Experimentally decoupling reproductive investment from energy storage to test the functional basis of a life-history trade-off

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Summary

1. The ubiquitous life-history trade-off between reproduction and survival has long been hypothesized to reflect underlying energy-allocation trade-offs between reproductive investment and processes related to self-maintenance. Although recent work has questioned whether energy-allocation models provide sufficient explanations for the survival cost of reproduction, direct tests of this hypothesis are rare, especially in wild populations.

2. This hypothesis was tested in a wild population of brown anole lizards (*Anolis sagrei*) using a two-step experiment. First, stepwise variation in reproductive investment was created using unilateral and bilateral ovariectomy (OVX) along with intact (SHAM) control. Next, this manipulation was decoupled from its downstream effects on energy storage by surgically ablating the abdominal fat stores from half of the females in each reproductive treatment.

3. As predicted, unilateral OVX (intermediate reproductive investment) induced levels of growth, body condition, fat storage and breeding-season survival that were intermediate between the high levels of bilateral OVX (no reproductive investment) and the low levels of SHAM (full reproductive investment).

4. Ablation of abdominal fat bodies had a strong and persistent effect on energy stores, but it did not influence post-breeding survival in any of the three reproductive treatments. This suggests that the energetic savings of reduced reproductive investment do not directly enhance post-breeding survival, with the caveat that only one aspect of energy storage was manipulated and OVX itself had no overall effect on post-breeding survival.

5. This study supports the emerging view that simple energy-allocation models may often be insufficient as explanations for the life-history trade-off between reproduction and survival.

Key-words: *Anolis*, cost of reproduction, energy allocation, estradiol, fat body, lizard, mark–recapture, natural selection, phenotypic manipulation, survival

Introduction

The concept of a trade-off between reproduction and survival is central to life-history theory (Stearns 1992; Roff 2002), but we still have a very fragmentary understanding of the underlying mechanisms that link these two components of fitness. One of the most widely invoked explanations is based on the assumption that reproduction and self-maintenance compete over limited energy and nutrients (van Noordwijk & Jong 1986; Zera & Harshman 2001; Harshman & Zera 2007). In support of this idea, reproductive investment has been shown to impact metabolism and energy storage (Vézina & Williams 2005; Hatle *et al.* 2008; Cox *et al.* 2010) as well as DNA repair, resistance to oxidative stress, immune function, resistance to parasites and wound healing (Gustafsson *et al.* 1994; Hosken 2001; Bonneaud *et al.* 2003; Alonso-Alvarez *et al.* 2004; Partridge, Gems & Withers 2005; French, DeNardo & Moore 2007; Zera, Harshman & Williams 2007; Knowles, Nakagawa & Sheldon 2009; Cox *et al.* 2010; Christe *et al.* 2012). Because these processes are thought to be both energetically expensive and intimately tied to self-maintenance, they represent promising intermediaries that could link reproduction, energetics and survival (Lochmiller & Deerenberg 2000; Martin, Scheuerlein & Wikelski 2003). However, it is generally unknown whether the energetic cost of reproduction *per se* is responsible for reduced

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self-maintenance and survival. Indeed, it has recently been questioned whether simple models of energy allocation between competing processes provide a sufficiently complex framework for explaining life-history trade-offs (Råberg *et al.* 1998; Barnes & Partridge 2003; Eraud *et al.* 2005; Williams 2005; Edward & Chapman 2011).

Our understanding of how energetic costs shape the trade-off between reproduction and survival is hindered by a lack of studies building direct causal links among all components of this proposed pathway. For example, many of the above studies manipulated reproductive investment and found effects on energetics, self-maintenance and/or survival. However, none conclusively demonstrated that the downstream effects of reproductive investment on survival were the result of its upstream effects on energetics and self-maintenance. This is no idle concern, as many of the physiological changes typically interpreted as 'costs' of reproduction may actually serve adaptive functions (e.g. immunosuppression may prevent inappropriate autoimmune responses against embryos; Råberg *et al.* 1998) or could be neutral by-products of reproductive physiology (e.g. reduced haematocrit may result from osmoregulatory adjustments to increased levels of yolk precursors; Williams *et al.* (2004)). Moreover, these changes may often arise from pleiotropic modulation by reproductive hormones, rather than from energetic trade-offs with reproduction *per se* (Råberg *et al.* 1998; Barnes & Partridge 2003; Williams 2005; Edward & Chapman 2011). An ideal demonstration of an energetically mediated trade-off would show that (i) reproduction impacts energetics, (ii) reproduction impacts survival and (iii) reproduction does not impact survival when it is decoupled from its effects on energetics. The present study addresses these issues by experimentally altering reproductive investment and then decoupling this manipulation from its effects on energy storage in a wild population of brown anoles, *Anolis sagrei*.

The brown anole is a small, short-lived lizard in which the elimination of reproduction via bilateral ovariectomy (OVX) increases both breeding-season and post-breeding survival relative to SHAM controls (Cox & Calsbeek 2010b). OVX also increases fat storage, body condition, haematocrit and immune function (Cox *et al.* 2010). This suggests that reproduction leaves females energetically compromised and therefore unable to fully support maintenance functions that could otherwise improve their survival. The present study tests this hypothesis by experimentally decoupling reproductive investment from energy storage through a combination of (i) surgical ovariectomy and (ii) subsequent ablation of the fat bodies, paired storage organs that increase dramatically in size following OVX. If the increased post-breeding survival of OVX females is related to this surfeit of stored energy, then the surgical removal of these fat stores should reduce survival to levels characteristic of SHAM females.

Although bilateral OVX provides a powerful tool for measuring costs of reproduction, this approach has at least two important caveats. First, the complete elimination of reproduction creates a level of investment that is well below the range of natural variation. Interpretations about the relevance of experimental results with respect to natural evolutionary and demographic processes must therefore be made with caution (Dunham & Beaupre 1998). Secondly, the removal of both ovaries eliminates a source of steroid hormones that may influence behaviour (Whittier & Tokarz 1992; Woodley & Moore 1999; Williams 2005) and the mobilization of stored energy (Hahn 1967; Greenberg & Gist 1985; Shanbhag & Prasad 1992). This study addresses these caveats by including a unilateral OVX treatment to induce levels of reproductive investment that are similar to the low end of natural variation and intermediate between bilateral OVX and SHAM. This experimental gradient of reproductive investment is predicted to result in parallel gradients with respect to growth, body condition, fat storage and survival. The preservation of a single ovary is also predicted to compensate for any effects of bilateral OVX on steroid production, thus partially controlling for any direct endocrine effects.

Materials and methods

STUDY SPECIES AND REPRODUCTIVE BIOLOGY

This study focuses on a population of *A. sagrei* at February Point, near Georgetown on Great Exuma, the Bahamas (23° 29′N, 75°45′W). Reproductive investment of females in this population has previously been manipulated over 3 years (2007–2009), revealing consistent stimulatory effects of bilateral OVX on growth, fat storage and survival (Cox & Calsbeek 2010b, 2011; Cox *et al.* 2010). The reproductive season of *A. sagrei* extends from April through October (Lee *et al.* 1989), over which time females repeatedly lay single eggs at intervals of 1–2 weeks (Andrews & Rand 1974; Cox & Calsbeek 2010b). Only one follicle per ovary matures at a given time, with the cycle of ovulation offset between right and left ovaries (Crews 1980). Although *A. sagrei* females exhibit a genetically based, dorsal-pattern polymorphism (Calsbeek, Bonneaud & Smith 2008; Calsbeek, Bonvini & Cox 2010), the three alternative female morphs do not differ in (i) frequency of egg production, (ii) number, frequency, size or sex ratio of their offspring or (iii) the extent to which OVX improves their survival, growth, immune function or haematocrit (Cox & Calsbeek 2011). Hence, the present study does not distinguish among female morphs.

