Seasonal Shifts in Reproduction Depend on Prey Availability for an Income Breeder

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ABSTRACT

The evolution of reproductive strategies depends on local environmental conditions. When environments are seasonal, selection favors individuals that align changes in key reproductive traits with seasonal shifts in habitat quality. Offspring habitat quality can decline through the season, and increased maternal provisioning to late-produced offspring may compensate. This shift, however, may depend on environmental factors that influence reproduction and are, themselves, subject to temporal changes (e.g., food abundance). We studied the brown anole lizard (*Anolis sagrei*) to demonstrate how prey abundance modifies seasonal changes in key reproductive traits. We bred lizards in controlled laboratory conditions across the reproductive season and manipulated the availability of food by providing some breeding pairs high prey availability and some low. Halfway through the season, we switched half of the breeding pairs to the opposite treatment. We measured growth of male and female lizards as well as latency to oviposit, fecundity, egg size, egg content (yolk, water, shell mass), and egg quality (steroid hormones, yolk caloric content) over this period. Higher prey availability enhanced lizard growth and some key reproductive traits (egg size, fecundity) but not others (egg content and quality). Moreover, we found that seasonal patterns of reproduction were modified by prey treatment in ways that have consequences for offspring survival. Our results demonstrate that seasonal changes in reproduction are dependent on fluctuations in local environmental conditions. Moreover, researchers must account for seasonal shifts in environmental factors and reproductive traits (and their interactions) when designing experiments and drawing conclusions about how the environment influences reproduction.

Keywords: life-history evolution, seasonality, parental investment, trade-offs, *Anolis*, reproductive effort, nutrition.

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Introduction

A fundamental goal of life-history theory is to explain the evolution of reproductive strategies (Hirshfield and Tinkle 1975; Monaghan and Nager 1997). A reproductive strategy is a suite of heritable traits—such as size and age at maturity or clutch size—that collectively increase reproductive success in a given environment (Stearns 1976). How selection shapes reproductive strategies is dependent on population-specific phenotypes and local environmental factors (Giesel 1976; Ricklefs 1977; Promislow and Harvey 1990; Jönsson 1997; Espírito-Santo et al. 2013; Pyron and Burbrink 2014; Thomann et al. 2015). As a result, reproductive strategies typically vary within and between species (Tuomi 1980; Shine 2003). For example, key reproductive traits (e.g., size or age at maturity, fecundity, clutch size, brood size) can predictably change across geographic gradients (e.g., latitude or altitude), both within (Ballinger 1979; Morrison and Hero 2003; Ji and Wang 2005; Blanck and Lamouroux 2007; Boyce et al. 2015) and between (Denno and Dingle 1981; Conover 1992; Martin et al. 2006) species. Thus, it is imperative that we understand how local environmental factors impact reproductive success to explain the evolution of reproductive strategies. An additional layer of complexity emerges, however, when we consider that local environments are not stable through time.

Because of seasonal changes in resource availability or climatic factors, habitat quality often declines through the reproductive season, reducing reproductive success and/or offspring survival for late-season breeding attempts (Varpe et al. 2007; Öberg et al. 2014; Harriman et al. 2017). Consequently, aspects of the reproductive strategy can shift within a single season to compensate for changing environmental factors. An extreme example comes from species of aphids that change from asexual to sexual reproduction during late summer because eggs produced by genetic recombination are frost resistant and have higher overwinter survival (Simon et al. 2010). More subtle changes occur in a wide variety of taxa and usually involve seasonal changes in clutch/brood mass and size (e.g., spiders [Iida et al. 2016], fish [Heins et al. 2004], frogs [Williamson and Bull 1995], lizards [Nussbaum 1981; DeMarco 1989], birds [Rowe et al. 1994; Du

et al. 2014], and mammals [Dobson and Myers 1989; Singleton et al. 2001]). These changes may reflect the basic trade-off between the number and quality of offspring (Lack 1947): if the late-season environment is poor, females should invest relatively more energy in fewer offspring during that time (Smith and Fretwell 1974; Brockelman 1975). Although seasonal changes in patterns of reproduction have been described for many study systems, we know comparatively little about the mechanisms that drive these patterns or the environmental factors that may constrain them.

Food abundance, which determines the amount of energy available for reproduction, can fluctuate through time in ways that affect reproduction (Wright et al. 2013). For example, in birds, supplemental feeding positively impacts clutch size, chick body mass, and breeding success and results in an advancement of laying date (Hogstad 2005; Ruffino et al. 2014). Conversely, food scarcity may result in a reduction in reproductive effort. An individual's response to seasonal variation in food resources is constrained by ancestral adaptation to the relative predictability of food: if food is predictably abundant when conditions are also optimal for reproduction, populations might evolve to utilize current food supplies for reproduction (i.e., income breeders). Conversely, if food is not predictably abundant during this time, populations might evolve to rely on stored energy for reproduction (i.e., capital breeders; Jönsson 1997). Seasonal changes in food resources may result in immediate changes in reproductive effort for income but not capital breeders (Ruffino et al. 2014). Thus, the source of energy that fuels reproduction (income vs. capital) is an important aspect of a reproductive strategy that determines how individuals respond to seasonal changes in resource availability.

Manipulative studies under controlled conditions are helpful to demonstrate how seasonal variation in food supply can interact with a reproductive strategy (Ruffino et al. 2014), because some environmental factors (e.g., rainfall, temperature) correlate with both food availability and other aspects of a species' reproductive life history. Moreover, for species that exhibit parental care, such as birds and mammals, studying the influence of food availability on clutch or litter size is problematic since energy must be expended to produce offspring and then subsequently to care for them. Organisms that lack parental care (e.g., most nonavian reptiles) might make more suitable models for addressing these issues because excess food during the reproductive season can be allocated to current reproduction without compromising the survival of offspring. Reptiles often supplement capital reserves with income during reproduction (Bonnet et al. 2001; Warner et al. 2008; Luo et al. 2010), so we can expect food availability to modify aspects of their seasonal reproductive strategies.

To understand how food availability may constrain seasonal changes in patterns of reproduction, we conducted a manipulative laboratory experiment with brown anoles (*Anolis sagrei*). Brown anoles are relatively small (2–6 g) tropical lizards with high reproductive effort and relatively short life spans (~2 yr). They are generalist feeders, consuming a wide variety of terrestrial invertebrates (e.g., arthropods), and they produce single-

egg clutches about once per week during the breeding season. A single egg clutch allows females to rapidly produce eggs and maximize reproductive effort when conditions are favorable (Andrews and Rand 1974). Moreover, egg production alternates between the left and right ovaries, so each egg is yolked and shelled independent of other eggs, allowing females to make fine-scale adjustments to energy allocation among offspring (Crews 1977). Studies conducted in the laboratory and field indicate that increases in food availability have an immediate, positive effect on anole reproduction (Guyer 1988; Wright et al. 2013; Warner and Lovern 2014; Warner et al. 2015). Thus, we expect they can rapidly alter reproduction in response to fluctuations in food abundance (i.e., income breeders).

The reproductive strategy of *A. sagrei* in Florida is characterized by seasonal shifts in reproductive effort and allocation among offspring: egg production is greatest at the beginning of the season, and egg size and quality often increase with successive eggs (Warner and Lovern 2014; Warner et al. 2015; Delaney et al. 2016; Mitchell et al. 2018; Pearson and Warner 2018). Additionally, in laboratory breeding experiments, the allocation of yolk testosterone (T) increases with each egg females produce (Warner and Lovern 2014; Delaney et al 2016). Like many lizard species, offspring survival is higher for individuals that hatch early in the season compared to those that hatch late (brown anoles [Pearson and Warner 2018], other lizards [Olsson and Shine 1997; Warner and Shine 2007; Uller and Olsson 2010]). Therefore, increased energy or steroid allocation to fewer offspring late in the season might partially compensate for the seasonal decline in habitat quality (Mitchell et al. 2018). Variation in food availability, however, could modify or constrain this reproductive strategy. For example, egg quality may only be greater at the end of the season if food is relatively abundant during the same time. Conversely, even if food is scarce, females may reduce fecundity and concomitantly increase egg size or steroid content. Although many studies have sought to characterize the seasonality of anole reproduction (e.g., variation between wet and dry season; Gorman and Licht 1974; Lee et al. 1989), little attention has been given to the ways that intraseasonal variation in food resources affect seasonal changes in reproductive traits (e.g., Wright et al. 2013). Pulses in food resources are an important influence on the reproductive ecology of brown anoles because such changes (1) can be common in their environment and (2) can influence lifetime reproductive success and population dynamics (Wright et al. 2013; Kenny et al. 2017).

