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Phenotypic Diversity and Costs of Early Hatching in Lizards

Zachary Winstead
University of South Florida

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Phenotypic Diversity and Costs of Early Hatching in Lizards

by

Zachary Winstead

A thesis submitted in partial fulfillment
of the requirements for the degree of
Master of Science in Conservation Biology
Department of Biological Sciences
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University of South Florida

Major Professor: Jeremiah Sean Doody PhD
Alison Gainsbury PhD
Lisa Ganser PhD

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Abstract

Early hatching is a form of environmentally cued hatching in which embryos can emerge early to improve chances of survival and is considered a means of antipredator defense. Early hatching as a response to predation has been well documented in amphibians, but its prevalence among other animal groups is unknown. Moreover, any trade-offs between early hatching and other traits, and thus survival, are not well understood. There is anecdotal evidence that lizards exhibit early hatching behavior, but quantitative evidence is limited to one study. In the present study, I tested for the presence of early hatching in six species of lizards spanning six families including: crested geckos (*Correlophus ciliatus*), red tegus (*Salvator rufescens*), brown basilisks (*Basiliscus vittatus*), eastern glass lizards (*Ophisaurus ventralis*), green iguanas (*Iguana iguana*), and Mediterranean geckos (*Hemidactylus turcicus*). Specifically, I surveyed for early hatching behavior and any potential trade-offs in body size at hatching (hatchling mass, hatchling snout to vent length (SVL) and residual yolk) or growth rates (in one species, for four weeks). Predation was simulated by rubbing a chopstick along the surface of the hatching-competent egg for two minutes, while control eggs were allowed to hatch spontaneously. Two of the six species (*green iguanas* and *brown basilisks*) did not respond to the simulated predation and hatched spontaneously, while the other four species (*crested geckos*, *red tegus*, *Mediterranean geckos*, and *eastern glass lizards*) did respond and hatched during experimental simulations. Three of those four species

(*crested geckos*, *red tegus* and *Mediterranean geckos*) exhibited 'explosive' early hatching, whereby hatchlings emerged from the egg and immediately attempted to escape by running away from the perceived threat. Eastern glass lizards showed no evidence of early hatching. Though embryos pipped during predator simulation trials, they remained in their eggs for hours to days. Hatching response in this species may reflect synchronous hatching, whereby vibrations stimulate simultaneous pipping to promote hatchlings escaping the nest together. In *crested geckos*, earlier hatching (treatment) lizards were significantly smaller, shorter, and left a greater amount of residual yolk than spontaneously hatching (control) lizards. Growth trials with *crested geckos* showed that the treatment lizards were significantly shorter for the first week. However, trends suggest that the differences in length and size are distinct between treatment and controls and narrow over time eventually converging after four weeks. In *red tegus*, treatment lizards were significantly shorter and left a greater amount of residual yolk than control lizards. In *brown basilisks*, treatment lizards were significantly smaller and shorter than control lizards. While incubation periods for *red tegus* and *green iguanas* suggested early hatching, small sample sizes precluded confirmation, and the small sample size for *Mediterranean geckos* (N=5) also precluded analysis. The present study demonstrates early hatching in three new species and suggests its presence in two more. Collectively, my results and those of others suggest that early hatching is common in lizards, particularly in geckos, and that a smaller body size is likely a general cost of early hatching in lizards and perhaps other animals. Further research should examine how long those costs persist along with potential fitness consequences.

Chapter 1: Introduction

Phenotypic Plasticity

Phenotypic Plasticity as an Adaptive Advantage

Phenotypic plasticity is a phenomenon in which biotic and abiotic influences can alter the phenotypic expression of individuals with the same genotype (DeWitt and Scheiner 2004). Plasticity can influence behavioral, physiological, and morphological traits as a means of providing some adaptive advantage (Price et al. 2003). For example, *Littorina obtusata*, an intertidal snail, displays plasticity in foot and shell size in response to hydrodynamic stress. When exposed to greater hydrodynamic stress, snails show decreased shell size and increased foot size to help prevent dislodgement by predators (Trussell 1997). Orangespotted sunfish (*Lepomis humilis*) exhibit plasticity in body truss, gill raker and pharyngeal jaw measurements in response to their diets (Hegrenes 2001). Finally, in larval wood frogs (*Rana sylvatica*), predator cues induce several changes including behavior, morphology, and mass (Schoeppner and Relyea 2009).

The threat of predation is a powerful influence on the adaptive advantage of phenotypic plasticity. For example, several models demonstrate plasticity in the metamorphosis of some larval organisms in response to predation. These include alterations to the timing of metamorphosis and to the size at which an organism will

metamorphose due to predation pressures. Empirical studies testing these predator-induced phenotypic plasticity models found that their predictions are frequently met. Additionally, models display instances of common but unpredicted responses to predator pressure such as a delay in metamorphosis (Bernard 2004).

Phenotypic Plasticity in Eggs: Environmentally Cued Hatching

Remarkably, plasticity of behavior can begin while developing embryos are still in the egg, influencing the timing of hatching. Due to this plasticity in hatching timing, an embryo may experience an alteration to the duration of the embryonic period, hatch at an entirely different developmental stage, or both (Warkentin 2011a). Plasticity of hatching behavior has been recorded in a wide range of different animal taxa including reptiles, amphibians, fish, and invertebrates (Doody 2011, Warkentin 2011a). Additionally, hatching responses have been recorded as a result of a wide variety of stimuli, including waterborne chemical cues, physical disturbance or vibrations, hypoxia, and acoustic cues (Warkentin and Caldwell 2009).

Since survival is the variable that has the most immediate effect on the fitness of eggs and hatchlings, behaviorally plastic responses can be an essential part of a successful developmental environment (Warkentin and Caldwell 2009). Nesting and hatching behaviors are important components of an organism's life history and its ability to successfully adapt to the environment around it. The time an organism spends both developing in an egg and hatching are moments of substantial vulnerability. As a result, hatching at the ideal time is pivotal to the survivability and fitness of the organism. The ideal time needed for development, however, does not always match the ideal time for

the organism to have the greatest chances of survival in its environment (Warkentin 2011a). Several species respond to environmental stimuli through alterations in the timing of hatching. Plasticity in hatching timing allows an organism to hatch at a time with the greatest benefit to cost ratio and in turn increased survivability (Warkentin and Caldwell 2009). This plasticity in hatching timing due to stimulus is referred to as environmentally cued hatching and comes in three different types.

The first type of environmentally cued hatching is delayed hatching, whereby, in the presence or absence of some environmental stimulus, the embryo will delay the timing of hatching to occur in the presence or absence of an environmental stimulus. For example, two species of salamanders (*Ambystoma texanum* and *Ambystoma barbouri*) will delay hatching in the presence of predators and chemicals associated with those predators (Sih and Moore 1993). A second type of environmentally cued hatching involves individuals synchronizing their hatching with that of their clutch mates (Doody 2011). Quantitative analyses show that pig-nosed turtles (*Carretochelys insculpta*) synchronize hatching in response to anoxia as a cue (Doody 2011; Doody et al. 2012). *C. insculpta* will hatch when introduced to hypoxic conditions to ensure that they hatch and emerge during the wet season flooding (Webb et al. 1986; Doody et al. 2001). Additionally, laboratory experiments suggest that *C. insculpta* may detect and respond to the vibrations created by the hatching of siblings and that groups of eggs hatch more quickly than solitary eggs (Doody et al. 2012). The third type of environmentally cued hatching, early hatching, involves hatching prior to the spontaneous hatching window in response to environmental conditions, including the threat of predation.

Early Hatching

Early Hatching: Trade-offs

Early hatching as a response to physical stimuli allows an organism to increase its survivability in the face of environmental threats (Warkentin 2011 a). Early hatching as a response to predation risk was first documented in amphibians in 1993 (Sih and Moore 1993). The most thoroughly studied amphibian species for environmentally cued hatching is the red-eyed tree frog (*Agalychnis callidryas*). *A. callidryas* exhibits early hatching behavior in response to predation by snakes and wasps (Warkentin 1995, 2000, 2002; Warkentin et al. 2001). Females of *A. callidryas* deposit eggs on leaves and vegetation above bodies of water, and predator cues will stimulate hatching in developing embryos, allowing them to escape to safety in the water below. (Warkentin 1995, 2000). Individual embryos will hatch quickly when attacked directly by wasps or snakes. However, the rate at which the rest of the clutch hatches is dependent on the predator. The rest of the clutch is likely to hatch within a shorter window of time when being attacked by the more rapidly eating snake, which could be considered a more immediate threat to the entire clutch than the slower wasp (Warkentin et al. 2006, 2007). Vibrations appear to be the cue inducing early hatching in most cases, though chemical, visual, and auditory cues cannot be ruled out (Warkentin 2000). Vibrations alone are a sufficient stimulus to induce early hatching in *A. callidryas* embryos. Furthermore, evidence suggests that embryos can differentiate between vibrations caused by predators and vibrations caused by non-threatening stimuli such as rain or wind (Warkentin 2005). The stage at which early hatching occurs also appears to be variable, depending on the specific stimulus. Developmental stage and the development of mechanosensory organs appear to be constraints on the timing that *A. callidryas* can

hatch early. Anoxic conditions induce early hatching at an earlier developmental stage than physical disturbance, which requires the development of mechanosensory organs (Warkentin et al. 2017).

