a few days before oviposition until we took the blood samples during egg brooding (see “Sampling Regime” below), we housed females in brooding chambers to minimize disturbance and avoid clutch abandonment. At oviposition, we briefly removed each female from her clutch to determine clutch mass (colony A only: absolute clutch mass [g of eggs] and relative clutch mass [g of eggs/female postoviposition mass]), clutch size, and female postoviposition mass. To determine reproductive rate and year-to-year dynamics of reproductive output, we assessed the clutch (but not the maternal) characteristics in this manner for three consecutive years in colony A. We sampled morphological and physiological variables during the second year. All data from colony F were collected during a single

year. For more information, see “Sampling Regime” below and “Reproductive Husbandry” in the appendix, available online.

After we collected our final blood samples during brooding (see “Sampling Regime” below), we either (1) removed females from their clutches and incubated the eggs artificially on moistened perlite at $31.5 \pm 0.3^\circ\text{C}$ (colony A) or (2) allowed females to brood their clutches until hatchlings emerged (colony F; fig. 1). In colony F, we also determined hatching success. We then housed these females individually in a manner identical to that used during gravity. We offered each postreproductive female one pre-killed (frozen, then thawed) mouse approximately weekly for 14 wk. In colony A, we measured postreproductive snakes’ assimilation efficiency (the efficiency by which ingested nutrients are absorbed) for energy, crude fat, and crude protein under two dietary intake treatments—low (one mouse per week for 4 wk) and high (two mice per week for 2 wk)—because assimilation efficiency of energy varies due to dietary intake (low > high; see Stahlschmidt et al. 2011 for details). Before taking postreproductive blood samples (≥ 16 wk postreproduction), females had recovered >50% of the mass they had lost during reproduction (fig. 2a).

Sampling Regime

We used a repeated-measures design to control for interindividual variation. An optimal design would have included a control group of females that were not allowed to breed but that were fasted similar to reproductive females. Although our sample size precluded this design, recent research in *A. childreni* used this approach and demonstrated that fasted nonreproductive females exhibit greater musculature and contractile strength than fasted reproductive females sampled at the same time (Lourdais et al. 2013). Thus, we have confidence that any

