ORIGINAL ARTICLE

SEVERE COSTS OF REPRODUCTION PERSIST IN *ANOLIS* LIZARDS DESPITE THE EVOLUTION OF A SINGLE-EGG CLUTCH

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A central tenet of life-history theory is that investment in reproduction compromises survival. We tested for costs of reproduction in wild brown anoles (*Anolis sagrei*) by eliminating reproductive investment via surgical ovariectomy and/or removal of oviductal eggs. Anoles are unusual among lizards in that females lay single-egg clutches at frequent intervals throughout a lengthy reproductive season. This evolutionary reduction in clutch size is thought to decrease the physical burden of reproduction, but our results show that even a single egg significantly impairs stamina and sprint speed. Reproductive females also suffered a reduction in growth, suggesting that the cumulative energetic cost of successive clutches constrains the allocation of energy to other important functions. Finally, in each of two separate years, elimination of reproductive investment increased breeding-season survival by 56%, overwinter survival by 96%, and interannual survival by 200% relative to reproductive controls. This extreme fitness cost of reproduction may reflect a combination of intrinsic (i.e., reduced allocation of energy to maintenance) and extrinsic (i.e., increased susceptibility to predators) sources of mortality. Our results provide clear experimental support for a central tenet of life-history theory and show that costs of reproduction persist in anoles despite the evolution of a single-egg clutch.

KEY WORDS: Growth, life history, locomotor performance, phenotypic manipulation, reproductive investment, survival, trade-off.

A central organizing theme in life-history theory is that investment in current reproduction reduces survival and, hence, future reproduction (Williams 1966; Gadgil and Bossert 1970; Stearns 1992). This inherent trade-off between the two primary components of Darwinian fitness is thought to explain diverse biological phenomena ranging from demography and population structure to the evolution of senescence and aging (Stearns 1992; Roff 2002). Artificial selection studies have shown that selection on age-specific survivorship results in correlated evolution of life-history traits associated with reproduction, and that selection on reproductive traits can similarly influence the evolution of life span (Rose and Charlesworth 1981; Reznick et al. 1990; Gasser et al. 2000; Harshman and Hoffmann 2000). These studies are essential for demonstrating life-history evolution in response to selection, but they often leave the mechanistic basis of the trade-off unresolved. Moreover, inferences regarding natural patterns of life-history evolution are limited because studies of experimental evolution are rarely feasible in wild populations (see Reznick et al. 1990 for a notable exception).

Instead, studies of wild populations have focused primarily on testing for negative correlations between reproductive investment and survival, either across species (Dunham and Miles 1985; Read and Harvey 1989; Martin 1995; Jervis et al. 2001, 2007), or among individuals (Reznick 1985; Stearns 1989; Schwarzkopf 1993, 1994). However, reproductive investment and survival are often correlated with other, unmeasured variables (e.g., age, body size, genetic quality, physiological condition) that can obscure any inherent trade-offs (Landwer 1994; Cox 2006). Thus, to accurately quantify the survival cost imposed by reproduction, it is often necessary to manipulate reproductive investment while

randomizing or controlling for variation in other potential determinants of survival. Here, we employ this experimental approach to test whether reproduction reduces survival in a wild population of lizards.

The trade-off between reproduction and survival shapes life-history evolution because its costs and benefits are measured directly in units of fitness (i.e., current vs. future reproductive success). However, the concept of a reproductive cost applies to a variety of phenotypic effects that are often measured in units other than fitness, such as time, energy, and performance (Shine 1980; Reznick 1985; Schwarzkopf 1994). These costs are of particular interest because they can help to clarify the mechanistic pathways that link survival with reproduction. For example, the physical burden of a clutch or litter impairs locomotor performance in terrestrial, aerial, and aquatic vertebrates, and may thereby increase susceptibility to predation (Shine 1980; Seigel et al. 1987; Cooper et al. 1990; Sinervo et al. 1991; Lee et al. 1996; Miles et al. 2000; Veasey et al. 2001; Ghalambor et al. 2004). Likewise, reductions in foraging and growth of reproductive females indicate underlying constraints on energy acquisition and allocation that may force analogous trade-offs with respect to somatic maintenance, thus decreasing survival (Landwer 1994; Weeks 1996; Cox 2006). In this study, we quantify the effects of reproductive investment on growth and locomotor performance to assess the potential mechanisms that structure the life-history trade-off between reproduction and survival.

