

Female investment in offspring size and number shifts seasonally in a lizard with single-egg clutches

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Abstract The timing of reproduction strongly influences reproductive success in many organisms. For species with extended reproductive seasons, the quality of the environment may change throughout the season in ways that impact offspring survival, and, accordingly, aspects of reproductive strategies may shift to maximize fitness. Life-history theory predicts that if offspring environments deteriorate through the season, females should shift from producing more, smaller offspring early in the season to fewer, higher quality offspring later in the season. We leverage multiple iterations of anole breeding colonies, which control for temperature, moisture, and food availability, to identify seasonal changes in reproduction. These breeding colonies varied only by the capture date of the adult animals from the field. We show that seasonal cohorts exhibit variation in key reproductive traits such as inter-clutch interval, egg size and hatchling size consistent with seasonal shifts in reproductive effort. Overall, reproductive effort was highest early in the season due to a relatively high rate of egg production. Later season cohorts produced fewer, but larger offspring. We infer that these results indicate a strategy for differential allocation of resources through the season. Females maximize offspring quantity when environments are favorable, and maximize offspring quality when environments are poor for those offspring. Our study also highlights that subtle differences in methodology (such as capture date of study animals) may influence the interpretation of results. Researchers interested in reproduction must be conscious of how their organism’s reproductive patterns may shift through the season when designing experiments or comparing results across studies.

Keywords Life-history evolution · Seasonality · Parental investment · Tradeoffs · Reproducibility

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Introduction

Animals have a number of adaptations to the predictable environmental changes that occur annually in seasonal environments (McNamara and Houston 2008; Varpe 2017). For example, the timing of reproduction can be a strong predictor of offspring success in seasonal environments. In organisms that reproduce once per year, selection has aligned reproductive events with temporally shifting environmental factors, such as food availability, in ways that optimize fitness (Conover 1992; Post 2003). However, organisms that produce multiple clutches or litters over extended breeding seasons may produce offspring across a wide range of environmental conditions that are not particularly well-aligned with environmental shifts (Tucker et al. 2008). When temporal variation in environmental quality is predictable, life history theory predicts that aspects of reproductive strategies should shift through the season (Lack 1947; Sinervo et al. 1992; Conover 1992; Sinervo and Doughty 1996). Indeed, a wide variety of taxa display seasonal increases or decreases in clutch or brood size in response to seasonal changes in environmental conditions (Nussbaum 1981; Dobson and Myers 1989; Rowe et al. 1994; Williamson and Bull 1995; Singleton et al. 2001; Heins et al. 2004; Du et al. 2014)

In many instances, seasonal changes in offspring size and number reflect changes in environmental factors to which reproductive females are exposed. For example, seasonal variation in clutch and egg size is related to ambient temperature the month prior to egg laying in an Australian frog; bigger eggs and clutches are produced mid-season, when temperatures are warmest (Williamson and Bull 1995). However, the quality of offspring habitat also changes as the season progresses, and accordingly, this should alter the optimal offspring size and number. Late hatched/birthing individuals often have shorter growing seasons and face greater competition from earlier hatched/birthing conspecifics (Verhulst and Nilsson 2008; Harriman et al. 2017), and in such instances, individuals produced later in the season have lower fitness (Varpe et al. 2007, Warner & Shine 2007, Anderson et al. 2010, Uller and Olsson 2010, Munguía-Rosas et al. 2011). Thus, females of many animals may shift from producing more offspring early in the year to fewer, better-provisioned offspring later in the season (Demarco 1989). Is this pattern best explained by strategic shifts in parental investment, such that females are maximizing offspring number when offspring environments are good, and maximizing offspring quality to compensate for later season declines in offspring environment (“parental investment hypothesis”, Nussbaum 1981)? Or does this pattern of decreasing reproductive effort through the season instead reflect seasonal deterioration in maternal environments, such that food-limited females produce fewer offspring (“bet-hedging hypothesis”, Nussbaum 1981)? While numerous field studies have observed such patterns, experimenters must control for seasonal changes in maternal habitat quality to disentangle these alternative hypotheses for seasonal shifts in reproductive allocation. If maternal habitat quality is controlled and seasonal changes in reproductive allocation persist, this would suggest shifts are intrinsic efforts to maximize offspring number in the early season when environments are good and improve offspring survival later in the season when environments are poor (supporting the parental investment hypothesis). However, if reproductive shifts are not apparent under controlled conditions, it would indicate that reproductive shifts observed in the field result from the immediate environmental factors acting on females (Nussbaum 1981).

Lizards are excellent models for studying seasonal shifts in reproductive allocation. Unlike birds and mammals, most lizards provide no parental care to offspring after oviposition, thus, measures of clutch mass vs egg mass can serve as indicators of total reproductive

effort vs effort allocated per offspring, respectively (Warner 2014). Moreover, the timing of hatching is an important determinant of offspring fitness because hatching early allots more time for growth prior to winter and reduces competition with conspecifics (Sinervo et al. 1992; Qualls and Shine 2000; Warner and Shine 2007). As a consequence, early season hatching may enhance survival (Le Henanff et al. 2013) and/or enable lizards to reproduce earlier or at a larger size (Uller and Olsson 2010). Many lizards produce multiple clutches across an extended breeding season and, therefore, can adjust egg size and number per clutch over successive reproductive events as the environment changes.

To test for seasonal shifts in reproductive allocation, we studied a non-native population of *Anolis sagrei* (brown anole) in Florida. Females produce successive, single egg clutches over an extended reproductive season that spans from March to October (Lee et al. 1989). Eggs are yolked and shelled over a ~4–10-day period, thus immediate maternal environmental conditions can have significant impacts on the size and quality of each egg (Warner and Lovern 2014; Warner et al. 2015). Field studies demonstrate that the timing of hatching is a strong predictor of offspring survival: early season individuals have a survival advantage over those that hatch later (Pearson and Warner 2018). Intraspecific competition is particularly important in anoles (Calsbeek and Cox 2010) as they are found in extremely high densities, and the competitive environment, particularly for hatchlings, increases markedly throughout the reproductive season (Warner, unpublished; Schoener and Schoener 1980). Additionally, prior research has shown that egg mass increases with egg order (eggs get larger successively) in *A. sagrei* in the laboratory (Warner and Lovern 2014; Delaney et al. 2016), indicating similar shifts in the field are plausible.