We collected adult pairs of brown anoles from a naturalized population in Florida and manipulated their prey availability over a 30-wk period, the typical length of their reproductive season in Florida (March–October; Lee et al. 1989). We measured key reproductive traits (e.g., fecundity, egg size, egg quality, yolk caloric content, and yolk testosterone and corticosterone [CORT] levels) to see how seasonal changes in reproduction would be modified by food availability. We changed the diet of some individuals halfway through the study to observe how quickly reproductive traits shift with changing food conditions. Because brown anoles are short-lived income breeders, we predicted that both males and females would utilize abundant food

to maximize current reproductive success: when prey are abundant, females will produce more and larger eggs while males will grow to a larger size. If prey availability is low, we expect the opposite. We also anticipated that both sexes would rapidly respond if diets were changed. Studies of *Anolis* yolk T and CORT have found that neither respond much to variation in maternal food resources (Lovern and Adams 2008; Warner and Lovern 2014); however, these studies have not been conducted across the full length of a reproductive season. Given that T is often associated with positive phenotypic effects (e.g., higher growth rates; reviewed by Groothuis et al. 2005) and CORT is associated with negative effects (Hayward and Wingfield 2004), we predict that T yolk content will increase seasonally for females on a high-prey diet and CORT will increase for females provided low prey.

Finally, we predicted that seasonal shifts in key reproductive traits would be dependent on food abundance. For example, egg size should increase through the season if food remains abundant but may remain constant or decrease if food is scarce. Our design affords a novel assessment of the way local environmental conditions can influence reproductive strategies and impact fitness (i.e., reproductive success). Such relationships between the environment and reproduction are important to understand how reproductive strategies evolve.

Methods

Housing

Adult brown anoles (*Anolis sagrei*) were collected from Palm Coast, Florida, on October 9 and 10, 2012. At this location, females stop producing eggs in early October (Mitchell et al. 2018), so we are certain the lizards we collected were finished breeding for the year. Forty male/female pairs were transported to the University of Alabama at Birmingham and housed in cages (29 cm × 26 cm × 39 cm) containing three wooden perches for basking, a plant pot filled with soil for nesting, and artificial plants for hiding and climbing. Reptile cage carpet (ZooMed) lined the bottom of each cage. Cages were illuminated with Reptisun 5.0 UVB bulbs (ZooMed), and plant grow bulbs (model F40, General Electric) were set on a 12L:12D photoperiod. Lizard pairs were fed crickets (dusted with calcium and vitamins) three times per week and misted with water daily before the onset of the experiment.

Experimental Design

On January 7, 2013, ambient conditions were gradually changed over a 1-wk period to mimic a spring/summer temperature (28°C) and photoperiod (14L:10D) to stimulate breeding and egg production. These conditions were maintained throughout the experiment (30 wk). Each lizard was measured (snout-vent length [SVL] to the nearest 1 mm) and weighed (to the nearest 0.01 g), and pairs were randomly assigned to high- and low-prey treatments. Lizards in the high-prey treatment were given five crickets/cage three times each week, and those in the low treatment were given two crickets/cage three times each week. Therefore, the

high-prey treatment was given a diet 3.3 times greater in caloric content than the low-prey treatment (Warner et al. 2015). Lizards remained on these prey treatments for 15 wk. We refer to this time as the early season. On April 24, 2013, each lizard was measured and weighed again, and half the lizards in each treatment were switched to the opposite treatment. The remaining half experienced no change, and the experiment continued for another 15 wk; final measures of SVL and mass were collected for each lizard on August 9, 2013. We refer to this second 15-wk period as the late season. This design created four treatments: a high-prey-availability treatment for 30 wk ($n = 10$ pairs); a low-prey-availability treatment for 30 wk ($n = 10$ pairs); a high-prey treatment for 15 wk changed to a low-prey treatment for 15 wk ($n = 10$ pairs); and a low-prey-availability treatment for 15 wk that was switched to high for 15 wk ($n = 10$ pairs). Although we randomly selected females for each treatment, one group of females was smaller in SVL than the others at the start of the experiment (high-to-low treatment; see fig. 1). We compared early season growth rates between these females and those of the constant high-prey treatment (both groups received the high-prey treatment in the early season). The difference in growth rate was slightly greater for the smaller group of females (0.0096 mm per week ± 0.05 SE); however, this was not significant ($F_{1,16} = 0.038$; $P = 0.85$). These groups also did not differ in egg size ($t = 1.45$, df $= 15.33$, $P = 0.17$) or fecundity ($t = 0.91$, df $= 15.77$, $P = 0.37$); thus, this size difference had little, if any, influence on our results.

Egg and Embryo Collection

We checked nesting pots three times each week for freshly laid eggs and massed each egg (to the nearest 0.0001 g) immediately on collection. Eggs produced during the early season ($n = 238$), before the treatment switch, were incubated at a constant 28°C and allocated to another study (Warner et al. 2015); however, we include them in this study to analyze latency to oviposit, fecundity, egg mass, and hatchling mass throughout the entire 30-wk period. Many eggs from the late season were also incubated at a constant 28°C until hatching ($n = 96$); however, we systematically selected eggs for dissection ($n = 65$; yolk, water, shell content, and yolk caloric content analyses) and yolk steroid hormone analyses ($n = 64$) in such a way that each experimental group was represented as equally as possible and maternal biases were minimized. Eggs designated for incubation were placed individually in glass jars (59 mL) half-filled with moist vermiculite (-150 kPa). All jars were covered with plastic wrap and sealed with a rubber band to prevent desiccation. At hatching, we measured the mass of each hatchling to the nearest 0.0001 g. Eggs designated for dissection were opened, and the embryo was removed from the yolk. The remaining yolk and eggshell were each massed, dried thoroughly in an oven (over 24 hr), and remeasured. This procedure allowed us to quantify dry yolk mass, dry shell mass, and total water mass. The dried yolk was analyzed for energy content with bomb calorimetry. Eggs used for steroid hormone analysis were massed, marked, and then stored in a freezer for future analysis.
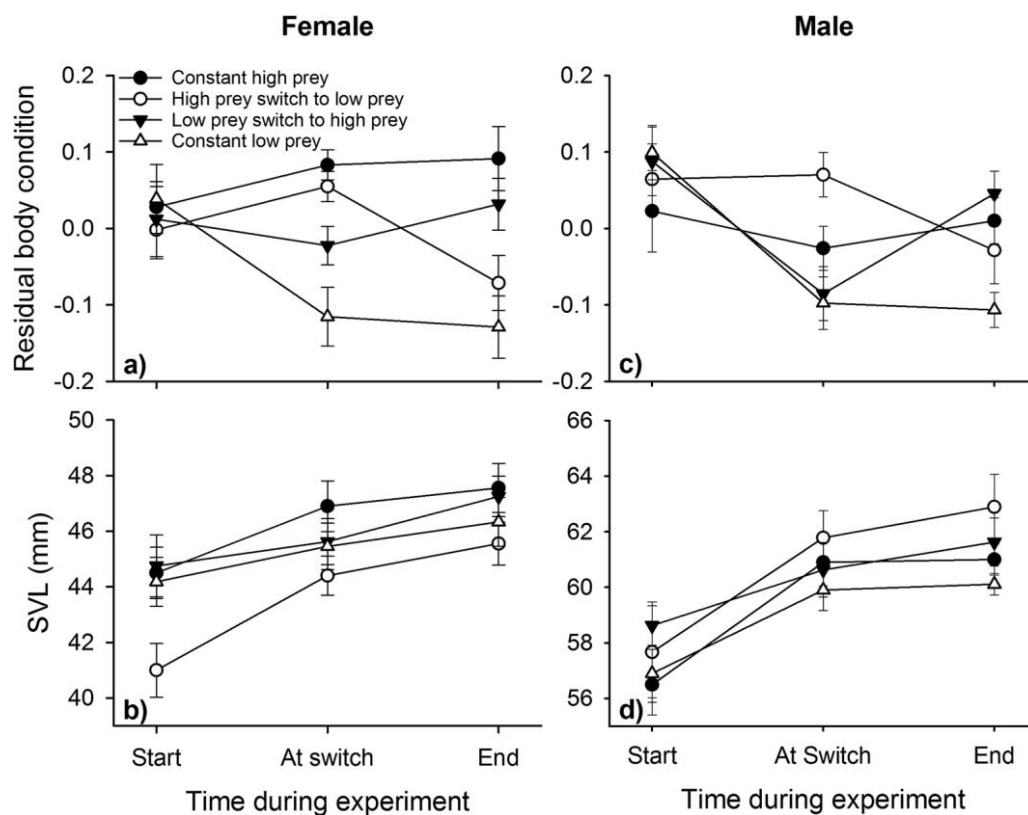
Figure 1. Changes in body condition and snout-vent length (SVL) of female (*a*, *b*) and male (*c*, *d*) brown anoles resulting from high or low prey availability across the 30-wk study. Body condition and SVL were measured at the start of the study (week 1), when the diet treatments were switched (week 15), and at the end of the study (week 30). The early season was from the start of the study to the switch, and the late season was from the switch until the end of the study. Symbols show raw means, and bars represent standard error.

