Reproductive and physiological response to environmental stress in *Anolis sagrei* and *Anolis carolinensis*

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ABSTRACT

Some species appear to be more well-suited than others for invasion success; *Anolis sagrei* (the brown anole) is one such species. What makes an invasive species like the brown anole apparently so well adapted to colonizing new environments? There have been different methods of invasion studied, but it is believed that brown anoles do not deal with stress the same as native species like *Anolis carolinensis* (the green anole), who's native territory the brown anole invades. Previous work demonstrated that while green anole males react to a simulated stress response with reduced territory guarding, brown anole males showed no such reduction. In the present study we tested whether female brown anoles would show the same trend with reproductive fitness (measured by egg production) when exposed to a simulated stress. We used the same method of transdermal application of corticosterone to both green anoles and brown anoles as used previously. We dosed a control group of both species with sesame oil and a treatment group of both species with a mixture of sesame oil and corticosterone. We found the brown anoles showed the expected trend where the females had the same high reproductive

output whether stressed or not. The green anoles, though the reproductive output was very low, had no significant difference in egg production between the control and the treatment groups. We expected the green anoles to show a decrease in reproduction when exposed to stress, but that was not supported. We propose that because the green anoles may have been stressed beforehand during capture and shipping, and because we received our green anoles near the end of the breading season, the results we obtained for green anoles may be unreliable. Further experimentation needs to be done on female green anoles specifically to determine their reproductive response to stress.

***Keywords: Anolis carolinensis*; *Anolis sagrei*; Green anole; Brown anole; reproduction; stress; invasive; egg production**

INTRODUCTION

Green anoles (*Anolis carolinensis*) are native to the southeastern United States and are our only endemic anole (Kolbe et. al. 2013). Brown anoles (*Anolis sagrei*) are native to Cuba and the Bahamas (Angetter et. al. 2011), but they are an invasive species to the southeastern United States (Kolbe et. al. 2013). The brown anole displaces other species of lizards including the green anole (Losos & Spiller 1999). Many invasive species show an ability to rapidly adapt to new environments (Prentis et. al. 2008) and the brown anole is among these.

The stress response is a suite of physiological and behavioral processes that activate in response to a stressor in an attempt to return to a homeostatic state. An individual with this response will increase circulating glucocorticoids and the sympathetic nervous system will be activated (Lucas &French 2012). This causes a mass gluconeogenesis and glycogenolysis, mobilizing glucose to the blood stream to enhance muscle function. This has numerous physiological effects including the redistribution of immune cells to the periphery, an increase in blood pressure, and decreased reproductive activity.

In general, most species respond to stress (like a sickness or an injury) by reallocating energy from nonessential functions like reproduction and territorial aggressing to focus more on resource-intensive activities (Lucas & French 2012). It is possible that brown anoles are successful invaders in part because they prioritize reproduction over other resource-intensive activities even when challenged with physiological stress. Previous work demonstrated that while green anole males react to a simulated stress response (transdermal application of the hormone corticosterone) with reduced territory guarding, brown anole males showed no such reduction (Parikh & Lovern, Unpubl.). In the present study, we tested whether brown and green anole females, respectively, would show a similar difference in stress responsiveness.

We hypothesized that brown anole females would show no change in egg production (our measure of energy allocation to reproduction) after exposure to stress compared to control females. Thus, brown anoles will not reallocate energy to deal with the stress and will continue to produce high numbers of eggs. We also hypothesized that, in contrast to the brown anoles, green anole females would show reduced egg production after exposure to stress compared to control females, thus reallocating energy away from reproduction and reducing egg production.

MATERIALS AND METHODS

Females (*A. sagrei* n=10 and *A. carolinensis* n=14) were housed in breeding pairs; one female and one male per 110-liter cage (Figures 1-2). Brown anoles were laboratory raised and had been in the lab for a minimum of one year. Green anoles were wild-caught adults shipped by a commercial supplier from LaPlace, LA, USA. There was an initial group of green anoles, also lab raised like the brown anoles, which we attempted to use but unfortunately they did not produce eggs. That group was dropped and the new, wild-caught group described above was used instead. This resulted in us starting the green anole experiment several weeks later than the brown anole experiment, but otherwise all conditions were held constant for the study.