VALIDATION OF UNILATERAL OVX IN CAPTIVITY

The first objective was to confirm that unilateral OVX reduces reproductive investment. Removal of one ovary should extend the interval between ovulation of successive eggs, thereby decreasing the number of eggs produced over the breeding season. In other *Anolis* species, unilateral OVX does not detrimentally impact egg production by the remaining ovary (Lovern & Passek 2002), although it can induce compensatory growth of the contralateral follicle (Jones *et al.* 1997). Compensation could offset the expected decrease in reproductive frequency by accelerating folliculogenesis in the remaining ovary. Moreover, experimental

reductions in clutch size can result in increased allocation to the remaining eggs or embryos (Bleu *et al.* 2012). Therefore, effects of unilateral OVX on the frequency and size of offspring were characterized prior to interpreting this manipulation as a reduction in reproductive investment.

In May of 2010, 36 wild females were captured and transported to Dartmouth College. Brown anoles can produce viable eggs from stored sperm for up to 4 months following isolation (Calsbeek & Bonneaud 2008; Cox & Calsbeek 2010a), so no further access to mates was provided. Pairs of females were housed together in 10-gal glass terraria (50 × 25 × 30 cm) containing mulch, a potted plant for oviposition, a 40W incandescent bulb and two Repti Glo 5.0 fluorescent bulbs (5% UVB; Hagen Inc., Montreal, QC, Canada). Terraria and plants were sprayed with water daily, and diurnal temperatures spanned a gradient from 26–35 °C. Each cage was provisioned with 4–6 crickets per day (Fluker Farms, Port Allen, LA, USA), dusted weekly with Fluker's Repta-Vitamin dietary supplement.

Snout–vent length (SVL, nearest 1·0 mm with a ruler) and body mass (nearest 0·02 g with an electronic balance) were used to divide females into two size-matched treatment groups: (i) unilateral OVX and (ii) intact SHAM control. Both females within a terrarium were assigned to the same treatment to avoid confounding progeny between treatments. Prior to surgery, local anaesthesia was administered at the incision site with a 2-μL injection of 2% lidocaine HCl (Phoenix Pharmaceuticals Inc., St. Joseph, MO, USA). Females were cooled at −20 °C for 5 min, and surgeries were performed atop a slightly thawed chemical ice pack. For unilateral OVX, a single ventral incision was made, the right ovary was ablated, and the oviduct was cauterized (Cox 2006; Cox & Calsbeek 2010b). For SHAM, the right ovary was physically manipulated with forceps and left intact. Incisions were closed with Vetbond™ tissue adhesive (3M Animal Care Products, St. Paul, MN, USA).

Once eggs began to hatch, each terrarium was searched every 2 days and the sex, SVL and body mass of each new hatchling were recorded. Although offspring were still hatching at 3·5 months post-surgery, this point was used as a cut-off for data collection to minimize the potential for sperm limitation to impact estimates of fecundity. This experiment included $n = 18$ females per treatment, but pairing females within terraria resulted in $n = 9$ terraria per treatment as units of observation. To test whether unilateral OVX reduced the frequency of reproduction, the number of offspring produced per female/month was calculated by dividing the total number of offspring produced in a terrarium by the sum of the number of months that each female was in the terrarium. To assess the potential for increased investment per egg following unilateral OVX, body mass and SVL of hatchlings were compared using ANOVA with each individual hatchling as an observation and terrarium as a random effect. Finally, sex ratio was compared between surgical treatments using generalized linear models with binomial error distributions and logit links (Wilson & Hardy 2002). This analysis was conducted because males grow more quickly than females after hatching (Cox, Stenquist & Calsbeek 2009) and may therefore reflect greater maternal investment (Cox & Calsbeek 2011).

HORMONE ASSAYS

The second objective was to evaluate any hormonal changes induced by the removal of ovaries and to determine whether unilateral OVX compensates for any effects of bilateral OVX on circulating 17β-estradiol (E_2) levels. At the conclusion of the captive study (3·5 months post-treatment), 30–60 μL of blood was collected from $n = 10$ unilateral OVX and 13 SHAM females via decapitation. At the conclusion of the field study (12 months post-treatment), blood was collected from $n = 15$ bilateral OVX, 22 unilateral OVX and 13 SHAM females. Samples were held on ice until centrifugation within 1 h of collection. Separated plasma was stored at −20 °C.

Plasma E_2 levels were measured using radioimmunoassay (RIA) (Wingfield & Farner 1975; Wack *et al.* 2008). For each sample, 4–20 μL of plasma was mixed with 0·5 mL ddH$_2$O and 1000 cpm of ^{3}H-E$_2$ (NET-317; PerkinElmer Life Sciences, Inc., Boston, MA, USA) for individual recovery determination, then stored overnight at 4 °C. Samples were extracted twice with 2 mL diethyl ether, dried in a 37 °C water bath under nitrogen gas, reconstituted in 0·3 mL assay buffer and stored overnight at 4 °C. RIA was performed using ^{3}H-E$_2$, E_2 standard (E8875; Sigma-Aldrich, St. Louis, MO, USA) and a highly specific antibody for E_2 (7010-2650, Biogenesis, Ltd., Poole, UK). The standard curve ranged from 0·98 to 250 pg and was run in duplicate. Samples were run singly and adjusted for individual recovery (average = 75%, range = 61–82%) and sample volume. All samples were run in a single assay. The intra-assay coefficient of variation was 9%. Four samples were below the assay detection limit, all of which were from the field experiment ($n = 1$ SHAM, 1 unilateral OVX and 2 bilateral OVX). These samples were assigned a value of 0·1 pg and corrected for recovery and sample volume as described above. Results did not differ when excluding these samples or assigning them values of zero.

MANIPULATION OF REPRODUCTION IN THE WILD

The third objective was to test whether unilateral OVX induces intermediate levels of growth, body condition, fat storage and breeding-season survival with respect to bilateral OVX and SHAM. A total of 504 females were captured near the beginning of the reproductive season (13–25 May 2010). Each was given a unique toe clip for identification, measured for SVL and body mass, and haphazardly assigned to one of three treatments: (i) removal of both ovaries (bilateral OVX, $n = 170$), (ii) removal of a single ovary (unilateral OVX, $n = 166$) and (iii) intact control (SHAM, $n = 168$). One day after surgery, each individual was released to its exact location of capture. In September (4 months post-treatment), the site was searched exhaustively in an attempt to recapture all survivors (Fig. 1). SVL and mass of each recaptured female were measured to assess treatment effects on growth in SVL, growth in mass and body condition (residuals from regression of $\log_{10}$ mass on $\log_{10}$ SVL).

MANIPULATION OF ENERGY STORES IN THE WILD

The fourth objective was to test the hypothesis that the increased energy stores of OVX females are responsible for their increased post-breeding survival. Many lizards store energy in abdominal fat bodies that vary in size as energy is accumulated and then drawn upon to support reproduction, growth or maintenance (Licht & Gorman 1970; Derickson 1974, 1976). Ovariectomy has a stimulatory effect on the size of fat bodies (Smith 1968; Shanbhag & Prasad 1992; Cox *et al.* 2010), and surgical ablations of the fat bodies have been used to investigate their role in reproduction (Hahn & Tinkle 1965; Smith 1968; Greenberg & Gist

1985). Following recapture in September, the surviving females in each reproductive treatment (bilateral OVX, unilateral OVX and SHAM) were divided into two further treatments: (i) surgical removal of both fat bodies and (ii) control surgery (n = 17–22 per treatment; Fig. 1). Surgical procedures were identical to those above, with the fat bodies manipulated, rather than the ovaries. Wet mass of excised fat bodies was recorded to test the effects of OVX on energy storage during the breeding season.