Yolk Energy Content

We determined the energy content of dried yolks using bomb calorimetry. Yolks were placed individually into weighed gelatin capsules (size 00, Parr Instruments, Moline, IL), with each reweighed to determine yolk mass. Capsule and yolk were ignited in a bomb calorimeter (model 1266, Parr Instruments) to provide the mass-specific energy content of the combined capsule and yolk. We subtracted capsule energy (19.48 kJ g^{-1} × capsule mass) from total energy (mass-specific energy × mass of capsule and yolk) to determine yolk energy (mean ± SE 26.68 ± 0.17 kJ g^{-1}).

Yolk Steroid Content

Concentrations of T and CORT steroid hormones in the yolk were measured by radioimmunoassay following extraction and isolation of T and CORT by column chromatography (Schwabl 1993; Lovern and Adams 2008). We collected 8–57 mg of yolk, recorded to the nearest milligram for each sample, from each frozen egg following separation of the whole yolk from the rest of the egg and thorough homogenization with a spatula. These samples were mixed in 1.0 mL of ddH$_2$O and equilibrated with tritiated T and CORT overnight at 4°C with ~1,000 cpm of ³H-T

(NET-370) and ³H-CORT (NET-399) from PerkinElmer Life Sciences (Boston) for determination of individual recoveries. Samples of yolk were extracted twice with 4.0 mL of a 30 : 70 mixture of petroleum ether : diethyl ether, after which they were dried with nitrogen in a 37°C water bath, reconstituted in 1.0 mL of 95% ethanol, and stored at −20°C overnight. The following day, yolk samples were spun at 2,000 rpm for 5 min in a 0°C centrifuge, and the supernatant was transferred to new test tubes and dried with nitrogen in a 37°C water bath. Samples of yolk were then reconstituted in 0.5 mL of 10% ethyl acetate in isooctane for chromatographic isolation of T and CORT.

Chromatography columns consisted of a filter agent : ethylene glycol : propylene glycol upper phase (4 : 1 : 1, m : v : v) and a filter agent : ddH$_2$O (3 : 1, m : v) lower phase. The filter agent (Celpure P300) was purchased from Sigma-Aldrich (St. Louis). Neutral lipids and dihydrotestosterone were removed with isooctane and 10% ethyl acetate in isooctane, respectively, and then discarded. Column fractions containing T and CORT were collected with 20% and 52% ethyl acetate in isooctane, respectively. These samples were dried with nitrogen in a 37°C water bath, resuspended in phosphate buffer, and refrigerated at 4°C overnight.

Radioimmunoassays were performed using the appropriate tritiated steroid tracer (see above), antibodies from Research Diagnostics for T (T-3003; Flanders, NJ) and Sigma-Aldrich for CORT

(C8784), and steroid standards from Sigma-Aldrich. The standard curves were run in duplicate, and samples were run singly and adjusted for individual recovery and initial sample mass. Average recovery for T and CORT was 39% for both steroids, comparable to previous work with this species (Warner et al. 2013; Warner and Lovern 2014). Yolk T and CORT were detectable in 100% of samples. Intra-assay CVs, based on four aliquots from a standard pool for each steroid were 18% and 8% for yolk T and CORT, respectively.

Statistical Analyses

For each dependent variable of adult body size and reproduction, we performed two separate mixed-model ANCOVAs to quantify the effect of prey availability. The first analysis quantified the effect of prey availability during the early season, before the treatment switch (i.e., low- versus high-prey treatments were compared). The second analysis quantified the effect of both early and late-prey treatments (and their interaction) on late-season measurements. We also included time (number of days since the start of the study) and interactions between prey treatment and time for each model to assess how reproductive traits shifted through the season. For analyses of body condition and SVL, we used the most recent previous measure of body condition or SVL, respectively, as covariates. In addition, individual was a random effect, and males and females were analyzed separately. To calculate body condition, the mass and SVL of each lizard from each time of measure (start, middle, end of study) were combined into a single data set and residual scores for each lizard were obtained from a single linear regression of log body mass by log SVL (Warner et al. 2016). This calculation was performed separately for males and females. Because most females laid their first egg within the first 15 wk, we only tested the effect of the early season prey treatment on latency of oviposition. We also included initial maternal SVL and initial body condition as covariates in this analysis. Latency was the number of days from the start of the study (Jan. 7) until the first egg was laid. Five males (three from high-prey and two from low-prey treatments) and four females (two from high and two from low) died during the second half of the study. These individuals were included in the analyses for body size for the first but not the second half of the study.

For fecundity, we included both initial body condition and initial SVL as covariates. Egg mass and hatchling mass were analyzed with repeated measure ANCOVAs including mother as a random effect. Covariates in both egg mass analyses were the most recent measure of maternal SVL for each egg and time (number of days from the start of our study until oviposition). Egg mass was a covariate for analyzing hatchling mass. Finally, the effects of early and late-season treatments on egg production were assessed through time: separate regression analyses were performed for each prey availability treatment to assess the number of eggs produced per female for each treatment group through time. We modeled both linear and nonlinear (quadratic) functions for each treatment and chose the model that explained the most variance by comparing scores of corrected Akaike information criteria.

We used two-way mixed-model ANCOVAs to assess the effect of both early and late-season prey treatments, their interaction, and time (oviposition date) on yolk caloric content, egg contents (water, yolk, shell), and yolk steroids (T, CORT). We also included interactions between prey treatment and time in all models. For egg contents (grams of water, yolk, shell), egg mass was considered a covariate. Values for testosterone were log transformed to satisfy the assumption of homogeneity of variance (Games and Howell 1976). To analyze egg survival (hatching success), we used two generalized linear mixed effects models. One analyzed the effect of prey treatment in the early season, and the other analyzed the effects of early and late-prey treatments and their interaction as fixed effects. Egg mass was a covariate. Each egg analysis included maternal ID as a random effect. All analyses were performed in R 3.1.3 (R Development Core Team 2015). We utilized the following R packages: nlme for general linear mixed models, lme4 and car for generalized linear mixed models, and piecewiseSEM to estimate r^2 values for nlme models.

Results

Body Size and Condition

For females, the high-prey-treatment-enhanced body condition compared to the low-prey treatment in both the early and late seasons (fig. 1a; table 1); however, the high-prey treatment only enhanced growth in SVL during the early season (fig. 1b; table 1). Snout-vent length did not significantly differ between groups at the end of the study (fig. 1b). Switching prey availability from high to low resulted in a rapid decline of body condition, while switching from low to high resulted in increases (fig. 1a); however, this seasonal diet switch had little effect on growth in SVL (fig. 1b; table 1). We found no interaction between early and late-season prey treatments on female body size or condition (table 1).

Like females, male body condition was enhanced by the high-prey treatment compared to low in both the early and late season (fig. 1c; table 2). Unlike females, male initial body condition positively covaried with final body condition (table 2): males that were initially more robust tended to remain relatively robust, regardless of season or diet (fig. 2c, 2d).

In the early season, males on a high-prey diet experienced more growth in SVL; however, in the late season, this trend was reversed, and males maintained on a low-prey diet experienced more growth than those given high prey (fig. 1d; table 2). We also observed a significant interaction between early and late-season prey treatments for male SVL (table 2): lizards fed a high-prey diet during the late season increased in SVL only if they were fed a low-prey diet in the early season; lizards fed a low-prey diet during the late season increased in SVL only if they were fed a high-prey diet in the early season (fig. 1d).

Reproductive Output

Females given a high-prey diet started laying eggs earlier (i.e., shorter latency; fig. 3a) and had greater fecundity (fig. 4a) than those on a low-prey diet (table 3). Fecundity was about twice as

Table 1: Effect of early and late-season prey availability and their interaction on female body condition and body size (snout-vent length [SVL])

Independent variable	Female residual body condition				Female SVL (mm)			
	Early season		Late season		Early season		Late season	
	$\beta \pm$ SE	F; P	$\beta \pm$ SE	F; P	$\beta \pm$ SE	F; P	$\beta \pm$ SE	F; P
Early prey treatment	**.15 ± .03**	**$F_{1,36} = 24.19$; $P < .0001$**	.013 ± .057	$F_{1,30} = .051$; $P = .82$	**1.32 ± .47**	**$F_{1,36} = 8.02$; $P = .0075$**	−.64 ± .56	$F_{1,30} = 1.32$; $P = .26$
Late prey treatment	⋯	⋯	**.15 ± .051**	**$F_{1,30} = 8.7$; $P = .0061$**	⋯	⋯	.13 ± .56	$F_{1,30} = .05$; $P = .83$
Early × late treatment interaction	⋯	⋯	.016 ± .074	$F_{1,30} = .049$; $P = .83$	⋯	⋯	.73 ± .77	$F_{1,30} = .88$; $P = .36$
Previous body condition	.07 ± .12	$F_{1,36} = .32$; $P = .58$	.36 ± .22	$F_{1,30} = 2.76$; $P = .11$	⋯	⋯	⋯	⋯
Previous SVL	⋯	⋯	⋯	⋯	.72 ± .072	**$F_{1,36} = 98.25$; $P < .0001$**	**.85 ± .078**	**$F_{1,30} = 117.84$; $P < .0001$**

Note. Treatment estimates show high prey availability minus low. Boldface indicates statistical significance.