Experimental manipulations of clutch size, offspring size, and reproductive investment have made invaluable contributions to our understanding of life-history evolution (Ketterson et al. 1992; Sinervo et al. 1992; Sinervo and DeNardo 1996; Sinervo 1999). The power of such manipulations lies in their ability to generate controlled phenotypic variation when natural covariance with other traits obscures causal effects on fitness, or when natural variation is minimal (Sinervo et al. 1992; Landwer 1994; Cox 2006). For example, unlike most other reptiles, lizards in the genus *Anolis* produce a single-egg clutch (Andrews and Rand 1974; Kratochvil and Kubicka 2007). This absence of natural variation in clutch size precludes traditional methods of assessing reproductive costs through correlated variation in survival (Stearns 1989). Anoles also present an interesting study system because their single-egg clutch is thought to be an adaptation to reduce the physical constraints of an egg burden on arboreal locomotion (Andrews and Rand 1974; Kratochvil and Kubicka 2007). This suggests that anoles may have evolved to circumvent the fitness costs that typically accompany reproduction in other reptiles (Shine 1980; Seigel et al. 1987; Cooper et al. 1990; Sinervo et al. 1991; Landwer 1994; Miles et al. 2000). However, given that anoles iteratively produce several single-egg clutches per month throughout a lengthy reproductive season (Andrews and Rand 1974; Lee et al. 1989), the cost of reproduction may

actually be quite substantial in these lizards. Here, we experimentally manipulate reproduction in a wild population of brown anoles (*Anolis sagrei*) to generate extreme variation in reproductive investment and test for its effects on survival, growth, and locomotor performance.

Materials and Methods
STUDY SPECIES AND NATURAL HISTORY

The brown anole (*A. sagrei*) is a small, semi-arboreal lizard that is native to islands throughout the West Indies. We studied a wild population of brown anoles at February Point, near Georgetown on the island of Great Exuma, The Bahamas (23°29′N, 75°45′W). The habitat at this site consists primarily of landscaped shrubs and small trees (e.g., bougainvillea, *Bougainvillea* sp.; gum elemi, *Bursera simaruba*; sea grape, *Coccoloba uvifera*; buttonwood, *Conocarpus erectus*; oleander, *Nerium oleander*; palms, *Pseudophoenix* sp.). Potential predators on this site include numerous birds (green herons, *Butorides virescens*; smooth-billed anis, *Crotophaga ani*; mangrove cuckoos, *Coccyzus minor*; American kestrels, *Falco sparverius*; mockingbirds, *Mimus polyglottos*), snakes (Bahamian racers, *Alsophis vudii*; boas, *Epicrates striatus*), and possibly other lizards (ameivas, *Ameiva auberi*). Survivorship is low in this and other nearby populations, with 85–95% of adult females typically dying from one reproductive season to the next (Cox and Calsbeek, 2009).

Most oviparous lizards produce multiegg clutches and exhibit both intra- and interspecific variation in clutch size, but the genus *Anolis* is atypical in that all anoles produce a single-egg clutch (Andrews and Rand 1974). This occurs because only one follicle per ovary matures at any given time. The cycle of follicular maturation and ovulation is offset between right and left ovaries, such that the oviposition of an egg produced by the right ovary coincides with the ovulation of a mature follicle from the left ovary. These events often overlap, such that females may contain zero, one, or two oviductal eggs at any given time. Although we have not measured the frequency of oviposition in wild *A. sagrei*, the hatching interval for eggs laid in captivity suggests that females can oviposit as frequently as once per week, a figure that agrees with measures from several other *Anolis* species (Andrews and Rand 1974). Hence, wild females in our population likely produce one egg every 1–2 weeks (Fig. 1), although this rate probably varies among individuals and in response to environmental factors such as rainfall or food availability. For our experiments, we excluded all females below 38 mm snout-vent length (SVL), the minimum size at which oviductal eggs are present. The reproductive season for *A. sagrei* females typically extends from April through October (Lee et al. 1989), although its precise duration is not known for our population (Fig. 1).
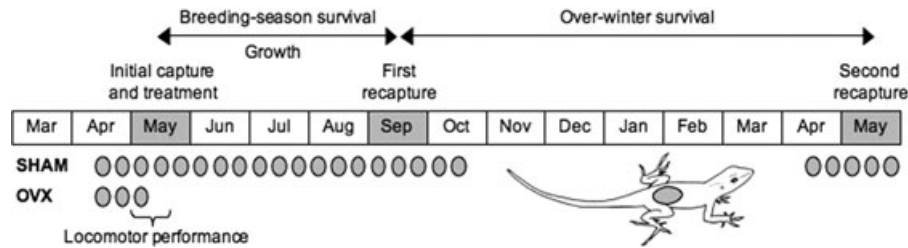
Figure 1. Timing of surgical manipulations and data collection with respect to the reproductive cycle of *A. sagrei*. Females are assumed to begin reproduction in April and iteratively produce single eggs at 10-day intervals through October. The drawing illustrates the approximate size of a single-egg clutch relative to an adult female.