One day after surgery, each individual was released to its exact location of capture. Two subsequent recapture censuses were conducted: one in December (3 months after fat removal, 7 months after initial OVX treatments), and one in May, early in the subsequent breeding season (8 months after fat removal, 12 months after initial OVX treatments; Fig. 1). In the final census (May), females were euthanized to obtain blood for steroid assays and to validate the long-term effects of OVX and fat-removal treatments on the wet mass of abdominal fat bodies.

ANALYSES OF SURVIVAL

Program MARK (version 6.1) was used to construct Cormack–Jolly–Seber capture–recapture models estimating the probability of survival (ϕ) and of recapture (p) for each treatment (White & Burnham 1999). Effects of reproductive manipulations on breeding-season survival were tested with fully time-dependent models allowing ϕ and p to differ for each interval (May–September, September–December, December–May) and corresponding recapture census (September, December, May). Four candidate models were generated by variably including or excluding treatment effects on ϕ and p. A bootstrapping procedure (1000 iterations) was used to estimate the variance inflation factor ($\hat{c}$) for the fully parameterized model. This yielded an estimate of $\hat{c}$ = 0·73, indicating modest under-dispersion with respect to the fully parameterized model. Because there is no clear consensus on whether likelihood comparisons should correct for under-dispersion, all subsequent

analyses were conservatively conducted both with ($\hat{c}$ = 0·73) and without ($\hat{c}$ = 1) such correction. These analyses always converged on the same preferred models, albeit with differences in relative support.

The four candidate models were evaluated on the basis of Akaike Information Criterion (AIC_c) and associated measures of model weight and likelihood. For situations in which $\hat{c}$ = 0·73, quasi-likelihood adjusted AIC_c scores ($QAIC_c$) were calculated. These analyses do not distinguish between individuals subsequently assigned to 'fat-removal' and 'control' treatments when estimating parameters for the initial SHAM, unilateral OVX and bilateral OVX treatments. Given the lack of any effect of fat-body manipulations on ϕ and p (see Results) and that the primarily goal of this analysis was to estimate ϕ and p only during the first survival interval and recapture period (prior to fat-body manipulations), this approach is unlikely to have introduced substantial error into estimates of breeding-season survival.

Cormack–Jolly–Seber models were also used to jointly assess the effects of reproductive manipulations and fat-removal treatments on post-breeding survival. Fully time-dependent models were constructed by allowing ϕ and p to differ across each interval (September–December, December–May) and corresponding recapture census (December, May). A total of 16 candidate models were generated by variably including or excluding effects of reproductive manipulation, fat manipulation and their interaction on ϕ and p. A bootstrapping procedure (1000 iterations) was used to estimate $\hat{c}$ = 1·81 for the fully parameterized model. This value was used to obtain $QAIC_c$ scores, weights and likelihood values for all candidate models. The overall support for different scenarios was evaluated by summing the $QAIC_c$ weights across subsets of models containing each possible combination of treatment effects on ϕ or p. Because these analyses provided no support for interactive effects of reproductive manipulations and fat removals with respect to survival and recapture success, they were not used to derive parameter estimates for treatment effects on ϕ
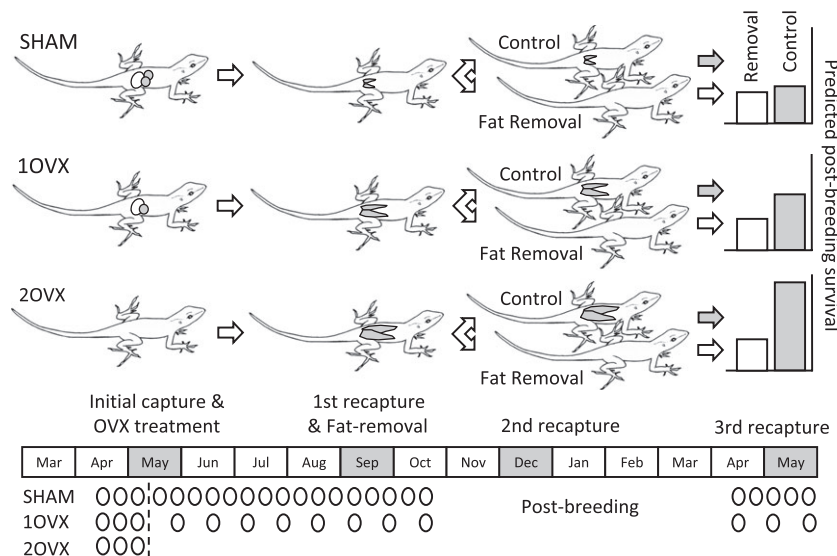

Fig. 1. Timeline, experimental design and predictions of this experiment. Females were assigned to SHAM, unilateral OVX (1OVX) and bilateral OVX (2OVX) groups in May and recaptured in September, at which point fat bodies were manipulated using control and fat removal treatments. Post-breeding survival was measured via recaptures in December and the following May. Approximate differences in the frequency of egg production for each group are shown below the timeline. Females are non-reproductive from November through March.

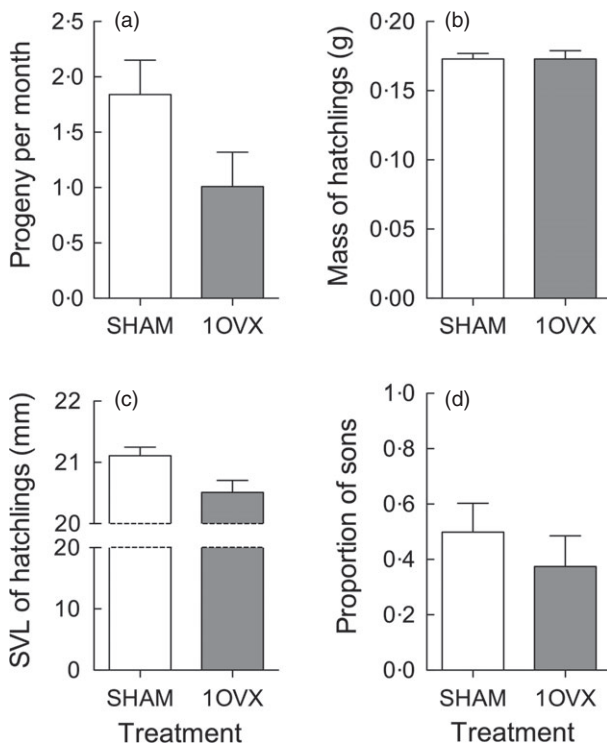and *p*. Instead, observed levels of survival are reported for each treatment group.

Results

VALIDATION OF UNILATERAL OVX IN CAPTIVITY

Unilateral OVX reduced the frequency of reproduction by 45% relative to SHAM (one-tailed $t = 1.88$; d.f. = 16; $P = 0.039$; Fig. 2a). Offspring of unilateral OVX and SHAM were nearly identical in mass at hatching ($F_{1,13} = 0.13$; $P = 0.73$; Fig. 2b), and offspring of unilateral OVX were slightly smaller than offspring of SHAM with respect to SVL at hatching ($F_{1,13} = 6.31$; $P = 0.026$; Fig 2c). Sex ratio of progeny did not differ between treatments ($\chi^2 = 0.27$; $P = 0.60$; Fig. 2d). Collectively, these results indicate that the removal of a single ovary results in a substantial reduction in reproductive investment.