Here we quantify reproduction in three temporally replicated breeding colonies of *A. sagrei* that differ in the dates of capture and reproduction (early-, mid-, late-season) of breeding adults from the field. We tested for differences in key reproductive traits such as egg size, hatchling size, inter-clutch interval and total reproductive effort among these cohorts to make inferences on seasonal shifts in reproduction. Although many studies have carefully monitored seasonal variation in anole reproduction in the field (Licht and Gorman 1970; Gorman and Licht 1974; Lee et al. 1989; Otero et al. 2015), measuring egg size at oviposition and offspring mass at hatching is logistically unfeasible as anoles nest frequently and inconspicuously. Therefore, our study will utilize lab-based breeding assays to provide the first test for seasonal shifts in key reproductive traits in anoles. Given the strong seasonal decline in habitat quality for offspring (Pearson and Warner 2018), we expect females to produce more offspring early in the season, and better provisioned offspring later in the season (i.e. parental investment hypothesis; Nussbaum 1981). If we do not detect seasonal shifts in reproduction, it would indicate that shifts either do not occur in the field, or that shifts in the field are driven by immediate environmental factors such as food availability (Nussbaum 1981; Warner et al. 2015). However, if we detect seasonal shifts in reproductive patterns, it would indicate such shifts are at least partially intrinsic and support the parental investment hypothesis, given that we control the short-term environmental differences between seasonal cohorts.

Materials and methods

Study system

Anoles are a speciose group (~400 sp) that inhabit a diverse array of niches and represent one of the largest vertebrate adaptive radiations. Nearly all species that have been studied exhibit some capacity for seasonal reproduction such that egg production peaks during the warm or wet season and subsides during the cool or dry season (examples, Gorman and Licht 1974; Crews 1977; Lee et al. 1989; Lister and Aguayo 1992). Importantly, their invariant clutch size of one egg and their frequent oviposition across the season is important for two reasons. First, the production of successive single-egg clutches allows females the opportunity to allocate resources to each egg independently of other eggs. This is useful for quantifying how maternal reproductive effort impacts fitness of individual offspring (Warner 2014). Conversely, for species that produce multi-egg clutches, the trade-off between egg size and number limits how females allocate resources toward individual offspring independent of clutch mates. Second, successive single-egg clutches potentially allow for fine-scale adjustments to the allocation of yolk quantity and content to each egg across a broad reproductive season. As local conditions (e.g. temperature, food availability, competition) gradually change through the season, key reproductive traits can continuously shift as well. Thus, we suggest that anoles are poised to serve as a model system for studying how predictable seasonal fluctuations in environmental quality drive the evolution of life history traits.

The brown anole (*A. sagrei*) is native to Cuba and the Bahamas but is naturalized in Florida. The annual routine of brown anoles in Florida includes a prolonged reproductive season from March to October. Outside this time virtually no reproduction occurs; however, during the winter months, lizards remain active and feed, building up fat reserves that are subsequently depleted across the following breeding season (see Lee et al. 1989 for description of *A. sagrei* reproduction in Florida). Anoles are primarily income breeders, but, like other reptiles, they may utilize fat reserves at the beginning of the breeding season to begin yolking follicles (Bonnet et al. 2001; Price 2017): in lab experiments, body condition or body mass often covary with important reproductive traits (i.e., fecundity, latency to oviposition, Warner and Lovern 2014; Warner et al. 2015) and field studies demonstrate that fat reserves cycle with seasonal reproduction (Licht and Gorman 1970; Lee et al. 1989).

Anolis sagrei lay a single egg clutch approximately once every 4–10 days. Both lab and field studies suggest that, when food is abundant, females will lay eggs for the entirety of the breeding season (March–October) (Lee et al. 1989; Warner et al. 2015). Likewise, we have conducted several studies in the lab which involved breeding females for the entire breeding season (30–40 weeks) and all females continuously laid eggs during this time. In the field, most lizards hatch sometime between May and October; however, in our population, late-hatching individuals have lower survival than earlier-hatching individuals (Pearson and Warner 2018) likely because of increased competition at the end of the breeding season. Age at maturity varies since maturity is based on body size (~34 mm for females; Lee et al. 1989); but, we estimate the females in our population often reach maturity within 3–6 months after hatching. Growth is indeterminate but slows once maturity is reached; however, females experience stabilizing selection on body size since a larger size enhances survival and a small size at maturity increases fecundity (Cox and Calsbeek 2010). Larger body size at hatching confers a survival advantage in the lab (Warner and Lovern 2014)

and field (Reedy unpublished, Warner unpublished); however, timing of hatching appears to be a stronger predictor of survival in the field (Pearson and Warner 2018). Once mature, females rarely live more than 2 years (Schoener and Schoener 1982), so most of the lifetime reproductive effort comes in the first (often the only) reproductive season.

Laboratory methods

We captured *Anolis sagrei* for three temporally-replicated breeding colonies by noose or by hand near Palm Coast, FL USA. We targeted both males and females that appeared to be sexually mature and sought to capture twice as many females as males. Given that our field proxy for sexual maturity is size, we targeted individuals that we perceived as large enough to be reproductively mature (females, SVL > 33 mm; males, SVL > 38; Lee et al. 1989), though we did not measure until they were placed in the breeding colonies. We captured lizards on 21–22 Feb-2015 (cohort 1; n=40 females), 16–17 Jun-2015 (cohort 2; n=95 females), and 3–4 Sep-2015 (cohort 3; n=84 females). Because our goal was to obtain reproductively mature individuals, we biased our capture efforts towards larger females during all three collecting trips. Thus, our size measurements do not reflect average female size in the field during those time periods, but rather suggest that the availability of larger females in the population increases through the season (likely due to growth through the season).

