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Experimental evidence for physiological costs underlying the trade-off between reproduction and survival

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Summary

1. A central tenet of life-history theory is that investment in reproduction compromises survival. However, the underlying physiological mechanisms that link reproduction to survival are poorly understood, particularly in wild populations.

2. Previous experiments in the brown anole lizard (*Anolis sagrei*) show that the elimination of reproduction via surgical ovariectomy results in a dramatic increase in the survival of wild females. We hypothesized that this trade-off reflects underlying differences in energy allocation between reproduction and physiological processes that influence survival.

3. To test this hypothesis, we compared ovariectomized (OVX) females to reproductive controls (SHAM) with respect to four physiological parameters that are thought to influence survival: energy storage, haematocrit, immune function and parasitemia.

4. Consistent with previous studies, we found that OVX females exhibited increased survival and growth relative to reproductive SHAM females. At the end of the breeding season, OVX also exceeded SHAM with respect to energy storage, haematocrit and immune response to phytohemagglutinin challenge.

5. Contrary to our predictions, OVX were more likely than SHAM to exhibit high levels of parasitemia. However, growth and parasite load were positively correlated in OVX and negatively correlated in SHAM, suggesting that reproductive investment may compromise parasite tolerance rather than parasite resistance.

6. Collectively, our results provide direct experimental evidence that reproductive investment affects several key physiological traits that likely interact to influence survival in wild populations.

Key-words: costs of reproduction, energy allocation, haematocrit, immune function, parasitemia, reproductive investment

Introduction

One of the most ubiquitous patterns in life-history evolution is the tendency for species and individuals that invest heavily in current reproduction to exhibit low levels of survival and future reproduction. This trade-off is central to life-history theory (Williams 1966; Gadgil & Bossert 1970; Stearns 1992; Roff 2002), yet we still lack a detailed understanding of its underlying physiological basis in all but a few model systems (Zera & Harshman 2001; Partridge *et al.* 2005; Harshman & Zera 2007). This is particularly true in wild populations, where a historical reliance on correlative approaches has largely precluded direct, causal tests of the physiological links between reproduction and survival (Reznick 1985; Landwer 1994; Roff 2002).

Physiological explanations for the trade-off between reproduction and survival are typically based on the assumption that these two components of fitness compete for limited energy and nutrients (Reznick 1992; Zera & Harshman 2001; Harshman & Zera 2007). When resources are limiting, experimental manipulations of reproductive

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investment (e.g. via artificial selection or phenotypic engineering) are therefore predicted to impact survival by altering energy allocation to intermediate physiological processes that regulate somatic maintenance and resistance to disease. Recent laboratory experiments have found support for this prediction with respect to physiological processes such as nutrient and energy storage, metabolism, DNA repair, resistance to oxidative stress, wound healing, and immune function (Hosken 2001; Zera & Harshman 2001; Bonneaud *et al*. 2003; Partridge *et al*. 2005; Harshman & Zera 2007; Zera *et al*. 2007; Hatle *et al*. 2008; French *et al*. 2009). However, most of this research has been conducted in captivity, which can alter physiological interactions and eliminate the primary ecological drivers of survival in the wild (Calisi & Bentley 2009). To the extent that researchers are interested in explaining natural variation in survival, it is critical to explore the physiological links between reproduction and survival in their natural ecological context (Roff 2002).

One promising approach for dissecting the mechanistic basis of reproductive trade-offs in wild populations is to manipulate reproductive investment and measure downstream effects on both survival and the intermediate physiological traits that are thought to impact survival (Landwer 1994; Ardia *et al*. 2003; Kalmbach *et al*. 2004). For example, in brown anole lizards (*Anolis sagrei*, Fig. 1), reducing reproductive investment via surgical ovariectomy dramatically increases survival (Cox & Calsbeek 2010). Ovariectomy also increases growth, suggesting that reproduction is energetically expensive and that this energy can be diverted to other physiological processes when reproductive demands are eliminated (Cox & Calsbeek 2010). However, the specific physiological processes that influence survival in this species, as well as their sensitivity to changes in reproductive investment, are presently unknown.

Fig. 1. A female brown anole (*Anolis sagrei*) in The Bahamas.