HORMONE ASSAYS

Plasma concentrations of E_2 were 34% lower for unilateral OVX relative to SHAM at 3 months post-treatment in captivity (Fig. 3a), a difference that was marginally significant using a one-tailed test ($t = 1.79$; d.f. = 22; $P = 0.044$). However, comparison of bilateral OVX, uni-

lateral OVX and SHAM in the field at 12 months post-treatment revealed no difference in plasma E_2 levels ($F_{2,26} = 0.44$; $P = 0.65$; Fig. 3b). Fat removal had no effect on circulating E_2 in field-active females, either as a main effect ($F_{1,26} = 1.68$; $P = 0.21$) or via interaction with OVX treatment ($F_{2,26} = 0.48$; $P = 0.62$). The wet mass of fat bodies was uncorrelated with circulating E_2 across all field-active females ($r^2 < 0.01$; $P = 0.99$; $n = 50$) and within each reproductive treatment group (all $P > 0.42$). Circulating E_2 levels were not correlated with SVL ($r^2 = 0.01$; $P = 0.43$) or body mass ($r^2 < 0.01$; $P = 0.50$), so size covariates were not included in these analyses. Circulating E_2 levels were not correlated with growth or body condition, irrespective of whether treatment effects on growth were included in models (all $P > 0.10$).

REPRODUCTIVE MANIPULATION IN THE WILD

A total of 170 bilateral OVX, 166 unilateral OVX and 168 SHAM were released in May, and 43 bilateral OVX, 43 unilateral OVX and 39 SHAM were recaptured in the fall for analyses of growth, body condition and fat storage. An additional five SHAM, nine unilateral OVX and 13 bilateral OVX were not recaptured in the fall but were recaptured in the winter or spring and included in analyses of survival. As predicted, the stepwise experimental reduction in reproductive investment was mirrored by a stepwise increase in growth (Fig. 4). These treatment effects were highly significant when measured as either change in SVL (ANCOVA: $F_{2,121} = 10.51$; $P < 0.0001$; SVL covariate: $F_{1,121} = 72.18$; $P < 0.001$; Fig. 4A) or change in body mass (ANCOVA: $F_{2,121} = 23.64$; $P < 0.0001$; mass covariate: $F_{1,121} = 44.29$; $P < 0.001$; Fig. 4B). Measures of body condition revealed a similar stepwise increase from SHAM to unilateral OVX and bilateral OVX (ANOVA: $F_{2,121} = 12.35$; $P < 0.0001$; Fig. 4C), as did levels of fat storage (ANCOVA: $F_{2,58} = 58.12$; $P < 0.0001$; Fig. 4D). Although these treatment effects followed the predicted stepwise patterns, unilateral OVX were generally more similar to SHAM than
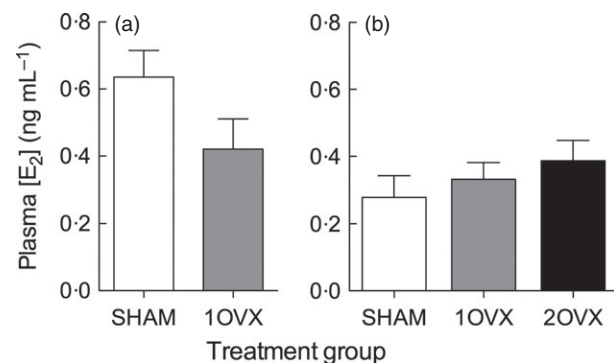

Fig. 2. Comparison of unilateral ovariectomy (1OVX) and intact control (SHAM) with respect to (a) frequency of offspring production, (b) mass of offspring at hatching, (c) snout–vent length (SVL) of offspring at hatching and (d) sex ratio of offspring at hatching. Data are treatment means (± 1 SE) calculated from $n = 9$ individual terraria per treatment (a, d), or $n = 22$ 1OVX and $n = 60$ SHAM hatchlings (b, c).

Fig. 3. Mean (± 1 SE) levels of plasma estradiol (E_2) for (a) intact control (SHAM) and unilateral ovariectomy (1OVX) at 3 months post-treatment in captivity and (b) SHAM, 1OVX and bilateral ovariectomy (2OVX) at 12 months post-treatment in the wild.

Table 1. Comparison of four fully time-dependent (*t*) Cormack-Jolly-Seber models variously including or excluding effects of ovariectomy (SHAM, unilateral OVX and bilateral OVX) on the probability of survival (ϕ) and recapture (*p*). General models including treatment effects on ϕ and *p* are preferred over reduced models excluding these treatment effects on the basis of quasi-likelihood adjusted Akaike Information Criterion (lower QAIC$_c$ scores, greater weight and likelihood). Comparisons are presented using variance inflation factors of $\hat{c} = 1{\cdot}00$, which does not correct for under-dispersion; and $\hat{c} = 0{\cdot}73$, which was estimated from 1000 bootstrap simulations and reflects moderate under-dispersion relative to the most general model

| $\hat{c}$	Model	Parameters	QAIC$_c$	Δ QAIC$_c$	Weight	Likelihood
1·00	$\phi_{t*ovx} \, p_{t*ovx}$	15	1086·26	0·00	0·766	1·000
	$\phi_t \, p_{t*ovx}$	11	1090·32	4·06	0·101	0·131
	$\phi_t \, p_t$	5	1090·82	4·56	0·078	0·102
	$\phi_{t*ovx} \, p_t$	11	1091·55	5·29	0·054	0·071
0·73	$\phi_{t*ovx} \, p_{t*ovx}$	15	1478·65	0·00	0·980	1·000
	$\phi_t \, p_{t*ovx}$	11	1487·31	8·67	0·013	0·013
	$\phi_t \, p_t$	5	1488·99	10·35	0·006	0·006
	$\phi_{t*ovx} \, p_t$	11	1492·58	13·93	0·001	0·001

to bilateral OVX and were not always statistically distinct from SHAM when assessed via Tukey's *post hoc* tests (Fig. 4).

Cormack–Jolly–Seber models revealed similar treatment differences in breeding-season survival. Likelihood comparison of four candidate models that variously included and excluded treatment effects on ϕ and *p* strongly favoured the model including separate ϕ and *p* parameters for each treatment (Table 1). This model was 7·6 times more likely than the next best model when assuming $\hat{c} = 1{\cdot}0$ and 77 times more likely when accounting for observed under-dispersion by modelling $\hat{c} = 0{\cdot}73$ (Table 1). Estimates of ϕ from this preferred model indicate a strong stepwise increase in

breeding-season survival from SHAM (0·29) to unilateral OVX (0·40) and bilateral OVX (0·55; Fig. 5a). In part, these pronounced treatment differences in probability of survival reflect the sharp decrease in estimated probability of recapture from SHAM (0·82) to unilateral OVX (0·65) and bilateral OVX (0·47; Fig. 5b).

ENERGY MANIPULATION IN THE WILD

A total of 62 fat removals (20 bilateral OVX, 22 unilateral OVX and 20 SHAM) and 57 controls (21 bilateral OVX, 19 bilateral OVX and 17 SHAM) were treated and released. Of these females, 19 fat removals and 15
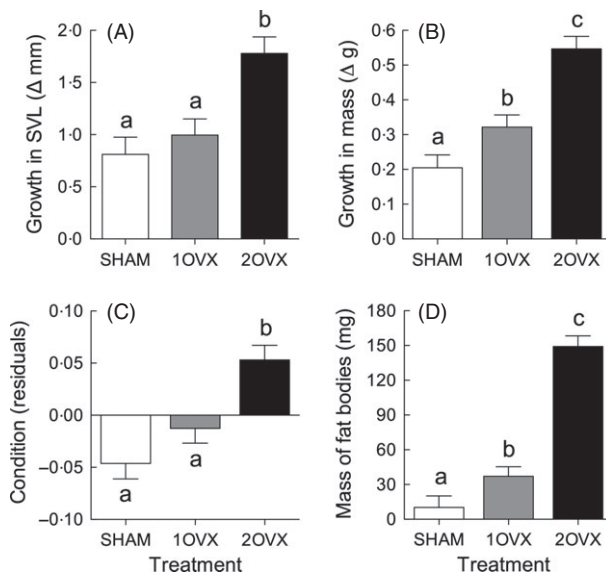