EXPERIMENTAL DESIGN AND SURGICAL TREATMENTS

We used a hand-held noose to capture adult *A. sagrei* females near the beginning of the reproductive season (early May; Fig. 1) in each of two years (2007–2008). We marked each female with a unique combination of colored elastomer tags for permanent identification (Calsbeek and Irschick 2007; Calsbeek et al. 2008; Calsbeek and Smith 2008b). These tags were injected into the underside of each limb so that they were invisible to predators. We then measured snout-vent length (SVL, nearest 1.0 mm) and body mass (nearest 0.1 g) and randomly assigned each female to a treatment group. We conducted two different surgical manipulations: one in which we permanently eliminated reproduction via surgical removal of the ovaries (OVX), and one in which we temporarily eliminated the physical burden of reproduction by removing oviductal eggs while leaving the ovaries intact (EGGX). For each of these manipulations, we included a similar number of control females that received sham surgeries in which eggs and ovaries were left intact (SHAM).

Prior to surgery, we administered local anesthesia with a 2-μl subcutaneous injection of 2% lidocaine HCl (Phoenix Pharmaceuticals Inc., St. Joseph, MO). We then immobilized females by holding them at −20°C for 5 min and performing surgeries atop a slightly thawed chemical ice pack. For OVX treatments, we made a single ventral incision, ligated each oviduct, ablated each ovary, and cauterized each oviduct (Cox 2006). If any oviductal eggs were present, we removed them via a small incision in the oviduct. For EGGX treatments, we made identical incisions and removed oviductal eggs, but left the ovaries intact. For SHAM treatments, we used the same anesthesia and cold-immobilization procedure, made identical incisions, and physically manipulated the eggs and ovaries with forceps, but left them intact. We closed the incisions with Nexaband surgical glue (Veterinary Products Laboratories, Phoenix, AZ) and allowed females to recover overnight. The following day, each female received a temporary spot of white paint on her dorsum to prevent immediate recapture and was released at her exact site of capture.

SURVIVAL

We measured survival on the basis of an initial sample of $n = 194$ OVX and 188 SHAM females. We attempted to recapture all surviving females at the end of the breeding season (September, 4 months post-treatment) and again at the beginning of the following breeding season (May, 12 months posttreatment; Fig. 1). Although we searched the entire site exhaustively for 7–10 consecutive days at each recapture census, we occasionally recaptured animals in May that we had failed to recapture the previous September. Thus, our recapture success slightly underestimated actual breeding-season survival. This should not affect inferences regarding the survival cost of reproduction, unless the likelihood of recapture was biased by experimental treatment. Nonetheless, we conducted analyses of survival in two ways. First, we analyzed survival directly from recapture data by considering an individual to have survived over a given interval if it was recaptured in any subsequent census. This provides a minimum estimate of survival for each group. We measured survival over three time intervals (Fig. 1): breeding-season survival (May–September), overwinter survival (September–May), and the cumulative period encompassing both of these successive intervals, which we refer to as interannual survival (May–May). For each interval, we tested for survival costs of reproduction using generalized linear models with a logit link function including surgical treatment (OVX, SHAM) as the main effect and survival as a binomial response variable (live, die). These models included effects for year (2007, 2008) and the year × treatment interaction. To establish a natural baseline, we compared the survival of experimental females to that of a nearby (1 km), unmanipulated population on Kidd Cay, Great Exuma (23°30′N, 75°45′W; $n = 339$ females) that has been subject to intensive mark–recapture studies since 2003 (Calsbeek and Irschick 2007; Calsbeek 2008; Calsbeek and Bonneaud 2008; Calsbeek et al. 2008; Calsbeek and Smith 2008a).

Second, we used the program MARK (version 5.1) to construct a standard Cormack–Jolly–Seber (CJS) model to estimate the probability of survival (φ) and of recapture (p) for each treatment group (White and Burnham 1999). This analysis differs from

the method presented above in that it uses data from those females that were not recaptured in September but were known to be alive (i.e., they were recaptured the following May) to estimate actual survival probabilities in light of our imperfect recapture success. For all analyses, we constructed fully time-dependent CJS models by allowing both ϕ and p to differ for the two separate survival intervals (breeding-season, overwinter) and corresponding recapture points (September, May). We then generated four alternative candidate models by variably including or excluding treatment effects on ϕ and p. Estimating both ϕ and p separately for each treatment creates a saturated model in which the number of parameters is sufficient to account for any observed pattern in the data. By definition, a saturated model exhibits no overdispersion and therefore requires no adjustment with a variance inflation factor ($\hat{c}$). Hence, we evaluated our four candidate models directly on the basis of Akaike's information criterion (AIC_c) and associated measures of model weight and likelihood, as calculated by the program MARK.