To address these issues, we manipulated reproductive investment in wild *A. sagrei* females and then measured downstream effects on a suite of interrelated physiological traits that likely influence survival: energy storage, haematocrit, immune function and parasitemia. We measured energy stored as fat tissue to test the overarching hypothesis that reproductive trade-offs are mediated by changes in energy allocation among competing processes (i.e. growth, storage, reproduction and maintenance). We measured haematocrit, immune function and parasitemia as potential 'proximate effectors' that could link patterns of energy allocation to survival (Harshman & Zera 2007). For example, allocation of energy to egg production is thought to lower haematocrit by impairing the production of red blood cells, thereby reducing oxygen transport and compromising aerobic performance, which could impact survival (Kalmbach *et al*. 2004; Williams *et al*. 2004; Wagner *et al*. 2008). Likewise, energy allocation to reproduction may reduce immune function, which is energetically expensive but important for survival in the face of pathogens (Demas *et al*. 1997; Ardia *et al*. 2003; Martin *et al*. 2003; Hanssen *et al*. 2005; French *et al*. 2007). Reduced allocation to immune function could also lower parasite resistance (the ability to limit parasite burden) or tolerance (the ability to limit harm caused by a given burden) and thereby decrease survival (Norris *et al*. 1994; Sheldon & Verlhust 1996; Nordling *et al*. 1998; Martin *et al*. 2008; Raberg *et al*. 2009). Therefore, we predicted that experimentally reducing reproductive investment via ovariectomy would increase energy storage, haematocrit, parasite resistance and/or tolerance, and immune function, relative to reproductive controls.

Materials and methods

STUDY SPECIES AND NATURAL HISTORY

The brown anole (*Anolis sagrei*, Fig. 1) is a small, semi-arboreal lizard that is native to islands throughout the West Indies. Unlike most oviparous lizards, anoles produce a single-egg clutch (Andrews & Rand 1974). The interval between successive clutches is short, typically ranging from 1 to 2 weeks, and the reproductive season extends from April through October (Lee *et al*. 1989). Thus, per-clutch reproductive investment is low, but cumulative reproductive effort can be quite high (Cox & Calsbeek 2010). We studied a wild population of brown anoles at February Point near Georgetown, Great Exuma, The Bahamas (23°29′N, 75°45′W). Further details on the reproductive biology and natural history of *A. sagrei* females from this population are available elsewhere (Cox & Calsbeek 2010).

SURGICAL TREATMENTS AND EXPERIMENTAL DESIGN

We captured adult *A. sagrei* females near the beginning of the reproductive season (early May of 2009) and marked each animal

with a unique toe-clip for permanent identification. We then measured snout-vent length (SVL, nearest 1·0 mm) and body mass (nearest 0·1 g) and randomly assigned each female to one of two treatment groups: (i) permanent elimination of reproduction via surgical removal of the ovaries (OVX), or (ii) sham (i.e. control) surgery in which the ovaries were left intact (SHAM). We first administered local anaesthesia with a 2-μL intraperitoneal injection of 2% lidocaine HCl (Phoenix Pharmaceuticals Inc., St Joseph, MO, USA). We then cooled females at −20 °C for 5 min and performed surgeries atop a slightly thawed chemical ice pack. For OVX surgeries, we made a single ventral incision, ablated each ovary, and cauterized each oviduct (Cox 2006; Cox & Calsbeek 2010). For SHAM surgeries, we physically manipulated the eggs and ovaries with forceps, but left them intact. We closed the incisions with Vetbond™ tissue adhesive (3M Animal Care Products, St Paul, MN, USA) and allowed females to recover overnight prior to release.

A total of $n = 211$ females (106 OVX, 105 SHAM) were captured at February Point and released to their exact location of capture following surgical treatment. A second group of $n = 600$ females (300 OVX, 300 SHAM) were captured at February Point and released to several offshore islands as part of a separate study investigating effects of predation on survival (R. Cox and R. Calsbeek, unpublished data). Our analyses of survival are based on the first group of $n = 211$ females at February Point, and our analyses of growth and body condition are based on the subset of females from this population that survived until the end of the breeding season. Prior to surgery, females from February Point did not differ as a function of treatment (OVX or SHAM) with respect to SVL ($F_{1,209} = 0{\cdot}28$, $P = 0{\cdot}60$), body mass ($F_{1,209} = 0{\cdot}11$, $P = 0{\cdot}74$), or body condition ($F_{1,209} = 1{\cdot}38$, $P = 0{\cdot}24$).

Our analyses of energy storage, haematocrit, immune function, and parasitemia are based on those females from both sites (February Point and offshore islands) that survived to the end of the breeding season, at which point we measured these physiological parameters. Effects of study site (February Point versus offshore islands) were examined and, as necessary, accounted for in subsequent analyses. Those surviving females for which we measured physiological traits did not differ by treatment (OVX or SHAM) with respect to initial SVL ($F_{1,261} = 0{\cdot}31$, $P = 0{\cdot}58$), initial body mass ($F_{1,261} = 1{\cdot}97$, $P = 0{\cdot}16$), or initial body condition ($F_{1,261} = 1{\cdot}65$, $P = 0{\cdot}20$).