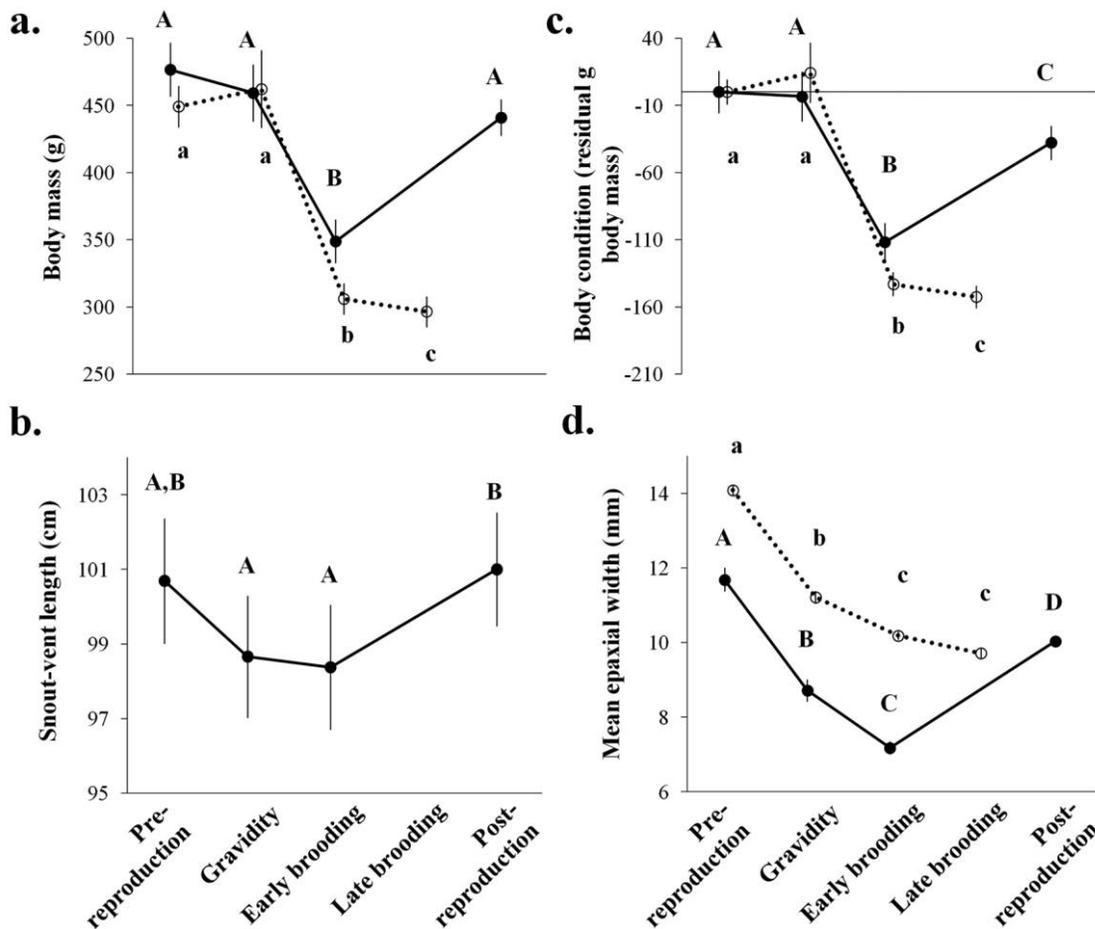


Figure 2. Morphological traits at the onset of reproduction, during the three stages of reproduction, and ≥ 16 wk after reproduction in *Antaresia childreni*. We present data from two colonies of *A. childreni* (filled symbols = colony A, open symbols = colony F; see text for details), both of which exhibited similar morphological patterns. The graphs show (a) body mass, (b) snout-vent length, (c) body condition, and (d) mean epaxial muscle width. Data are displayed as mean \pm SEM, and significant within-colony differences are denoted by different letters (e.g., for colony A, A \neq B; for colony F, a \neq b).

differences among reproductive stages are primarily due to the effects of reproduction rather than the effects of sampling time point or fasting.

We measured morphological and physiological variables from females of both colonies. However, our sampling regimes varied between the two colonies because of logistical constraints (fig. 1). We measured morphological traits of all female *A. childreni* at the onset of reproduction (immediately after an overwintering period and before vitellogenesis), during gravidity (after ovulation [mean \pm SEM]: 13 ± 2 d before oviposition), and during early egg brooding (7 d postoviposition; see “Morphology” below). We also measured morphology in colony F females during late egg brooding (40 d postoviposition) because prolonged egg brooding is associated with increased mortality and reduced rate of reproduction in free-ranging pythons (*Liasis fuscus*; Madsen and Shine 1999). We measured morphological traits after reproduction (≥ 16 wk postbrooding) in colony A females.

We collected serial blood samples from a random subset of

females in colony A ($n = 10$, due to the size of our biochemical assay kits) and from all females in colony F ($n = 15$) during gravidity, early egg brooding, and after reproduction (≥ 16 wk postbrooding) within 5 min of initiating manual restraint. From colony F, we took an additional blood sample during late egg brooding (fig. 1). We used 10–50 μ L to make thin blood smears on glass microscope slides, which we air-dried at ambient temperature and stored until fixation (see “Leukocyte Profile” below). We kept the remainder of the blood on ice for < 1 h before centrifuging (750 g, 5 min at room temperature), removing the plasma, and storing the plasma at -80°C until we performed the assays described briefly below and in more detail in “Sample Collection and Processing” in the appendix.

Morphology

We measured body mass, SVL, body condition (residual index [residuals from a regression of body mass on SVL]), and epaxial (dorsal) muscle width to determine the effects of reproduction

on morphology (fig. 1). See “Sample Collection and Processing” in the appendix for more information.

Antioxidant Defenses and Oxidative Damage

Reactive oxygen species (ROS) are free radicals produced as by-products of oxidation-reduction reactions. ROS are important signaling molecules at low levels, but they can cause oxidative damage to lipids, proteins, and nucleic acids at high levels (reviewed in Dowling and Simmons 2009). Oxidative damage occurs when ROS production exceeds antioxidant defenses (Costantini et al. 2010). Information about antioxidant capacity alone is insufficient to make inferences about oxidative status; rather, antioxidant capacity should be coupled with a measure of oxidative damage (Costantini and Verhulst 2009). Thus, we measured total antioxidant capacity (TAC) in colony A and lipid peroxidation (thiobarbituric acid-reactive substances [TBARS], a proxy for ROS-induced damage to lipids) in both colonies. See “Sample Collection and Processing” in the appendix for more information.

Glucocorticoids

In vertebrates, stress is typically associated with an elevation of circulating glucocorticoids (e.g., CORT) due to the activation of the hypothalamic-pituitary-adrenal (HPA) axis (Sapolsky et al. 2000). The HPA axis mobilizes nutrient stores and alters behaviors, and these effects are adaptive in the short term but detrimental in the long term (Sapolsky et al. 2000). Thus, we measured plasma concentrations of CORT in colony F snakes. See “Sample Collection and Processing” in the appendix for more information.