GROWTH

For those females recaptured at the end of the breeding season, we measured growth as change in SVL or body mass from premanipulation (May) to recapture (September; Fig. 1). Sample sizes for analyses of growth consisted of $n = 86$ OVX and 59 SHAM females. Our pretreatment measures of body mass included the mass of ovaries and oviductal eggs prior to surgery. Therefore, the change in mass that we measured for the OVX group actually underestimates their growth because it does not account for the additional mass gain required to offset the surgical loss of ovaries and eggs. Hence, our estimates of the growth cost of reproduction in terms of body mass are actually conservative. Growth was negatively correlated with initial size for both SVL and mass, and animals grew more in 2007 than in 2008 (see Results). For these reasons, we compared change in size as a function of surgical treatment (OVX or SHAM) using analysis of covariance (ANCOVA) with initial size (SVL or mass) as a covariate and including effects for year and the year $\times$ treatment interaction. Growth responses were interpreted on the basis of least-square means from these analyses. Interactions for initial size $\times$ surgical treatment, initial size $\times$ year, and initial size $\times$ surgical treatment $\times$ year were never significant and were omitted from our final statistical models. We also assessed body condition by comparing regressions of body mass on SVL for each treatment group. Body mass and SVL were $\log_{10}$-transformed prior to this analysis.

LOCOMOTOR PERFORMANCE

We tested whether the physical burden of an egg constrains locomotor performance by comparing stamina and sprint speed for $n = 34$ EGGX and 28 SHAM females. Individuals without any oviductal eggs at the time of surgery were omitted from both treatment groups. We measured stamina by running females to exhaustion (i.e., loss of righting reflex) on a motorized treadmill inclined at $20°$ and rotating at 0.4 km/h. We measured maximal sprint speed by chasing females up an inclined dowel ($20°$ inclination, 1 m length, 2.5 cm diameter), recording the trials on a video camera, and digitizing and analyzing the video using MotionAnalysis software (http://warthog.ucr.edu/). We recorded three trials per female and estimated sprint speed as the maximal speed achieved over any 10-cm interval in any of the three trials. These performance assays are described in detail elsewhere (Calsbeek and Irschick 2007; Calsbeek 2008; Calsbeek and Bonneaud 2008). Stamina and sprint speed for each individual were measured both prior to and one day following surgery (Fig. 1). Effects of surgical treatment on performance were inferred from time $\times$ surgery interactions using repeated-measures ANOVA. Because the elimination of reproductive burden via egg removal is only a temporary manipulation, we did not follow the long-term growth or survival of these females.

Results

At initial capture in May, most (78%) females contained at least one oviductal egg and many (22%) contained two eggs. The number of oviductal eggs did not differ between treatment groups prior to surgery ($\chi^2 = 2.55$; $P = 0.28$). Upon recapture in September, abdominal palpation verified that surgical manipulations produced their intended treatment effects. We never detected a shelled egg within an OVX female ($n = 86$), but the majority of SHAM females were still producing eggs when recaptured in September ($n = 32$ of 59, 54%).

SURVIVAL

As predicted, breeding-season survival was substantially higher for nonreproductive OVX females (0.53) relative to reproductive SHAM females (0.34) ($\chi^2 = 13.27$; $P < 0.001$; Fig 2A). Remarkably, overwinter survival was nearly twice as high for OVX females (0.45) relative to SHAM females (0.23) ($\chi^2 = 8.46$; $P = 0.004$; Fig. 2B). Interannual survival, the product of breeding-season and overwinter survival, was threefold higher for OVX females (0.24) relative to SHAM females (0.08) ($\chi^2 = 18.53$; $P < 0.001$; Fig. 2C). We did not find significant effects of year or the year $\times$ treatment interaction on survival over any of these three intervals, indicating that results were congruent between 2007 and 2008 (Fig. 2). Moreover, breeding-season survival of SHAM females was nearly identical to that of unmanipulated females at a nearby reference site in both 2007 (0.36 vs. 0.37; $\chi^2 = 0.04$; $P = 0.84$) and 2008 (0.33 vs. 0.32; $\chi^2 = 0.03$; $P = 0.87$). This confirms that treatment effects were due to the increased survival of OVX females and that surgery itself had no detrimental effect on survival.
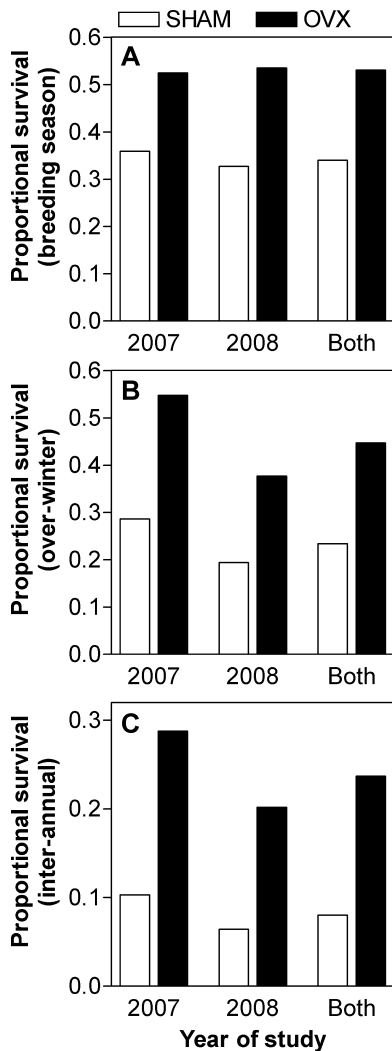
Figure 2. Survival of nonreproductive OVX females exceeded that of reproductive SHAM over three separate intervals: (A) breeding-season (May–September), (B) overwinter (September–May), and (C) interannual (May–May) in each of two independent years of study.