Most life-history strategies, however, come with important trade-offs. Early hatching is no exception. Young that hatch early are likely to be smaller, less developed, and unable to absorb all the yolk in the egg. These costs could leave individuals more vulnerable to predation post-hatching when compared to individuals that were able to hatch spontaneously (Doody and Paull 2013; Warkentin 1999, 2011b). Similarly, hatching early in *A. callidryas* is not without trade-offs. Side effects of hatching timing were detectable across the larval and metamorph life stages. Individuals that hatched early exhibited increased larval growth but were less likely to survive to metamorphosis (Touchon et al. 2013). Additionally, hatchling morphology varies by embryonic age as development of feeding, digestive, respiratory, and locomotor structures occur over the plastic period. For example, early hatched tadpoles lose external gills and grow relatively smaller tails. There is, however, no difference in size at the time of metamorphosis as hatched tadpoles develop quicker than embryos (Warkentin 1999). The presence of early hatching as a response to predation is not well studied for most groups of organisms, and the associated trade-offs are not fully understood (Warkentin 2011b; Doody 2011).

Early Hatching in Lizards

While early hatching has been studied relatively extensively in amphibians, it has only recently been documented in reptiles. However, quantitative data is limited.

Evidence is mostly anecdotal, with biologists observing the seemingly coincidental hatching of eggs during handling (reviewed in Doody 2011). Unfortunately, quantitative data are limited. Early hatching has been reported in each order of reptiles excluding *Sphenodontia* (Doody 2011). In lizards, early hatching has been recorded in response to vibrations and other forms of physical disturbance in addition to fungal growth. Fungal growth on the eggs, for instance, stimulates early hatching in Iberian rock lizards (*Iberolacerta monticola*). However, *I. monticola* hatchlings that emerged earlier were smaller, lighter, and left yolk remaining in the egg. The actual mechanism behind this hatching response is currently unknown, but it may have been due to decreased oxygen levels or changes in water potential as a result of the fungal growth (Moreira and Barata 2005). Hatching in response to vibrations and physical disturbance is most likely due to perceived predation risk. While lizards often hatch slowly under normal conditions, they have also demonstrated a rapid hatching response in response to handling and perceived predation (Vitt 1991; Doody and Paull 2013). Since sluggish, slow-hatching embryos are more likely to be caught by a predator, lizards that hatch early may need to escape the egg quickly to avoid being eaten.

Among lizards, early hatching has been qualitatively demonstrated in 16 species (Table 1). However, only one quantitative study has demonstrated early hatching in response to simulated predator cues (Doody and Paull 2013). The delicate skink (*Lampropholis delicata*) hatched about three days earlier than clutch mates in response to vibrations used to emulate a predator cue. Early hatching was measured in both the laboratory and *in situ*. Laboratory tests simulated predator cues by placing eggs collected from the field on a laboratory shaker, while control eggs were allowed to hatch

spontaneously. Tests in the field simulated predator cues by prodding eggs in nest sites. Offspring hatched rapidly (within 30 seconds) and often sprinted from the egg and nest site simultaneously. Young that hatched early were smaller on average and left more residual yolk in the egg than those that hatched spontaneously (Doody and Paull 2013).

Project Proposal

Objectives

The prevalence of early hatching as a response to predation among lizards is unknown. Although, several anecdotal observations suggest that early hatching may actually be widespread in lizards. However, a more thorough understanding of the prevalence of this behavior would provide further insight into the evolutionary significance of early hatching in lizards. Additionally, analyzing early hatching behavior in lizards could help predict and inform management decisions for conservation-listed species, especially in the face of a novel egg predator. The present study aimed to take advantage of the high diversity of invasive lizard species in Florida (Krysko et al. 2005) to reveal the taxonomic diversity of early hatching in lizards. The present study investigated early hatching in 10 different species representing nine different families (though only 6 species and 6 families were able to be analyzed) and predicts, based on previous research and observations (Table 1) that early hatching is ubiquitous among these species investigated. Additionally, the present study tested the hypothesis that there is a tradeoff in body size and growth rate associated with early hatching.

Table 1: Lizard species recorded to hatch early.

Species	Scientific Name	Source
Green anole	<i>Anolis carolinensis</i>	Godfrey et al. 2018
Brown anole	<i>Anolis sagrei</i>	Losos et al. 2003; Doody et al. 2018
Cuban Knight Anole	<i>Anolis equestris</i>	Hernandez et al. 2017
Marbled southern gecko	<i>Christinus marmoratus</i>	Doody 2011 (M. Thompson, pers. com.)
Iberian rock liizard	<i>Iberolacerta monticola</i>	Moreira and Barata 2005
Delicate skink	<i>Lampropholis delicata</i>	Doody and Paull 2013
Common garden skink	<i>Lampropholis guichenoti</i>	Doody 2011 (S. Doody, pers. obs.)
Common five-lined skink	<i>Plestiodon fasciatus</i>	Doody 2011 (L. Vitt, pers. com.)
Southeastern five-lined skink	<i>Plestiodon inexpectatus</i>	Doody 2011 (L. Vitt, pers. com.)
Broadhead skink	<i>Plestiodon laticeps</i>	Doody 2011 (L. Vitt, pers. com.)
Red-throated Skink	<i>Acritoscincus platynota</i>	Doody and Schembri 2014a
Robust Rainbow Skink	<i>Carlia schemltzii</i>	Doody and Schembri 2014b
Plica plica	<i>Plica plica</i>	Vitt 1991
Lace monitor	<i>Veranus varius</i>	Doody 2011 (L. Vitt, pers. com.)
Tree Dtella	<i>Gehyra australis</i>	Doody et al. 2015
Pilbara Dtella	<i>Gehyra pilbara</i>	Doody et al. 2015
Crested Gecko	<i>Correlophus ciliatus</i>	present study
Mediterranean Gecko	<i>Hemidactylus turcicus</i>	present study
Brown Basalisk	<i>Basaliscus vittatus</i>	present study
Green Iguana	<i>Iguan iguana</i>	present study
Red Tegu	<i>Salvator rufescens</i>	present study

Chapter 2: Materials and Methods

Collection and Care of Gravid Females

The sampling and capture of lizards took place in several different locations across Florida between early April and late June. Mediterranean geckos (*Hemidactylus turcicus*) were captured in Saint Petersburg (Latitude 27.7627389, Longitude -82.6361822). Brown basilisks (*Basiliscus vittatus*) (Latitude 25.677900, Longitude -80.284850) were captured in Snapper Creek Miami. Northern curly-tailed lizards (*Leiocephalus carinatus*), and rainbow whiptails (*Cnemidophorus lemniscatus*) were captures in Hialeah, Florida (Latitude 25.841750, Longitude -80.265020). Giant ameivas (*Ameiva ameiva*) were captured in Naples Florida (Latitude 26.267120, Longitude -81.738840). Butterfly lizards (*Leiolepis belliana*) were captured in Fort Myers (Latitude 26.564303, Longitude -81.907604). Lastly, nests of eastern glass lizards (*Ophisaurus ventralis*) were found in Fort Myers (Latitude 26.190930, Longitude -81.797280) and St. Petersburg (Latitude 27.767590, Longitude -82.691370) with the mother guarding the nest.

Eastern glass lizard eggs were placed in a Tupperware® container with several layers of moist paper towels for transport to the laboratory. Lizards were caught via noose or by hand and inspected to confirm that they were gravid females (in most lizard species, females will appear conspicuously plump when gravid). Lizards were then

transported to the laboratory in pillowcases. Once in the lab, lizards were weighed with an Adam® CQT202 scale and placed in 10-gallon terrariums equipped with a 45-watt spotlight for heat, a fluorescent 10.0 uvb zoomed bulb, a hide (6"L x 5"W x 2.5"H, 8.5"L x 7"W x 3.3"H or 10.5"L x 8.8"W x 4.25"H to fit the size of the lizard), paper towels as a substrate and a water bowl. Lights were set to a timer to provide 14 hours of light and 10 hours of darkness. Humidity was measured with a hygrometer placed outside the enclosures. Lizards were fed every other day with crickets dusted with calcium powder. Enclosures and lizards were checked daily to spot clean the enclosures, ensure proper health, refresh water, and to determine if any eggs were laid.