SURVIVAL

We attempted to recapture all surviving females at the end of the breeding season (September, 4 months post-treatment) and also performed a second census the following year (May, 12 months post-treatment). Previous studies using Cormack-Jolly-Seber mark–recapture models have shown that, although recapture rates underestimate actual survival rates in our population, there is no bias in probability of recapture between OVX and SHAM (Cox & Calsbeek 2010). Hence, treatment differences in survival can be reliably inferred from recapture data. We tested for treatment effects on survival using generalized linear models with a logit link function including surgical treatment (OVX, SHAM) as the main effect with survival as a binomial response variable (live, die). Survival did not differ as a function of initial SVL ($\chi^2 = 3{\cdot}25$; $P = 0{\cdot}07$; treatment × SVL: $\chi^2 = 0{\cdot}05$; $P = 0{\cdot}82$), body mass ($\chi^2 = 1{\cdot}95$; $P = 0{\cdot}16$; treatment × mass: $\chi^2 = 0{\cdot}02$; $P = 0{\cdot}90$), or body condition ($\chi^2 = 0{\cdot}03$; $P = 0{\cdot}85$; treatment × condition: $\chi^2 = 0{\cdot}08$; $P = 0{\cdot}77$), so these factors were omitted from subsequent analyses of treatment effects on survival. To assess inter-annual variation in survival, we compared our data from the present experiment (2009) with published results from two previous temporal replicates (2007, 2008) at this same study site ($n = 194$ OVX, 188 SHAM females, see Cox & Calsbeek 2010). Collectively, these three temporal replicates allowed us to assess the repeatability of treatment effects on survival across inter-annual variation in overall survival. All other measures of growth and physiology described below were collected during the 2009 experiment and have not previously been reported.

GROWTH AND BODY CONDITION

For those females recaptured at the end of the breeding season, we measured growth as change in SVL or body mass from pre-manipulation (May) to recapture (September). Because our initial measures of body mass were collected prior to removal of eggs and ovaries, the mass gained by OVX females during the experiment actually underestimates the true growth cost of reproduction because it does not account for the additional mass required to offset the loss of eggs and ovaries (see Cox & Calsbeek 2010). Change in size was negatively correlated with initial size (see Results), reflecting the asymptotic growth characteristic of this species (Cox *et al.* 2009). Hence, we compared growth between OVX and SHAM treatments using ANCOVA with initial size (SVL or mass) as a covariate. Prior to conducting ANCOVA, we verified homogeneity of slopes between treatment groups (treatment × SVL: $F_{1,46} = 0{\cdot}20$; $P = 0{\cdot}66$; treatment × mass: $F_{1,46} = 2{\cdot}31$; $P = 0{\cdot}14$). Our results were qualitatively identical and statistically significant even without accounting for this scaling of growth. We assessed body condition by comparing regressions of body mass on SVL for each treatment group. Body mass and SVL were $\log_{10}$-transformed for this analysis.

ENERGY STORAGE

Anoles and other lizards store energy in paired abdominal structures known as fat bodies (Derickson 1976). The size of these fat bodies typically fluctuates seasonally as energy is stored during periods of surplus and then drawn upon to support reproduction and/or overwinter maintenance (Derickson 1976). We assessed treatment effects on energy storage by dissecting females at the end of the breeding season and comparing the wet mass of abdominal fat bodies between OVX and SHAM. We used ANCOVA with body mass (adjusted by subtracting the wet mass of fat bodies, eggs, and ovaries from total body mass) as a covariate for these analyses. We also measured the wet mass of reproductive tissues (eggs and ovaries) from SHAM females to compare patterns of allocation to storage versus allocation to reproduction.

HAEMATOCRIT

We used heparinized microhaematocrit tubes to collect 20–60 μL of blood from each female. After centrifugation at 1300 *g* for 10 min, we measured the length of the tube that contained plasma versus the length that contained blood cells. Buffy coat was typically negligible. We calculated haematocrit as the proportion of blood cells to total blood volume. We compared haematocrit between OVX and SHAM using ANOVA. To determine whether any differences in haematocrit were due to underlying treatment effects on size and/or condition, we constructed separate models including SVL, body mass, or body condition as effects interacting with treatment.