Estimates of survival using standard CJS models also revealed pronounced differences between OVX and SHAM females. Of the four candidate models we examined, the two that include separate survival parameters (φ) for each treatment group receive the majority of support (summed weight = 0.92; data for both years combined, Table 1). In each separate year, and when combining data from both years, the candidate model with the highest likelihood was that which included a separate φ for each treatment group and a single recapture probability (p) for both groups (Table 1). We used this model ($\phi_{treatment}\, p_.$) to estimate breeding-season survival in light of our imperfect recapture success ($p = 0.66$ for both years combined). Hence, minimum estimates of survival based on observed recaptures (Fig. 2) are approximately two-thirds as large as model estimates that ac-

count for p (Table 2). However, because p was modeled as equivalent for OVX and SHAM, treatment differences in survival were pronounced using either approach (Table 2). In fact, the saturated model ($\phi_{treatment}\, p_{treatment}$) generated slightly higher recapture probabilities for SHAM ($p = 0.73$) relative to OVX ($p = 0.63$). Thus, the magnitude of the survival difference between treatments generally increased when p was modeled separately for each group (Table 2).

GROWTH

Growth was negatively correlated with initial size (SVL: $F_{1,137} = 118.20$; $P < 0.0001$; body mass: $F_{1,137} = 99.97$; $P < 0.0001$) and growth in SVL was greater in 2007 than in 2008 (year: $F_{1,137} = 7.43$; $P = 0.007$). Hence, we tested for treatment effects on growth using ANCOVA with initial size (SVL or body mass) as a covariate. As predicted, experimental removal of reproductive investment increased growth in SVL ($F_{1,139} = 9.47$; $P = 0.003$; Fig. 3) and more than doubled growth in body mass ($F_{1,139} = 14.43$; $P < 0.0001$; Fig. 3). Although these effects were observed in both years of study, they were more pronounced in 2007 than in 2008 (treatment × year effect on SVL: $F_{1,137} = 3.52$; $P = 0.06$; body mass: $F_{1,137} = 7.03$; $P = 0.009$). For any given length, OVX females also had significantly greater body mass than SHAM females ($F_{1,139} = 9.39$; $P < 0.003$). Thus, despite the fact that more than half of the SHAM females were gravid at recapture, nonreproductive OVX females still consistently outweighed SHAM females, indicating substantially greater mass per unit SVL.

LOCOMOTOR PERFORMANCE

Repeated-measures analysis of variance (ANOVA) revealed significant time × treatment interactions for both stamina ($F_{1,60} = 11.20$; $P = 0.001$) and sprint speed ($F_{1,60} = 4.22$; $P = 0.044$). Removing the physical burden of reproduction increased stamina by 9% and sprint speed by 12% relative to pretreatment values for EGGX females (Fig. 4). By contrast, SHAM females with an egg burden exhibited no change in sprint speed and a slight, nonsignificant decrease in stamina (Fig. 4).

Discussion

By surgically preventing reproduction in wild female anoles, we quantified the costs of reproduction in terms of survival, growth, and locomotor performance. Despite the evolution of a single-egg clutch, anoles clearly suffer severe costs of reproduction with respect to each of these fitness-related traits. The dramatic increase in survival following removal of reproductive investment is of particular significance because it demonstrates a trade-off between the two primary components of Darwinian fitness. Given that anoles iteratively produce single-egg clutches throughout a lengthy breeding season (Fig. 1), adult longevity should be closely

Table 1. MARK maximum-likelihood comparison of four candidate models estimating the probability of survival (ϕ) and recapture (p) for *Anolis sagrei* females. Models allowing ϕ to differ by treatment but estimating a single p for both groups consistently received the greatest support (i.e., lowest AIC$_c$, highest weight and likelihood).