Collection and Care of Eggs

Eggs of crested geckos (*Correlophus ciliatus*), red tegus (*Salvator rufescens*), and green iguanas (*Iguana iguana*) were obtained directly from reptile breeders. Eggs were then transported to the laboratory in a Tupperware® container. Eggs collected from breeders or from mothers in the laboratory were then marked with a Sharpie® to indicate the top of the egg and prevent embryonic mortality due to rolling. Eggs were then placed in dressing containers (2 oz. size for crested geckos, brown basilisks, Mediterranean geckos, rainbow whiptails, northern curly-tailed lizards and eastern glass lizards and 4 oz. size for green iguanas and red tegus) with vermiculite and water (2:1 by weight, respectively) and transferred to a ZooMed ReptiBator® Digital Reptile Egg Incubator. Green iguanas were incubated at 30°C, red tegus were incubated at 32°C, rainbow whiptails and brown basilisks were incubated at 29°C, Mediterranean geckos were incubated at 31°C, northern curly-tailed lizards were incubated at 27°C and crested geckos were incubated at 23.5°C. Incubation temperatures were selected by

recommendation of breeders when applicable. Humidity was measured by the hygrometers built into the incubator and humidity ranges were kept between 80-100%. The eggs of crested geckos the first season were not placed in an incubator, but rather left to incubate at the ambient temperature of the laboratory (25-27°C). For this reason, crested gecko eggs from the first season were not used in comparisons of hatchling SVL, mass, yolk remaining, or growth trials. No eggs were obtained from butterfly lizards or giant ameivas. In some cases, as with Mediterranean geckos, no breeder recommendation could be found, so the eggs were incubated at a temperature previously used in literature (Rose and Barbour 1968). Eggs were checked daily for hatching and for mold.

Trials

Simulated Predator Hatching Trials

Trials with treatment eggs began once eggs were six weeks old for most species, ten weeks old for green iguanas and five weeks old for Mediterranean geckos. A predator was simulated using a chopstick. This was done by gently holding the top of the egg with the pointer finger and the bottom of the egg with the thumb. A timer was set for two minutes and watched. The egg was then scratched with the tip of the chopstick back and forth around the egg at a steady rate for 30 seconds, followed by 10 seconds of rest and repeated until the two-minute timer was finished. If hatching did not occur, the egg was carefully placed back in its dressing cup and returned to the incubator. When hatching did occur, length of incubation was recorded as the number of days from the lay date. The hatchling weight, snout to vent length (SVL) and the weight

of the residual yolk were also obtained. The SVL was measured by flattening out the hatchlings against a flat surface and measuring from the tip of the snout to the vent with a ruler along the flat surface. Residual yolk weight was determined by taking the mass of the egg with the yolk and white inside and then removing the yolk and taking the weight to find the difference. Hatchlings were then placed in an enclosure that was outfitted similarly to the adult enclosures. Crested gecko hatchlings were placed in enclosures without the 45-watt spotlight bulb for heat due to a lower ideal temperature range that closely matches room temperature (Aparicio Ramirez et al. 2020).

Growth Trials

In order to investigate the costs of early hatching and how long those costs persist, hatchling crested geckos were placed in enclosures and fed *ad libitum* (ZooMed crested gecko food, watermelon flavor). Hatchling SVLs and weights were then measured once per week for one month following hatching, with the first measurement being taken on the day of hatching. Four crested geckos were excluded from growth trials due to hatching before growth trials were set to begin.

Statistical Analyses

All statistical analyses were completed in R version 4.0.3 (R Core Team 2020) in RStudio version 1.4.1103 (RStudio Team 2021) with the packages “ggpubr” (Kassambara 2020), “tidyverse” (Wickham et al. 2019), “rstatix” (Kassambara 2021), “growthrates” (Petzoldt 2020) and “lme4” (Bates et al. 2015). In order to compare the difference of means between treatment and control groups, Welch Two Sample t-tests

were used for days until hatching, hatchling mass, SVL, residual yolk after hatching and to compare each week of the growth trials to find at which point they were no longer significant. Bartlett tests were run to test the heterogeneity assumption of a Welch Two Sample t-test, in which all data presented with a p-value greater than 0.05, meaning that the data was heterogeneous. Shapiro-Wilk normality tests were run to test the normality assumption of a Welch Two Sample t-test. When this was not met, such as in the case of brown basilisk mass, eastern glass lizard SVL, green iguana mass, green iguana days until hatching and green iguana SVL, Wilcoxon rank sum tests with continuity correction were run to compare the difference of the medians. ANOVAR tests were performed to determine the effect of the treatment on the SVL growth and mass growth of crested geckos for the four weeks following hatching. The assumption of sphericity was tested with Mauchly's test with Greenhouse-Geisser corrections made. Lastly, mean differences were calculated for each variable and are displayed with the correlating standard deviation.

Chapter 3: Results

Crested Geckos

Mean number of days until hatching differed significantly between treatment and control hatchlings of crested geckos ($t = 4.828$, $df = 16.045$, $p < 0.0001$), as did hatchling SVL ($t = 2.787$, $df = 13.225$, $p = 0.015$), hatchling mass ($t = 3.081$, $df = 15.619$, $p = 0.007$), and mass of residual yolk ($t = -2.870$, $df = 11.203$, $p = 0.015$). On average, treatment embryos hatched 6.8 ± 2.503 days earlier, were 0.25 ± 0.235 cm shorter, were 0.30 ± 0.232 g lighter and had 0.15 ± 0.128 g more residual yolk after hatching, than control embryos (Figure 3). Additionally, 13 out of 16 (81%) treatment eggs exhibited 'explosive' early hatching in which the hatchling pipped and escaped the egg during the course of the two-minute trial. Lastly, treatment hatchlings were neither significantly shorter ($f = 1.619$, $df = 15$, $p = 0.223$) (Fig. 7) nor lighter ($f = 2.816$, $df = 15$, $p = 0.114$) (Fig. 6) over the course of four weeks while being fed *ad libitum*. However, t-tests demonstrated that treatment crested geckos hatch smaller and shorter. Additionally Fig. 6 and Fig. 7 demonstrate clear differences in the size of control and treatment lizards that narrows slightly over time. Subsequent t-tests comparing treatment and control groups at each week demonstrated statistical differences in length ($t = 2.399$, $df = 13.933$, $p = 0.031$) (Table 2) are only present for week zero, while statistical differences in size

(Table 3) are not present. The lack of a statistical difference is likely in part due to sample size (n=19).

Red Tegus

Hatchling mass differed significantly between treatment and control hatchlings of red tegus ($t= 3.397$, $df= 4.495$, $p= 0.023$) as did residual yolk ($t= -3.051$, $df= 3.364$, $p= 0.048$) with an average difference of 2.42 ± 1.001 g and 1.52 ± 0.968 g respectively. Neither time until hatching ($t= 1.319$, $df= 4.567$, $p=0.250$) nor SVL ($t=0.038$, $df=4.615$, $p=0.971$) were significantly different between treatment and control hatchlings. However, there was a trend that suggests fewer days until hatching with treatment eggs hatching 3.38 ± 4.152 days earlier on average. Additionally, one of seven (14%) treatment hatchlings demonstrated 'explosive' early hatching and another one hatched immediately after receiving the stimulus regimen after showing no hatching behavior during the treatment.

Brown Basilisks

Hatchling length differed significantly between treatment and control brown basilisks ($t= 2.948$, $df= 5.475$, $p=0.029$) with treatment individuals being an average of 0.34 ± 0.096 cm shorter. However, treatment and control hatchlings did not differ significantly in the mass ($W=17.5$, $n=9$, $p=0.0851$) (0.29 ± 0.091 g mean difference) or residual yolk ($t= -0.258$, $df= 1.047$, $p=0.839$) (0.06 ± 0.061 g mean difference) compared to controls. The mass and mass of yolk remaining demonstrated trends toward significance that could be strengthened with a greater sample size (Fig. 1). I also observed that brown basilisk eggs did not tolerate trials well and 12 of 16 (75%) total

treatment eggs developed large dark bruises, became dented over the course of testing and never hatched.

Green Iguanas

Green iguanas demonstrated no significant differences in number of days until hatching ($t= 2.342$, $df= 3.294$, $p=0.093$), SVL ($W=5$, $n=5$, $p=0.387$), or hatchling mass ($W=7$, $n=6$, $p=0.383$). There was an apparent trend for all three attributes demonstrating a mean difference of 3.58 ± 2.986 days, 0.41 ± 0.530 cm and 1.23 ± 1.772 g respectively.

Eastern Glass Lizards

Comparisons between control and treatment eastern glass lizard eggs yielded no significant differences in SVL ($t= 15.5$, $n=11$, $p=1$), hatchling mass ($t= 0.252$, $df= 8.059$, $p= 0.808$) or residual yolk mass ($t=-0.802$, $df= 2.973$, $p=0.482$). There was an apparent trend with yolk weight remaining (mean difference of 0.08 ± 0.141 g) (Fig. 4). Six of eight (75%) treatment hatchlings pipped during the trial, though none of them demonstrated 'explosive' early hatching and instead finished hatching at a later time, sometimes days later.

Other Study Organisms

Only five Mediterranean geckos reached hatchling stage. Two of the three treatment hatchlings demonstrated 'explosive' early hatching. The treatment hatchlings hatched at 38, 42 and 43 days, while the controls hatched at 40 and 43 days. For two

individuals, SVL and mass could not be measured. The two remaining treatment lizards weighed 2.1 g and 2.4 g, while the remaining control lizard weighed 2.3 g. Yolk weight was not obtained for any of the Mediterranean gecko hatchlings. Four eggs were obtained from rainbow whiptails and only one, treatment control egg, hatched. All eight northern curly-tailed lizard eggs molded before trials began.