IMMUNE FUNCTION

We used two separate assays to assess immune function. First, we used the phytohemagglutinin (PHA) skin-swelling technique, an *in vivo* assay that is commonly used in wild vertebrates, including lizards (Svensson *et al.* 2001; Oppliger *et al.* 2004; Calsbeek *et al.* 2008). Injection of PHA induces a series of cellular responses comprising both innate and acquired immune defenses (Kennedy & Nager 2006). These responses include influxes of lymphocytes, heterophils, thrombocytes, basophils and macrophages, which collectively manifest as localized swelling at the site of injection (Martin *et al.* 2006). We measured the thickness of each female's right hind foot to the nearest 0·01 mm at a standardized location (between the first and fifth digits) and then injected 0·1 mg PHA (PHA-P, L8754; Sigma-Aldrich Inc., St Louis, MO, USA) dissolved in 0·01 mL phosphate-buffered saline (PBS) into the foot (Calsbeek *et al.* 2008). We measured the foot again at 24 h post-injection and calculated swelling as the proportional change in foot thickness between pre- and post-injection measurements. We used the mean of three replicate measurements of foot thickness at each time point. We tested for treatment effects on swelling response to PHA using ANOVA. To determine whether any differences in swelling response were due to underlying treatment effects on size and/or condition, we constructed separate models including SVL, body mass, or body condition as effects interacting with treatment.

We also assessed immune function by measuring the ability of lizard plasma to kill *Escherichia coli* bacteria *in vitro* (Millet *et al.* 2007). We used a recent modification of this assay in which bacteria were quantified with spectrophotometry (Liebl & Martin 2009). Following centrifugation of whole blood, plasma fractions were frozen for 7–10 days until assay. We diluted thawed plasma (1·5 μL) 1 : 20 with sterile PBS added to 12·5 μL of an *E. coli* solution (10^5 bacteria per mL) and incubated this mixture at room temperature for an hour to allow the complement activity of the plasma to kill the microbes. After incubation, we added 250 μL of sterile tryptic soy broth and incubated the entire solution for 12 h at 37 °C. We measured bacterial density using a Nanodrop spectrophotometer. We handled control samples in the same way with the exception that we added an additional 1·5 μL PBS in place of plasma. We calculated percent killing by dividing the absorbance of each sample by the absorbance of the control and subtracting this value from one (Liebl & Martin 2009).

PARASITEMIA

We prepared blood smears by spreading a thin film of blood on a microscope slide. We fixed smears in absolute methanol, stained them with 1 : 10 Giemsa, and scanned them for infected cells at 1000× magnification. We scored parasitemia as the number of infected cells per 1000 red blood cells. We tentatively identified most parasites as *Plasmodium azurophilum,* although *P. floridense* and other apicomplexans are observed in Caribbean anoles (Schall 1992; Staats & Schall 1996) and it is possible that these contributed to our counts.

To test whether reproduction compromised parasite resistance, we compared total parasitemia (parasites per 1000 cells) and the incidence of parasitism (presence/absence) between OVX and SHAM. Parasitemia levels were not normally distributed, so we used nonparametric Wilcoxon tests. We assessed treatment effects on the incidence of parasitism using logistic regression. Parasite tolerance is operationally defined as the slope of the regression of host fitness on parasite burden (Raberg *et al.* 2009). To determine whether reproduction compromised parasite tolerance, we tested for treatment differences in the slope of host growth regressed on $\log_{10}$ parasitemia.

Results

SURVIVAL

We recaptured 30 OVX females and 21 SHAM females from our February Point study site in September of 2009. We recaptured an additional six OVX and three SHAM during May of 2010, yielding a total of 36 OVX females (34% survival) and 24 SHAM females (23% survival; Fig. 2). Although this indicates a 48% increase in survival following OVX, this effect was not significant ($\chi^2 = 3{\cdot}21$; $P = 0{\cdot}073$). In part, this reflects the atypically high mortality that occurred in 2009 (Fig. 2), which resulted in low statistical power. Combining these 2009 survival estimates with published data from two previous years (Cox & Calsbeek 2010) revealed a strong effect of treatment on survival ($\chi^2 = 18{\cdot}80$; $P < 0{\cdot}001$) and a significant difference in overall survival between years ($\chi^2 = 10{\cdot}76$; $P = 0{\cdot}001$). However, we found no interaction between treatment and year ($\chi^2 = 0{\cdot}15$; $P = 0{\cdot}70$), indicating that the treatment effect on survival was consistent across all three temporal replicates of the experiment, despite substantial inter-annual variation in overall survival (Fig. 2).