Fig. 4. Effects of altered reproductive investment on (A) growth in snout–vent length (SVL), (B) growth in body mass, (C) body condition and (D) wet mass of abdominal fat bodies. Most data are least square means (± 1 SE) from ANCOVAs including (a) pre-treatment SVL, (b) pre-treatment mass or (d) post-treatment mass as covariates (see text for details). Data in (c) are residuals from regressions of $\log_{10}$ body mass on $\log_{10}$ SVL. Lowercase letters denote statistical separation of treatment groups based on Tukey's *post hoc* tests.
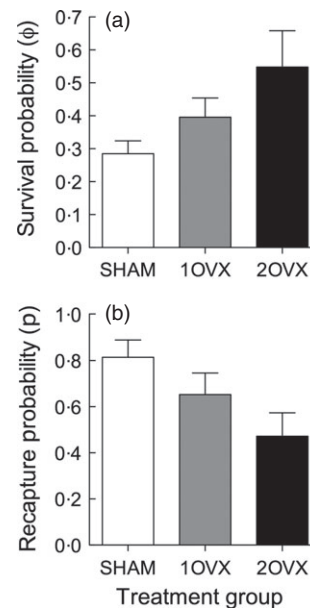
Fig. 5. Mean (± 1 SE) estimates of (a) probability of breeding-season survival (May–September) and (b) probability of recapture (in September) for females assigned to intact control (SHAM), unilateral ovariectomy (1OVX) and bilateral ovariectomy (2OVX) treatments. Probabilities were estimated from the preferred, time-dependent, Cormack-Jolly-Seber model that included separate treatment effects for ϕ and *p* (Table 1).

controls were recaptured in the spring for measurement of fat bodies, growth and survival. Removal of fat bodies in September had a strong and persistent effect on energy stores, such that treatment differences in the mass of fat bodies were still evident in May (8 months following removal of fat bodies; Fig. 6a). The wet mass of fat bodies in May was related to body mass (covariate: $F_{1,26} = 4.24$; $P = 0.049$), OVX treatment ($F_{2,26} = 8.18$; $P = 0.002$), fat-removal treatment ($F_{1,26} = 15.99$; $P < 0.001$) and the interaction of OVX and fat-removal treatments ($F_{2,26} = 4.30$; $P = 0.024$). Even one full year after initial OVX treatment, females in the control group (fat bodies intact) still exhibited clear stepwise variation in fat mass as a function of OVX treatment (compare Fig. 4d with control group in Fig. 6a), whereas females in the fat-removal group had dramatically reduced energy stores.
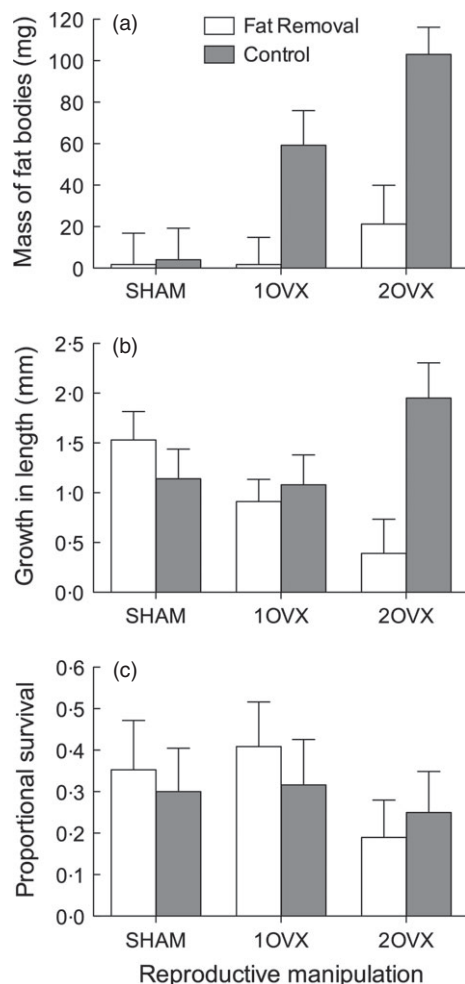
Fig. 6. Combined effects of reproductive manipulation (SHAM, 1OVX, 2OVX) and fat-body manipulation (fat removal, control) on (a) mean (±1 SE) mass of abdominal fat stores in spring (May), 8 months after manipulation of fat bodies, (b) least square mean (±1 SE) growth in SVL over this 8-month period (with initial SVL as a covariate); and (c) mean (±1 SE) survival during this same 8-month period. Survival in (c) is calculated directly from observed survivors rather than from CJS model estimates of survival probability.

This confirms that fat removal impacted energy stores throughout the period over which survival was measured. Moreover, fat removal had a modest inhibitory effect on growth in SVL ($F_{1,22} = 4.03$; $P = 0.054$; SVL covariate: $F_{1,22} = 5.17$; $P = 0.032$). Whereas this effect was pronounced in bilateral OVX, it was minor in unilateral OVX and absent in SHAM (OVX × fat removal: $F_{2,22} = 5.17$; $P = 0.031$; Fig. 6b), suggesting that the effect of fat removal on growth scaled roughly with the amount of stored energy that was removed. Fat removal also inhibited growth in mass ($F_{1,22} = 6.11$; $P = 0.022$; initial body mass, corrected for mass of excised fat bodies, as a covariate: $F_{1,22} = 6.70$; $P = 0.017$), but this effect did not differ among OVX treatments (OVX × fat removal: $F_{2,22} = 2.38$; $P = 0.12$). On average, females in the fat-removal group did not change in mass over the non-breeding season (least squares mean $= -0.001 \pm 0.04$ SEM), whereas females in the control group gained mass (least squares mean $= 0.185 \pm 0.061$ g). Females in the control group were slightly (but not significantly, all $P > 0.13$) more massive than females in the fat-removal group upon recapture in the spring (least squares mean $= 2.00 \pm 0.06$ vs. 1.88 ± 0.04 g body mass), even when subtracting the mass of the fat bodies from the body mass of each individual (1.91 ± 0.05 vs. 1.85 ± 0.05 g), suggesting that females in the fat-removal group did not directly compensate for the loss of fat bodies by storing an equivalent amount of fat elsewhere (e.g. tail).

Despite these effects of OVX and fat removal on stored energy and growth, post-breeding survival did not differ as a function of OVX treatment, fat removal or their interaction (Fig. 6c). Comparison of 16 time-dependent Cormack–Jolly–Seber models variously including or excluding effects of OVX and fat removal on both ϕ and p did not reveal strong support for any single model (Table S1, Supporting information). However, the four models including an interaction between OVX and fat removal for ϕ were the four least preferred models (Table S1, Supporting information), contrary to the hypothesis that effects of fat removal on survival would become increasingly pronounced from SHAM to unilateral OVX and bilateral OVX (Fig. 1). Indeed, the majority of support in these analyses favoured models that estimated a single ϕ or p parameter across all treatments (Table 2).