Leukocyte Profile

We examined blood smears to determine the effects of reproduction on the ratio of heterophils to lymphocytes (H : L, or leukocyte profile), which is typically higher in individuals experiencing chronically elevated plasma glucocorticoid concentrations resulting from stress (reviewed in Davis et al. 2008). See “Sample Collection and Processing” in the appendix for more information.

Innate Immune Function

The landmark article of Matson et al. (2005) provides a thorough overview of the function and utility of the hemolysis-hemoagglutination assay of innate immune function. Briefly, the degree of natural antibody (NAb) agglutination is a function of the amount of NAb, which is produced before antigen exposure and may be instrumental in the initial stages of controlling bacterial and viral challenges (Ochsenbein and Zinkernagel 2000). Complement-mediated lysis, which is a function of both NAb and complement presence, is a fundamental component of the immune response that can result in the elimination of both bacteria and viruses (Ochsenbein and Zinkernagel 2000). Additionally,

because this assay benefits from not requiring species-specific secondary antibodies, it can be used across several taxa, including birds (e.g., Matson et al. 2005; Parejo and Silva 2009) and reptiles (e.g., Sparkman and Palacios 2009). To assess NAb agglutination and lysis, we modified a previously described protocol (Matson et al. 2005) that quantifies the most dilute plasma that can still generate an agglutination or lytic response to foreign red blood cells. See “Sample Collection and Processing” in the appendix for more information.

Morphological Condition, Physiological Condition, and Digestive Efficiency Index

We examined how initial variables (e.g., body condition or TBARS) varied due to reproductive stages (see “Statistical Analyses” below). However, because of our relatively low sample sizes ($n = 10\text{--}16$) and the relative abundance of independent variables of interest ($n > 10$), we also used principal components analysis (PCA) to reduce the number of independent variables and, in turn, our Type I error. Without PCA, we would have had limited insight into our first hypothesis (reproduction entails correlated effects on morphology and self-maintenance). With PCA, we were able to examine relationships within morphological condition, physiological condition, and digestive efficiency index (e.g., the relationship between leukocyte profile and antioxidant defenses at each sampling point; see below). We were also able to examine relationships between condition (morphological or physiological) and aspects of reproduction, such as fecundity (e.g., clutch size and mass in both colonies) and reproductive success the following breeding season in colony A (see below).

Specifically, we used PCA to generate principal component (PC) scores for each individual’s morphological condition from initial morphological variables (body mass, epaxial muscle width, and SVL) and physiological condition from initial physiological variables (colony A: antioxidant defenses, leukocyte profile, innate immune function, and oxidative damage; colony F: glucocorticoid concentration, leukocyte profile, and oxidative damage) at each sampling point, as well as for each individual’s digestive efficiency index from raw variables (assimilation efficiency for energy, crude fat, and crude protein under low and high dietary intake) after reproduction. We included PCs with eigenvalues >1 , which resulted in one to three PCs for morphological condition, physiological condition, and digestive efficiency index depending on reproductive stage (figs. A1, A2). For information about how PCs loaded onto each initial trait, see “Eigenvector Loadings for Principal Components” and figs. A1 and A2 in the appendix.

Statistical Analyses

We analyzed all data with SPSS (ver. 19; IBM, Armonk, NY) or SAS software (ver. 9.2; SAS Institute, Cary, NC), and we determined significance at $\alpha < 0.05$ for all tests. We performed analyses on each colony independently because colonies differed significantly in prereproductive SVL (colony A [mean \pm

SEM]: 101 ± 2 cm; colony F: 93 ± 1 cm; $t_{29} = 3.4$, $P = 0.0020$), prereproductive mean epaxial muscle width (colony A: 11.7 ± 0.3 mm; colony F: 14.1 ± 0.1 mm; $U_{29} = 7.0$, $P < 0.001$), clutch size (colony A: 7 ± 1 eggs; colony F: 10 ± 0 eggs; $t_{20} = 2.8$, $P = 0.012$), and variance in age (colony A: 12 yr; colony F: 3 yr). To determine the effect of reproductive stage on morphological and physiological variables (e.g., body condition and TBARS), we used repeated-measures ANOVA (rmANOVA) tests on data meeting the assumptions of parametric statistics. If the assumption of sphericity was violated, we used Greenhouse-Geisser epsilon adjustments. We used Bonferroni corrections for pairwise post hoc tests. Because agglutination and lysis values were not normally distributed and could not be transformed to achieve normality, we ran Friedman's tests (a nonparametric analog to rmANOVA) to control for individual. To determine where significant differences existed, we then performed sign-rank tests within each variable that significantly differed by reproductive stage.