Lizards were transported to a temperature-controlled room at the University of Alabama at Birmingham where we weighed, measured snout-vent length (SVL), and marked lizards with toe clips. We housed 1 female per cage in plastic cages (29×26×39 cm; H×W×D) outfitted with two bamboo perches, plastic vegetation, reptile cage carpet (Zoo Med Inc.), and a nesting pot filled with a moistened soil/peat moss mixture. Cages were illuminated with a Reptisun 5.0 UVB bulb and a Tropisun bulb (Zoo Med Inc) with a 12:12 h light/dark cycle. Room temperatures were maintained at 28–29 °C. We had half as many males as females, and males were rotated between female cages approximately every other week to ensure all females were mated (females can store sperm for months; Calsbeek et al. 2007). Twice per week, we fed lizards 3 crickets each, dusted with vitamin and calcium powder.

Adult lizards were in cages from 23-Feb-2015–27-Apr-2015 (cohort 1), 18-Jun-2015–30-Jul-2015 (cohort 2), and 5-Sep-2015–15-Oct-2015 (cohort 3). The first cohort was captured in February, prior to the onset of reproduction (Lee et al. 1989). Thus, the eggs laid by those females certainly represent the first eggs of the season. These lizards were held in captivity for substantially longer than the lizards in the latter two cohorts to reach our target egg sample size for the field experiment. Observations from dissections of females captured near our field site (~28 km away, Tomoka State Park, FL) suggest that egg production ceases at our field site in early-mid October (30-Sep-2014, 11 of 13 dissected females gravid; 1-Oct-2014, 12 of 18 gravid; 10-Oct-2014, 0 of 17 gravid), such that the third cohort represents the tail end of the breeding season. Because females were field caught, we cannot control for environmental factors acting prior to capture. Photoperiod and temperature are important for programming certain traits, like circannual rhythm (Lee et al. 1989; Bertolucci et al. 1999). However, increases in food availability rapidly boost reproduction in anoles (Guyer 1988; Wright et al. 2013; Warner and Lovern 2014; Warner et al. 2015) and their short inter-clutch interval allows them to be especially responsive to rapidly changing environmental conditions (Andrews and Rand 1974). Thus, we believe any field effects on reproduction were minimal compared to those induced by lab conditions.

Twice per week we checked the nesting pots for eggs. Upon collection, we weighed each egg and transferred it to a 59 ml glass jar with moistened vermiculite (-150 kPa), which we covered in plastic cling wrap secured with a rubber band to prevent desiccation. Eggs were incubated in programmable incubators (Memmert IPP 400) at temperatures that represent the typical diel fluctuation experienced at nest sites near the study area. The thermal profile had a mean of 27.73 °C, and fluctuated from a low of 25.5 °C at 0600 to a high of 32.1 °C at 1300. This regime was based on data collected from August and October 2013 from Thermocron iButtons placed adjacent to *A. sagrei* eggs in the field at a location near our study site (Ormond Beach, FL; Hulbert et al. 2017). Eggs were checked daily for hatchlings. Upon hatching, SVL, tail length, and hatchling mass were recorded.

Statistical analysis

Initial female size and latency to first egg: We tested whether females from the three cohorts differed in SVL, mass, body condition and latency to first egg with separate ANOVAs using cohort as the explanatory variable. Latency was the date animals were brought into captivity subtracted from the date the first egg was laid. Female body condition was calculated from a regression of log transformed body mass on log transformed SVL of all females pooled (Warner et al. 2016). Thus, positive residual values represent females that were relatively heavy for their length. We made pairwise comparisons between cohorts using Tukey–Kramer post hoc tests.

Fecundity and reproductive effort: Females from the three cohorts were not given the same amount of time to breed. The females from the first cohort did not produce eggs immediately (i.e. had larger latency to first egg) because we captured them in the field prior to the onset of ovulation, and were consequently held in captivity longer than the later cohorts (which began reproducing soon after being brought to the lab). Therefore, we calculated a reproductive span to utilize as a covariate in our analyses. We calculated the reproductive span for each individual as the difference (in days) between the final egg collection date for that cohort and the date each female laid her first egg in captivity. We calculated reproductive effort during the captive period as the sum of the egg masses of all the eggs laid by an individual female, whereas fecundity was the total number of eggs. Given that these two response variables are not independent, we ran a MANOVA, which was significant (Wilk's Lambda $F_{4,392} = 20.43$; $P < 0.0001$). Therefore, to assess differences in fecundity and reproductive effort between cohorts, we performed separate ANCOVAs with reproductive span as the covariate and cohort as the categorical variable. We made pairwise comparisons between cohorts using Tukey–Kramer post hoc tests.

Egg mass, hatchling mass, and inter-clutch interval: To assess differences in egg mass and inter-clutch interval (number of days between successive eggs) between cohorts, we performed separate mixed model ANCOVAs with either egg mass or inter-clutch interval as the response variable, cohort as the explanatory variable, initial female SVL as a covariate, and female nested within cohort as a random factor. We also performed an ANCOVA with hatchling size as the response variable, cohort as the explanatory variable, egg mass as a covariate and female nested within cohort as a random factor. This analysis will provide an egg-size controlled proxy for egg quality (hatchlings that are relatively large for their given egg size may indicate higher quality yolk provisions). We made pairwise comparisons between cohorts using Tukey–Kramer post hoc tests.

Regressions within cohorts: We tested whether egg mass, inter-clutch interval, and hatchling mass shifted through time within each cohort. For each of these three response

variables, we ran separate mixed models for each cohort with oviposition date as the predictor variable and maternal ID as the random factor. For our egg mass analysis, we used maternal SVL as a covariate, and for our hatchling mass analysis we used egg mass as a covariate.

Several methodological inconsistencies arose among our cohorts that require brief discussion. During the first cohort, we inadvertently used improperly moistened vermiculite during one day of egg checking that resulted in 34 eggs being incubated in vermiculite that was too dry until the mistake was noticed. During the second cohort, one incubator malfunctioned for several days, resulting in the culling of 94 eggs from the experiment prior to hatching. These latter two issues resulted in the removal of these eggs from the analyses of hatchling traits, though these eggs were included for the maternal reproductive traits (i.e., egg size, and inter-clutch interval which are unaffected by the methodological inconsistencies). Several eggs partially desiccated prior to incubation, and these were removed from analyses of egg mass or subsequent hatchling analyses.

