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The brown anole (*Anolis sagrei*) as a model for studying life-history adaptation to seasonality

Introduction

In seasonal environments, the timing of reproduction can impact offspring fitness. Offspring produced late in the season often experience decreased survival and lower growth rates than earlier-produced individuals. This trend has been studied across a variety of taxa (e.g. Varpe et al. 2007; Warner and Shine 2007; Öberg et al. 2014; Pearson and Warner 2018). The seasonal decline in fitness-relevant phenotypes of offspring may be due to a concomitant decline in the quality of the offspring environment. Late-produced offspring may suffer from increased

competition from earlier-produced conspecifics that are larger and better able to acquire resources. Late-produced offspring may, independent of competition, have access to a poorer pool of resources during a critical early-life stage or simply have less time to grow prior to winter or dry seasons. Moreover, during winter, environmental conditions (e.g. reduced temperature and rainfall) may favor survival of larger individuals. Thus, late-produced offspring may not attain a body size or fat reserves/water supply that would ensure overwinter survival. For this reason, late-produced offspring tend to be of lesser reproductive value to their

Figure 1. A brown anole egg uncovered in the field. Eggs are often laid underneath cover objects (e.g. rocks. leaf litter) and left to develop under prevailing environmental conditions.

parents than earlier-produced offspring (see Varpe 2017 for review of adaptation to seasonality). When the reproductive value of offspring is season-dependent, life-history theory predicts that females will invest differently in early- vs late-produced offspring. This may be accomplished by altering the investment in offspring size vs number as the season progresses (Lack 1947; Smith and Fretwell 1974; Brockelman 1975). Early in the season, females should invest lots of energy

in many, smaller offspring; however, late in the season, they should invest less total energy but divide it among fewer, better provisioned (i.e. larger) offspring (Nussbaum 1981).

Reptiles have played an important role in studying seasonal shifts of parental investment towards offspring size versus number (e.g. Nussbaum 1981; DeMarco 1989; Du et al. 2014).
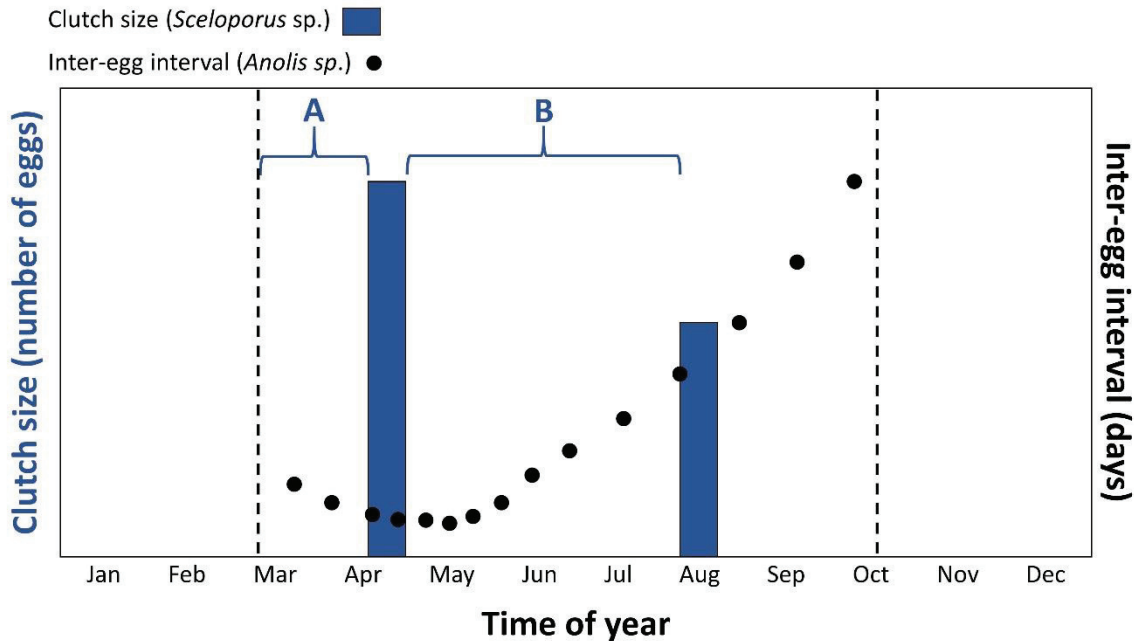