GROWTH AND CONDITION

Over the course of the breeding season, OVX females grew substantially more than SHAM females (Fig. 3). This treatment effect was evident for length (ANCOVA treatment effect: $F_{1,47} = 11{\cdot}89$, $P = 0{\cdot}001$; SVL covariate: $F_{1,47} = 33{\cdot}58$, $P < 0{\cdot}001$) and body mass (ANCOVA treatment effect: $F_{1,47} = 6{\cdot}36$, $P = 0{\cdot}015$; mass covariate: $F_{1,47} = 16{\cdot}38$, $P < 0{\cdot}001$). Body condition was also significantly higher for OVX than for SHAM females ($F_{1,48} = 6{\cdot}93$; $P = 0{\cdot}011$).

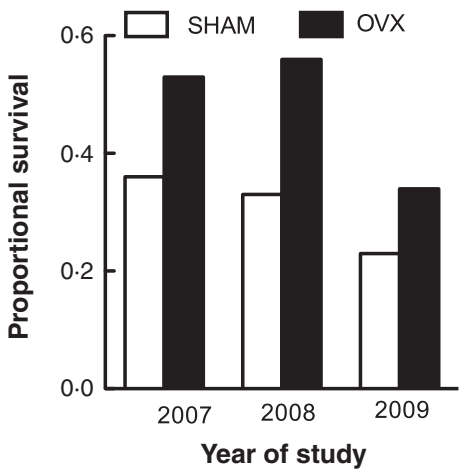

Fig. 2. Elimination of reproduction (OVX) increased breeding-season survival relative to reproductive controls (SHAM) across 3 years of study (2007–2009). Survival data from 2007 and 2008 were previously reported by Cox & Calsbeek (2010) and are included here to illustrate that treatment effects on survival are consistent despite inter-annual variation in overall population survival.

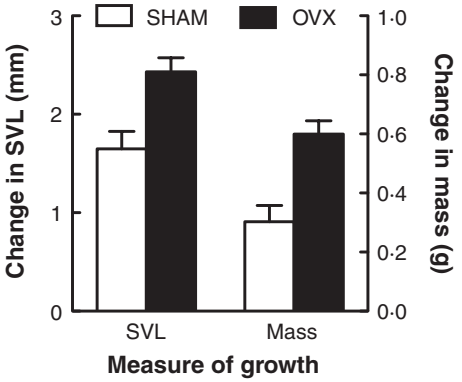

Fig. 3. Elimination of reproduction (OVX) increased growth in (a) SVL, and (b) body mass, relative to reproductive controls (SHAM). Data are least squares means (±1SE) from models with initial size (SVL or mass) as a covariate.

Body condition was correlated with wet mass of fat bodies in OVX females (r^2 = 0·35; $F_{1,26}$ = 14·29 P < 0·001). However, the treatment effect on condition actually became stronger after subtracting the wet mass of fat bodies and reproductive tissues from estimates of total body mass ($F_{1,44}$ = 7·78; P = 0·008; degrees of freedom differ because tissues were not obtained from all females).

ENERGY STORAGE

Wet mass of abdominal fat bodies averaged 153 mg for OVX females, compared to only 18 mg for SHAM females (ANCOVA treatment effect: $F_{1,44}$ = 75·08, P < 0·001; body mass covariate: $F_{1,44}$ = 8·24, P = 0·006; Fig. 4a). Whereas the abdominal cavities of SHAM females contained an average of 147 mg of reproductive tissues (eggs and ovaries), those of OVX females were filled by an equivalent amount of fat.

HAEMATOCRIT

Haematocrit was significantly higher in OVX relative to SHAM females ($F_{1,108}$ = 40·40; P < 0·001; Fig. 4b). Haematocrit was unrelated to SVL ($F_{1,107}$ = 0·48; P = 0·49; treatment × SVL: $F_{1,107}$ = 0·09; P = 0·77) or body mass ($F_{1,107}$ = 0·86; P = 0·35; treatment × body mass: $F_{1,107}$ = 1·56; P = 0·21), and treatment effects on haematocrit were still highly significant (P < 0·001) when including these size effects and their interactions. Haematocrit was positively related to body condition ($F_{1,106}$ = 4·64; P = 0·033), although this relationship differed between treatment groups (treatment × condition: $F_{1,107}$ = 7·93; P = 0·006). Whereas haematocrit increased with condition in OVX females (r^2 = 0·16; P = 0·002), it was unrelated to condition in SHAM females (r^2 = 0·01; P = 0·62). Treatment effects on haematocrit remained highly significant ($F_{1,106}$ = 28·17; P < 0·001) even when accounting for these effects of condition and treatment × condition.