Results

The first cohort of 40 females produced 188 eggs, the second cohort of 95 females produced 507 eggs and the third cohort of 84 females produced 319 eggs. Some females from each cohort never reproduced (cohort 1, $n=7$; cohort 2, $n=3$, cohort 3, $n=9$), but were still included in the analyses. Female SVL increased with each successive cohort (Table 1). Initial female SVL ranged from 34–50 mm in cohort 1, 36–51 mm in cohort 2, and 41–51 mm in cohort 3. Females in cohort 1 were less massive than those from cohorts 2 and 3, while female mass from cohorts 2 and 3 did not differ (Table 1). All three cohorts of females differed in body condition, with cohort 2 having the highest body condition and cohort 1 having the lowest (Table 1).

Females in the first cohort were in captivity for ~9 weeks, and those from the latter two cohorts were in captivity for ~6 weeks, and there were differences in reproductive span. For example, females from the first cohort spent ~5.5 weeks in captivity prior to laying their

Table 1 Initial body size and reproductive traits of female *Anolis sagrei* from three temporally replicated breeding colonies. Least-square means \pm SE estimated from the statistical models are reported

Trait	Cohort		
	1	2	3
Initial female SVL (mm)	40.12 \pm 0.43 (A)	44.78 \pm 0.28 (B)	46.23 \pm 0.29 (C)
Initial female mass (g)	1.43 \pm 0.06 (A)	2.38 \pm 0.04 (B)	2.41 \pm 0.04 (B)
Initial female condition	-0.11 \pm 0.017 (A)	0.065 \pm 0.011 (B)	-0.021 \pm 0.012 (C)
Latency to first egg (d)	37.32 \pm 1.37 (A)	6.92 \pm 0.65 (B)	12.27 \pm 0.79 (C)
Fecundity (eggs)	7.22 \pm 0.36 (A)	4.67 \pm 0.21 (B)	4.55 \pm 0.22 (B)
Reproductive effort (g)	1.22 \pm 0.06 (A)	0.77 \pm 0.04 (B)	0.83 \pm 0.39 (B)
Inter-clutch interval (d)	4.40 \pm 0.35 (A)	6.80 \pm 0.22 (B)	6.81 \pm 0.28 (B)
Egg mass (g)	0.177 \pm 0.0031 (A)	0.167 \pm 0.0014 (B)	0.179 \pm 0.0019 (A)
Hatchling mass (g)	0.1642 \pm 0.001 (A)	0.167 \pm 0.001 (B)	0.184 \pm 0.001 (C)

Letters in parentheses denote statistically significant ($P < 0.05$) differences from Tukey–Kramer pairwise comparisons

first egg (i.e., ~3 weeks reproducing), whereas the second and third cohort spent about 1 and 2 weeks prior to laying (5 and 4 weeks reproducing), respectively (latency to first egg; Table 1). The reproductive spans (mean \pm SD) of the three cohorts were 22.7 ± 8.3 days for cohort 1, 35.3 ± 4.7 days for cohort 2, and 28.9 ± 7.4 days for cohort 3. After correcting for reproductive span, the females in the first cohort had ~50% greater fecundity and reproductive effort than the latter two cohorts (Fig. 1; Table 1). Accordingly, the inter-clutch interval was about 35% shorter for the first cohort compared to the latter two (Table 1). Thus, early season females had relatively higher effort than later cohorts due to a more rapid rate of egg production.

Because females in the first cohort were comparatively small, egg size did not differ between the first and third cohort when correcting for SVL, while the second cohort produced smaller eggs (Table 1). The unadjusted (raw) mean egg size (\pm SE) was 0.166 g (± 0.002) for cohort 1, 0.168 g (± 0.001) for cohort 2, and 0.183 g (± 0.001) for cohort 3. Hatchling mass increased through the season, suggesting increases in egg quality: even after correcting for egg size, hatchling mass became progressively larger from the first to third cohort (Fig. 1; Table 1). Thus, as the season progressed, hatchlings increased in mass relative to their egg mass by ~12%. Results for these statistical tests are reported in Table 2.

In addition to the seasonal trends among cohorts, we tested for shifts in reproductive traits within each cohort. Inter-clutch interval did not change within cohort 1 but increased by ~3.3 and 3.94 days from the first to last egg for cohort 2 and 3, respectively. Egg mass increased through time by ~8.9% for cohort 1 and ~6.3% for cohort 2 (as a percentage of raw mean egg mass for each cohort) but did not change within cohort 3. Similarly, hatchling mass increased within cohort 1 and 2 by magnitudes like those for egg mass. Cohort 3 exhibited no substantial change (Table 3).

Discussion

The optimal balance between offspring quality and number can shift as environments change (Lack 1947), particularly in organisms with long reproductive periods that extend across seasons. Thus, optimal reproductive strategies change seasonally because of shifting maternal and/or offspring environments (Nussbaum 1981). By using replicated laboratory-breeding colonies of field caught *Anolis* lizards, we tested whether reproductive patterns shifted among cohorts while controlling for maternal environmental factors. Specifically, our design enabled us to test whether seasonal reproductive investment is mostly driven by intrinsic factors (as predicted by the parental investment hypothesis) or extrinsic factors (as predicted by the bet-hedging hypothesis). Our results showed that females have high reproductive effort early in the season by producing many small hatchlings when environmental quality for those hatchlings is likely best. By mid-season, females produced fewer, slightly larger hatchlings and by late season, hatchling size increases substantially while egg-laying rate remains low. This demonstrates that female anole reproductive patterns shift seasonally in ways generally predicted by life-history theory, and that these shifts are not wholly due to short-term environmental effects on females, but likely due to a strategy that optimizes fitness (e.g., parental investment hypothesis).