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Table 2: Effect of early and late-season prey availability and their interaction on male body condition and body size (snout-vent length [SVL])

Independent variable	Male residual body condition				Male SVL (mm)			
	Early season		Late season		Early season		Late season	
	$\beta \pm$ SE	F; P	$\beta \pm$ SE	F; P	$\beta \pm$ SE	F; P	$\beta \pm$ SE	F; P
Early prey treatment	**.13 ± .02**	$F_{1,37}$ = **28.76**; **$P <$ .0001**	−.07 ± .037	$F_{1,30}$ = 3.4; P = .075	**1.71 ± .47**	$F_{1,37}$ = **13.096**; **P = .0004**	−.83 ± .56	$F_{1,30}$ = 2.25; P = .14
Late prey treatment	…	…	**.11 ± .039**	$F_{1,30}$ = **8.1**; **P = .0079**	…	…	**−1.16 ± .55**	$F_{1,30}$ = **4.47**; **P = .043**
Early × late treatment interaction	…	…	.017 ± .055	$F_{1,30}$ = .093; P = .76	…	…	**1.81 ± .79**	$F_{1,30}$ = **5.14**; **P = .031**
Previous body condition	**.5 ± .09**	$F_{1,37}$ = **30.61**; **$P <$ .0001**	**.68 ± .15**	$F_{1,30}$ = **20.68**; **P = .0001**	…	…	…	…
Previous SVL	…	…	…	…	**.67 ± .075**	$F_{1,37}$ = **82.28**; **$P <$ .0001**	**.81 ± .079**	$F_{1,30}$ = **103.11**; **$P <$ .0001**

Note. Treatment estimates show high prey availability minus low. Boldface indicates statistical significance.
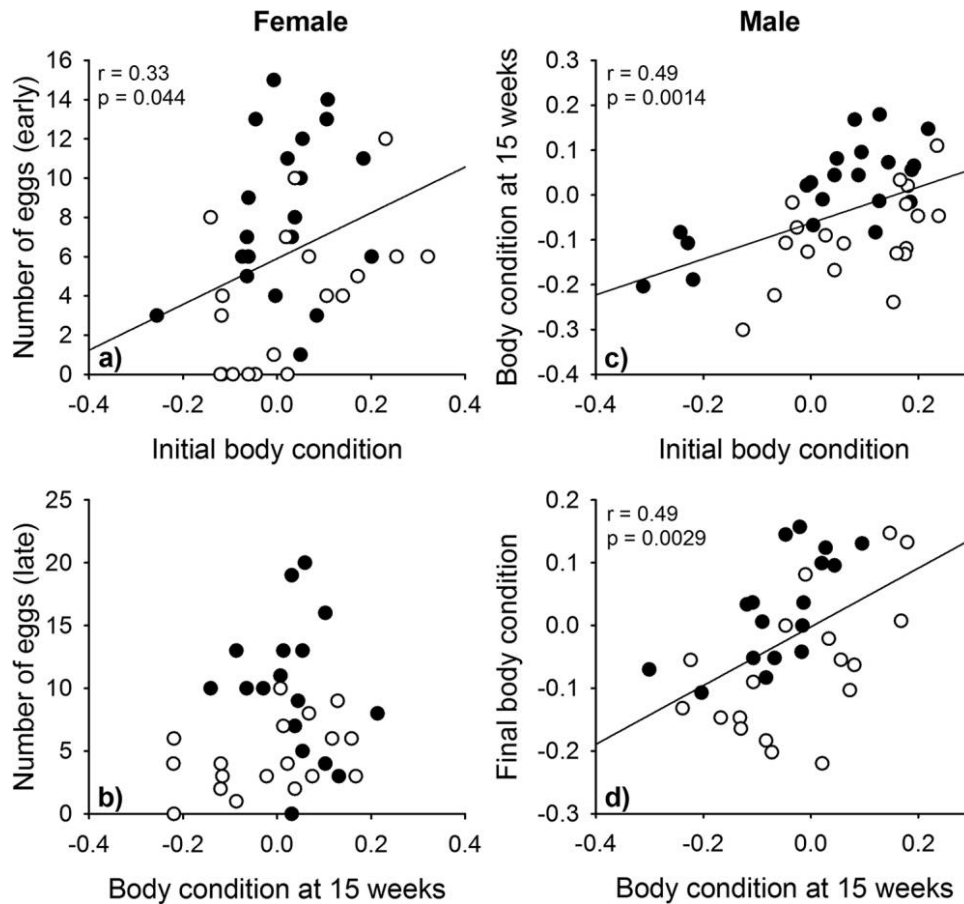
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Figure 2. Relationship between body condition and fitness-relevant phenotypes. Female body condition positively covaried with fecundity early (a; $F_{1, 35} = 5.29$; $P = 0.028$) but not late in the season (b; $F_{1, 30} = 0.0092$; $P = 0.92$). Initial male body condition positively covaried with final body condition early (c; $F_{1, 37} = 30.61$; $P < 0.0001$) and late (d; $F_{1, 30} = 20.68$; $P = 0.0001$) in the season, regardless of treatment. Initial body condition was measured at the start of the study, and final body condition was measured at the end of the study (week 30). Body condition at week 15 corresponds with the time in the study when the prey treatments were changed. Closed and open circles show data for high- and low-prey treatments, respectively. Regression lines are for illustration; r and P values given in each panel are for raw values of high and low treatments combined. See tables 2 and 3 for model estimates.

high for females that received abundant prey (fig. 4a). When the diets were switched, fecundity changed such that females that were moved from a low- to high-prey diet increased fecundity and those that were changed from high prey to low decreased fecundity (fig. 4a). Previous body condition positively covaried with fecundity early in the season: females with greater initial body condition produced more eggs than those with lower condition (fig. 2a); however, body condition was not related to fecundity during the late season (fig. 2b; table 3). Moreover, females with a relatively high initial body condition started laying eggs earlier (shorter latency) than those with relatively lower body condition (fig. 3b; table 3).

Egg production through time differed among treatments (fig. 5; table A1). For females given a high-prey diet through the entire reproductive season, egg production rapidly increased and peaked relatively early in the study and then declined (fig. 5a). This pattern was similar for females in the high-prey treatment that were switched to low prey (fig. 5b); however, the decline was rapid due to the change in prey treatment. Females maintained

on a low-prey diet had consistently low egg production (fig. 5c), but egg production increased for those females switched from a low- to high-prey treatment (fig. 5d).

Egg mass was 0.0138 g (± 0.0099 SE) greater (7.77% larger) for females in the high-prey treatment than those in the low treatment in the early season (fig. 4b), but this was not significant (table 4). Egg mass increased through time, but females in the high-prey treatment exhibited more increase in egg mass than those in the low treatment (significant time $\times$ treatment interaction; fig. 6; table 4). During the late season, egg mass was 0.038 g (± 0.022 SE) greater (22% larger) for the high-prey treatment, but this was not significant (table 4; fig. 4b); however, we observed an interaction between prey treatment and time: late-season egg mass declined for females on a low-prey diet irrespective of which diet was provided to them in the early season, but egg mass increased through time for females given a high-prey diet (table 4; fig. 6). Due to the interaction, we split the data into high and low treatments both before and after the diet switch to assess how egg size changed through time. Egg size increased for females pro-
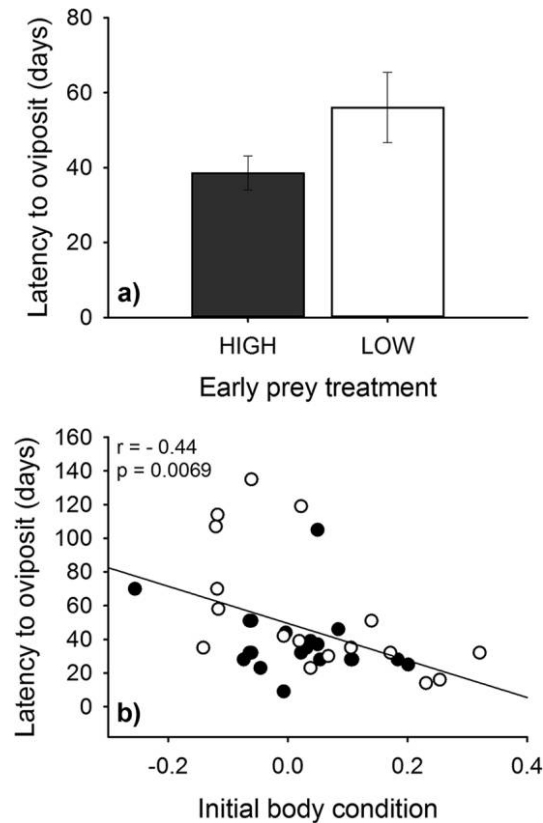
early but not late in the season (table 4). We found no effect of prey treatment on hatching success in the early season ($\chi^2 = 0.36$; df $= 1$; $P = 0.55$). In the late season, there was no effect of early ($\chi^2 = 1.28$; df $= 1$; $P = 0.26$) or late ($\chi^2 = 0.12$; df $= 1$; $P = 0.73$) prey treatments or their interaction ($\chi^2 = 0.54$; df $= 1$; $P = 0.46$) on hatching success.