Females recaptured from offshore islands had slightly lower haematocrit than those recaptured from the February Point population (ANOVA site: $F_{1,108}$ = 4·63; P = 0·034), but differences between OVX and SHAM were consistent across sites (ANOVA site × treatment: $F_{1,108}$ = 1·49; P = 0·23). After accounting for differences in haematocrit between OVX and SHAM by including treatment as an effect, we found that haematocrit was unrelated to either immune response to PHA ($F_{1,57}$ = 0·69; P = 0·41; treatment × PHA: $F_{1,57}$ = 0·01; P = 0·97), or $\log_{10}$ parasitemia ($F_{1,31}$ = 2·13; P = 0·15; treatment × parasitemia: $F_{1,31}$ = 0·44; P = 0·51). However, haematocrit was positively related to growth in OVX females, but negatively related to growth in SHAM females (treatment × change in SVL: $F_{1,108}$ = 5·28; P = 0·024).

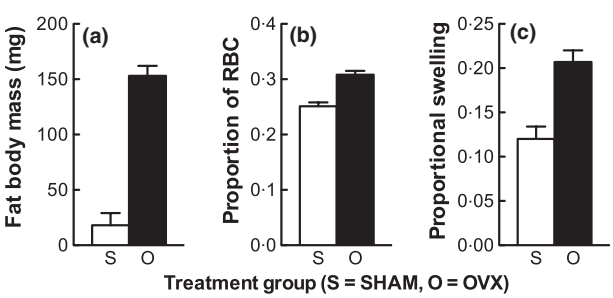

Fig. 4. At the end of the breeding season, OVX and SHAM females differed in (a) energy storage, as measured by the wet mass of fat bodies, (b) haematocrit, as measured by the proportion of red blood cells (RBC) to total blood volume and (c) immune function, as measured by swelling response to PHA. Data in (a) are least squares means (±1SE) from models with body mass as a covariate. Data in (b) and (c) are mean (±1SE).

IMMUNE FUNCTION

Swelling response to PHA was twofold greater in OVX relative to SHAM females ($F_{1,199}$ = 21·07; P < 0·001; Fig. 4c). Response to PHA was unrelated to either SVL ($F_{1,196}$ = 1·91; P = 0·17; treatment × SVL: $F_{1,196}$ = 0·73; P = 0·39), body mass ($F_{1,196}$ = 0·01; P = 0·93; treatment × mass: $F_{1,196}$ = 0·42; P = 0·52) or condition ($F_{1,196}$ = 1·20; P = 0·27; treatment × condition: $F_{1,196}$ = 0·22; P = 0·64). Treatment effects on response to PHA were still highly significant (all P < 0·001) when including these factors. Thus, treatment effects on response to PHA were not simply the result of the larger size or better condition of OVX females. After accounting for differences in immune function between OVX and SHAM by including treatment as an effect, we found that response to PHA was unrelated to growth ($F_{1,196}$ = 0·17; P = 0·68; treatment × growth: $F_{1,196}$ = 0·42; P = 0·52), haematocrit (see above), or $\log_{10}$ parasitemia ($F_{1,135}$ = 2·12; P = 0·15; treatment × parasitemia: $F_{1,135}$ = 0·16; P = 0·69).

The ability of lizard plasma to kill *E. coli* did not differ between OVX and SHAM females ($F_{1,66}$ = 0·78; P = 0·39). However, lizard plasma exhibited extremely low killing capacity for *E. coli* (mean proportion killed = 0·013), and insufficient plasma remained to re-assay samples with lower bacterial densities.

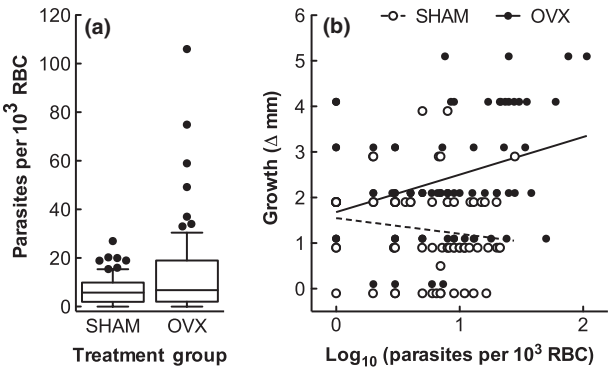

Fig. 5. (a) Box-and-whisker plots depict the median (bar), 25–75% quartiles (box), 10–90% quantiles (whiskers), and outliers (points) for parasite burdens in OVX and SHAM females. (b) Growth and parasitemia are positively correlated in OVX females, but negatively correlated in SHAM females. Symbols for OVX and SHAM are vertically offset at each 1-mm growth increment for clarity.