Discussion

In female brown anoles, the elimination of reproduction substantially increases growth, body condition, fat storage, haematocrit, immune function and survival (Cox & Calsbeek 2010b, 2011; Cox *et al.* 2010). In the present study, unilateral OVX induced intermediate levels of reproductive investment while maintaining endogenous steroid production and producing intermediate effects on growth, body condition, fat storage and breeding-season

Table 2. Summed $QAIC_c$ weights from likelihood comparison of 16 fully time-dependent Cormack-Jolly-Seber models variously including or excluding treatment effects of ovariectomy (OVX), fat removal (FAT) and their interaction (OVX*FAT) on the probability of post-breeding survival (ϕ) and recapture (*p*). Each value reflects the total weight summed across the subset of four models with that particular combination of effects and can be interpreted as the proportional support for models including those effects. See Table S1 for model weights and likelihoods

Effects on ϕ	Summed weight	Effects on *p*	Summed weight
None	0·492	None	0·436
FAT	0·339	OVX	0·429
OVX	0·168	FAT	0·130
OVX*FAT	0·001	OVX*FAT	0·005

survival. Collectively, these results suggest a functional link between reproduction and survival that is potentially mediated by underlying energetic trade-offs (Cox *et al.* 2010). However, experimentally decoupling reproductive investment from its downstream effects on one important component of energy storage provided no support for this hypothesis. Removal of fat bodies had no effect on post-breeding survival, either alone or in interaction with OVX treatment. While this suggests that the energetic savings of reduced reproductive investment do not directly enhance post-breeding survival, post-breeding survival was also unrelated to OVX treatment in this study, contrary to previous studies of this same population (Cox & Calsbeek 2010b). Below, these results are discussed in the context of mechanisms potentially underlying the trade-off between reproduction and survival in brown anoles and other organisms.

UNILATERAL OVX INDUCES INTERMEDIATE EFFECTS

Unilateral OVX reduced reproductive frequency by 45% relative to SHAM and induced levels of growth, body condition and fat storage that were intermediate between SHAM and bilateral OVX. Although unilateral OVX and SHAM differed markedly in reproductive investment, differences in growth, body condition and fat storage were only marginally significant (or non-significant) in *post hoc* comparisons (Fig. 4). By contrast, bilateral OVX always differed substantially and significantly from the other two treatments. The rationale for including the unilateral OVX treatment was based in part on the concern that bilateral OVX induces effects that are beyond the range of natural variation in reproductive investment and may therefore lead to inferences of limited biological significance. However, from a statistical point of view, this study underscores the pragmatic counterpoint that more modest experimental reductions in reproductive investment may produce effects that are difficult to detect empirically, even with experimental designs (such as this) that include hundreds of individuals per treatment. We suggest that the inclusion of stepwise experimental variation in reproductive investment, potentially culminating in the complete

elimination of reproductive function (e.g. bilateral OVX), may therefore be a profitable tactic in other systems in which more modest experimental reductions produce effects that appear to be biologically relevant, but are difficult to confirm statistically (Bleu *et al.* 2012).

Extreme manipulations of reproductive investment may be particularly useful when the response of interest is survival, typically measured as a binary outcome with potential error due to emigration and/or incomplete sampling of those survivors that are present. In this study, survival probabilities estimated from Cormack–Jolly–Seber models strongly supported the prediction of increasing survival from SHAM to unilateral OVX and bilateral OVX (Fig. 5a). In part, this is because the probability of recapture (Fig. 5b) was atypically high for SHAM ($p = 0·82$) and low for bilateral OVX ($p = 0·47$) relative to estimates from two previous years ($p = 0·66$ for both groups; Cox & Calsbeek 2010b). This difference in recapture probability could reflect treatment effects on activity, potentially corresponding to differences in foraging and/or thermoregulatory behaviour related to gravidity (Shine 1980; Schwarzkopf & Shine 1991), or to direct effects of ovarian steroids on reproductive behaviour and activity patterns (Whittier & Tokarz 1992; Woodley & Moore 1999). Behavioural responses to staged introductions of same-sex territorial intruders did not differ among bilateral OVX, unilateral OVX and SHAM females, nor did E_2 implants alter display rates of captive females (E. Parker, N. Brown, R. Cox and R. Calsbeek, unpublished data), but we lack comparable data on other relevant behaviours such as activity, foraging and thermoregulation of females in the wild. Irrespective of why recapture probability differed across treatments, these results support the prediction that unilateral OVX should induce intermediate levels of survival, relative to SHAM and bilateral OVX. Given that mean levels of reproductive investment in the unilateral OVX group are similar to the lower bounds of natural variation for intact females, this result supports the biological relevance of the trade-off between reproduction and survival that has previously inferred from more extreme manipulations (Cox & Calsbeek 2010b, 2011; Cox *et al.* 2010).

In contrast to the clear stepwise variation among treatments with respect to growth, body condition, fat storage and survival, no clear differences in circulating E_2 were evident, particularly among field-active females. Whether the lack of a treatment effect on plasma E_2 in field-active females reflects extra-ovarian production of E_2, maintenance or regeneration of small amounts of steroidogenic ovarian tissue following OVX, or the inability of our sampling and assay procedures to detect biologically meaningful variation is currently unclear. However, we tentatively conclude that the effects of our manipulations on circulating E_2 may not be as dramatic as previously assumed, and note that circulating E_2 in both unilateral and bilateral OVX females is detectable and within the range of natural physiological variation in intact females.

Although the measurement of one ovarian steroid at a single time point captures only a small portion of the complex endocrine dynamics of reproductive investment, this measure was chosen in part because E_2 has been shown to mobilize stored energy from the fat bodies in other lizards (Hahn 1967; Greenberg & Gist 1985; Shanbhag & Prasad 1992). However, the results of this study suggest that effects of unilateral and bilateral OVX on fat storage are not simply the result of altered E_2 levels. Specifically, strong effects of OVX on fat storage were observed in the absence of corresponding changes in circulating E_2 and no correlation was observed between the size of fat bodies and circulating E_2 levels when measured across individuals. Thus, the seasonal decrease in fat storage that accompanies reproduction in anoles (Licht & Gorman 1970) is most parsimoniously viewed as a consequence of the energetic cost of egg production, rather than as an obligate response to altered E_2 levels (Greenberg & Gist 1985; Shanbhag & Prasad 1992). Of course, fat mobilization by E_2 is part of an integrated physiological system for the modulation of reproductive investment, so endocrine regulation and energetic trade-offs should not be considered as strict alternatives (Harshman & Zera 2007).

LINKING REPRODUCTION, ENERGETICS AND SURVIVAL

The results of this experiment agree with those from other species in which manipulations of reproductive investment have impacted both energetics and survival (Reznick 1985; Reid 1987; Landwer 1994; Nilsson & Svensson 1996; Koivula *et al.* 2003). However, direct experimental evidence that energetic trade-offs are responsible for the effects of reproduction on survival is scarce, particularly in wild populations (Williams 2005). Despite strong effects of reproductive investment on both energy storage and survival in brown anoles, the subsequent elimination of energy stores accumulated during the reproductive season had no effect on post-breeding survival (Fig. 6c). Although females in the bilateral OVX group may have partially offset the effects of fat removal on their energy stores by accumulating fat over the non-breeding season (Fig. 6a, fat removal), no such effect was observed in unilateral OVX, nor did accumulation of fat over the non-breeding season homogenize the pronounced effects of reproduction on fat reserves (Fig. 6a, Control). Collectively, these results suggest that the cumulative energetic cost of reproduction, or at least that portion of it that is captured by experimental variation in the size of fat bodies, is insufficient to explain the reduction in post-breeding survival that has previously been documented in this experimental system (Cox & Calsbeek 2010b).

This conclusion is tempered by the fact that neither OVX treatment had a significant effect on post-breeding survival in the current experiment (Fig. 6c), although a significant effect on breeding-season survival was evident

(Fig. 5a). Statistical power to detect survival effects decreased at each stage of this experiment due to mortality, such that only about 20 females per each of the six OVX × fat-removal groups were released in September (Fig. 1). However, not even a weak trend towards predicted survival patterns was observed (compare predictions in Fig. 1 with results in Fig. 6c). In light of the fact that post-breeding survival did not differ among reproductive treatments with fat bodies intact, inferences about the interactive effects of reproduction and fat storage should be made with caution. Indeed, a more direct approach may be to focus on the role of energetics in shaping survival costs during the breeding season, when the trade-off between survival and reproduction is pronounced. Nonetheless, it is somewhat surprising that the removal of fat bodies had no discernable effect on survival. This suggests that the primary role of the fat body is to fuel reproduction at the beginning of the breeding season, rather than to support maintenance and survival (Licht & Gorman 1970; Greenberg & Gist 1985). It is also important to consider that energy is stored in sites other than the fat bodies (e.g. tail, liver), so the present experiment provides only a partial test of energy-allocation hypotheses for the trade-off between reproduction and survival.