The differences in cohort latency times are likely due to seasonal differences in time of capture. Early-season lizards were caught prior to the onset of natural reproduction in the field, whereas the later season cohorts were both captured during the reproductive season. Therefore, the long latency time observed for early-season lizards reflects the time

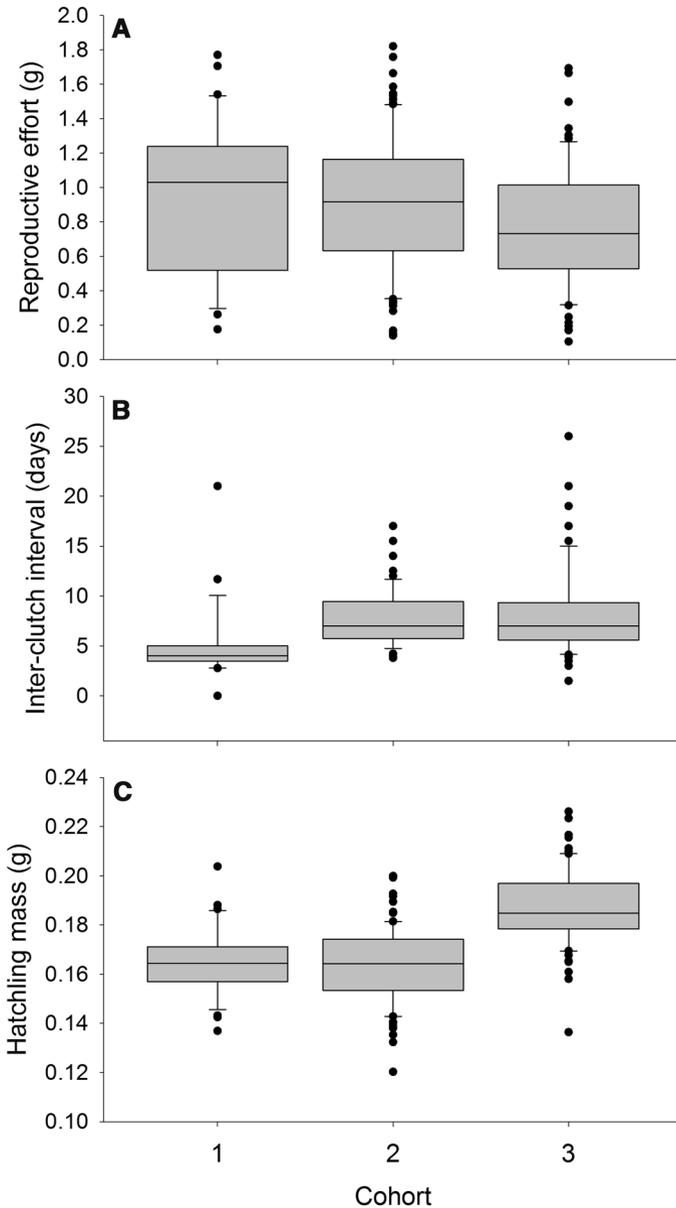


Fig. 1 Differences in key reproductive traits between seasonal cohorts of captive-bred *A. sagrei*. Boxplots show raw data, the center horizontal bars are the median, upper and lower bounds of the box are the 75th and 25th percentile, respectively. Whiskers show the 10th and 90th percentile and closed circles are data points more extreme than the 10th and 90th percentile

associated with physiologically preparing for reproduction (~4-10 days to yolk and shell an egg). Given the initial smaller sizes of early-season lizards (range 34-50 mm), it is possible that some lizards were not reproductively mature as they were captured, and therefore the

Table 2 Statistics from mixed model ANOVAs comparing differences between cohorts

Trait	Cohort main effect		Covariate	Covariate main effect	
	Statistic	<i>P</i> value		Statistic	<i>P</i> value
SVL	$F_{2,218} = 70.84$	$P < 0.001$	–	–	–
Mass	$F_{2,212} = 97.66$	$P < 0.001$	–	–	–
Condition	$F_{2,212} = 39.40$	$P < 0.001$	–	–	–
Latency	$F_{2,199} = 195.52$	$P < 0.001$	Female SVL	$F_{2,199} = 12.38$	$P < 0.001$
Fecundity	$F_{2,200} = 22.40$	$P < 0.001$	Reprod. span	$F_{1,200} = 86.84$	$P < 0.001$
Reproductive effort	$F_{2,200} = 18.26$	$P < 0.001$	Reprod. span	$F_{1,200} = 90.64$	$P < 0.001$
Inter-clutch interval*	$F_{2,189} = 18.94$	$P < 0.001$	–	–	–
Egg mass*	$F_{2,196} = 14.90$	$P < 0.001$	Female SVL	$F_{1,771} = 34.08$	$P < 0.001$
Hatch mass*	$F_{2,193} = 34.83$	$P < 0.001$	Egg mass	$F_{1,595} = 35.07$	$P < 0.001$

Analyses with an asterisk (*) denote analyses where female ID was nested within cohort as a random effect

long latency times observed may partially reflect additional time needed for some individuals to reach sexual maturity. Moreover, even after the onset of reproduction, smaller females tend to allocate more energy toward growth than to reproduction compared to larger females (Andrews and Rand 1974).

Early season females had high total reproductive effort, which was largely driven by the production of more, rather than larger, eggs (~2.6 more eggs per female; Fig. 1, Table 1). Moreover, egg size relative to female body size was slightly larger (~6%) for early-season females than those from the mid-season, and comparable to those produced in the late season. Body size at hatching, however, was smallest in the early season and grew progressively larger with each cohort (Table 1). While it seems initially puzzling that the early season females produced relatively large eggs but small hatchlings, this result is due to the use of different covariates between the two analyses (the egg mass analysis controlled for maternal SVL, while the hatchling body mass analysis used egg mass as a covariate). Why wouldn't these early season females produce smaller eggs if it allowed them to produce even more eggs? There are two possible constraints that could explain this discrepancy from our prediction. First, it is possible that these early season females were producing eggs at their maximum rate. Female anoles produce a single egg alternately from their left and right ovaries (Smith et al. 1972), and it is possible that physiological constraints prevent them from decreasing the inter-clutch interval even further (Smith et al. 1972; Crews 1977). Second, there is likely a minimum size threshold for eggs to be viable. Warner and Lovern (2014) showed a reduction in egg survival, hatchling quality, and hatchling survival after a manual 22% reduction in yolk quantity, supporting this supposition. Thus, these early season lizards are likely producing the maximum amount of viable eggs, as we predicted. Though the eggs are relatively large for the animal's body size, the unadjusted mean egg size of early season lizards is the smallest of the three cohorts. By later season, the inter-clutch interval lengthens, and unadjusted egg size and hatchling size increases, which matches predictions.