Year	Model	Parameters	AIC$_c$	Δ AIC$_c$	Weight	Likelihood
2007	$\phi_{treatment} \, p_.$	5	351.52	0.00	0.600	1.000
	$\phi_. \, p_{treatment}$	5	353.80	2.28	0.192	0.320
	$\phi_{treatment} \, p_{treatment}$	7	354.17	2.65	0.160	0.266
	$\phi_. \, p_.$	3	356.57	5.05	0.048	0.080
2008	$\phi_{treatment} \, p_.$	5	463.45	0.00	0.488	1.000
	$\phi_. \, p_{treatment}$	5	464.22	0.77	0.332	0.681
	$\phi_{treatment} \, p_{treatment}$	7	465.50	2.05	0.175	0.359
	$\phi_. \, p_.$	3	472.95	9.50	0.005	0.009
Both	$\phi_{treatment} \, p_.$	5	809.30	0.00	0.623	1.000
	$\phi_{treatment} \, p_{treatment}$	7	810.80	1.50	0.294	0.472
	$\phi_. \, p_{treatment}$	5	813.34	4.04	0.082	0.131
	$\phi_. \, p_.$	3	827.34	18.04	0.001	0.001

tied to fecundity. Thus, investment in current reproduction incurs a direct cost with respect to future reproduction. To the extent that traits such as egg size or clutch frequency are heritable, this trade-off can structure the evolution of alternative life-history strategies (Stearns 1989, 1992; Sinervo et al. 2000). Lizards have long been know to follow the classic life-history axis ranging from species with low reproductive investment and high survival to those with high reproductive investment and low survival (Tinkle 1969; Dunham and Miles 1985; Clobert et al. 1998). We have extended the scope of previous work by providing strong experimental evidence for this trade-off in a species that exhibits the atypical reproductive adaptation of a single-egg clutch.

The magnitude of the survival cost that we observed suggests that mortality due to reproduction can have a considerable impact on the demography and age structure of a population. Breeding-season survival of SHAM females was reduced to approximately two-thirds that of nonreproductive OVX females, a calculation that was robust to three alternative methods of estimating survival (Table 2). Overwinter survival of SHAM females was only half that of OVX females, which is somewhat surprising given that brown anoles do not reproduce through most of the winter (Lee et al. 1989). Our recapture intervals did not bracket the entire reproductive season, so this could simply reflect mortality due to reproduction that occurred after our September census or prior to our May census (see Fig. 1). Alternatively, this could indicate that a lengthy reproductive season leaves females energetically depleted and thus subject to increased probability of overwinter mortality. In other lizards, energetic costs of reproduction are known to persist even after the cessation of reproduction (Landwer 1994; Cox 2006). Overall, the markedly higher survival of OVX

Table 2. Estimates of breeding-season survival for OVX and SHAM females using three alternative methods: (1) Recaptures—minimum estimate based directly on observed recaptures; (2) $\phi_{treatment} \, p_.$—CJS mark–recapture model with a single recapture probability (p) for each treatment; and (3) $\phi_{treatment} \, p_{treatment}$—CJS mark–recapture model with a separate p for each treatment. The inferred survival cost of reproduction is alternatively expressed as the difference between treatments (absolute cost) and as the fractional survival of SHAM relative to OVX=1.0 (relative survival).

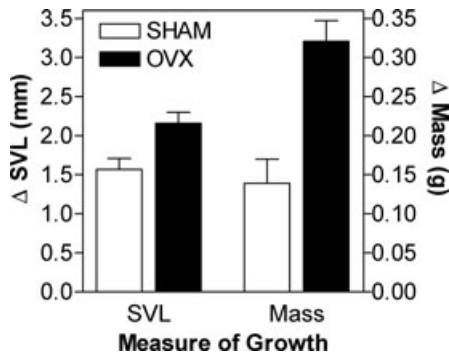| Year	Method	Survival probability		Absolute cost	Relative survival
		OVX	SHAM	(OVX–SHAM)	(SHAM/OVX)
2007	Recaptures	0.53	0.36	0.17	0.68
	$\phi_{treatment} \, p_.$	0.62	0.46	0.16	0.74
	$\phi_{treatment} \, p_{treatment}$	0.65	0.40	0.25	0.62
2008	Recaptures	0.54	0.33	0.21	0.61
	$\phi_{treatment} \, p_.$	0.76	0.50	0.26	0.66
	$\phi_{treatment} \, p_{treatment}$	0.75	0.53	0.22	0.71
Both	Recaptures	0.53	0.34	0.19	0.64
	$\phi_{treatment} \, p_.$	0.69	0.48	0.21	0.70
	$\phi_{treatment} \, p_{treatment}$	0.70	0.44	0.26	0.63