PARASITEMIA

Incidence of parasite infection was high in both OVX (67 of 75 females, 89%) and SHAM (61 of 71, 86%) and did not differ between treatments (logistic: $\chi^2 = 0{\cdot}39$; $P = 0{\cdot}53$). However, OVX exhibited threefold higher variance in parasitemia relative to SHAM (two-sided *F*-test: $F_{75,70} = 8{\cdot}85$; $P < 0{\cdot}001$), primarily due to the greater tendency for some OVX females to exhibit high parasite loads (Fig. 5a). Mean levels of parasitemia were twofold higher in OVX relative to SHAM females (Welch ANOVA for unequal variances: $F_{1,146} = 8{\cdot}94$; $P = 0{\cdot}004$), but median values were only marginally different (Wilcoxon: $\chi^2 = 3{\cdot}69$; $P = 0{\cdot}055$). Whereas $\log_{10}$ parasitemia was positively correlated with growth across OVX females, this relationship was negative in SHAM females (ANOVA treatment × growth: $F_{1,143} = 5{\cdot}65$; $P = 0{\cdot}018$; Fig. 5b).

Discussion

In brown anoles (*Anolis sagrei*), experimentally reducing reproductive investment via ovariectomy dramatically increased survival over both the immediate breeding season and the subsequent post-breeding period (Cox & Calsbeek 2010). In the present study, we found that OVX also increased energy storage, haematocrit, and one aspect of immune function, as predicted by resource allocation models for reproductive trade-offs. Contrary to our predictions, we also found that OVX females were more likely to have high levels of parasitemia than were SHAM females, although our growth data suggest that SHAM females suffered reduced parasite tolerance. Below, we discuss these treatment effects in the context of resource-allocation trade-offs and explore their implications for survival.

Although ovariectomy does not necessarily abolish the total energetic cost of reproduction (Chinzei & Wyatt 1985; Hatle *et al.* 2008), it clearly results in a substantial energetic savings in brown anoles, as evidenced by the increased growth, improved condition, and enlarged fat bodies that we observed in OVX females. This does not provide direct evidence that ovariectomy increases allocation to maintenance, which is distinct from growth and storage. Moreover, it is possible that some of the difference in fat storage between OVX and SHAM could reflect the limited abdominal space available for fat deposition in gravid SHAM females. However, at the onset of the reproductive season, we typically observe much larger fat bodies in gravid females than those measured in SHAM at the end of the breeding season (R. M. Cox, pers. obs.), suggesting that spatial constraints alone cannot explain the low fat storage of SHAM females. Given that lizards often draw upon stored energy to support over-winter maintenance (Derickson 1976), the enlarged fat bodies of OVX females may explain why their post-breeding survival is twofold greater than that of SHAM females, despite the cessation of reproduction (Cox & Calsbeek 2010). In other lizards, analogous manipulations of reproductive investment can impact growth and survival well beyond the actual reproductive season (Landwer 1994; Cox *et al.* 2006). Collectively, these studies indicate that reproduction often leaves females energetically compromised and therefore susceptible to post-reproductive mortality. This underscores the importance of identifying the underlying physiological functions to which stored energy may be allocated to improve survival.

The immune system has been proposed as a fundamental physiological link between reproductive investment and survival (Sheldon & Verlhust 1996; Deerenberg *et al.* 1997; French *et al.* 2007, 2009; Harshman & Zera 2007). Consistent with this idea, we found that OVX increased immune response to PHA nearly twofold relative to SHAM females. In birds, manipulations of reproductive effort via egg removal and/or addition have been shown to produce similar effects on various measures of immune function (Deerenberg *et al.* 1997; Nordling *et al.* 1998; Moreno *et al.* 1999; Ardia *et al.* 2003; Hanssen *et al.* 2005). Because immune function is energetically expensive (Demas *et al.* 1997; Lochmiller & Deerenberg 2000; Bonneaud *et al.* 2003; Martin *et al.* 2003), its characteristic negative association with reproductive investment is thought to reflect underlying energetic trade-offs (Sheldon & Verlhust 1996; Deerenberg *et al.* 1997; French *et al.* 2007, 2009; Martin *et al.* 2008). Although we have only characterized a single component of the complex vertebrate immune system, our results are nonetheless consistent with this interpretation, raising the question of whether immune function influences survival in wild anoles.