Table 2: Temporal Comparison of SVL T-Tests Per Week During Crested Gecko Growth Trials

Week	Group 1	Group 2	n1	n2	statistic	df	p	significance (95%)
0	control	treatment	10	9	2.399	13.933	0.031	*
1	control	treatment	10	9	1.134	15.315	0.274	
2	control	treatment	10	9	1.145	11.778	0.275	
3	control	treatment	10	9	1.268	10.554	0.232	
4	control	treatment	10	9	1.071	9.291	0.311	

Table 3: Temporal Comparison of Mass T-Tests Per Week During Crested Gecko Growth Trials

Week	Group 1	Group 2	n1	n2	statistic	df	p	significance (95%)
0	control	treatment	10	9	1.882	13.271	0.082	
1	control	treatment	10	9	2.048	12.406	0.062	
2	control	treatment	10	9	1.679	11.000	0.121	
3	control	treatment	10	9	1.706	11.033	0.116	
4	control	treatment	10	9	1.764	11.760	0.104	

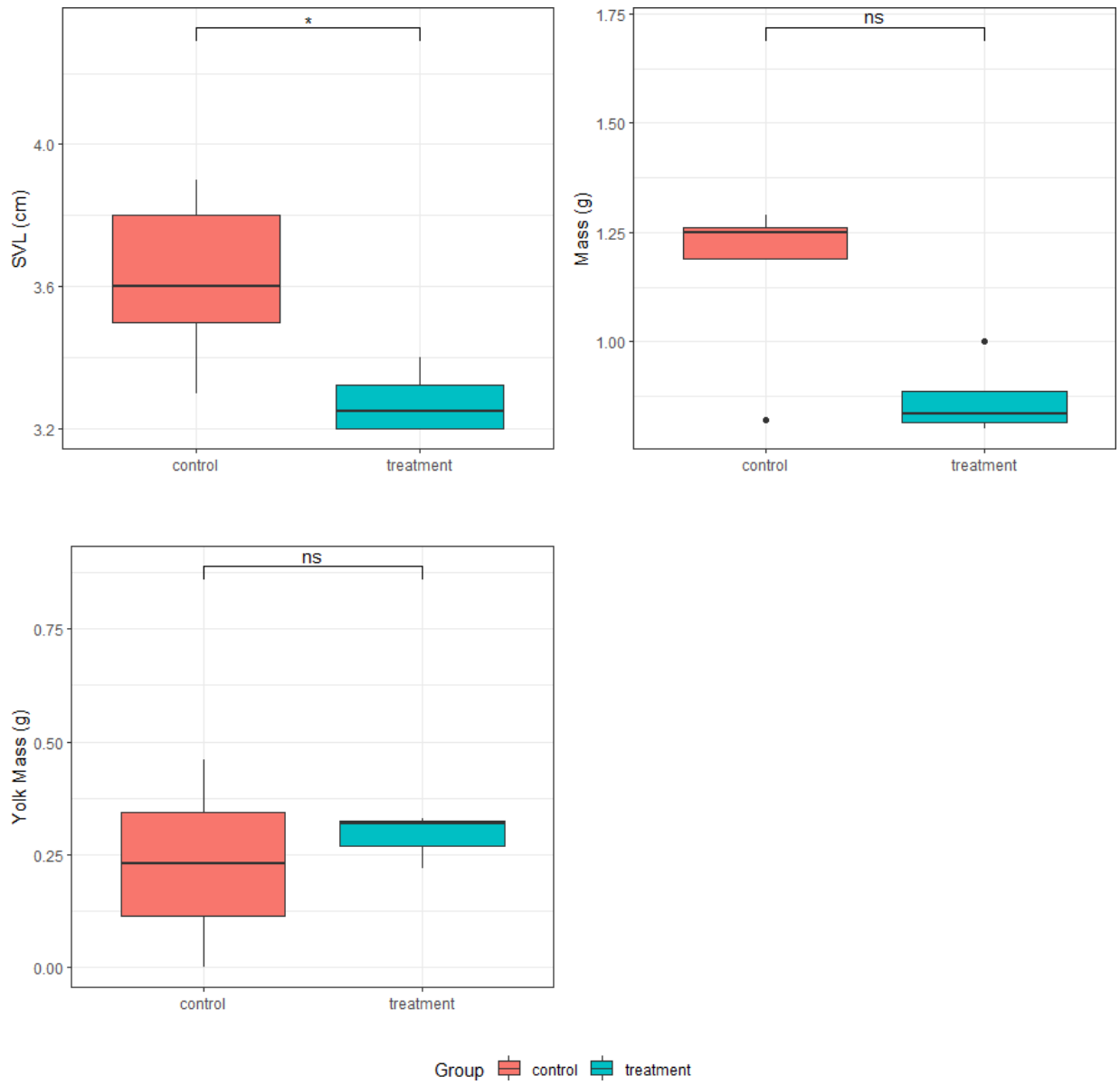


Figure 1: Differences in brown basilisk control and treatment groups.

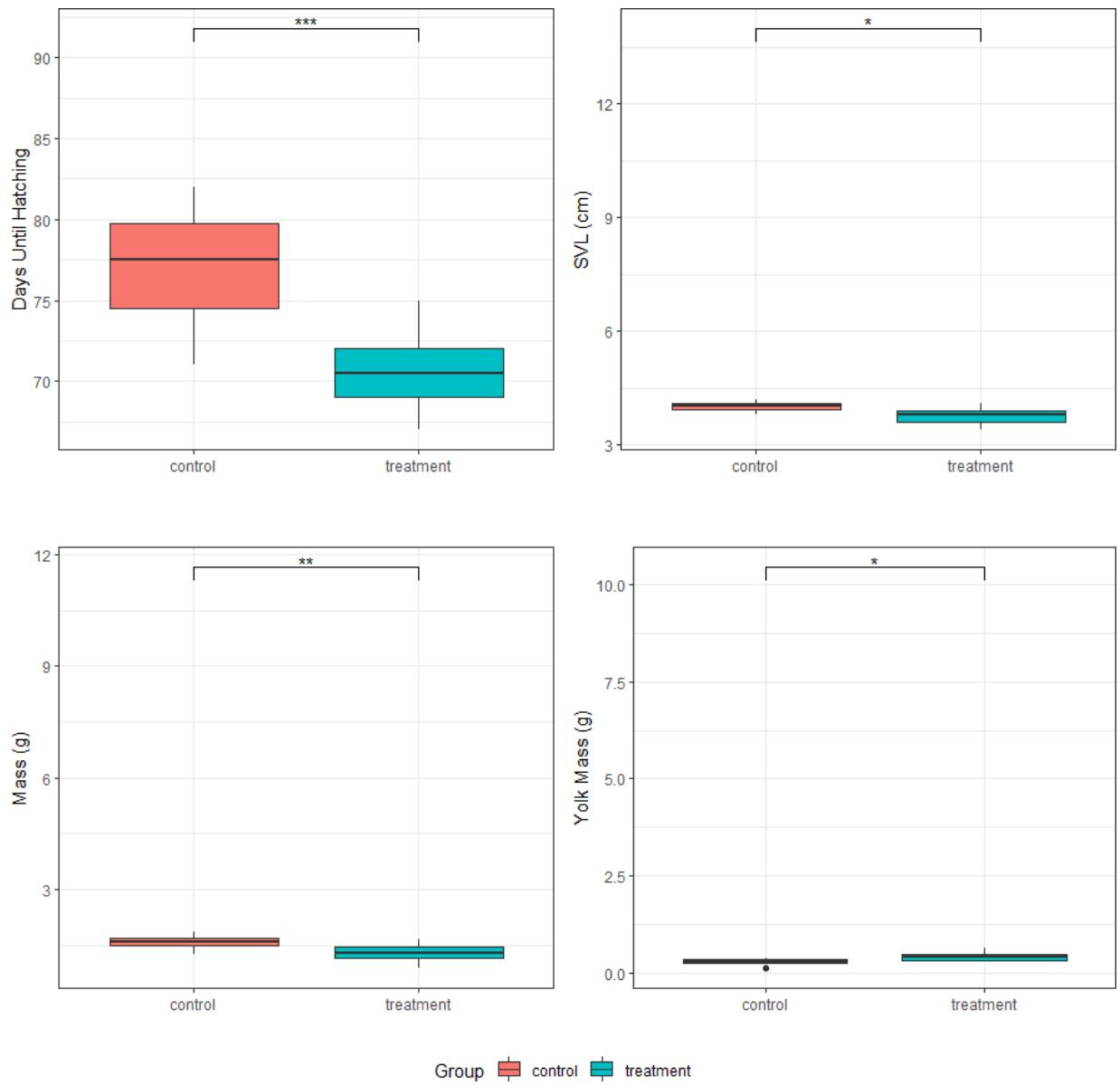


Figure 2: Differences in crested gecko control and treatment groups.