Figure 3. Mean (+1SEM) breeding-season growth in snout-vent length (SVL) and body mass of OVX females exceeded that of SHAM females. Data are least-square mean treatment effects from statistical models accounting for effects of initial size, year, and the year × treatment interaction. Log$_{10}$-transformed data were used for statistical analyses, but raw values are shown here for illustrative purposes.

relative to SHAM over both the breeding-season and overwinter recapture intervals translated into a threefold difference in inter-annual survival. Whereas the brown anole is effectively an annual species under natural levels of mortality, nearly one-third of the female population survived to a second breeding season in the absence of reproductive investment. Thus, elimination of reproductive investment increased survival well beyond what is typical of a natural population.

This extreme survival cost of reproduction could arise from at least two distinct, nonexclusive processes: reduced allocation of energy to maintenance and survival, or increased susceptibility to predation. This distinction bears on the fundamental question of how intrinsic and extrinsic sources of mortality interact to shape life-history evolution (Reznick et al. 2004; Williams et al. 2006). Our data cannot address this question directly, but treatment effects on growth and locomotor performance may have
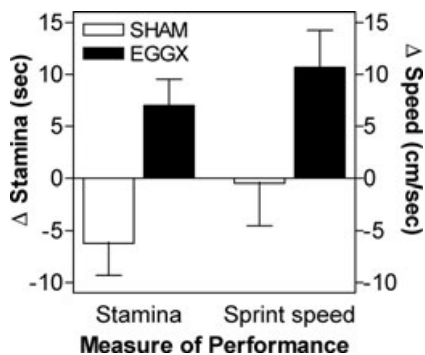

Figure 4. Mean (+1SEM) changes in stamina (left) and sprint speed (right) were greater following experimental egg removal (EGGX) than in control females (SHAM) that retained the physical burden of an egg. Change in performance is based on repeated measurements conducted prior to and one day following surgery.

implications for intrinsic and extrinsic mortality, respectively. For example, a treatment effect on growth would verify that energy-allocation decisions could be altered in response to surgical manipulations. This would suggest that analogous energetic trade-offs could potentially constrain allocation to somatic maintenance and thereby influence levels of intrinsic mortality. In the brown anole, eliminating reproductive investment increased growth in length by 37% and growth in mass by 128% relative to reproductive females. In a viviparous lizard (*Sceloporus jarrovii*) with a single annual litter, the same ovariectomy manipulation increased growth in length by 25% relative to pregnant females (Cox 2006). Hence, the magnitude of the growth cost of reproduction is comparable or perhaps even larger in *A. sagrei*, which iteratively lays single eggs at frequent intervals, than in a species that bears a large litter (2–14 offspring) once per year.

Reproductive investment clearly forces an energy-allocation trade-off with growth, but further research is required to determine whether analogous trade-offs influence other physiological processes that directly influence survival. For example, immune function influences the survival of female anoles (Calsbeek and Bonneaud 2008; Calsbeek et al. 2008) and is known to be both energetically expensive and functionally compromised during reproduction in other animals (Ardia et al. 2003; Hanssen et al. 2005; Martin et al. 2006; French et al. 2007). However, we still lack a clear demonstration that reduced immune function itself accounts for the survival cost of reproduction in any wild population. Other aspects of maintenance, such as DNA repair and resistance to oxidative stress, could also be compromised by energy-allocation trade-offs with reproduction. Inter- and intraspecific variation in these components of maintenance have recently been shown to correlate with variation in longevity of other squamate reptiles (Bronikowski 2008). Brown anoles provide a promising system with which to extend this research by experimentally disentangling the energetic trade-offs between reproduction and maintenance that presumably shape life-history variation.