Both mid- and late-season females produced eggs at a similar rate, which was a ~35% increase in inter-clutch interval than observed during the early-season. Moreover, because our hatchling size analysis used egg size as a covariate, the larger hatchling size apparent in the late-season cohort represents an increase in egg quality while controlling for egg

Table 3 Statistics from regressions of key reproductive traits through time within each cohort (oviposition date is predictor variable)

Trait	Cohort					
	1	2	3			
	$\beta \pm SE$	$\beta \pm SE$	$\beta \pm SE$			
	F;p	F;p	F;p			
Inter-clutch Interval (days)	0.033 ± 0.034	0.093 ± 0.020	0.141 ± 0.027	$F_{1,133} = 1.00; P = 0.318$	$F_{1,330} = 21.85; P < 0.0001$	$F_{1,176} = 27.41; P < 0.0001$
Egg Mass (g)	0.0007 ± 0.0001	0.0003 ± 0.0001	0.0002 ± 0.0001	$F_{1,148} = 24.04; P < 0.0001$	$F_{1,393} = 17.42; P < 0.0001$	$F_{1,227} = 3.50; P = 0.062$
Hatch Mass (g)	0.0007 ± 0.0001	0.0004 ± 0.00005	0.00002 ± 0.00007	$F_{1,85} = 30.05; P < 0.0001$	$F_{1,298} = 46.68; P < 0.0001$	$F_{1,1214} = 0.1; P = 0.74$

The egg mass analysis used maternal SVL as a covariate, and the hatching mass analysis used egg mass as a covariate

size. Thus, the late season females produced the largest hatchlings by producing larger and higher quality eggs. The increase in egg quality is plausibly due to changes in the composition or relative quantity of yolk. For example, laboratory research on *A. sagrei* demonstrates that concentrations of yolk testosterone within a given female progressively increase with subsequent eggs, and reducing egg yolk quantity substantially reduces hatchling mass (Warner and Lovern 2014). Though we use adjusted hatchling mass as a proxy for quality, size is not the only metric for offspring quality and it is possible that other unmeasured quality traits differed as well (e.g. immunity). Shifts in other aspects of yolk quality (e.g. nutrient content) are not well studied in anoles.

The patterns we observed within cohorts are in accord with those we observed between cohorts. The slopes for interclutch interval over time get progressively larger with each cohort while those for egg/hatchling mass over time grow progressively smaller (Table 3); thus, the rate of egg production (i.e. investment in offspring number) continually slows down through the season while the investment per individual offspring grows toward some asymptote. Our collective results show that seasonal patterns of reproductive effort generally support the parental investment hypothesis predicted by life history theory (Nussbaum 1981; Sinervo et al. 1992). When offspring environmental quality is high in the early season, mothers reproduce rapidly, but produce fewer, larger offspring later in the season. By controlling for maternal environments, we show this pattern is likely not due only to shifting maternal environmental conditions (i.e., bet hedging hypothesis).

Given that we controlled the immediate maternal environment, yet still detected seasonal shifts in reproduction, it is likely that short-term maternal environments (i.e. moisture, temperature, food) did not drive the patterns we see. We recognize, however, the exceptionally important role prior female environmental conditions can have on animal reproduction (Williamson and Bull 1995; Warner et al. 2015; Mitchell et al. 2017). Additionally, we acknowledge that the females in our study experienced environmental differences prior to capture when they were in the field. Therefore, traits like females' circannual rhythm were possibly set by photoperiod and/or temperature prior to capture (Lee et al. 1989; Bertolucci et al. 1999). Though tracking individual anole reproduction in the field is challenging, our design produces an excellent proxy for how we expect reproduction to occur in the field. Therefore, we are confident that the seasonal patterns we report here are commensurate with those that could be observed from an ideal field study of reproduction. Though we speculate that the shifts we observe are innate, our data cannot confirm or refute that observation. We are currently undertaking complementary experiments that house females prior to and across the entire reproductive season under controlled conditions, which will allow us to test for similar patterns within individual females throughout the season, and explicitly test whether these shifts are intrinsic.

This experiment enhances our understanding of life-history theory and the tradeoff between offspring size and number in anoles, but it does not provide a complete picture. If we were able to non-invasively track reproduction in the field, there are many other factors that could influence these reproductive patterns. For example, abiotic conditions, prey availability, mortality rates, photoperiod, tradeoffs between growth and reproduction, and competition with conspecifics are all important factors that do or may shift seasonally and that can influence patterns of reproduction (Brown and Sexton 1973; Rose 1982; Lister and Aguayo 1992). Future studies can expand upon ours to provide a more complete picture of how seasonal variation drives life-history evolution. For example, longitudinal studies that track individual growth and egg production throughout the entire reproductive season will further our understanding of seasonal shifts in reproduction, while also examining tradeoffs with growth. Additionally, field studies that quantify the influences of hatching date

on offspring growth rates, survival, (Qualls and Shine 2000; Warner and Shine 2007; Pearson and Warner 2018), and patterns of reproduction are warranted. These types of studies are currently underway and should advance our understanding of how seasonality influences life-history evolution of anoles.

Our results can also provide a useful lesson on the reproducibility of scientific results for those looking to repeat experiments or draw inferences between different published studies. Empirical studies in ecology and evolution are challenging to replicate, particularly when there is a field component. Spatial and temporal variation in diverse environmental factors can alter numerous variables of interest, and therefore biologists must be conscious of how such variation may impact their own study (Warner et al. 2010). For example, Demarco (1989) found that seasonal shifts in key reproductive traits were observable in some years, but not others and this difference was associated with important environmental factors. Within our study, the capture date of our breeding colonies significantly influenced their initial size and reproductive allocation. While we were explicitly testing for seasonal shifts in reproduction, seasonal variation in reproductive patterns could be an easy factor to overlook if an experiment is designed for other purposes, or if one is trying to draw inferences from multiple experiments (e.g. meta-analysis).