For all females, initial snout-vent length and mass were recorded to the nearest mm and 0.01 g, respectively. Females were randomly chosen for control or treatment groups. Each female was either dosed transdermally with sesame oil (5 ul; control) or a mixture of corticosterone dissolved in sesame oil (5 ug in 5 ul; treatment). Both the corticosterone and the sesame oil were purchased from Sigma (St. Louis, MO). Treatment was daily for 5 days and consisted of using a pipettor to place 5 ul of sesame oil or 5 ul of corticosterone dissolved in sesame oil on the back of each female. We then monitored egg production for six weeks. There were 4 control individuals for both the brown and the green anoles (but unfortunately one green anole died after beginning the study, bringing that number down to 3). There were 6 treatment brown anoles and 10 treatment green anoles.

Care of females was identical across species and treatments as follows. Nest boxes were checked daily and lizard enclosures were sprayed with water daily; feeding was every other day with vitamin-dusted crickets, mealworms, or waxworms. In addition to the daily spraying with water, each cage also contained a water dish providing a constant water source. Any eggs that we found were weighed to the nearest mg and incubated at 28 C. Hatchlings were weighed to the nearest mg, snout-vent length was recorded to the nearest mm, and sex was recorded as male or female. Data were analyzed with t-tests and Pearson correlations (SigmaPlot v. 11). All procedures were conducted according to our Animal Care and Use Protocol (AS1312).

Figure 1: The housing set up for all tanks.

Figure 2: A brown anole female and male in housing.

RESULTS

Brown anoles

Over the course of the study, female brown anoles lost mass and this decline was not statistically different between control and treatment groups ($t = -0.067$, $p=0.95$). There was not a statistically significant difference in the number of eggs produced ($t=0.16$, $p=0.88$; Table 1), the average egg mass ($t=0.77$, $p=0.47$), or the proportion of hatched eggs (t= -1.82, p=0.11) between control and treatment groups of brown anole females. There was a statistically significant positive correlation between female beginning mass and total eggs produced for brown anoles (r=0.74, p=0.01; Figure 3).

Green anoles

Over the course of the study, female green anoles lost mass and this decline was not statistically different between control and treatment groups (t= -0.41, p=0.69). There was not a statistically significant difference in the number of eggs produced (t=0.25, p=0.81; Table 1), the average egg mass (t=0.63, p=0.55), or the proportion of hatched eggs (t=0.63, p=0.55) between control and treatment groups of the green anole females. There was not a statistically significant positive correlation between female beginning mass and the total eggs produced for green anoles (r=0.20, p=0.52; Figure 4).

	# Females	# Eggs produced	Average # of eggs per female ± 1 SE
Brown control	4	27	6.8 ± 2.5
Brown treatment	6	38	6.3 ± 1.4
Green control	3	5	1.7 ± 0.3
Green treatment	10	15	1.5 ± 0.4

Table 1: The average number of eggs produced per female of each treatment group for both species: Brown anoles in general produced a much larger number of eggs than green anoles.

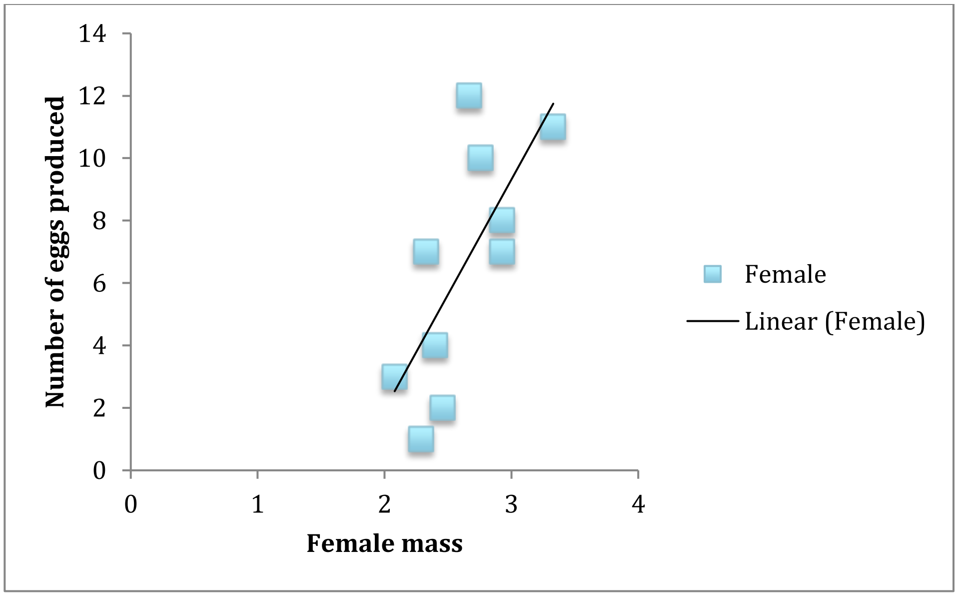

Figure 3: Initial mass in brown anole females compared to the number of eggs produced; there was a sharp positive correlation between female mass and the number of eggs produced in brown anoles. Larger females produced more eggs r=0.74, p=0.01.