Water content was 0.066 g (± 0.022 SE) greater (a 1.7% increase) in eggs from mothers given low prey in the early season than those provided with high prey availability; however, the late-season prey treatment had no effect (table 4). Moreover, we observed an interaction between time and early season prey treatment for egg water content: eggs from mothers initially provided with low prey availability increased water content through time, while those initially provided with high prey availability experienced no change (fig. A1; table 4).

We found no effect of prey treatment on yolk mass, egg shell mass, yolk caloric content, T, or CORT. Moreover, we observed no significant interactions between early and late-season prey availability for any measure of egg content; however, both dry yolk content and water content positively covaried with egg mass (table 4). For each 1 g increase in egg mass, water content increased by 0.89 g (± 0.11 SE) and yolk content increased by 0.097 g (± 0.046 SE). For context, our 10 largest eggs (mean $= 0.2355$ g) should contain approximately 0.102 g more water and 0.011 g more yolk than our 10 smallest eggs (mean $= 0.1211$ g).

Figure 3. Effect of prey treatment (*a*) and initial body condition (*b*) on latency to oviposit for brown anoles. Latency to oviposit was lower for females provided a high-prey diet in the early season (*a*) and negatively covaried with initial body condition (*b*; $F_{1,33} = 7.89$; $P = 0.0083$). *a*, Bars show the standard error. *b*, Regression line is for illustration. Values *r* and *P* are for raw data of high- and low-prey treatments combined. See table 3 for model estimates.

vided with abundant prey in the early season by 0.0022 g per week (± 0.00091 95% confidence interval [CI]; $F_{1,145} = 22.7$; $P < 0.0001$; conditional $R^2 = 0.37$), but those given low prey at this time had no change ($F_{1,58} = 0.06$; $P = 0.81$; fig. 6). However, in the late season, egg size decreased for females on a low-prey diet by 0.0017 g per week (± 0.0013 95% CI; $F_{1,37} = 7.45$; $P = 0.0096$; conditional $R^2 = 0.33$) and those given high prey increased by 0.0006 g per week (± 0.00065 95% CI), but this was not significant ($F_{1,153} = 3.24$; $P = 0.07$; fig. 6). Finally, egg size increased with female body size both early and late in the season (marginally not significant in the late season; $P = 0.051$; table 4): for every 1-mm increase in maternal SVL, egg mass increased by 0.0022 g (± 0.001 SE). Thus, the largest females (~ 50 mm SVL) produced eggs roughly 20% more massive than the smallest females (~ 40 mm).

Egg Quality and Composition

Prey availability had little influence on egg quality and composition. Hatchling mass was unaffected by prey treatment when egg size was included as a covariate, but it increased with time
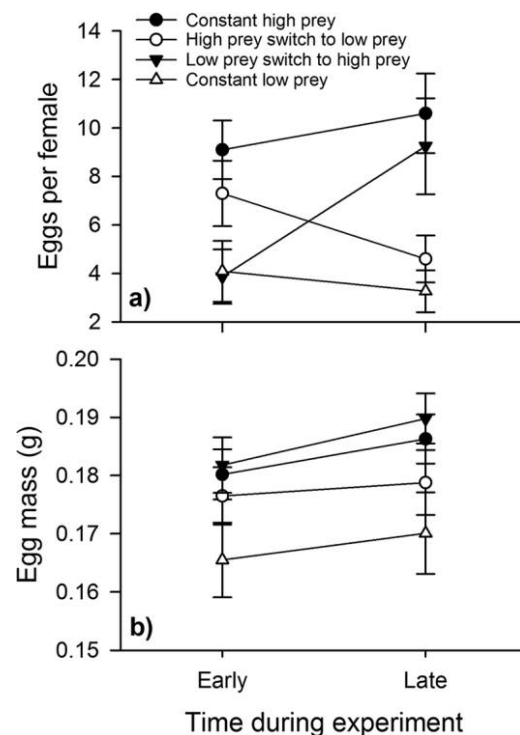

Figure 4. Average fecundity (*a*) and egg mass (*b*) of each female brown anole that was provided high or low prey availability in both the early and late seasons. Symbols show mean values, and bars are standard error.

Table 3: Effect of early and late-season prey availability and their interaction on fecundity and latency to oviposit

	Fecundity (no. eggs)				Latency (d)	
	Early season		Late season		Early season	
Independent variable	$\beta \pm$ SE	F; P	$\beta \pm$ SE	F; P	$\beta \pm$ SE	F; P
Early prey treatment	**4.9 ± 1.16**	**$F_{1,35} =$ 17.88; $P =$ .0002**	1.22 ± 2.34	$F_{1,30} =$ .27; $P =$ .61	**22.78 ± 8.89**	**$F_{1,33} =$ 6.57; $P =$ .015**
Late prey treatment	...	...	**5.12 ± 2.15**	**$F_{1,30} =$ 5.65; $P =$ .024**	...	...
Early × late treatment interaction	...	...	−.29 ± 3.03	$F_{1,30} =$ .0092; $P =$ .92	...	...
Previous body condition	**10.96 ± 4.8**	**$F_{1,35} =$ 5.29; $P =$ .028**	−.85 ± 8.89	$F_{1,30} =$ .0092; $P =$ .92	**−104.9 ± 37.33**	**$F_{1,33} =$ 7.89; $P =$ .0083**
Previous SVL	.32 ± .18	$F_{1,35} =$ 3.08; $P =$ .088	.17 ± .31	$F_{1,30} =$ .32; $P =$ .57	−2.0 ± 1.45	$F_{1,33} =$ 1.9; $P =$ .18

Note. Treatment estimates show high prey availability minus low. Boldface indicates statistical significance. SVL = snout-vent length.

These values equal 55% and 6.1% of the average egg mass (mean = 0.182 g) for water and yolk, respectively.

Discussion

Reproductive strategies often include seasonal shifts in reproductive traits (i.e., egg production, egg size, steroid hormone allocation); however, seasonal changes in reproduction can potentially be modified by resource abundance. Moreover, resource availability can also influence body size, which in turn impacts reproduction (e.g., larger males have better mating success; larger females lay larger eggs). In the brown anole lizard, we demonstrated that egg size and frequency rapidly change with shifts in prey abundance, but nearly all aspects of egg quality were unaffected. Furthermore, we showed that the effect of prey abundance on growth is dependent on seasonal timing: high prey availability influenced growth early but not late in the season. These results demonstrate that the relationship between resource abundance and seasonal timing has consequences for fitness.

Body Size and Condition

Body size and condition are important for fitness in many animals. For example, relatively large body size is associated with increased mating success in male anoles (Trivers 1976). In females, large body size equates to greater survival, fecundity, and clutch or litter size, as well as greater size of individual offspring. Such relationships have been demonstrated across diverse taxa (Blueweiss et al. 1978; Tuomi 1980; Honěk 1993) and have important implications for fitness: large offspring may have increased probability of survival to maturity (Sinervo et al. 1992). Many studies report that food abundance impacts growth and body size (Stamps and Tanaka 1981; Madsen and Shine 2000; Wright et al. 2013; Warner et al. 2015); however, we show that the effect of food abundance on body size of anoles depends on seasonal timing: high prey availability resulted in substantial growth early but not late in the reproductive season. Andrews

and Rand (1974) report that small female anoles allocate more energy to growth than do large females when reproductively active; therefore, prey abundance may have more influence on growth earlier in the breeding season when few individuals have reached their maximum body size. Once females reach reproductive maturity, the energy they consume must be split among growth, maintenance, and egg production. Females that attain larger body size early in the breeding season may increase fecundity because they will have more time during the season to shunt energy toward reproduction rather than growth (Wright et al. 2013).

Body condition seems to be more sensitive to prey treatment than body size. Female body condition, in particular, was determined by current food supplies during the reproductive season. The trends we observed were driven primarily by a reduction in condition of females receiving few prey rather than an increase in the condition of those receiving abundant prey (fig. 1a). This result suggests that, during the breeding season, females use excess energy to fuel reproduction rather than store it as capital (i.e., greater body condition).