Figure 22. Hypothetical seasonal change in clutch size for an individual Sceloporus lizard (e.g. Sceloporus woodi; Jackson and Telford 1974; blue bars) and hypothetical seasonal change in inter-egg interval for an individual Anolis lizard (black circles). A greater inter-egg interval equates to a slower rate of egg production. Vertical dashed lines represent the hypothetical beginning (March) and end (October) of the breeding season for both species. Both individuals display the same general trend in reproduction: more eggs are produced earlier in the season than later. However, for the Sceloporus female, there are long periods of time during the breeding season (periods A and B) during which there is great uncertainty in how the environment impacts reproduction. The continuous reproduction of anoles, however, allows researchers to monitor changes in reproduction at a finer scale.

Unlike birds and mammals, oviparous reptiles rarely exhibit parental care (Fig 1). For this reason, measuring maternal investment into offspring is as straightforward as quantifying the amount of energy that a female invests into a single egg vs a clutch of eggs. Often, a simple measure of egg mass vs clutch mass will suffice. Although many oviparous reptiles have a prolonged reproductive season, there are often long intervals between reproductive events for a given individual. For example, seasonal shifts in offspring size vs number have been well studied in *Sceloporus* lizards (e.g. DeMarco 1989; Du et al. 2014); however, females may only produce 1 or 2 clutches (rarely 3) per year. Large periods of time pass between each clutch, making it difficult to determine which seasonally-shifting environmental factors (e.g. temperature,

photoperiod, food availability) drive seasonal changes in reproduction and precisely how those factors impact reproductive physiology (Fig 2).

The unique reproductive biology of *Anolis* lizards makes this group an excellent model for studying seasonal shifts in maternal investment. Anoles lay a single-egg clutch once every 4-14 days (depending on the species) across a broad reproductive season. They alternate egg production between ovaries, so each egg is yolked, shelled, and laid separate from every other egg (Crews 1977). The rapid, independent production of eggs allows females to adjust their reproductive effort among offspring as the environment changes. This continuous production of eggs has great potential to demonstrate how changes in maternal investment subtly shift through the season. In contrast, for lizards that produce 2-3 multi-egg clutches per season (e.g. *Sceloporus*), changes in maternal investment can only be measured discretely by observing mean differences between early and late season clutches (Fig 2). Furthermore, species that produce multiple eggs in a clutch are limited in their ability to differentially allocate resources among individual offspring within each clutch.

Figure 3. Differences in key reproductive traits between three seasonal cohorts of captive-bred A. sagrei. Cohorts 1, 2, and 3 were collected early-, mid-, and late-season, respectively. This figure was taken from Mitchell et al. (2018).