A positive association between immune function and survival is typically assumed and has been demonstrated in some systems (Lochmiller & Deerenberg 2000; Ardia *et al.* 2003; Kilgas *et al.* 2006). However, other studies show that mounting an immune response to non-pathogenic antigens can actually reduce survival (Hanssen *et al.* 2004). Similar complexity is evident in lizards, where the relationship between immune function and survival can vary with respect to population density, sex and genetic morph (Svensson *et al.* 2001, 2009; Calsbeek & Bonneaud 2008; Calsbeek *et al.* 2008). Despite this complexity, data from female brown anoles indicate that response to PHA is positively correlated with survival over

the breeding season (Calsbeek & Bonneaud 2008). This is consistent with our interpretation that reduced immune function may contribute to the low survival of reproductive females.

One fundamental way in which the immune system may impact survival is by combating parasites (Sheldon & Verlhust 1996; Deerenberg *et al.* 1997; Martin *et al.* 2008). Manipulations of reproductive effort have been shown to increase parasite loads in other vertebrates (Norris *et al.* 1994; Nordling *et al.* 1998), and parasitemia is known to be energetically costly in lizards (Schall *et al.* 1982). However, we found that OVX females were actually more likely to exhibit high parasite loads than were SHAM females. One possibility is that ovariectomy altered behaviour (Whittier & Tokarz 1992; Woodley & Moore 1999), such that OVX females were more likely to encounter parasites (Olsson *et al.* 2000). However, the prevalence of infection was similarly high in each treatment group, suggesting that both OVX and SHAM females experienced similar exposure to parasites. An alternative interpretation of this result is that the energetic demands of reproduction left SHAM females more susceptible to parasite-induced mortality, such that only those with low levels of parasitemia were likely to survive the breeding season. Indeed, animal ecologists have recently called attention to the importance of parasite tolerance, in addition to parasite resistance, as an adaptive host defense (Raberg *et al.* 2009). The operational measure of tolerance is the slope of the regression of host fitness on parasite burden (Raberg *et al.* 2009). In support of this interpretation, we observed a significant difference in slopes between OVX and SHAM when using growth as a fitness-related measure. Thus, our results are broadly consistent with the idea that OVX females are better able to mitigate the harmful effects of high parasite loads, and that reduced parasite tolerance may be an important cost of reproduction.

One way in which parasites can influence lizard fitness is by lowering haematocrit and reducing the concentration of haemoglobin in the blood (Schall *et al.* 1982; Schall 1992; Dunlap & Mathies 1993; Salvador *et al.* 1996). Although SHAM females had significantly lower haematocrit than OVX females, we did not detect any correlation between haematocrit and parasitemia across individuals. This suggests that treatment effects on haematocrit were not simply caused by upstream effects on parasite burden. Instead, treatment effects on haematocrit may occur because reproductive investment limits the energy available for production of red blood cells, as hypothesized for birds (Kalmbach *et al.* 2004; Williams *et al.* 2004; Wagner *et al.* 2008). The 16% relative difference in haematocrit that we observed between OVX and SHAM is likely to impair organismal performance (Schall *et al.* 1982), but the relevance of this cost to survival requires further study. We also found that OVX and SHAM females differed in the slopes of the relationships between haematocrit and body condition and between haematocrit and growth. A negative correlation between haematocrit and growth, which was observed only in SHAM females, could indicate that trade-offs between these two functions are only observed when reproductive investment limits available energy.

In summary, we have presented direct experimental evidence for pronounced costs of reproduction with respect to survival, growth, body condition, energy storage, haematocrit, and immune function in a wild lizard population. Our results also suggest that reproductive investment reduces parasite tolerance, supporting the emerging view that both resistance and tolerance should be assessed when measuring host defenses against parasites. Unlike many previous studies, we have simultaneously investigated multiple physiological mechanisms with a powerful experimental approach performed in a natural environmental context. The physiological effects that we observed are broadly consistent with energy-allocation models for the trade-off between reproduction and survival and suggest several interrelated mechanisms that may structure this trade-off. Future studies should clarify whether experimentally induced variation in physiology is directly responsible for the difference in survival of OVX and SHAM females. In particular, it would be informative to alter energy stores, immune function, haematocrit, and parasite burdens independently of reproductive investment and track subsequent survival in the wild. Due to their natural abundance and amenability to manipulation, brown anoles provide an excellent natural system in which to conduct such studies and further elucidate the mechanistic basis of this fundamental life-history trade-off.

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