Female brown anoles likely experience stabilizing selection on body size, while males experience directional selection for larger size (Cox and Calsbeek 2010b). Larger males can better defend territories and secure mating opportunities than smaller males (Trivers 1976; Tokarz 1985), but females that mature earlier have greater fecundity (Wright et al. 2013): egg laying is energetically expensive and limits further growth (Cox and Calsbeek 2010a). Cox and Calsbeek (2010b) predict that male growth should be more sensitive to environmental variation than female growth due to this sex-specific selection on growth rates. These selection pressures might explain the sex-specific effects we observed on body size and condition: prey abundance influenced male body size throughout the study, but female size was affected only during the early season. Moreover, a general negative trend between male body condition and time (fig. 1c) suggests that males were utilizing excess energy for growth in SVL rather than for body mass (i.e., fat) accumulation.
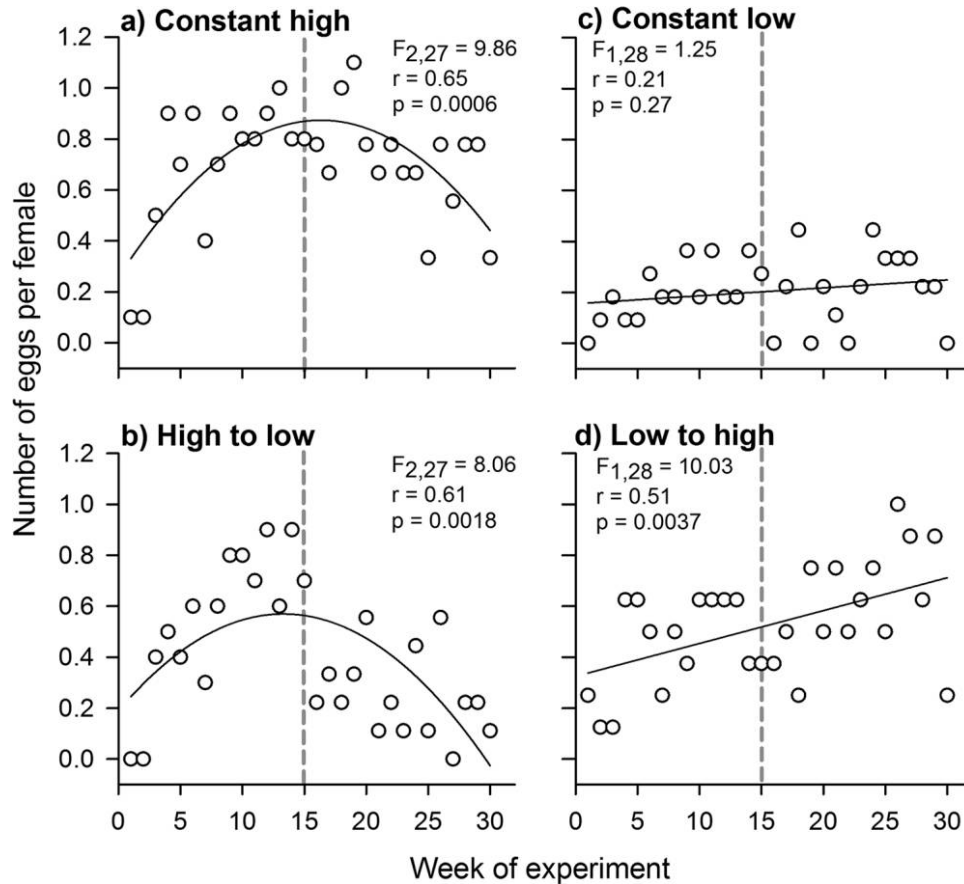
Figure 5. Relationship between brown anole egg production and time across four feeding treatments: females in high-prey-abundance treatment (*a*); females switched from high to low prey (*b*); females in low prey-abundance treatment (*c*); and females switched from low to high prey (*d*). Open circles show the number of eggs per week per female. The statistics provided are from regressions of egg production through time. Solid lines show the best fit of egg production through time. Vertical dotted lines refer to the point in the study when diets were changed for two groups (*c*, *d*). Estimates (β [SE]) of regressions and associated statistics are provided in table A1.

Regardless of diet, males that are more robust when breeding begins will be relatively robust throughout the season (fig. 2*c*, 2*d*), which will likely influence fitness. For females, however, initial body condition appears to influence fitness through its effect on latency to oviposit: females with higher initial body condition started laying eggs sooner and thus had higher fecundity. Females of many reptile species may rely on capital reserves to initiate ovulation (Bonnet et al. 2001; Price 2017). This may be particularly important in seasonal habitats where females utilize fat reserves before the onset of breeding (i.e., late winter/early spring) to initiate vitellogenesis (Price 2017). Food intake, however, may be a more important determinant of reproductive effort thereafter (Bonnet et al. 2001). One caveat of our study is that males did not have opportunities to interact with other males. In the wild, anoles expend great deals of energy patrolling and defending territories, and this activity would cause declines in fat reserves (Husak et al. 2016). Another is that body condition indices may not perfectly correlate with fat reserves (Warner et al. 2016). Ultimately, preseason body condition appears to be important for fitness-relevant phenotypes of both males and fe-

males; however, at least for females, the importance of body condition may change through the season. These patterns highlight the complex sex-specific influences of income and capital on reproductive success (Bonnet et al. 2001).

Reproduction and Egg Composition

In seasonal environments, the amount of time that offspring can grow before winter (temperate regions) or the dry season (tropical regions) often determines survival (Warner and Shine 2007). As a response, females may produce more offspring early in the season but shift allocation so that fewer, better provisioned offspring are produced later (lizards: Brockelman 1975; Nussbaum 1981; Ferguson et al. 1982; Sinervo et al. 1992; brown anole: Pearson and Warner 2018; Mitchell et al. 2018). We show that an individual's ability to provide such differential investment is mediated by food resources. Therefore, the fitness benefits associated with seasonal shifts in reproduction may only be realized when food is relatively abundant. This could explain why

Table 4: Effect of early and late-season prey treatments and their interaction on egg size, quality, and composition

Independent variable	Egg mass		Hatchling mass		Egg contents (late season)			Yolk energy and steroids (late season)		
	Early season	Late season	Early season	Late season	Water content	Yolk dry mass	Shell dry mass	Calories	T	CORT
Early prey treatment	$F_{1,30} = 1.95$; $P = .17$	$F_{1,187} = .11$; $P = .75$	$F_{1,27} = .64$; $P = .43$	$F_{1,37} = .005$; $P = .94$	$F_{1,25} = 9.15$; $P = .0057$	$F_{1,25} = .80$; $P = .38$	$F_{1,25} = 1.02$; $P = .32$	$F_{1,27} = .47$; $P = .50$	$F_{1,24} = 1.77$; $P = .20$	$F_{1,24} = .11$; $P = .74$
Late prey treatment	…	$F_{1,29} = 3.14$; $P = .087$	…	$F_{1,21} = .35$; $P = .56$	$F_{1,25} = .92$; $P = .35$	$F_{1,25} = 1.28$; $P = .27$	$F_{1,25} = .58$; $P = .45$	$F_{1,27} = .10$; $P = .75$	$F_{1,24} = .20$; $P = .66$	$F_{1,24} = 1.86$; $P = .18$
Early × late treatment interaction	…	$F_{1,29} = .0001$; $P = .99$	…	$F_{1,21} = .079$; $P = .78$	$F_{1,25} = 3.73$; $P = .065$	$F_{1,25} = 1.35$; $P = .26$	$F_{1,25} = 1.64$; $P = .21$	$F_{1,27} = .04$; $P = .84$	$F_{1,24} = 1.34$; $P = .245$	$F_{1,24} = 2.13$; $P = .16$
Time	$F_{1,203} = 21.07$; $P < .0001$	$F_{1,187} = 2.02$; $P = .16$	$F_{1,92} = 9.01$; $P = .004$	$F_{1,37} = 3.03$; $P = .089$	$F_{1,29} = .032$; $P = .86$	$F_{1,29} = 1.05$; $P = .31$	$F_{1,29} = .67$; $P = .42$	$F_{1,30} = .32$; $P = .58$	$F_{1,28} = 1.94$; $P = .17$	$F_{1,28} = .095$; $P = .761$
Time × early treatment	$F_{1,203} = 5.04$; $P = .026$	$F_{1,187} = .015$; $P = .90$	$F_{1,92} = 1.96$; $P = .17$	$F_{1,37} = .28$; $P = .60$	$F_{1,29} = 6.87$; $P = .014$	$F_{1,29} = .88$; $P = .36$	$F_{1,29} = 1.31$; $P = .26$	$F_{1,30} = .14$; $P = .71$	$F_{1,28} = 1.98$; $P = .170$	$F_{1,28} = .22$; $P = .65$
Time × late treatment	…	$F_{1,187} = 4.66$; $P = .032$	…	$F_{1,37} = .18$; $P = .67$	$F_{1,29} = .8$; $P = .38$	$F_{1,29} = .82$; $P = .37$	$F_{1,29} = .40$; $P = .53$	$F_{1,30} = .14$; $P = .71$	$F_{1,28} = .024$; $P = .88$	$F_{1,28} = 2.59$; $P = .12$
Time × early × late	…	$F_{1,187} = .09$; $P = .77$	…	$F_{1,37} = .17$; $P = .69$	$F_{1,29} = 2.51$; $P = .12$	$F_{1,29} = .95$; $P = .34$	$F_{1,29} = 1.49$; $P = .23$	$F_{1,30} = .01$; $P = .92$	$F_{1,28} = 1.42$; $P = .24$	$F_{1,28} = 2.17$; $P = .15$
Maternal SVL	$F_{1,30} = 8.65$; $P = .006$	$F_{1,29} = 4.17$; $P = .051$	…	…	…	…	…	…	…	…
Egg mass	…	…	$F_{1,92} = 71.51$; $P < .0001$	$F_{1,37} = 30.68$; $P < .0001$	$F_{1,29} = 69.2$; $P < .0001$	$F_{1,29} = 4.49$; $P = .043$	$F_{1,29} = 2.38$; $P = .13$			