Recent published results from the Warner lab

Recently-published work from the Warner Lab strongly suggests that the quality of the offspring environment declines seasonally for brown anoles (*Anolis sagrei*) in Florida: survival is higher for early-produced offspring and lower for late-produced offspring (in the field - Pearson and Warner 2018; Mitchell and Warner unpublished data; and in the lab - Warner and Lovern 2014). Thus, we predicted that reproductive investment should shift seasonally in ways predicted by life-history theory. In a recently published study (Mitchell et al. 2018), we found that females produced more but smaller eggs early in the season and fewer, but better provisioned, higher quality eggs later in the season
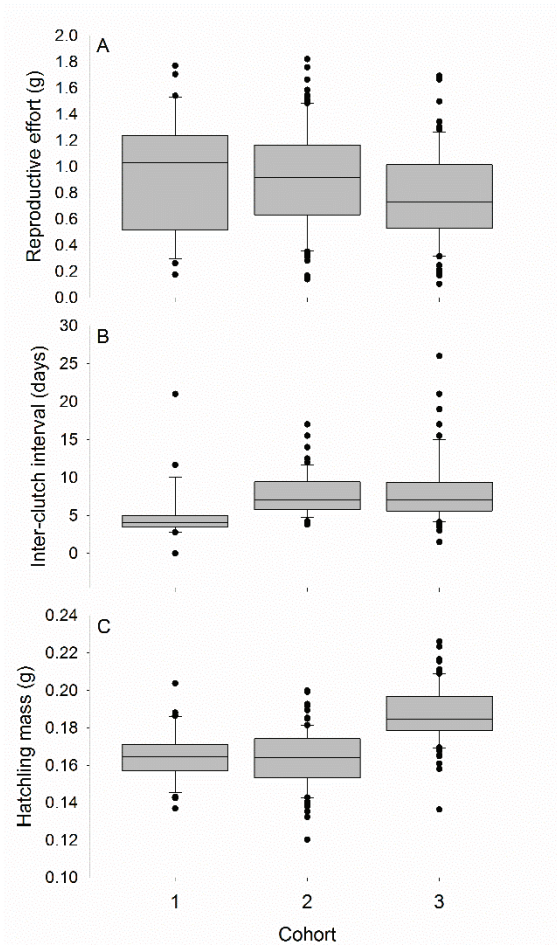
(Fig 3). Despite the larger size of late-produced eggs, reproductive effort was greatest early on. These are the patterns we would expect if selection favors females that shift investment in offspring size vs number throughout the season because the quality of the offspring environment declines. This result was also independently produced by another Warner Lab study (Pearson and Warner 2018).

Although these studies demonstrate that females shift reproductive investment in ways predicted by life-history theory, many important questions remain. For example, we still don't know to what extent these seasonal changes in reproduction are due to intrinsic factors (e.g. genes) and to what extent they are induced by proximate environmental cues. In these studies, we used separate cohorts of females that were each captured at different times during the same reproductive season (early-, mid-, and late-season cohorts in Mitchell et al 2018; early- and late-season cohorts in Pearson and Warner 2018). Although both studies controlled for factors that influence reproduction once the animals arrived in the lab (e.g. food abundance, temperature, humidity), each of these cohorts experienced a different environment in the field prior to capture. Thus, we can't say if these patterns are intrinsic or wholly induced by the environment. Additionally, existing studies have not explored how reproductive shifts differ among individual females. Such inter-individual variation is necessary for phenotypes to evolve via selection.

Current and future studies and preliminary results

To build upon these recent studies, we have another project underway that expands our knowledge of seasonal variation in reproduction of brown anoles. This project will determine if seasonal patterns of reproduction persist when females are housed in the lab for the entire
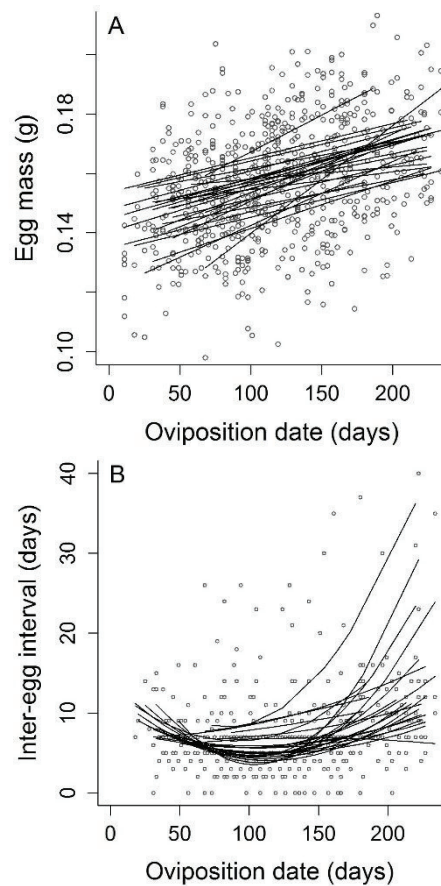