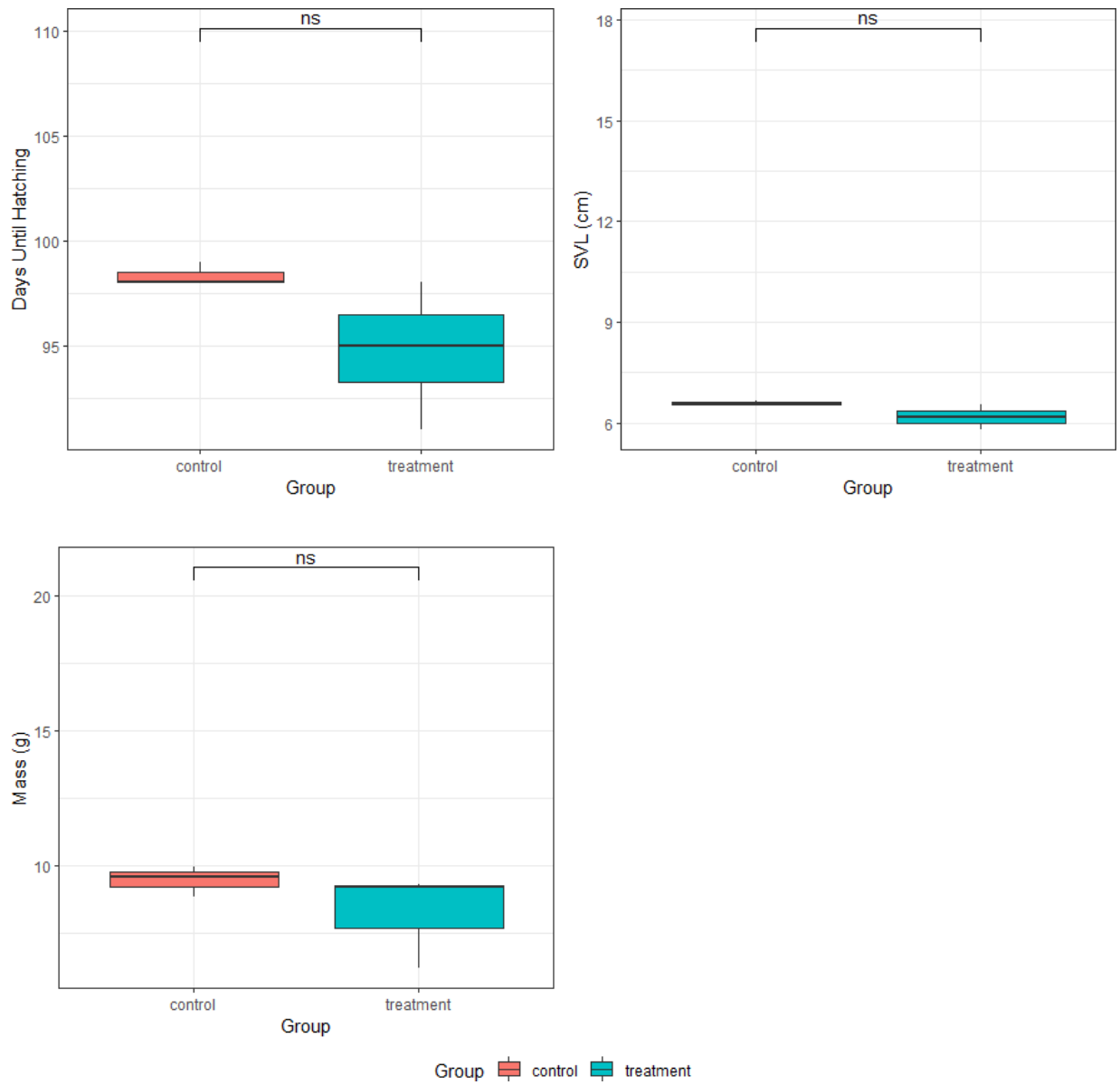


Figure 3: Differences in green iguana treatment and control groups

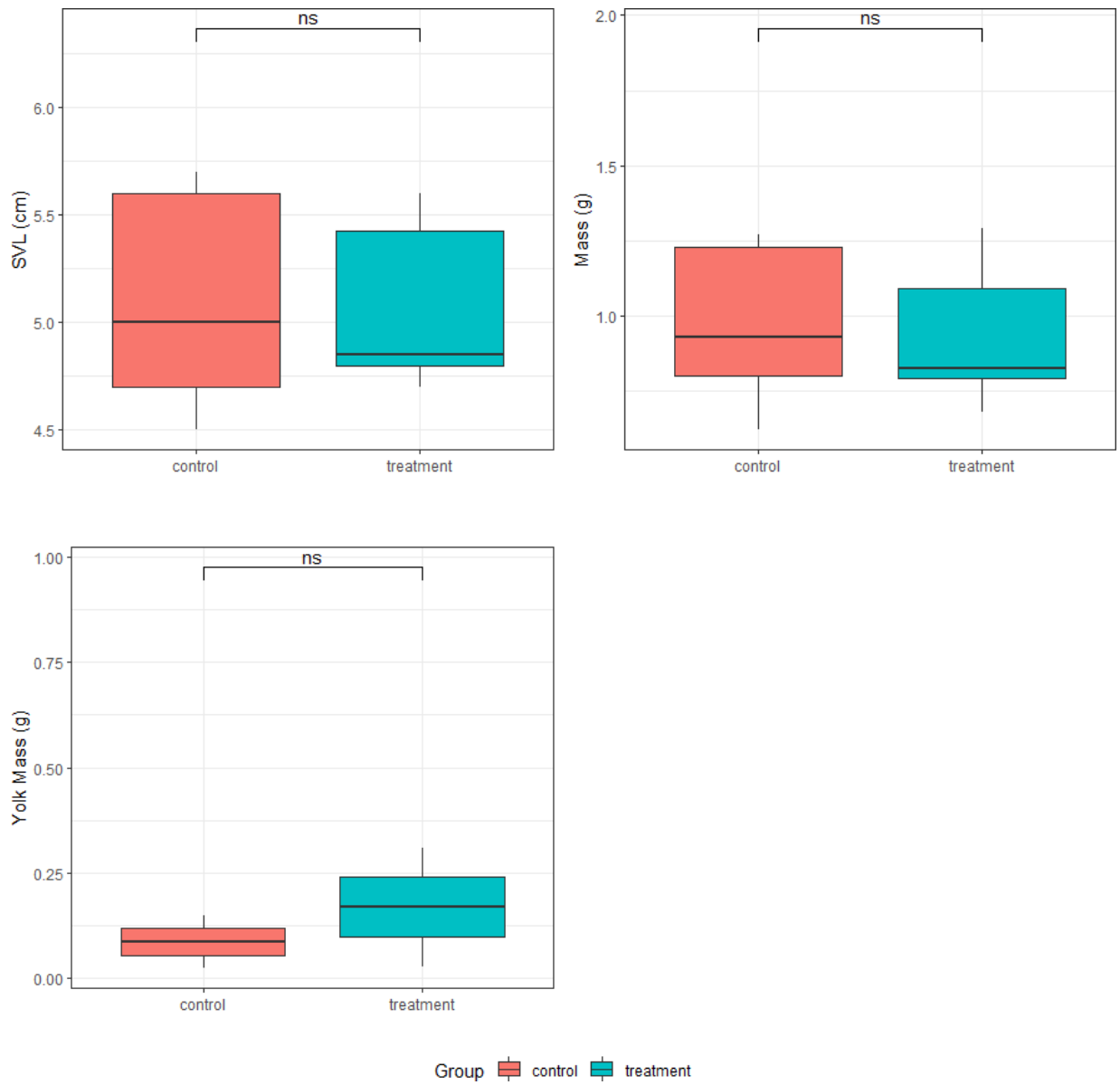


Figure 4: Differences in eastern glass lizard control and treatment groups.