To test relationships among individuals, we used simple linear regression analyses on variables we expected to have causal relationships with one another (e.g., we expected individuals with relatively large SVL to have relatively greater body mass), and we used correlation analyses on variables in which causal relationships were not known a priori (e.g., TBARS and CORT). We used Spearman's rank correlation tests (a nonparametric analog to the Pearson correlation test) to examine relationships between variables with nonparametric data sets. We used t -tests on postreproduction variables of physiology (PCs for physiological condition and digestive efficiency index) to determine whether there were physiological differences between females that did and did not reproduce the following breeding season.

Results

Effects of Age and Morphology

In colony A, snake age was related to only one morphological variable: a negative correlation with mean epaxial musculature at the onset of reproduction ($F_{1,14} = 4.9$, $P = 0.045$, $R^2 = 0.26$). Age was not related to clutch size ($F_{1,14} = 1.7$, $P = 0.21$) or clutch mass ($F_{1,14} = 3.3$, $P = 0.092$). Age was not typically related to physiological condition, except for physiological condition PC 2 during early brooding ($F_{1,8} = 11$, $P = 0.010$, $R^2 = 0.58$), where older females tended to have relatively high TBARS and low TAC.

Antaresia childreni in our study did not appear to exhibit size-dependent fecundity because while SVL was positively related to the number of hatchlings produced (colony F: $F_{1,13} = 4.8$, $P = 0.045$, $R^2 = 0.28$), SVL alone was not related to clutch size, clutch mass, or mean egg mass at any sampling point for either colony (all $P > 0.13$). Instead, *A. childreni* showed strong resource-dependent fecundity because morphological condition (colonies A and F: PC 1, index of body size, mass, and musculature) at the onset of reproduction was positively related to clutch size, mass, or both (all $P < 0.05$).

Effects of Reproductive Stage

Reproduction and the associated aphagia negatively affected morphology because snakes' body mass (colony A: $F_{3,42} = 52$, $P < 0.001$; colony F: $F_{1,16} = 66$, $P < 0.001$), body condition (colony A: $F_{3,42} = 47$, $P < 0.001$; colony F: $F_{1,16} = 66$, $P < 0.001$), and musculature (colony A: $F_{3,42} = 90$, $P < 0.001$; colony F: $F_{3,39} = 260$, $P < 0.001$) varied due to stage of reproduction (fig. 2). Although it also varied due to stage of reproduction statistically (colony A: $F_{1,19} = 5.4$, $P = 0.023$), the change in SVL was minimal (fig. 2b), suggesting that snakes in our study approached their asymptotic size limit and exhibited minimal growth. Reproduction was also associated with reduced self-maintenance (physiology) because snakes had lower TAC (colony A: $F_{2,18} = 6.5$, $P = 0.007$) and lytic capacity (colony A: $\chi^2 = 7.8$, $df = 2$, $P = 0.021$) and higher TBARS (colony F: $F_{3,33} = 5.8$, $P = 0.003$), H:L (colony A: $F_{2,18} = 3.8$, $P = 0.041$; colony F: $F_{3,39} = 3.8$, $P = 0.018$), and CORT (colony F: $F_{3,39} = 8.4$, $P < 0.001$) during reproduction (fig. 3). However, TBARS and agglutination capacity in colony A females did not differ among the time points we sampled (TBARS: $F_{2,18} = 2.2$, $P = 0.14$; agglutination: $F_{2,18} = 0.53$, $P = 0.60$; fig. 3). Unlike morphological variables, physiological variables did not vary due to stage within reproduction (figs. 2 and 3).

Effects of Fecundity

Reproductive output was generally associated with increased changes in morphology—for example, females with larger clutches lost more body condition during reproduction. Clutch size was positively related to reduction in body condition between the onset of reproduction and early brooding (colony A: $F_{1,14} = 18$, $P = 0.001$, $R^2 = 0.56$; colony F: $F_{1,13} = 6.1$, $P = 0.028$, $R^2 = 0.32$) and between gravidity and early brooding (colony A: $F_{1,14} = 17$, $P = 0.001$, $R^2 = 0.55$). In colony A, absolute and relative clutch mass were positively related to body condition reduction between the onset of reproduction and early brooding (absolute: $F_{1,14} = 13$, $P = 0.003$, $R^2 = 0.48$; relative: $F_{1,14} = 6.9$, $P = 0.020$, $R^2 = 0.33$) and between gravidity and early brooding (absolute: $F_{1,14} = 26$, $P < 0.001$, $R^2 = 0.65$; relative: $F_{1,14} = 5.0$, $P = 0.041$, $R^2 = 0.27$). The number of hatchlings produced by females was positively correlated with body condition lost from the onset of reproduction to late brooding (colony F: $r_{13} = 0.56$, $P = 0.030$).