Figure 4. Changes in egg mass (A) and inter-egg interval (B) of female brown anoles across the reproductive season. Open circles show raw data from all females. Lines show fitted values for each female. Day 0 of oviposition date is March 10, 2017. Values of 0 for inter-egg interval indicate that 2 eggs were collected from a nest pot on the same day – thus, one egg was assigned an inter-egg interval of 0 days. In this study, we collected eggs 3 times per week, so we cannot be certain that two eggs were laid on the same day. However, in a current, unrelated study, JMH is collecting eggs from brown anoles daily and on many occasions (n=25) has collected 2 eggs from a nest pot on the same day. This indicates that female brown anoles sometimes oviposit 2 eggs within a 24-hour period.

reproductive season. We collected females at the beginning of the breeding season (early March; Lee et al. 1989) and tracked their reproduction in the lab until the end of October when egg-laying ceases in the field (Mitchell et al. 2018). We carefully monitored reproduction and growth during this time. We anticipated that the patterns observed in previous studies (e.g. a seasonal shift toward fewer, better provisioned offspring) would be observed in the lab if these patterns were somehow intrinsic; however, if the expected patterns were not observed, they may only be induced by conditions in the field. We also considered that these patterns may differ among females. Such individual variation is suggestive of a genetic basis for reproductive traits, which is necessary for phenotypes to evolve in response to selection.

We observed that, even when females are housed in the lab for the entire season, relative egg size (egg mass relative to maternal body mass at oviposition) increases through time independent of snout-vent length (oviposition date: $t_{1,631}$=3.49; p=0.005; SVL: $t_{1,631}$=0.36; p=0.72; Fig 4A). Thus, females are increasing the relative effort per offspring as the reproductive season progresses. At the same time, the rate of egg production is lowest (i.e. highest inter-egg interval) at the end of the season (oviposition date: $t_{1,625}$=3.93; p <0.001; SVL: $t_{1,625}$=0.46; p=0.65; Fig 4B.). These data support our hypothesis; however, the trends observed in this study do not appear as strong as those observed in other studies that leveraged temporally separated cohorts of breeding adults (e.g. Mitchell et al. 2018). Likely, seasonal shifts in reproductive traits are strongest in the field where both extrinsic (e.g. temperature, photoperiod, diet) and intrinsic (e.g. genes) factors may work additively.

We also observed among-individual variation in how reproductive traits shift through time (Fig 4); however, there is remarkable consistency in the patterns. For example, although some females increased egg size more than others through the experiment, all the slopes for this trait are positive. Regardless, seasonal shifts in key reproductive traits seem to persist when females were kept in the lab for the entire season. Thus, we think that brown anoles have great potential to make important contributions to our understanding of life-history adaptations to seasonal environments.

One final (and monumental) challenge remains. We need to assess how seasonal shifts in reproduction occur in the field. Due to the inconspicuous nesting behavior of anoles, it is difficult to locate large numbers of freshly laid eggs in the wild. For perspective, JMH estimates that he has checked over 5980 nest pots for eggs (in the lab) over the last 3 years. He has only once observed a female anole in the process of digging a nest. Currently, Christopher Thawley, James Stroud, and JMH are collecting reproductive data on brown anoles and crested anoles (*Anolis cristatellus*) in Florida (via dissection). This study can potentially determine how egg size shifts seasonally for both species. Two major drawbacks to such a study are that euthanasia precludes the ability to obtain longitudinal reproductive data on individual females and egg size measurements from dissection will not perfectly reflect egg size at oviposition. Thus, to better

assess how reproduction shifts in the field, other experimental designs may need to be employed (e.g. outdoor caging and egg collection; use of an ultrasound to monitor reproduction during a mark-recapture study).

In conclusion, multiple studies from the Warner Lab demonstrate that seasonal shifts in reproduction of brown anoles conform to predictions from life-history theory: when the quality of the offspring environment declines through the year, females shift from producing many, smaller offspring to fewer, larger offspring as the season progresses. The nearly unique reproduction of anoles (compared to other lizards; Fig 2) should allow us to formulate studies that explore how seasonal shifts in reproduction evolve and determine how a changing environment can impact reproduction in ways that might drive or constrain evolution. Some of these studies are already underway in the Warner Lab and, hopefully, we will have many more answers (and questions) to present at the next *Anolis* Symposium.

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