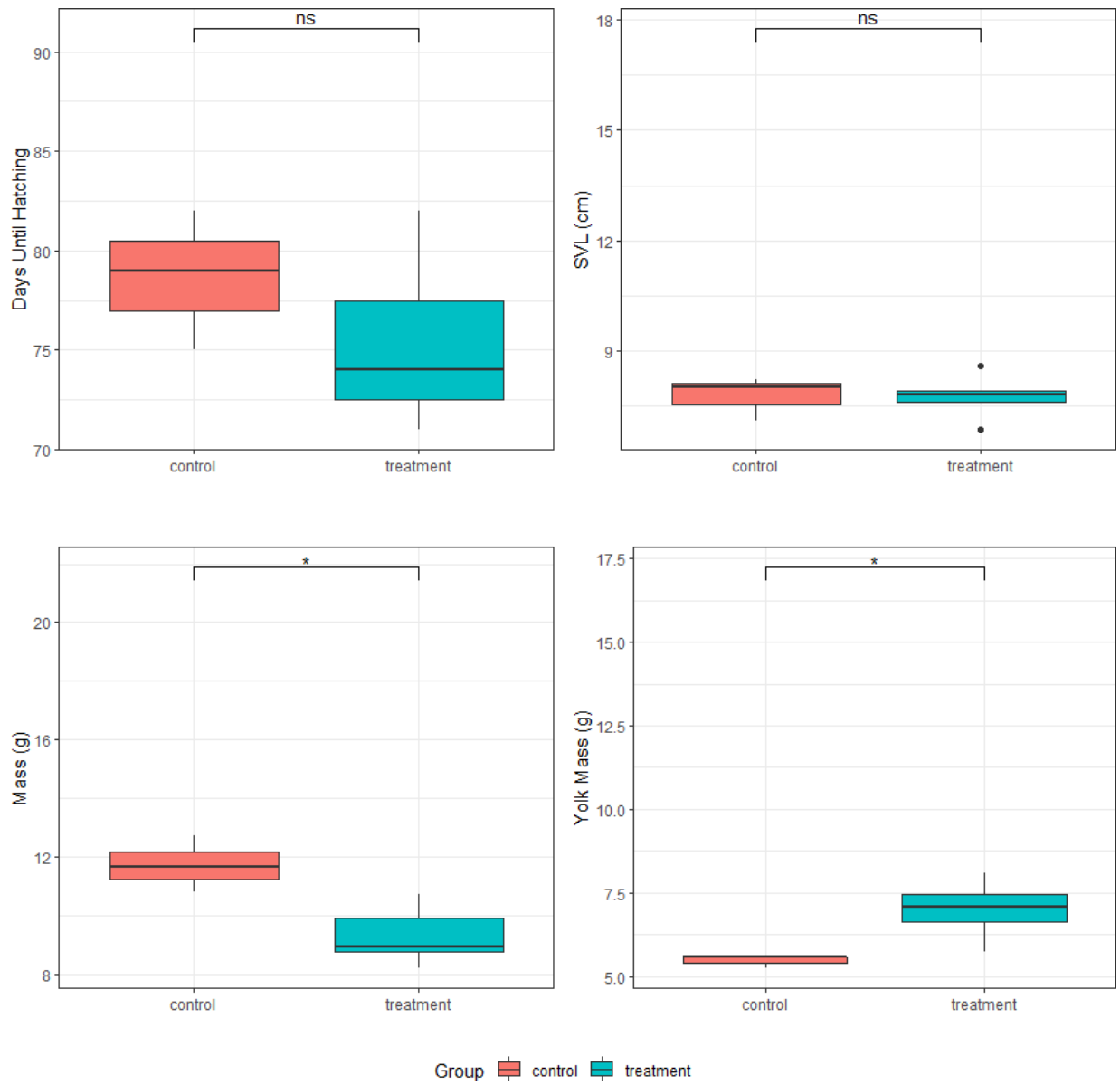


Figure 5: Differences in red tegu control and treatment groups.

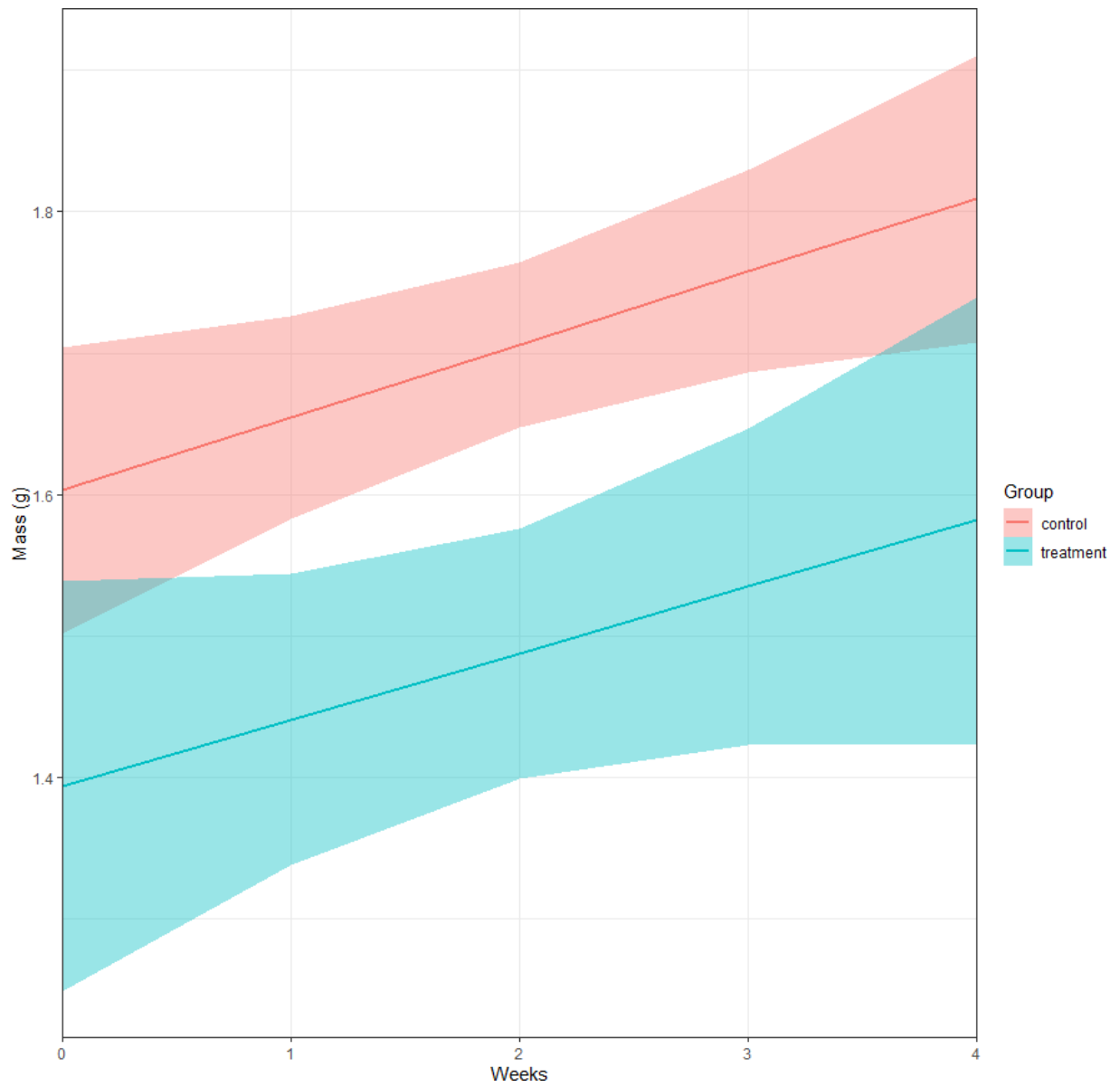


Figure 6: Temporal variation in mass between early hatching (treatment) and spontaneously hatching (control) lizards for crested geckos.

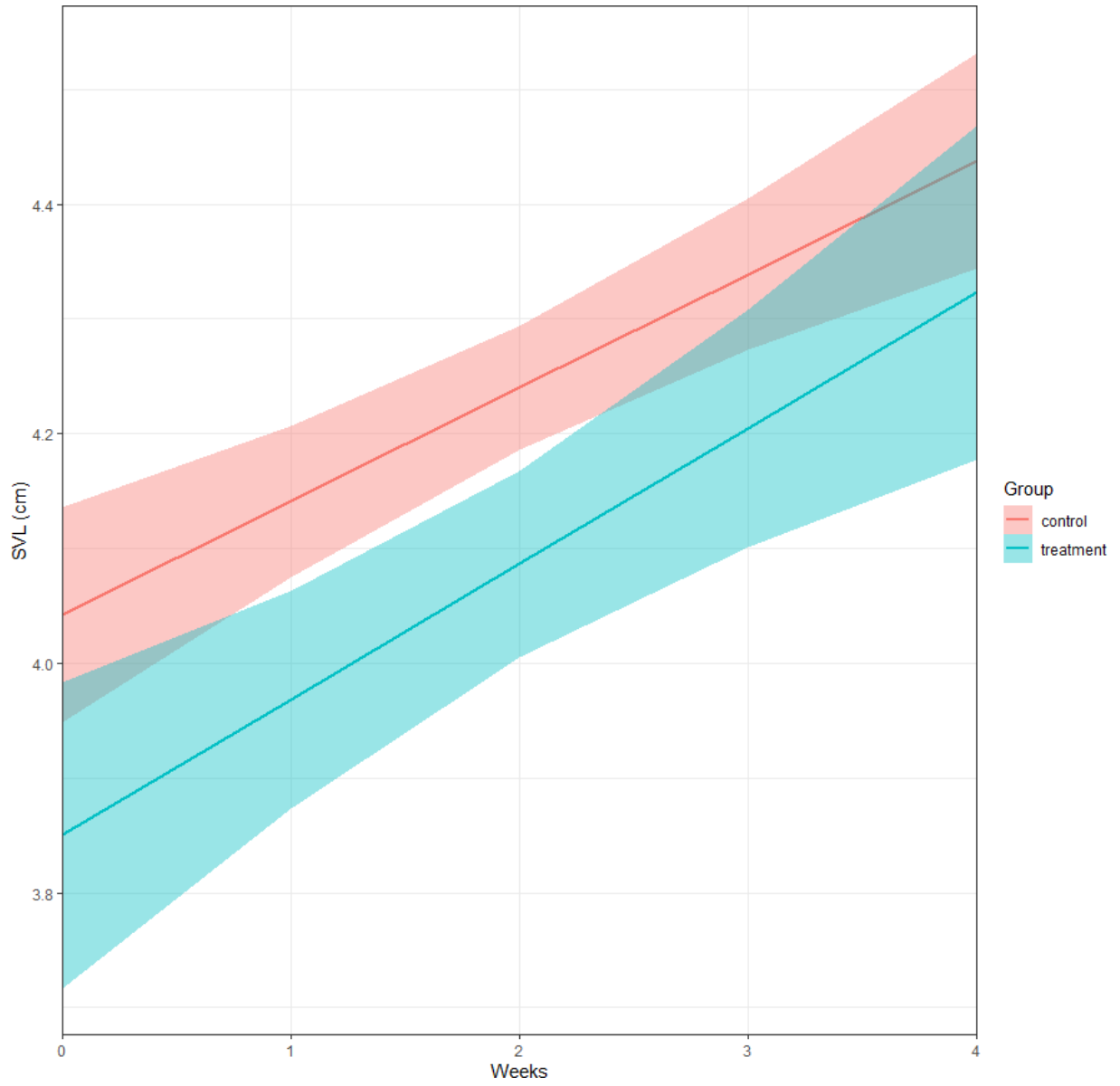


Figure 7: Temporal variation in length (SVL) between early hatching (treatment) and spontaneously hatching (control) lizards for crested geckos.