Reproductive output was generally negatively related to physiological changes that occurred over the course of reproduction. In colony A, clutch size and absolute clutch mass were negatively correlated with physiological condition PC 3 during early brooding (clutch size: $r_8 = 0.65$, $P = 0.041$; clutch mass: $r_8 = 0.69$, $P = 0.028$)—that is, females with large and heavy clutches had low H:L during early brooding. Clutch mass (absolute and relative) was negatively correlated with physiological condition PC 2 (absolute: $r_8 = 0.66$, $P = 0.039$; relative: $r_8 = 0.81$, $P = 0.005$), meaning that females with heavy clutches had low TBARS during early brooding. Because PCs 2 and 3 also loaded onto TAC during early brooding—although in opposite directions (fig.

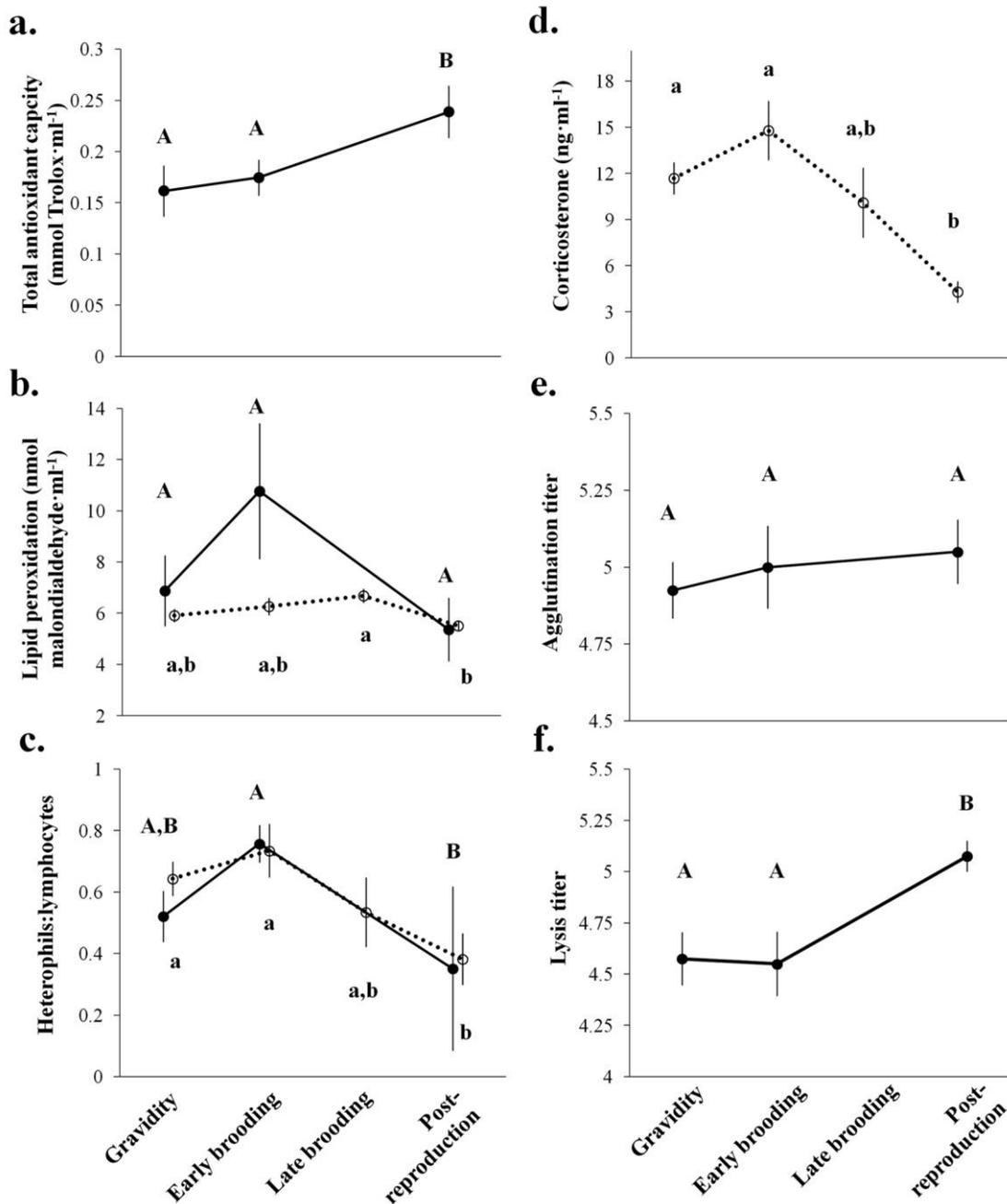


Figure 3. Physiological traits of plasma at three stages of reproduction and ≥ 16 wk after reproduction in *Antaresia childreni*. We present data on plasma from two colonies of *A. childreni* (filled symbols = colony A, open symbols = colony F; see text for details), both of which exhibited similar physiological patterns. The graphs show (a) total antioxidant capacity, (b) lipid peroxidation, (c) heterophil-to-lymphocyte ratio, (d) corticosterone, (e) agglutination titer, and (f) lysis titer. Data are displayed as mean \pm SEM, and significant within-colony differences are denoted by different letters (e.g., for colony A, A \neq B; for colony F, a \neq b).