Caveats aside, this experiment clearly illustrates the potential pitfalls associated with inferring that the trade-off between reproduction and survival is mediated by underlying energy-allocation trade-offs in the absence of an experimental design that explicitly links all components of this causal pathway. The same logic applies to experiments demonstrating effects of reproduction on physiological processes related to self-maintenance and survival, such as immune function and resistance to parasites (Lochmiller & Deerenberg 2000; Knowles, Nakagawa & Sheldon 2009). Although these processes are often energetically expensive, important for survival and causally related to reproduction, their roles as central mediators of the trade-off between reproduction and survival remain unclear (Råberg *et al.* 1998; Eraud *et al.* 2005; Williams 2005). In brown anoles, bilateral OVX impacts several such physiological parameters in a manner consistent with the idea that they may shape the trade-off between reproduction and survival (Cox *et al.* 2010). However, these physiological effects have yet to be causally linked to the energetic cost of reproduction, or to experimentally induced variation in survival, although selection analyses do suggest that natural variation in immune function is related to survival of females during the breeding season (Calsbeek, Bonneaud & Smith 2008).

In brown anoles, as in other organisms, a complete understanding of the energetic basis of reproductive trade-offs will likely require novel experimental approaches that go beyond the demonstration of energetic, physiological and survival costs of reproduction to link these mechanisms into clear causal pathways. Moreover, these studies must be synthesized with other

mechanisms linking reproduction and survival, such as hormonal pleiotropy (Williams 2005), adaptive signalling pathways (Barnes & Partridge 2003; Edward & Chapman 2011) and increased susceptibility to predation (Landwer 1994; Cox & Calsbeek 2010b). These alternatives may be particularly important given that the results of this study support the emerging view that simple 'Y models' of energy allocation (van Noordwijk & Jong 1986) may present an inappropriate and potentially misleading framework for the study of life-history trade-offs (Barnes & Partridge 2003; Edward & Chapman 2011; Stearns 2011).

Acknowledgements

Data accessibility

Data available from the Dryad Digital Repository: http://doi.org/10.5061/dryad.2vk43 (Cox, Lovern & Calsbeek 2014).

References

Alonso-Alvarez, C., Bertrand, S., Devevey, G., Prost, J., Faivre, B. & Sorci, G. (2004) Increased susceptibility to oxidative stress as a proximate cost of reproduction. *Ecology Letters*, **7**, 363–368.

Andrews, R.M. & Rand, A.S. (1974) Reproductive effort in anoline lizards. *Ecology*, **55**, 1317–1327.

Barnes, A.I. & Partridge, L. (2003) Costing reproduction. *Animal Behaviour*, **66**, 199–204.

Bleu, J., Massot, M., Haussy, C. & Meylan, S. (2012) Experimental litter size reduction reveals costs of gestation and delayed effects on offspring in a viviparous lizard. *Proceedings of the Royal Society B: Biological Sciences*, **279**, 489–498.

Bonneaud, C., Mazuc, J., Gonzalez, G., Haussy, C., Chastel, O., Faivre, B. *et al.* (2003) Assessing the cost of mounting an immune response. *American Naturalist*, **161**, 367–379.

Calsbeek, R. & Bonneaud, C. (2008) Postcopulatory fertilization bias as a form of cryptic sexual selection. *Evolution*, **62**, 1137–1148.

Calsbeek, R., Bonneaud, C. & Smith, T.B. (2008) Differential fitness effects of immunocompetence and neighborhood density in alternative female lizard morphs. *Journal of Animal Ecology*, **77**, 103–109.

Calsbeek, R., Bonvini, L.A. & Cox, R.M. (2010) Geographic variation, frequency-dependent selection, and the maintenance of a female-limited polymorphism. *Evolution*, **64**, 116–125.

Christe, P., Glaizot, O., Strepparava, N., Devevey, G. & Fumagalli, L. (2012) Twofold cost of reproduction: an increase in parental effort leads to higher malarial parasitaemia and to a decrease in resistance to oxidative stress. *Proceedings of the Royal Society B: Biological Sciences*, **279**, 1142–1149.

Cox, R.M. (2006) A test of the reproductive cost hypothesis for sexual size dimorphism in Yarrow's Spiny Lizard, *Sceloporus jarrovii*. *Journal of Animal Ecology*, **75**, 1361–1369.

Cox, R.M. & Calsbeek, R. (2010a) Cryptic sex-ratio bias provides indirect genetic benefits despite sexual conflict. *Science*, **328**, 92–94.

Cox, R.M. & Calsbeek, R. (2010b) Severe costs of reproduction persist in *Anolis* lizards despite the evolution of a single-egg clutch. *Evolution*, **64**, 1321–1330.

Cox, R.M. & Calsbeek, R. (2011) An experimental test for alternative reproductive tactics underlying a female-limited polymorphism. *Journal of Evolutionary Biology*, **24**, 343–353.

Cox, R.M., Lovern, M.B. & Calsbeek, R. (2014) Experimentally decoupling reproductive investment from energy storage to test the functional basis of a life-history tradeoff. *Dryad Digital Repository*. doi:10.5061/dryad.2vk43.

Cox, R.M., Stenquist, D.S. & Calsbeek, R. (2009) Testosterone, growth, and the evolution of sexual size dimorphism. *Journal of Evolutionary Biology*, **22**, 1586–1598.

Cox, R.M., Parker, E.U., Cheney, D.M., Leibl, A.L., Martin, L.B. & Calsbeek, R. (2010) Experimental evidence for physiological costs underlying the trade-off between reproduction and survival. *Functional Ecology*, **24**, 1262–1269.

Crews, D. (1980) Interrelationships among ecological, behavioral, and neuroendocrine processes in the reproductive cycle of *Anolis carolinensis* and other reptiles. *Advances in the Study of Behavior*, **11**, 1–74.

Derickson, W.K. (1974) Lipid deposition and utilization in the sagebrush lizard, *Sceloporus graciosus*: its significance for reproduction and maintenance. *Comparative Biochemistry and Physiology A*, **49**, 267–272.

Derickson, W.K. (1976) Lipid storage and utilization in reptiles. *American Zoologist*, **16**, 711–723.

Dunham, A.E. & Beaupre, S.J. (1998) Ecological experiments: scale, phenomenology, mechanism, and the illusion of generality. *Experimental Ecology: Issues and Perspectives* (eds W.J. Resetarits Jr & J. Bernardo), pp. 27–49. Oxford University Press, Oxford.

Edward, D.A. & Chapman, T. (2011) Mechanisms underlying reproductive trade-offs: costs of reproduction. *Mechanisms of Life History Evolution* (eds T. Flatt & A. Heyland), pp. 137–152. Oxford University Press, Oxford, UK.

Eraud, C., Duriez, O., Chastel, O. & Faivre, B. (2005) The energetic cost of humoral immunity in the Collared Dove, *Streptopelia decaocto*: is the magnitude sufficient to force energy-based trade-offs? *Functional Ecology*, **19**, 110–118.

French, S.S., DeNardo, D.F. & Moore, M.C. (2007) Trade-offs between the reproductive and immune systems: facultative responses to resources or obligate responses to reproduction? *The American Naturalist*, **170**, 79–89.

Greenberg, D.S. & Gist, D.H. (1985) Fat bodies and reproduction in female *Anolis carolinensis*. *Journal of Experimental Zoology*, **233**, 277–283.