Chapter 4: Discussion

Major Findings

My study provided four major findings: First, three of the six lizard species exhibited early hatching, representing three new families for which early hatching has been recorded. Second, in each of those three species, I found evidence of a cost of hatching early. On average, early hatching lizards were smaller and lighter than control lizards, in general. Third, three species hatched explosively, bursting from the egg and immediately sprinting away. Finally, growth trials with crested gecko hatchlings revealed that body size costs associated with early hatching were transient in the short-term, visually persisting over the course of a month post-hatching and only being present statistically at week zero. Prior to this study, only empirical evidence existed that one family of lizards exhibited early hatching behavior. However, with the addition of the three species from this study, representing three different families, it brings the total number of families on that list to four. Additionally, data trends and observed behaviors from the present study suggest potential early hatching behavior in two more species. With the addition of the five species from the present study, there is now documentation of early hatching behavior in 21 different species of lizard. These new data suggest that early hatching has deep evolutionary roots and may be a basal trait for squamate reptiles. Alternatively, early hatching may have evolved multiple times within lizards.

Evidence of Early Hatching

The present study provides empirical evidence of early hatching for three different species of lizards (crested geckos, brown basilisks, and red tegus) and suggests, based on data trends and observed behaviors, that early hatching may be present in two more species (Mediterranean geckos and green iguanas). However, these data trends need strengthening with a larger sample size. Crested geckos hatched on average 6.7 days earlier following simulated predation. Hatchlings were fully developed and viable, and this suggests that crested gecko embryos, after competent development, will hatch due to a substantial enough disturbance. Crested geckos hatched with significantly smaller mass, shorter SVL, and more residual yolk; red tegus hatched with a shorter SVL and more residual yolk; brown basilisks hatched with a shorter SVL. While hatching time was not significantly shorter for red tegus, treatment eggs did hatch earlier on average (mean difference of 3.38 ± 4.152 days), and this trend could likely be strengthened with a greater sample size ($n=10$). Additionally, I was not able to investigate how much earlier predator simulated brown basilisk eggs hatched because the lay dates were unknown (they were found in wild nests). However, the presence of reduced SVL, smaller mass and/or increased residual yolk suggests that the threat of predation caused the embryos to hatch at an earlier time than control eggs. A tradeoff between timing of hatching after a predation stimulus and body size has been demonstrated in frogs (Vonesh 2000, Warkentin 1995).

Hatching early due to disturbance has been shown as an effective last resort to escape a potential nest predator (Warkentin 1995). This type of environmentally cued

hatching has however rarely been documented in reptiles, including lizards (Doody 2011). The present study demonstrated that a wider array of lizards than previously thought are capable of hatching early as a means of predator defense; I documented three families of lizards not previously known to hatch early. The addition of 3-5 new families to the list of lizard species known to exhibit early hatching (Doody, 2011; Doody and Paull 2013) suggests that early hatching could be widespread among lizard species.

Immediate Explosive Hatching

Crested geckos, red tegus, Mediterranean geckos and eastern glass lizards all demonstrated hatching behavior during trials. All but eastern glass lizards displayed immediate explosive hatching, escaping the egg and vigorously trying to run away. This behavior directly supports that their escape is an attempt to get away from predators. However, eastern glass lizards had six out of eight (75%) treatment hatchlings pip their eggs during trials and then not attempt to flee or even finish hatching at that time. This suggests that there was hatching due to the disturbance with the egg but that the cue was not read as predation and instead was something else. J.S. Doody (2011) discussed that there are different types of environmentally cued hatching that can occur, one of which is synchronous hatching in which the young use the cues from others in the nest hatching to begin their own hatching. This would ensure that all young are able to hatch and escape while the nest gives off an odor that would attract potential predators. It is very possible that the cue to synchronous hatching is the vibrations of the other eggs hatching and the hatchlings crawling through the nest. This could be the

behavior displayed by eastern glass lizards since synchronous hatching does not pressure the hatchlings to immediately escape from the nest.

Costs of Early Hatching

The present study also revealed trade-offs associated with early hatching. Early hatching was associated with smaller body sizes and more residual yolk in three species (crested geckos, red tegus and brown basilisks). A smaller size in regard to both mass and SVL could make the hatchlings more susceptible to later predation. The residual yolk left behind when hatching represents energy that could have been metabolized and internalized for later use (Doody and Paull 2013). A greater amount of yolk remaining could in turn mean less developmental time, lower endurance and decreased vigor, potentially increasing the likelihood of predation (Warkentin 1995, 1999).

Feeding trials with crested geckos showed that the difference in size between early and spontaneously hatching lizards was transient in the short term. Treatment hatchlings hatched significantly shorter than control hatchlings and this statistical difference was present only for the first week of life. However, Fig.6 and Fig. 7 demonstrate a visual difference in the two groups with treatment lizards being consistently smaller and shorter for the first four weeks and these trends would likely become more prevalent with a greater sample size (n=19). The differences between the two groups narrows over time and there is likely a point in which the two lines meet representing the time in which the costs of early hatching have ended.

Conservation Implications

Understanding early hatching and its trade-offs could serve to influence conservation. Conservation is a field that encompasses many disciplines including behavioral ecology, and behaviors can be used to address ultimate questions like adaptive utility (Blumstein 2004). Early hatching behavior represents the ability of a given species to adapt to changes in predation risk in the earliest and most vulnerable stages of an organism's life history. This behavior could be the distinguishing factor of a species successfully adapting to the introduction of a novel nest predator in an environment. Additionally, the presence of early hatching brings to light the ability of the organism to respond to threats already present in its environment. Knowledge of this behavior in a species could help in strengthening conservation decisions.

Limitations

This study could be improved in several different ways. Firstly, the sample sizes for several species were relatively low, and the results of the study (or the significance of the results) would improve with a greater sample size. This is especially prevalent in the Mediterranean gecko group, because I was only able to obtain 5 eggs across two research seasons. Additionally, for giant ameivas and butterfly lizards, I was unable to obtain any eggs. Finally, for northern curly-tailed lizards, while several eggs were obtained, all of them molded and deceased before hatching. Viability could be improved by capturing more females for each of these three species in the hopes of obtaining more eggs. Though relative humidity was kept constant within the experimental setup, the eggs for northern curly-tailed lizards would also have likely experienced lower egg

mortality with a lower humidity in their dressing cups. Viability could be improved with a ratio of 1:1 vermiculite to water by weight or with increased air circulation in the dressing cups.

Some eggs also did not withstand the trials well. Brown basilisk eggs were most sensitive. Several brown basilisk eggs laid in the lab could not be used for the study because after even one session of simulated predation, the eggs would begin to look bruised and deteriorate. Notably, the shells of these eggs felt substantially thinner than the eggs of other species in the study, which could explain the reason behind their sensitivity to the disturbance. This sensitivity prevented me from getting any solid data on the incubation time of treatment and control hatchlings because the only clutches yielding substantial results ended up being from nests that were found, as opposed to clutches laid in the lab. The clutches found in the field were found in a subsequent research season that allowed me to be more aware of the difficulties with the eggs and be gentler potentially leading to greater success. However, a different method of testing them would probably be ideal. Eastern glass lizard eggs were also found in the field, rather than laid in the lab, preventing my analysis of incubation times for either group. Having mothers in the lab to lay eggs would be ideal, but the species is notoriously inconspicuous, which would make it difficult to find enough gravid females.

Future Studies

Future studies could focus on several things to help expand our understanding of early hatching. First, research into other families of squamate reptiles that do not yet have quantitative data supporting the presence early hatching would further our

understanding of the evolutionary significance of early hatching. The more clades that are studied, the greater our knowledge of the importance of this behavior will be. Future studies should also look to increase the scope of our understanding of the costs associated with early hatching. One method could be to increase the length of the feeding trials past four weeks, where hatchlings are fed *ad libidum* to find the time at which the mass and SVL of each group become more notably similar. Studies could also test the performance and endurance of hatchlings by running them on treadmills.