A2b)—the relationship between fecundity and TAC is unclear. In colony F, physiological condition PC 1 (index of CORT and H:L) was negatively correlated with hatching success (proportion of eggs that hatched; $r_{13} = 0.58$, $P = 0.025$) and the number of hatchlings produced ($r_{13} = 0.61$, $P = 0.016$)—that is, females with high CORT and H:L during early brooding had lower hatching success and produced fewer hatchlings.

Relationships between Morphological and Physiological Condition

Physiological condition was related to morphological condition and to shifts in morphology during reproduction. In colony A, morphological condition PC 1 was negatively correlated with physiological condition PC 1 ($r_8 = -0.83$, $P = 0.003$) during

early brooding, meaning that snakes with high body mass, condition, and musculature exhibited low innate immune function. In addition, the percentage of muscle lost (a proxy for protein allocation) from the onset of reproduction to early brooding was negatively correlated with innate immune function (agglutination: $r_8 = 0.68$, $P = 0.030$; lysis: $r_8 = -0.68$, $P = 0.029$), but it was also positively correlated with TBARS ($r_8 = 0.74$, $P = 0.014$) during early brooding. In colony F, body condition lost during brooding (between early and late brooding) was positively correlated with physiological condition PC 1 ($r_{13} = 0.67$, $P = 0.006$) during late brooding—that is, snakes that lost more body condition during brooding had elevated CORT and H:L toward the end of brooding.

Dynamics of Reproduction

In colony A, females' physiology (but not morphology) after reproduction was related to their future reproduction (reproductive status the following breeding season). Specifically, females that bred the following year had low physiological condition PC 1 after reproduction ($F_{1,8} = 6.8$, $P = 0.031$)—that is, only snakes with relatively low oxidative status (low TAC and high TBARS) but high immune function (high agglutination and lytic capacity) reproduced the following breeding season (fig. A2c). Females that bred the following year also had higher digestive efficiency index PC 1 (high assimilation rate of crude protein) after reproduction ($F_{1,13} = 6.2$, $P = 0.027$).

We also measured year-to-year dynamics of reproduction in colony A snakes, and we detected interannual variation in mean egg mass ($F_{2,10} = 6.7$, $P = 0.015$; post hoc: first year > second year) but not in clutch size ($F_{2,10} = 0.49$, $P = 0.63$) or clutch mass ($F_{2,10} = 2.2$, $P = 0.16$). Clutch size during the year of sampling was larger among females that reproduced the following breeding season ($F_{1,14} = 4.7$, $P = 0.047$). Reproductive rate varied from 0.33 to 1 clutches per year among females, and it was positively correlated with the mean size and mass of females' clutches over the 3 yr we measured (fig. 4; size: $r_{14} = 0.53$, $P = 0.034$; mass: $r_{14} = 0.52$, $P = 0.039$). Hatching success was measured only in colony F, and it was also positively correlated with clutch size ($r_{13} = 0.55$, $P = 0.035$). Reproductive rate was negatively correlated with the percentage of epaxial muscle lost during vitellogenesis (between the onset of reproduction and gravidity; $r_{14} = -0.51$, $P = 0.044$).

Discussion

Together, our results help to clarify changes associated with reproduction and the relationships between somatic function and reproductive performance in a pure capital breeder. In support of our hypotheses, reproduction in *Antaresia childreni* was associated with (and presumably responsible for) reduced somatic function (figs. 2 and 3), while aspects of somatic function were associated with (and thus may have contributed to) variation in reproductive performance. The first prediction from our first hypothesis stated that growth and self-maintenance would be associated with reproductive stage and fecun-