Note. Hatchling mass, egg contents, and yolk steroids were measured only in the late season. Time is the number of days from the beginning of the study to the date each egg was collected. Estimates (β [SE]) of effects are in table A2. Boldface indicates statistical significance. T = testosterone; CORT = corticosterone; SVL = snout-vent length.
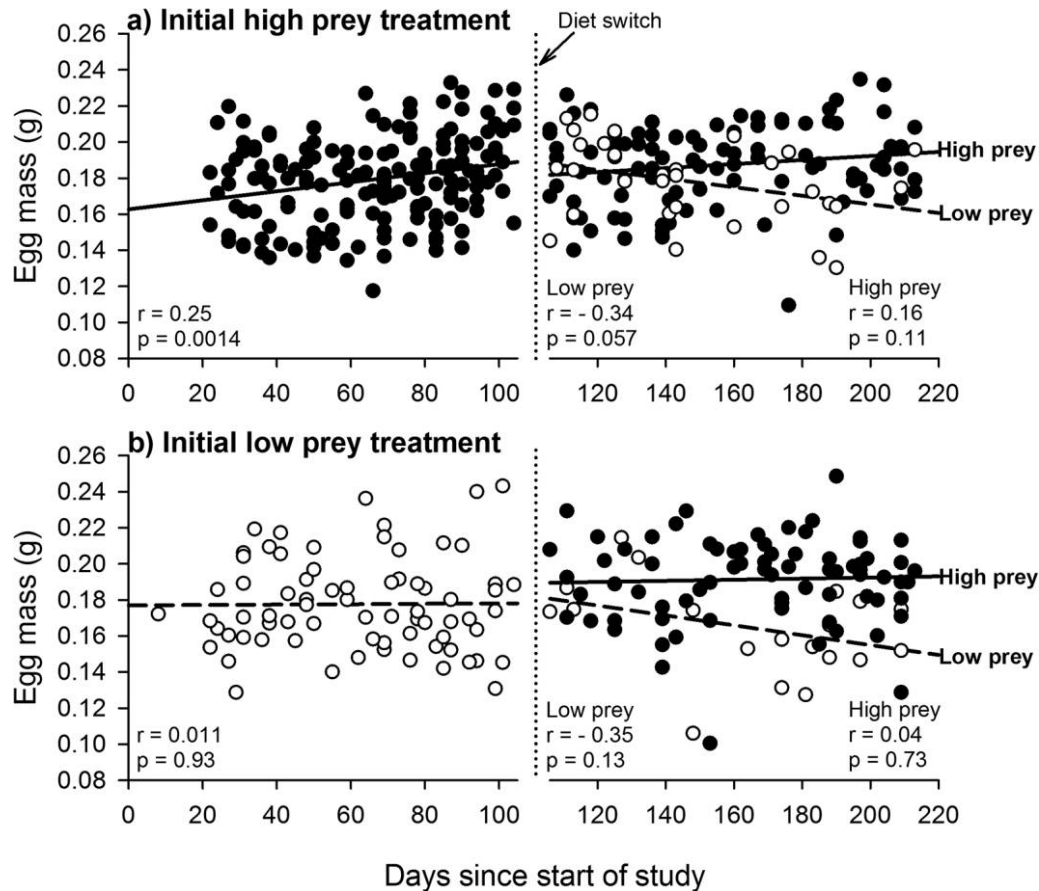
Figure 6. Relationship between egg mass of brown anoles and time across each feeding treatment: females initially provided with high-prey-abundance treatment (*a*) and females initially provided with low prey-abundance treatment (*b*). Closed circles and solid lines represent raw data and regression lines, respectively, for eggs laid by mothers in a high-prey treatment. Open circles and horizontal broken lines show raw data and regression lines for eggs laid by mothers in a low-prey treatment. Vertical dotted lines denote the point in the study when prey treatment was changed (day 105 from the start of the study). Regression lines are for illustration; *r* and *P* values in each panel are for raw values. See table 4 and the text for model estimates and statistics.

similar studies conducted in the field detect seasonal changes in reproduction in some years or populations but not in others (e.g., DeMarco 1989; Sinervo et al. 1992).

When food is continuously abundant, egg production may peak early in the season and egg size increases through time, a pattern expected when selection favors offspring that hatch early over those that hatch late (Brockelman 1975). Indeed, brown anoles that hatch earlier in the season have a survival advantage over those that hatch later (Pearson and Warner 2018); thus, some patterns of reproduction (e.g., relatively high early-season reproductive effort; fig. 5*a*) may be evolved responses that optimize reproductive success (see Mitchell et al. 2018 for further discussion specific to brown anoles). Conversely, when the quality of the maternal diet is poor, these patterns may change, and egg production and/or egg size will peak at suboptimal times for offspring. This interplay between resource availability and seasonal changes in reproduction can have consequences for fitness and population ecology. This is illustrated by comparing the two groups of females that experienced a midseason diet change

(fig. 5*b*, 5*d*). Even if intrinsic factors enable females to generate their greatest reproductive effort early in the season (as suggested by fig. 5*a*), only those capable of accruing adequate resources may do so. If we consider our treatments as resource conditions experienced in the wild, when food is abundant (e.g., fig. 5*a*, 5*c*), effort may peak early, and thus fitness is enhanced because effort aligns with a seasonal deterioration in habitat quality for offspring. However, if food is scarce (e.g., fig. 5*b*, 5*d*), effort may peak later during a time when habitat quality for offspring is poor. Thus, variation in local environmental factors may constrain the evolution of genetically canalized seasonal shifts in reproductive investment.

The quality and composition of eggs may shift seasonally (Mitchell et al. 2018) and be important for offspring survival (Warner and Lovern 2014); however, in our study, these factors were similar between prey treatments and consistent through time. Unfortunately, we do not have egg content and steroid hormone data for eggs laid early in the season because early produced eggs were allocated to another study. For this reason, we cannot

be certain that diet does not influence egg quality early in the season; however, variation in yolk content or quality should correspond with variation in hatchling mass (Warner and Lovern 2014), which was unaffected by diet treatment in our study. Indeed, experimentally removing egg yolk dramatically reduces egg and hatchling survival (Warner and Lovern 2014). Moreover, an increase in exercise and a decrease in calorie consumption results in lower fecundity but not egg size in anoles (Husak et al. 2016). Thus, there is likely some minimum provisioning that must be given to each egg for offspring to be viable. This would explain why low maternal prey availability does not decrease the quality of eggs, but abundant food, rather, allows females to increase fecundity and achieve a seasonal increase in egg size. Of course, some aspects of egg quality may only manifest after hatching (e.g., hatchling survival, hatchling growth). Indeed, survival and growth of hatchling brown anoles in low-prey environments is enhanced when mothers were also in a food-poor environment (Warner et al. 2015). Such thrifty phenotypes may be regulated by measures of egg quality not assessed in our study (e.g., gene expression).

Water content was the only aspect of egg quality that was impacted by prey treatment. Females provided with abundant prey in the early season allocated more water to eggs late in the season, and there was a significant interaction between early prey treatment and time (fig. A1); eggs from females with low food availability increased in water content during the late season, but this trend was not evident in eggs from the high food treatment. This interaction may have no biological significance; however, both water and yolk content contribute to the size and survival of hatchlings (Reedy et al 2012; Warner and Lovern 2014). Each of these positively covaried with egg size in our study; thus, although we did not observe an influence of diet on hatchling size per se, females that produce large eggs, for whatever reason, likely also produce large hatchlings. Thus, our treatments may influence hatchling size via their effect on egg size.

Nussbaum (1981) suggested that egg size may increase toward the end of the reproductive season for one of two reasons. First, females may better provision each late-season offspring, because the quality of the offspring environment is relatively poor during that time (i.e., parental investment). Second, the late-season environment is unpredictable, so females may reduce clutch size to ensure each offspring is minimally provisioned. If, however, excess energy is acquired during this time, it will thus be divided among fewer eggs, making them larger relative to eggs produced earlier in the year (i.e., bet hedging). Support for either hypothesis by the current study is equivocal. Our late-season, high-prey treatment supports the parental investment hypothesis: despite abundant food, females reduced egg production at the end of the experiment and concomitantly increased egg size, potentially investing in quality over quantity. However, parental investment would predict that females obtaining minimal late-season food resources should do the same. Rather, for low-prey treatments, egg production remained stable and egg size declined, supporting the bet-hedging hypothesis. One caveat is that we changed food availability abruptly, and this may not reflect how food resources shift in the wild. A gradual change in prey avail-

ability may be necessary for females to alter their investment among offspring late in the season.