Identifying the evolutionary pressures that optimize offspring size-number relationships is a major goal in evolutionary biology (Smith and Fretwell 1974). Environmental shifts across the season have important consequences for maternal reproductive investment and offspring fitness, and are critical in life-history models (McNamara and Houston 2008; Varpe 2017). Organisms with extended reproductive seasons and invariant clutch sizes (e.g., anoles) offer unique opportunities for evaluating the role of environmental seasonality in driving life-history evolution.

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References

- Anderson JH, Faulds PL, Atlas WI et al (2010) Selection on breeding date and body size in colonizing coho salmon, *Oncorhynchus kisutch*. *Mol Ecol* 19:2562–2573. <https://doi.org/10.1111/j.1365-294X.2010.04652.x>
- Andrews R, Rand AS (1974) Reproductive effort in Anoline lizards. *Ecology* 55:1317–1327. <https://doi.org/10.2307/1935459>
- Bertolucci C, Leorati M, Innocenti A, Foà A (1999) Circannual variations of lizard circadian activity rhythms in constant darkness. *Behav Ecol Sociobiol* 46:200–209
- Bonnet X, Naulleau G, Shine R, Lourdais O (2001) Short-term versus long-term effects of food intake on reproductive output in a viviparous snake, *Vipera aspis*. *Oikos* 92:297–308. <https://doi.org/10.1034/j.1600-0706.2001.920212.x>
- Brown K, Sexton O (1973) Stimulation of reproductive activity of female *Anolis sagrei* by moisture. *Physiol Zool* 46:168–172
- Calsbeek R, Cox RM (2010) Experimentally assessing the relative importance of predation and competition as agents of selection. *Nature* 465:613–616. <https://doi.org/10.1038/nature09020>
- Calsbeek R, Bonneaud C, Prabhu S et al (2007) Multiple paternity and sperm storage lead to increased genetic diversity in *Anolis* lizards. *Evol Ecol Res* 9:495–503
- Conover DO (1992) Seasonality and the scheduling of life history at different latitudes. *J Fish Biol* 41:161–178. <https://doi.org/10.1111/j.1095-8649.1992.tb03876.x>
- Cox RM, Calsbeek R (2010) Sex-specific selection and intraspecific variation in sexual size dimorphism. *Evolution* 64:798–809. <https://doi.org/10.1111/j.1558-5646.2009.00851.x>

- Crews D (1977) The annotated anole: studies on control of lizard reproduction: anoline lizards make excellent experimental animals for analyzing reproduction at the physiological, behavioral, and species levels. *Am Sci* 65:428–434
- Delaney DM, Lovern MB, Warner DA (2016) Does reduced perch availability affect reproduction in the brown anole? An experimental test in the laboratory. *J Herpetol* 50:227–232. <https://doi.org/10.1670/14-147>
- Demarco VG (1989) Annual variation in the seasonal shift in egg size and clutch size in *Sceloporus woodi*. *Oecologia* 80:525–532
- Dobson FS, Myers P (1989) The seasonal decline in the litter size of meadow voles. *J Mammal* 70:142–152. <https://doi.org/10.2307/1381677>
- Du W, Robbins TR, Warner DA et al (2014) Latitudinal and seasonal variation in reproductive effort of the eastern fence lizard (*Sceloporus undulatus*). *Integr Zool* 9:360–371. <https://doi.org/10.1111/1749-4877.12072>
- Gorman GC, Licht P (1974) Seasonality in ovarian cycles among tropical *Anolis* lizards. *Ecology* 55:360–369. <https://doi.org/10.2307/1935223>
- Guyer C (1988) Food supplementation in a tropical mainland anole, *Norops humilis*: effects on individuals. *Ecology* 69:362–369. <https://doi.org/10.2307/1940434>
- Harriman VB, Dawson RD, Bortolotti LE, Clark RG (2017) Seasonal patterns in reproductive success of temperate-breeding birds: experimental tests of the date and quality hypotheses. *Ecol Evol* 7:2122–2132. <https://doi.org/10.1002/ece3.2815>
- Heins DC, Baker JA, Guill JM (2004) Seasonal and interannual components of intrapopulation variation in clutch size and egg size of a darter. *Ecol Freshw Fish* 13:258–265. <https://doi.org/10.1111/j.1600-0633.2004.00064.x>
- Hulbert AC, Mitchell TS, Hall JM et al (2017) The effects of incubation temperature and experimental design on heart rates of lizard embryos. *J Exp Zool Part A Ecol Integr Physiol* 327:466–476. <https://doi.org/10.1002/jez.2135>
- Lack D (1947) The significance of clutch-size. *Ibis* 89:302–352
- Le Henanff M, Meylan S, Lourdaïs O (2013) The sooner the better: reproductive phenology drives ontogenetic trajectories in a temperate squamate (*Podarcis muralis*). *Biol J Linn Soc* 108:384–395. <https://doi.org/10.1111/j.1095-8312.2012.02005.x>
- Lee JC, Clayton D, Eisenstein S, Perez I (1989) The reproductive cycle of *Anolis sagrei* in southern Florida. *Copeia* 1989:930–937. <https://doi.org/10.2307/1445979>
- Licht P, Gorman GC (1970) Reproductive and fat cycles in Caribbean *Anolis* lizards. *Univ Calif Publ Zool* 95:1–52
- Lister BC, Aguayo AG (1992) Seasonality, predation, and the behaviour of a tropical mainland anole. *J Anim Ecol* 61:717–733. <https://doi.org/10.2307/5626>
- McNamara JM, Houston AI (2008) Optimal annual routines: behaviour in the context of physiology and ecology. *Philos Trans R Soc B Biol Sci* 363:301–319. <https://doi.org/10.1098/rstb.2007.2141>
- Mitchell TS, Refsnider JM, Sethuraman A et al (2017) Experimental assessment of winter conditions on turtle nesting behaviour. *Evol Ecol Res* 18:271–280
- Munguía-Rosas MA, Ollerton J, Parra-Tabla V, De-Nova JA (2011) Meta-analysis of phenotypic selection on flowering phenology suggests that early flowering plants are favoured. *Ecol Lett* 14:511–521. <https://doi.org/10.1111/j.1461-0248.2011.01601.x>
- Nussbaum RA (1981) Seasonal shifts in clutch size and egg size in the side-blotched lizard, *Uta stansburiana*. *Oecologia* 49:8–13
- Otero LM, Huey RB, Gorman GC (2015) A few meters matter: local habitats drive reproductive cycles in a tropical lizard. *Am Nat* 186:E72–E80. <https://doi.org/10.1086/682359>
- Pearson PR, Warner DA (2018) Early hatching enhances survival despite beneficial phenotypic effects of late-season developmental environments. *Proc R Soc B Biol Sci* 285:20180256. <https://doi.org/10.1098/rspb.2018.0256>
- Post E (2003) Timing of reproduction in large mammals. In: Schwartz MD (ed) *Phenology: an integrative environmental science*. Springer, Dordrecht, pp 437–449
- Price ER (2017) The physiology of lipid storage and use in reptiles. *Biol Rev* 92:1406–1426. <https://doi.org/10.1111/brv.12288>
- Qualls F, Shine R (2000) Post-hatching environment contributes greatly to phenotypic variation between two populations of the Australian garden skink, *Lampropholis guichenoti*. *Biol J Linn Soc* 71:315–341. <https://doi.org/10.1006/bjpl.2000.0445>
- Rose B (1982) Food intake and reproduction in *Anolis acutus*. *Copeia* 1982:322. <https://doi.org/10.2307/1444610>