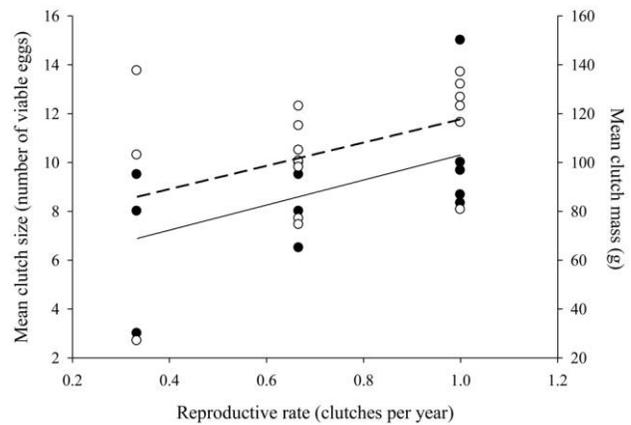


Figure 4. Over 3 yr of measurement, reproductive rate was significantly correlated with mean clutch size (filled symbols and solid line) and mean clutch mass (open circles and dashed line) in *Antaresia childreni* (colony A).

dity. In support of this, aspects of morphology and physiology in *A. childreni* were negatively related to reproductive stage (figs. 2 and 3). As in many taxa, reproduction in *A. childreni* obligates an array of morphological and physiological changes. Loss of body reserves (e.g., mass, condition, and musculature) during reproduction is experienced by other taxa (e.g., insects [Engqvist et al. 2011], fish [Sabat 1994], birds [Golet and Irons 1999], and mammals [Sand 1998]) and was expected in *A. childreni* given the combination of aphagia and the allocation of body resources to egg production. As in other taxa, reproduction was also associated with diminished oxidative status (fig. 3a, 3b; insects [Wang et al. 2001] and birds [Alonso-Alvarez et al. 2004; but see Beaulieu et al. 2011]), increased levels of circulating glucocorticoids (fig. 3d; amphibians, reptiles, and birds [reviewed in Romero 2002]), and reduced immune function (fig. 3e; insects [Fedorka et al. 2004; but for insects see Shoemaker and Adamo 2007], reptiles [French and Moore 2008], and birds [De Coster et al. 2010]). Further supporting our first prediction, we demonstrate that morphological effects were stage dependent (fig. 2). However, contrary to this prediction, physiological effects were similar between stages within reproduction (gravidity and brooding; fig. 3).

Also in support of the first prediction of our first hypothesis, morphological effects of reproduction appeared to be fecundity dependent in the short term because females with larger reproductive output (clutch size and mass, number of hatchlings) incurred greater losses in body mass and condition. However, similar to epaxial muscle loss, physiological effects did not appear to be dependent on fecundity—in fact, clutch characteristics were generally positively associated with physiological traits (highly fecund females had better physiological condition), in contradiction to our first prediction. The dichotomy between morphological and physiological results suggests that physiological changes during reproduction are fairly minimal in *A. childreni*. Morphological changes are inevitable given fe-

males' allocation of energy to egg production combined with aphagia. In contrast, physiological effects were not different among stages within reproduction (fig. 3) or related to fecundity, and this may be the result of pythons' dramatic ability to up- and down-regulate tissue (structure and performance) and metabolic rate (e.g., Ott and Secor 2007). Furthermore, glucocorticoid levels tended to drop from early to late egg brooding (fig. 3d). This finding may signify a particular emphasis on energy conservation toward the end of reproduction or that energy demand declines as reproduction progresses. In general, we found morphology to be more sensitive to reproduction than physiology in partial support for our first hypothesis.

The second prediction of our first hypothesis stated that morphological and physiological changes associated with reproduction would be correlated with one another. In support of this, allocation of muscle into reproduction was associated with reduced innate immune function and increased oxidative damage in *A. childreni*. Links between protein catabolism and aspects of physiology or self-maintenance occur in other vertebrate species. For example, the king penguin (*Aptenodytes patagonicus*) undergoes a breeding fast that depletes protein stores and is accompanied by a 40% reduction in plasma concentrations of immunoglobulins (Bourgeon et al. 2007). Additionally, oxidative damage due to fasting and, thus, protein catabolism has been documented in birds (Poljicak-Milas et al. 2007) and mammals (reviewed in Abdelmegeed et al. 2009). Recent research indicates that fasted nonreproductive female *A. childreni* lose musculature and contractile strength at a much slower rate than their fasted reproductive counterparts (Lourdais et al. 2013). Thus, if fasting alone cannot fully explain the effects of reproduction on morphology and performance, then shifts in self-maintenance (immune function and oxidative damage) during reproduction may similarly be due to more than fasting alone. Furthermore, the allocation of body resources into reproduction (loss of body condition) was associated with increased plasma concentrations of glucocorticoids toward the end of reproduction among *A. childreni*. Likely due to the role of glucocorticoids in energy mobilization, a similar negative relationship between body condition and glucocorticoids has been documented in other reptiles (Moore et al. 2000), birds (Gladbach et al. 2011), and mammals (Cabezas et al. 2007). Yet future research is required to clarify trade-offs between morphology and self-maintenance in *A. childreni* during reproduction.