Gustafsson, L., Nordling, D., Andersson, M.S., Sheldon, B.C. & Qvarnstrom, A. (1994) Infectious diseases, reproductive effort and the cost of reproduction in birds. *Philosophical Transactions: Biological Sciences*, **346**, 323–331.

Hahn, W.E. (1967) Estradiol-induced vitellogenesis and concomitant fat mobilization in the lizard *Uta stansburiana*. *Comparative Biochemistry and Physiology*, **23**, 83–93.

Hahn, W.E. & Tinkle, D.W. (1965) Fat body cycling and experimental evidence for its adaptive significance to ovarian follicle development in the lizard *Uta stansburiana*. *Journal of Experimental Zoology*, **158**, 79–86.

Harshman, L.G. & Zera, A.J. (2007) The cost of reproduction: the devil in the details. *Trends in Ecology and Evolution*, **22**, 80–86.

Hatle, J.D., Paterson, C.S., Jawaid, I., Lentz, C., Wells, S.M. & Fronstin, R.B. (2008) Protein accumulation underlying lifespan extension via ovariectomy in grasshoppers is consistent with the disposable soma hypothesis but is not due to dietary restriction. *Experimental Gerontology*, **43**, 900–908.

Hosken, D.J. (2001) Sex and death: microevolutionary trade-offs between reproductive and immune investment in dung flies. *Current Biology*, **11**, R379–R380.

Jones, R.E., Lopez, K.H., Maldonato, T.A., Summers, T.R., Summers, C.H., Propper, C.R. *et al.* (1997) Unilateral ovariectomy influences hypothalamic monoamine assymmetries in a lizard (*Anolis*) that exhibits alternation of ovulation. *General and Comparative Endocrinology*, **108**, 306–315.

Knowles, S.C.L., Nakagawa, S. & Sheldon, B.C. (2009) Elevated reproductive effort increases blood parasitaemia and decreases immune function in birds: a meta-regression approach. *Functional Ecology*, **23**, 405–415.

Koivula, M., Koskela, E., Mappes, T. & Oksanen, T.A. (2003) Cost of reproduction in the wild: manipulation of reproductive effort in the bank vole. *Ecology*, **84**, 398–405.

Landwer, A. (1994) Manipulation of egg production reveals costs of reproduction in the tree lizard (*Urosaurus ornatus*). *Oecologia*, **100**, 243–249.

Lee, J.C., Clayton, D., Eisenstein, S. & Perez, I. (1989) The reproductive cycle of *Anolis sagrei* in southern Florida. *Copeia*, **1989**, 930–937.

Licht, P. & Gorman, G.C. (1970) Reproductive and fat cycles in Caribbean *Anolis* lizards. *University of California Publications in Zoology*, **95**, 1–52.

Lochmiller, R.L. & Deerenberg, C. (2000) Trade-offs in immunology: just what is the cost of immunity? *Oikos*, **88**, 87–98.

Lovern, M.B. & Passek, K.M. (2002) Sequential alternation of offspring sex from successive eggs by female green anoles, *Anolis carolinensis*. *Canadian Journal of Zoology*, **80**, 78–82.

Martin, L.B., Scheuerlein, A. & Wikelski, M. (2003) Immune activity elevates energy expenditure of house sparrows: a link between direct and indirect costs? *Proceedings of the Royal Society, London B: Biological Sciences*, **270**, 153–158.

Nilsson, J.-A. & Svensson, E. (1996) The cost of reproduction: a new link between current reproductive effort and future reproductive success. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **263**, 711–714.

van Noordwijk, A.J. & Jong G.d. (1986) Acquisition and allocation of resources: their influence on variation in life history tactics. *The American Naturalist*, **128**, 137–142.

Partridge, L., Gems, D. & Withers, D.J. (2005) Sex and death: what is the connection? *Cell*, **120**, 461–472.

Räberg, L., Grahn, M., Hasselquist, D. & Svensson, E. (1998) On the adaptive significance of stress-induced immunosuppression. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, **265**, 1637–1641.

Reid, W.V. (1987) The cost of reproduction in the glaucous-winged gull. *Oecologia*, **74**, 458–467.

Reznick, D. (1985) Costs of reproduction: an evaluation of the empirical evidence. *Oikos*, **44**, 257–267.

Roff, D.A. (2002) *Life History Evolution*. Sinauer Associates Inc., Boston.

Schwarzkopf, L. & Shine, R. (1991) Thermal biology of reproduction in viviparous skinks, *Eulamprus tympanum*: why do gravid females bask more? *Oecologia*, **88**, 562–569.

Shanbhag, B.A. & Prasad, B.S.K. (1992) Fat body ovarian relationship in the garden lizard, *Calotes versicolor* (Daud.). *Journal of Experimental Zoology*, **264**, 454–460.

Shine, R. (1980) "Costs" of reproduction in reptiles. *Oecologia*, **46**, 92–100.

Smith, R.E. (1968) Experimental evidence for a gonadal-fat body relationship in two teiid lizards (*Ameiva festiva, Ameiva quadrilineata*). *Biological Bulletin*, **134**, 325–331.

Stearns, S.C. (1992) *The Evolution of Life Histories*. Oxford University Press, Oxford.

Stearns, S.C. (2011) Does impressive progress on understanding mechanisms advance life history theory? *Mechanisms of Life History Evolution: The Genetics and Physiology of Life History Traits and Trade-Offs* (eds T. Flatt & A. Heyland), pp. 365–374. Oxford University Press, Oxford, UK.

Vézina, F. & Williams, T.D. (2005) The metabolic cost of egg production is repeatable. *Journal of Experimental Biology*, **208**, 2533–2538.

Wack, C.L., Fox, S.F., Hellgren, E.C. & Lovern, M.B. (2008) Effects of sex, age, and season on plasma steroids in free-ranging Texas horned lizards (*Phrynosoma cornutum*). *General and Comparative Endocrinology*, **155**, 589–596.

White, G.C. & Burnham, K.P. (1999) Program MARK: survival estimation from populations of marked animals. *Bird Study*, **46**(Supplement), 120–138.

Whittier, J.M. & Tokarz, R.R. (1992) Physiological regulation of sexual behavior in female reptiles. *Biology of the Reptilia: Hormones, Brain, and Behavior* (eds C. Gans & D. Crews), pp. 24–69. University of Chicago Press, Chicago.

Williams, T.D. (2005) Mechanisms underlying the costs of egg production. *BioScience*, **55**, 39–48.

Williams, T.D., Challenger, W.O., Christians, J.K., Evanson, M., Love, O. & Vezina, F. (2004) What causes the decrease in haematocrit during egg production? *Functional Ecology*, **18**, 330–336.

Wilson, K. & Hardy, I.C.W. (2002) Statistical analysis of sex ratios: an introduction. *Sex Ratios: Concepts and Research Methods* (ed. I.C.W. Hardy), pp. 48–92. Cambridge University Press, Cambridge, UK.

Wingfield, J.C. & Farner, D.S. (1975) The determination of five steroids in avian plasma by radioimmunoassay and competetive protein binding. *Steroids*, **26**, 311–327.

Woodley, S.K. & Moore, M.C. (1999) Ovarian hormones influence territorial aggression in free-living female mountain spiny lizards. *Hormones and Behavior*, **35**, 205–214.

Zera, A.J. & Harshman, L.G. (2001) The physiology of life history trade-offs in animals. *Annual Review of Ecology and Systematics*, **32**, 95–126.

Zera, A.J., Harshman, L.G. & Williams, T.D. (2007) Evolutionary endocrinology: the developing synthesis between endocrinology and evolutionary genetics. *Annual Review of Ecology, Evolution, and Systematics*, **38**, 793–817.

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Table S1. Comparison of 16 candidate Cormack–Jolly–Seber models variously including or excluding effects of ovariectomy treatment (OVX) and fat-body manipulation (FAT) on the probability of post-breeding survival (ϕ) and recapture (p).