- Rowe L, Ludwig D, Schluter D (1994) Time, condition, and the seasonal decline of avian clutch size. *Am Nat* 143:698–722
- Schoener TW, Schoener A (1980) Densities, sex ratios, and population structure in four species of Bahamian *Anolis* lizards. *J Anim Ecol* 49:19–53. <https://doi.org/10.2307/4276>
- Schoener TW, Schoener A (1982) The ecological correlates of survival in some Bahamian *Anolis* lizards. *Oikos* 39:1. <https://doi.org/10.2307/3544525>
- Sinervo B, Doughty P (1996) Interactive effects of offspring size and timing of reproduction on offspring reproduction: experimental, maternal, and quantitative genetic aspects. *Evolution* 50:1314. <https://doi.org/10.2307/2410671>
- Sinervo B, Doughty P, Huey RB, Zamudio K (1992) Allometric engineering: a causal analysis of natural selection on offspring size. *Science* 258:1927–1930. <https://doi.org/10.1126/science.258.5090.1927>
- Singleton G, Krebs CJ, Davis S et al (2001) Reproductive changes in fluctuating house mouse populations in southeastern Australia. *Proc R Soc B Biol Sci* 268:1741–1748. <https://doi.org/10.1098/rspb.2001.1638>
- Smith CC, Fretwell SD (1974) The optimal balance between size and number of offspring. *Am Nat* 108:499–506
- Smith HM, Sinelnik G, Fawcett JD, Jones RE (1972) A Survey of the chronology of ovulation in Anoline lizard genera. *Trans Kansas Acad Sci* 75:107–120. <https://doi.org/10.2307/3627160>
- Tucker JK, Dolan CR, Lamer JT, Dustman EA (2008) Climatic warming, sex ratios, and red-eared sliders (*Trachemys scripta elegans*) in Illinois. *Chelonian Conserv Biol* 7:60–69. <https://doi.org/10.2744/CCB-0670.1>
- Uller T, Olsson M (2010) Offspring size and timing of hatching determine survival and reproductive output in a lizard. *Oecologia* 162:663–671. <https://doi.org/10.1007/s00442-009-1503-x>
- Varpe (2017) Life history adaptations to seasonality. *Integr Comp Biol* 57:943–960. <https://doi.org/10.1093/icb/ixc123>
- Varpe Ø, Jørgensen C, Tarling GA, Fiksen Ø (2007) Early is better: seasonal egg fitness and timing of reproduction in a zooplankton life-history model. *Oikos* 116:1331–1342. <https://doi.org/10.1111/j.2007.0030-1299.15893.x>
- Verhulst S, Nilsson J-A (2008) The timing of birds' breeding seasons: a review of experiments that manipulated timing of breeding. *Philos Trans R Soc B Biol Sci* 363:399–410. <https://doi.org/10.1098/rstb.2007.2146>
- Warner DA (2014) Fitness consequences of maternal and embryonic responses to environmental variation: using reptiles as models for studies of developmental plasticity. *Integr Comp Biol* 54:757–773. <https://doi.org/10.1093/icb/ucu099>
- Warner DA, Lovern MB (2014) The maternal environment affects offspring viability via an indirect effect of yolk investment on offspring size. *Physiol Biochem Zool* 87:276–287. <https://doi.org/10.1086/674454>
- Warner DA, Shine R (2007) Fitness of juvenile lizards depends on seasonal timing of hatching, not offspring body size. *Oecologia* 154:65–73. <https://doi.org/10.1007/s00442-007-0809-9>
- Warner DA, Jørgensen CF, Janzen FJ (2010) Maternal and abiotic effects on egg mortality and hatching size of turtles: temporal variation in selection over seven years. *Funct Ecol* 24:857–866. <https://doi.org/10.1111/j.1365-2435.2010.01714.x>
- Warner DA, Buckelew AM, Pearson PR, Dhawan A (2015) The effect of prey availability on offspring survival depends on maternal food resources. *Biol J Linn Soc* 115:437–447. <https://doi.org/10.1111/bjj.12519>
- Warner DA, Johnson MS, Nagy TR (2016) Validation of body condition indices and quantitative magnetic resonance in estimating body composition in a small lizard. *J Exp Zool Part A Ecol Genet Physiol* 325:588–597. <https://doi.org/10.1002/jez.2053>
- Williamson I, Bull CM (1995) Life-history variation in a population of the Australian frog *Ranidella signifera*: seasonal changes in clutch parameters. *Copeia* 105–113
- Wright AN, Piovio-Scott J, Spiller DA et al (2013) Pulses of marine subsidies amplify reproductive potential of lizards by increasing individual growth rate. *Oikos* 122:1496–1504. <https://doi.org/10.1111/j.1600-0706.2013.00379.x>