We found support for the prediction based on our second hypothesis, which stated that interrelated physiological traits would be associated with variation in reproductive performance. Females that bred the subsequent year had relatively low oxidative status (low TAC and high TBARS) but high immune function (high agglutination and lytic capacity), which suggests that more fecund females prioritize immunity over antioxidant defenses. This differs from related work that uncoupled condition from phenotypic correlation, which demonstrated a positive genetic correlation between antioxidant defenses at the onset of adulthood and the number of lifetime breeding events in the zebra finch (*Taeniopygia guttata*; Kim et

al. 2010). Female *A. childreni* with relatively high reproductive output also had a high assimilation rate for crude protein after reproduction, and they incurred fewer changes to some aspects of self-maintenance (e.g., lower CORT, H:L, and TBARS during brooding), at least one of which (CORT) is associated with increased energy expenditure (Brillon et al. 1995; Tataranni et al. 1996) and increased protein catabolism (Cherel et al. 1988). Yet robustness (morphological PC 1, a reflection of body mass, size, and musculature) was negatively related to innate immune function during reproduction, which suggests that physiological differences are complex. Together, these results suggest that individuals may have different reproductive strategies that are driven by underlying physiological differences. However, future research is necessary to better understand the role played by physiological networks in interindividual variation in reproductive strategies.

Related to this point, variation in reproductive strategies has been demonstrated in free-ranging *Liasis fuscus* (a python species sympatric with *A. childreni*), in which two distinct reproductive strategies are driven by behavioral differences between maternal care phenotypes (Madsen and Shine 1999; Stahlschmidt et al. 2012). Dichotomous maternal care phenotypes within this population of *L. fuscus* may be maintained by a trade-off between offspring number and quality. Meanwhile, physiological differences observed in captive *A. childreni* may be maintained by a trade-off between reproductive rate and longevity because females in our study that reproduced more frequently exhibited reduced oxidative status, which is associated with senescence in snakes (Robert et al. 2007). Thus, captive and free-ranging pythons may offer considerable insight into the factors underlying variation in reproductive strategies.

Although we measured reproduction over only 3 yr, we demonstrate that the rate of reproductive bouts was positively correlated with reproductive output per bout (clutch size and mass) and that clutch size in the year we sampled was related to reproductive success the following breeding season. Both results suggest a positive relationship between current and future reproduction, which is in apparent conflict with life-history theory (Williams 1966; Stearns 1992; Roff 2001). Yet others have also revealed a positive relationship between current and future reproduction (Bell 1984; Roff 1992). Under ad lib. resource conditions in the laboratory, Bell (1984) demonstrated that the correlation between current reproduction and future reproduction in asexual invertebrates is not negative but is instead zero or positive. Under natural conditions in the field, female ungulates often exhibit a positive relationship between current and future fecundity despite the energetic costs of lactation (Clutton-Brock et al. 1983; Festa-Bianchet 1989; Green and Rothstein 1991; Sand 1998). Such cases have not been interpreted as an absence of costs of reproduction but rather as the result of interindividual variation in quality (e.g., variation in resource acquisition) being more important to future reproduction than is current reproductive investment (Clutton-Brock 1984; van Noordwijk and de Jong 1986; Sand 1998; Beauplet and Guinet 2007). Herein we demonstrate links between reproductive output and both energy stores (e.g., females

with higher body condition at the outset of reproduction produce larger and heavier clutches) and resource assimilation (e.g., females assimilating protein more efficiently were more likely to reproduce the following breeding season).

Data from free-ranging pythons is generally comparable to our results in captive *A. childreni*, although we measured more variables more frequently in our study. For example, loss of body mass (but not epaxial musculature) due to reproduction was positively related to clutch size in free-ranging *L. fuscus* (Stahlschmidt et al. 2012). Leukocyte profile was positively correlated with blood parasite burden shortly after reproduction in *L. fuscus*, and changes in parasite burden during reproduction were positively correlated with relative clutch size—that is, females with relatively large clutches exhibited relatively large increases in parasitism over the course of reproduction (Stahlschmidt et al. 2012). Thus, morphological and physiological changes associated with reproductive activity and fecundity occur in captive and free-ranging pythons despite differing conditions.

In conclusion, our results provide insight into potential physiological aspects of pythons that enable them to be long-lived despite heavy allocation of resources into individual reproductive bouts. Experimental manipulations of reproductive effort have been conducted in short-lived and income-breeding vertebrates (e.g., Sinervo and DeNardo 1996; Cox et al. 2010). Further studies using similar approaches would be particularly relevant in long-lived capital breeders.

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