References

- Aparicio Ramirez, A., Perez, K., & Telemeco, R. S. (2021). Thermoregulation and thermal performance of crested geckos (*Correlophus ciliatus*) suggest an extended optimality hypothesis for the evolution of thermoregulatory set-points. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, 335(1), 86-95.
- Bates, Douglas, Maechler, Martin, Bolker, Ben, Walker, Steve (2015). Fitting Linear Mixed-Effects Models Using lme4. *Journal of Statistical Software*, 67(1), 1-48. doi:10.18637/jss.v067.i01.
- Benard, M. F. (2004). Predator-induced phenotypic plasticity in organisms with complex life histories. *Annu. Rev. Ecol. Evol. Syst.*, 35, 651-673.
- Blumstein, D., & Fernández-Juricic, E. (2004). The Emergence of Conservation Behavior. *Conservation Biology*, 18(5), 1175-1177.
- DeWitt, T. J., and Scheiner, S. M. (Eds.). (2004). *Phenotypic plasticity: functional and conceptual approaches*. Oxford University Press.
- Doody, J.S. (2011). Environmentally Cued Hatching in Reptiles. *Integrative and Comparative Biology*, 51(1): 49–61.
- Doody, J. S., Coleman, K. E., Coleman, L., and Stephens, G. 2018. *Anolis sagrei* (Brown Anole). Environmentally cued hatching. *Herpetological Review* (In press)
- Doody, J. S., Ellis, R., and Rhind, D. (2015). *Gehyra australis* (Tree Dtella) and *Gehyra pilbara* (*Pilbara dtella*). Environmentally Cued Hatching. *Herpetological Review* 46:46:257-258.

- Doody, J. S., & Paull, P. (2013). Hitting the ground running: environmentally cued hatching in a lizard. *Copeia*, 2013(1), 160-165
- Doody, J. S. and Schembri, B. (2014a). *Acritoscincus platynota* (Red-throated Skink). Environmentally cued hatching. *Herpetological Review* 45:693.
- Doody, J. S. and Schembri, (2014b). B. *Carlia schmeltzii* (Robust Rainbow Skink). Environmentally cued hatching. *Herpetological Review* 45:494.
- Doody, J. S., Stewart, B., Camacho, C., & Christian, K. (2012). Good vibrations? Sibling embryos expedite hatching in a turtle. *Animal Behaviour*, 83(3), 645-651.
- Godfrey S. T., Duberstein J. A., Mota J., Moore W. (2018). *Anolis carolinensis* (Green Anole). Nest Sites and Communal Nesting. *Herpetological Review* 49(1). 115.
- Hegrenes, S. (2001). Diet-induced phenotypic plasticity of feeding morphology in the orangespotted sunfish, *Lepomis humilis*. *Ecology of Freshwater Fish*, 10(1), 35-42.
- Hernandez, A., Villavicencio, W., Ljustina, O. and Doody, J. S. 2017. *Anolis equestris* (Cuban Knight Anole). Environmentally Cued Hatching. *Herpetological Review* 48:841.
- Kassambara, Alboukadel (2020). ggpubr: 'ggplot2' Based Publication Ready Plots. R package version 0.4.0. <https://CRAN.R-project.org/package=ggpubr>
- Kassambara, Alboukadel (2021). rstatix: Pipe-Friendly Framework for Basic Statistical Tests. R package version 0.7.0. <https://CRAN.R-project.org/package=rstatix>
- Krysko, K. L., Enge, K. M., Townsend, J. H., Langan, E. M., Johnson, S. A., & Campbell, T. S. (2005). New county records of amphibians and reptiles from Florida. *Herpetological Review*, 36(1), 85-87.
- Losos, J. B., Schoener, T. W., & Spiller, D. A. (2003). Effect of immersion in seawater on egg survival in the lizard *Anolis sagrei*. *Oecologia*, 137(3), 360-362.

- Moreira, P. L., & Barata, M. (2005). Egg mortality and early embryo hatching caused by fungal infection of Iberian rock lizard (*Lacerta monticola*) clutches. *The Herpetological Journal*, 15(4), 265-272.
- Petzoldt, Thomas (2020). growthrates: Estimate Growth Rates from Experimental Data. R package version 0.8.2. <https://CRAN.R-project.org/package=growthrates>
- Price, T. D., Qvarnström, A., & Irwin, D. E. (2003). The role of phenotypic plasticity in driving genetic evolution. *Proceedings of the Royal Society of London. Series B: Biological Sciences*, 270(1523), 1433-1440.
- R Core Team (2020). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL <https://www.R-project.org/>.
- Rose, F., & Barbour, C. (1968). Ecology and Reproductive Cycles of the Introduced Gecko, *Hemidactylus turcicus*, in the Southern United States. *The American Midland Naturalist*, 79(1), 159-168. doi:10.2307/2423161
- RStudio Team (2021). RStudio: Integrated Development Environment for R. RStudio, PBC, Boston, MA URL <http://www.rstudio.com/>.
- Sih, A., & Moore, R. D. (1993). Delayed hatching of salamander eggs in response to enhanced larval predation risk. *The American Naturalist*, 142(6), 947-960.
- Touchon, J. C., McCoy, M. W., Vonesh, J. R., & Warkentin, K. M. (2013). Effects of plastic hatching timing carry over through metamorphosis in red-eyed treefrogs. *Ecology*, 94(4), 850-860.
- Trussell, G. C. (1997). Phenotypic plasticity in the foot size of an intertidal snail. *Ecology*, 78(4), 1033-1048.
- Schoeppner, N. M., & Relyea, R. A. (2009). Phenotypic plasticity in response to fine-grained environmental variation in predation. *Functional Ecology*, 23(3), 587-594.
- Vitt, L. J. (1991). Ecology and life history of the scansorial arboreal lizard *Plica plica* (Iguanidae) in Amazonian Brazil. *Canadian Journal of Zoology*, 69(2), 504-511.

- Vonesh, J. R. (2000). Dipteran predation on the arboreal eggs of four *Hyperolius* frog species in western Uganda. *Copeia*, 2000(2), 560-566.
- Warkentin, K. M. (1995). Adaptive plasticity in hatching age: a response to predation risk trade-offs. *Proceedings of the National Academy of Sciences*, 92(8), 3507-3510.
- Warkentin, K. M. (1999). Effects of hatching age on development and hatchling morphology in the red-eyed tree frog, *Agalychnis callidryas*. *Biological Journal of the Linnean Society*, 68(3), 443-470.
- Warkentin, K. M. (2000). Wasp predation and wasp-induced hatching of red-eyed treefrog eggs. *Animal Behaviour*, 60(4), 503-510.
- Warkentin, K. M. (2002). Hatching timing, oxygen availability, and external gill regression in the tree frog, *Agalychnis callidryas*. *Physiological and Biochemical Zoology*, 75(2), 155-164.
- Warkentin, K. M. (2005). How do embryos assess risk? Vibrational cues in predator induced hatching of red-eyed treefrogs. *Animal Behaviour* 70:59–71.
- Warkentin KM. (2011a). Environmentally cued hatching across taxa: embryos respond to risk and opportunity. *Integr Comp Biol* 51:14–25.
- Warkentin, K. M. (2011b). Plasticity of hatching in amphibians: evolution, trade-offs, cues and mechanisms. *Integrative and Comparative Biology* 51:111–127.
- Warkentin, K. M., Buckley, C. R., & Metcalf, K. A. (2006). Development of red-eyed treefrog eggs affects efficiency and choices of egg-foraging wasps. *Animal Behaviour*, 71(2), 417-425.
- Warkentin KM, Caldwell MS. (2009). Assessing risk: embryos, information, and escape hatching. In: Dukas R, Ratcliffe J, editors. *Cognitive ecology II. The evolutionary ecology of learning, memory, and information use*. Chicago: University of Chicago Press. p. 177–200.

- Warkentin, K. M., Caldwell, M. S., Siok, T. D., D'Amato, A. T., & McDaniel, J. G. (2007). Flexible information sampling in vibrational assessment of predation risk by red-eyed treefrog embryos. *Journal of Experimental Biology*, 210(4), 614-619.
- Warkentin, K. M., Currie, C. R., & Rehner, S. A. (2001). Egg-killing fungus induces early hatching of red-eyed treefrog eggs. *Ecology*, 82(10), 2860-2869.
- Warkentin, K. M., Diaz, J. C., Güell, B. A., Jung, J., Kim, S. J., & Cohen, K. L. (2017). Developmental onset of escape-hatching responses in red-eyed treefrogs depends on cue type. *Animal Behaviour*, 129, 103-112.
- Webb, G. J., Choquenot, D., & Whitehead, P. J. (1986). Nests, eggs, and embryonic development of *Carettochelys insculpta* (Chelonia: Carettochelidae) from Northern Australia. *Journal of Zoology*, 1(3), 521-550.
- Wickham et al., (2019). Welcome to the tidyverse. *Journal of Open Source Software*, 4(43), 1686, <https://doi.org/10.21105/joss.01686>