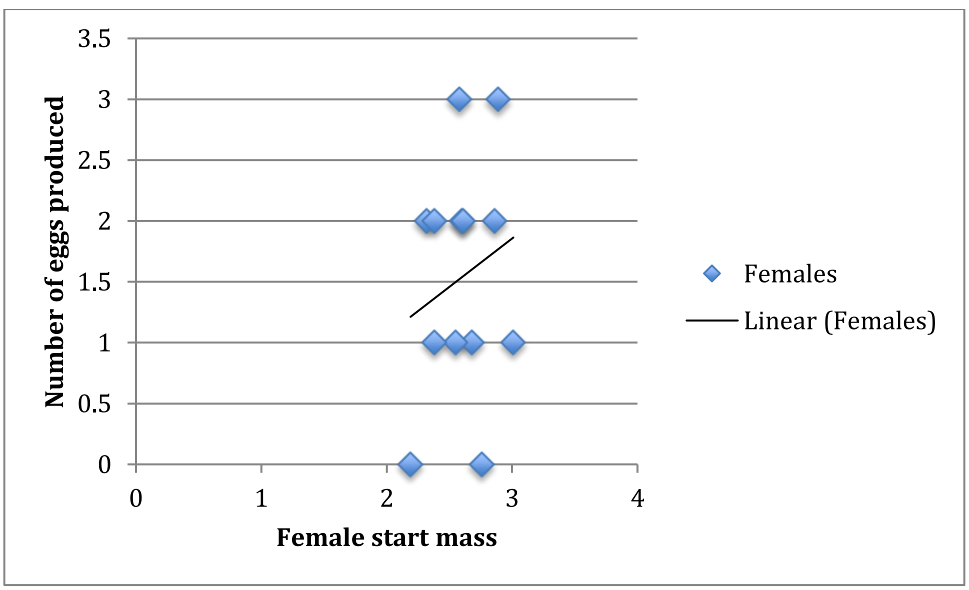

Figure 4: Initial mass in green anole females compared to the number of eggs produced; there was not a significant correlation between female mass and the number of eggs produced in green anoles. Larger females did not necessarily produce more eggs r=0.20, p=0.52.

DISCUSSION

Our hypothesis that brown anole females would show no change in egg production when challenged with a simulated stress response was supported. This is similar to the lack of a response to simulated stress shown by brown anole males in previous lab work. As expected, the brown anoles chose not to reallocate energy away from egg production to deal with the stress, but rather to continue to use energy to produce eggs.

Our hypothesis that green anole females would show reduced egg production when exposed to stress was not supported. This result differs from previous work in green anole males in which a simulated stress response caused a reduction in energy allocated to reproduction (territory guarding). It was expected that green anoles would have low egg production numbers but there was no difference between the control and treatment groups, which was not expected.

We speculate that the results for green anoles in the present study are not a strong rejection of our hypothesis for several reasons. First, green anoles were brought into the lab after collection in the field towards the end of the breeding season; thus the stress of capture and shipping at this stage may have caused reproduction to shut down (hence the low egg production we observed), regardless of which group (control or treatment) to which they were assigned once in the lab. Second, further indication of atypical reproductive behavior comes from the fact that in green anoles, there was no relationship between female mass and egg production. This is a standard, expected relationship across anoles (e.g., Losos, 2009); we observed it for our brown anoles, but not for our green anoles.

Regardless of the results for green anoles, the present data suggest that brown anole females, like males, are selected for prioritizing energy allocation to reproduction even when faced with a physiological stress response. The question remaining is where the trade-off is for the brown anoles. Whether there is a long-term consequence to this apparent short-term fitness gain remains to be determined. When it comes to brown anoles, there are numerous mechanisms that could be responsible for their response. We speculate brown anoles have a lower density or specificity of nuclear receptors for corticosterone. This would mean that higher levels of corticosterone would have a smaller effect on brown anoles. Also, brown anoles might have a higher level of baseline corticosterone, so a dose of the same concentration would raise a green anoles corticosterone levels proportionally more than a brown anole. Further studies need to be done on nuclear receptors and baseline corticosterone in both species to determine the mechanisms of the differences in stress response.

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