Income and Capital Breeding as a Continuum

Reptiles are thought to rely on capital energy for reproduction because the costs associated with storage and maintenance of energy reserves are relatively low for ectotherms (Bonnet et al. 1998). We know, however, that anoles are opportunistic reproducers: their single-egg clutch size allows them to maximize reproductive output when conditions are favorable (Andrews and Rand 1974). As such, they are probably income breeders, but income and capital breeding describe opposite ends of a continuum, and much recent work demonstrates that many reptiles utilize both capital and income to fuel reproduction (Bonnet et al. 2001; Warner et al. 2008). Our data support the assertion that both income and capital are important for reproduction in anoles, but the relative contribution of these energy sources shifts seasonally.

In many reptiles, fat reserves play a crucial role in initiating vitellogenesis (Price 2017), and this may be true for anoles. How capital and income each contribute to different aspects of reproduction is poorly understood, but we know relationships among capital, income, and reproduction can be complex (Bonnet et al. 2001). Both laboratory and field studies of *Anolis* lizards suggest that capital may be important for aspects of reproduction, though, to our knowledge, no direct test of this hypothesis has been executed. In the laboratory, body condition or body mass often covaries with important reproductive traits (i.e., fecundity, latency to oviposition; Warner and Lovern 2014; Warner et al. 2015; Hall and Warner 2017), and field studies reveal that fat reserves cycle in synchrony with reproduction (Gorman and Licht 1974; Lee et al. 1989). When females were fed a low-prey diet, egg size declined in the late season even for those that were provided with abundant food early on and likely had large fat reserves. Capital energy may play a role in the initiation of anole reproduction, but income is likely the primary determinant of reproduction (egg size, egg production, egg quality) thereafter.

Several food supplementation experiments have been conducted with anoles in the field (Guyer 1988; Wright et al. 2013) and in the laboratory (Lovern and Adams 2008; Warner and Lovern 2014; Warner et al. 2015), and many corroborate the positive relationship between current food abundance and reproductive output. Wright et al (2013) show that pulses in food abundance increase fecundity via increased growth rates, which decrease the time to maturity for females. This influences population ecology by increasing lizard density. Our data suggest that such pulses in food abundance may further impact population dynamics: not only would females mature earlier, but during pulses of increased food abundance, mature females increase egg production and produce larger eggs, which may increase survival probability for offspring.

Seasonality of Reproduction and Scientific Reproducibility

Studies of reproduction, even when conducted in controlled lab environments, must consider seasonal shifts in allocation to off-

spring (Mitchell et al. 2018). If, for example, we remove time and the interaction between time and treatment from our models for egg mass, we are unable to detect any differences between treatments (all P values > 0.22). Furthermore, we observed two shifts in measures of egg quality that were season dependent (different with respect to early vs. late timing). First, the relative size of hatchlings increased with time, but this was only true early in the study. Because we used egg size as a covariate in this analysis, this is an increase in egg quality rather than an increase in hatchling size per se (later-produced hatchlings were more massive with respect to their initial egg mass). Second, the nature of the interaction between egg mass and time was different in the early versus late season. Early, this interaction was driven by an increase in egg size for females on a high-prey diet; however, later, it was driven by a decrease in egg size for females on a low-prey diet. Failing to account for seasonal changes would impact our conclusions about how the environment influences reproduction in our study system; thus, we strongly recommend researchers consider how timing may impact study results or the interpretation of studies from the literature.

Conclusions

In seasonal environments, organisms must align changes in key reproductive traits with shifts in habitat quality to maximize fitness. Such relationships, however, will be modified by other environmental factors that influence reproduction and are, themselves, subject to temporal changes (e.g., food abundance). The evolution of life-history traits and reproductive strategies are likely shaped by changes in local environmental factors. We show that, for some traits, the effect of prey availability on reproduction depends on seasonal timing. This is of critical importance for two reasons. First, it illustrates the importance of conducting manipulative experiments for the full duration of the breeding season because of potential interactions between reproductive traits and time. Such interactions are of interest for reproductive measures that change through the season (e.g., egg size). Some interesting results would have been missed if our experiment had only been conducted for the initial 15 weeks.

Second, we emphasize the challenges involved in achieving scientific reproducibility when studying reproduction. Two researchers may perform the same experiment and observe different results if experiments were conducted at different times in the year, even in a controlled laboratory setting. Thus, researchers must account for seasonal shifts in environmental factors and reproductive traits (and their interactions) when designing experiments and drawing conclusions about how the environment influences reproduction.
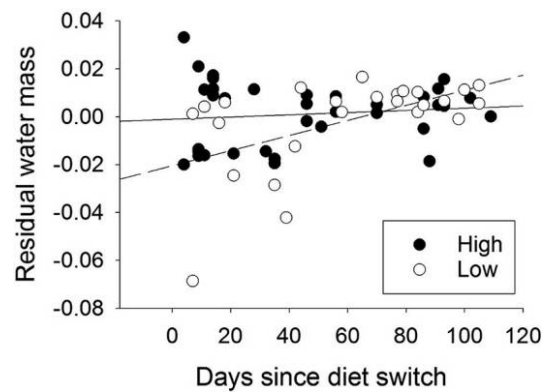
APPENDIX

Figure A1. Relationship between egg water content and time. Values show residuals corrected for egg mass. High- and low-prey treatments refer to the treatment that females received in the early season, though the data shown were collected from eggs laid during the late season. The solid and broken lines show trends for the high and low treatments, respectively.

Table A1: Results from linear and quadratic regressions relating egg production through time

	Linear				Quadratic				
	$\beta \pm SE$	r^2	AICc	P	Linear $\beta \pm SE$	Quadratic $\beta \pm SE$	r^2	AICc	P
Constant high	.0037 ± .052	.018	5.909	.48	**.076 ± .017**	**−.0023 ± .0005**	**.44**	**−8.022**	**.0004**
Constant low	**.0031 ± .0028**	**.043**	**−31.4**	**.27**	.019 ± .011	−.00051 ± .00035	.11	−30.95	.20
High to low	−.0094 ± .0056	.091	10.43	.11	**.057 ± .019**	**−.0021 ± .00061**	**.37**	**1.90**	**.0012**
Low to high	**.013 ± .004**	**.26**	**−8.67**	**.004**	.024 ± .02	−.00034 ± .00053	.28	−6.45	.013

Note. Boldface indicates the model we selected to describe the relationship between egg production and time for each treatment. If adding a quadratic term did not significantly improve model fit based on corrected Akaike information criterion (AICc), then we selected the linear model.

Table A2: Estimates (β [SE]) of effects on aspects of egg size and quality

Effect	Egg mass (g)		Hatchling mass (g)		Egg contents (late season)			Yolk energy and steroids (late season)		
	Early	Late	Early	Late	Water (g)	Yolk (g)	Shell (g)	Calories (kJ/g)	T (pg/mg)	CORT (pg/mg)
Early prey treatment	.0138	.0057	.002	−.00093	−.066	−.0093	−.29	−1.57	.99	.42
	(.0099)	(.017)	(.0014)	(.0018)	(.022)	(.01)	(.25)	(2.3)	(.75)	(1.26)
Late prey treatment	...	.038	...	−.0012	−.028	−.016	−.24	−.92	.41	2.08
		(.022)		(.0027)	(.03)	(.014)	(.38)	(2.9)	(.93)	(1.52)
Early × late treatment interaction	...	−.0000024	...	.0019	.097	−.027	.704	1.55	1.69	−3.53
		(.036)		(.0048)	(.05)	(.024)	(.576)	(7.82)	(1.46)	(2.42)
Time	.00031	.00009	.00025	−.00016	.000015	−.000041	−.0000098	−.0051	.0042	.0016
	(.000068)	(.000063)	(.000085)	(.000094)	(.000084)	(.00004)	(.000012)	(.0091)	(.003)	(.0051)
Time × early treatment	−.00028	−.000012	−.00016	.000072	.00035	.00006	.000022	.0052	−.0063	−.0035
	(.00013)	(.000097)	(.00011)	(.00013)	(.00013)	(.000064)	(.000019)	(.014)	(.0045)	(.0076)
Time × late treatment	...	−.00029	...	.000087	.00018	.000087	.000018	.0078	−.00096	−.016
		(.00013)		(.0002)	(.00021)	(.000096)	(.000029)	(.021)	(.0062)	(.01)
Time × early × late	...	−.000062	...	−.00015	−.00049	.00014	−.000053	−.0046	−.011	.023
		(.00021)		(.00036)	(.00031)	(.00015)	(.000044)	(.045)	(.0093)	(.015)
Maternal SVL	.0022	.0022	...	...	...	...	...	...	...	...
	(.00075)	(.0011)								
Egg mass	...	...	.43	.48	.89	.097	.021	...	...	...
			(.051)	(.087)	(.024)	(.046)	(.014)			

Note. Estimates are high treatment minus low. T values are estimates of transformed data. T = testosterone; CORT = corticosterone; SVL = snout-vent length.

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