Increased susceptibility to predation could also explain the survival cost of reproduction if anoles have greater difficulty escaping predators when encumbered with an egg. Predation is an important source of mortality that influences the strength and form of natural selection on *A. sagrei* females (Losos et al. 2004). Moreover, the physical burden of a clutch greatly impairs locomotion in other lizards (Cooper et al. 1990; Sinervo et al. 1991; Miles et al. 2000). Unlike these species, anoles have evolved a single-egg clutch that is thought to reduce the physical burden of reproduction on locomotion (Andrews and Rand 1974; Kratochvil and Kubicka 2007). Despite this reproductive adaptation, our manipulations revealed that gravid anoles suffer significant reductions in stamina and sprint speed, both of which are known to influence survival (Calsbeek and Irschick 2007; Calsbeek 2008; Calsbeek and Bonneaud 2008). However, the performance costs

that we observed are less pronounced than estimates from lizards with larger clutch sizes. For example, we found a 9% increase in sprint speed following the removal of an egg, whereas the difference between gravid and postreproductive western fence lizards (*Sceloporus occidentalis*) ranged from 20% to 45% across populations (Sinervo et al. 1991). Likewise, we found a 12% increase in stamina following egg removal, whereas gravid and postreproductive side-blotched lizards (*Uta stansburiana*) differed by 31% in stamina (Miles et al. 2000). Although female anoles clearly experience significant constraints on locomotion when gravid, these comparisons suggest that the evolution of a single-egg clutch has substantially reduced the magnitude of the locomotor costs associated with reproduction.

Our results agree with previous studies in which experimental reductions in reproductive investment were found to increase growth, locomotor performance, and survival (Landwer 1994; Sinervo and DeNardo 1996; Miles et al. 2000; Cox 2006). Although several of these studies provide indirect evidence that predation structures the trade-off between reproduction and survival, none has directly tested this hypothesis. Our demonstration that reproduction compromises stamina and sprint speed is consistent with this interpretation, but we have not yet directly linked either measure of performance to the difference in survival between OVX and SHAM females. To directly test this hypothesis, it will be necessary to manipulate both reproductive investment and predation intensity. Given the strong potential for energy-allocation trade-offs with maintenance, it is likely that some portion of the survival cost of reproduction also reflects intrinsic sources of mortality, or that it arises from interactions between intrinsic and extrinsic factors (e.g., immune function and parasites or pathogens).

Although surgical ovariectomy eliminates most components of reproductive investment, it also removes the primary source of steroid hormones that may influence growth, performance, and survival. For example, ovariectomy influences growth in some mammals by decreasing circulating estrogen levels (Ford and Klindt 1989). Testosterone is known to stimulate the growth of male brown anoles (Cox et al. 2009), and it is possible that estrogens or progestins also contribute to the development of sexual size dimorphism in this species by regulating the growth of females (Lerner and Mason 2001). Estrogens and progestins also regulate aggression and reproductive behaviors in female lizards (Whittier and Tokarz 1992; Woodley and Moore 1999; Rubenstein and Wikelski 2005). It is unclear how this might translate into the treatment effects on growth and survival that we observed, but reductions in aggression and mating behavior of OVX females could potentially increase their foraging time and/or reduce their exposure to predators. It is important to note that, in the broadest sense, reproductive investment and its associated costs should be viewed as inclusive of many of the sex-specific behaviors and activities modulated by ovarian steroids. Nonetheless, future studies would benefit from refined experimental approaches that partition the various costs of reproduction into those arising from behavioral and physiological effects of hormones, versus those arising from energetic trade-offs and/or the physical burden of a clutch.

We have taken a functional approach to assessing the cost of reproduction by manipulating reproductive investment and observing resultant phenotypic effects on growth, performance, and survival. However, many theoretical models are concerned primarily with the underlying genetic correlations between traits related to current reproduction versus those related to survival and future reproduction (Rose and Charlesworth 1981; Reznick 1985; Bailey 1992). Empirical approaches to this question have sought to estimate these genetic correlations or to measure the correlated evolution of life-history traits in response to selection (Rose and Charlesworth 1981; Reznick et al. 1990; Gasser et al. 2000; Harshman and Hoffmann 2000). We have not demonstrated a negative genetic correlation between survival and reproductive investment, nor have we assessed the genetic basis of any component of reproduction or survival. This limits our ability to predict life-history evolution in anoles, but our approach nonetheless confirms a functional link between reproduction and survival (Sinervo 1999).

In conclusion, we have shown that the elimination of reproductive investment via surgical ovariectomy results in a dramatic increase in the survival wild female anoles. This survival cost is evident during the breeding season and apparently increases thereafter, suggesting that the cumulative energetic cost of successive clutches leaves females energetically compromised and more susceptible to overwinter mortality. The energetic cost of reproduction also severely constrains the allocation of energy to growth. Future work should address the intriguing possibility that analogous energetic trade-offs with somatic maintenance (e.g., immune function, DNA repair) lead to increased intrinsic mortality and thereby contribute to the survival cost of reproduction. Despite the evolution of a single-egg clutch, gravid anoles also suffer significant reductions in locomotor performance, although the relative magnitude of this cost is apparently reduced relative to species with larger clutches. Manipulations of predation regime are required to determine whether this reproductive burden contributes to the survival cost of reproduction by increasing susceptibility to predation and, hence, extrinsic mortality.

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