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Group Housing and Social Stress in Side-Blotched Lizards (Uta stansburiana)

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GROUP HOUSING AND SOCIAL STRESS IN SIDE-BLOTCHED LIZARDS (*UTA STANSBURIANA*)

by

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Abstract

Stress in regards to an animal's social housing environment is well studied in mammals; however there are few studies examining this in reptiles and the results are thus far unclear. For example, behavioral evidence shows adverse effects from individual housing in juvenile chameleons, however physiological measures in whiptail lizards show adverse effects from group housing. Because some reptiles appear to be affected negatively by their social housing environment while others are impacted positively, if we can discover the reason for these differences, we will be able to implement the most effective housing regimes for reptiles. In this study, we tested if male and female side-blotched lizards (Uta stansburiana) experienced physiological changes such as 1) an increase in circulating corticosterone concentrations, an energy-mobilizing hormone indicative of stress, 2) changes in reproductive function and 3) decreasing immunocompetence when housed with and without a conspecific. We found that while there were no significant physiological changes in females, males housed alone had significantly higher corticosterone concentrations than males housed with females, and males housed with a female also had higher testosterone levels. Additionally, group-housed males had relatively reduced immune function when compared to individually-housed males. The difference among sexes might have been a result of stronger territoriality exhibited by males. Furthermore, the difference among species, when comparing this study to previous studies, may be because U. stansburiana are relatively more social reptiles, spending more time with conspecifics in nature, which indicates that the social structure of the species should be accounted for when making housing decisions. Our results suggest that housing male reptiles individually might be stressful, and that males group-housed with female conspecifics maintain higher levels of testosterone indicative of better reproductive function. This group housing is not

without cost, however, as animals with elevated testosterone also show reduced immune ability. Therefore, reptile husbandry facilities may wish to take the species in question into consideration as well as the reproductive-immune trade-off when determining housing regimes for reptiles in breeding programs compared to those on exhibit.

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Introduction

Social stress can have as extensive effects on an animal's physiology as physical stressors (Sapolsky 2004). For this reason it is important to consider an animal's psychological health with respect to its social environment when housing animals in captivity. Housing animals in inappropriate social situations can have drastic effects on an animal's behavior and overall health (Ballen, et al. 2014; McLeod, et al. 1997; Søndergaard and Ladewig 2004), potentially because of the physiological changes associated with stress (Sapolsky 2005).

Reptiles have widely been considered non-social animals despite many having very complex social hierarchies and extended interaction time with conspecifics (Doody, et al. 2013). Perhaps because of this misconception, few studies have examined social situations such as group and individual housing in reptiles (Ballen, et al. 2014; Gustafson and Crews 1981). Although these studies examined stress via behavioral changes and reproductive success, changes in circulating hormone concentrations can further elucidate the effects of different housing strategies, and have as yet remained understudied.

When confronted with a stressor, animals undergo a hypothalamic-pituitary-adrenal-axis hormone cascade that releases energy-mobilizing glucocorticoids (Sapolsky, et al. 2000). Specifically, reptiles release corticosterone (CORT) (Moore and Jessop 2003). When confronted with an acute stressor (such as a predator), glucocorticoids promote immune function, consequently increasing immediate survival. However, persistently high levels of CORT as a result of chronic stress can cause or a decrease in long term survival by suppression of the immune system (Dhabhar 2009), as well as a decrease in reproductive function (Moore 1991). Alternatively, animals may reallocate energy from the immune system to the reproductive

system in times of chronic stress in order to increase their immediate reproductive success (Adamo, et al. 2001; French, et al. 2007). This is not without cost as the animals in these studies experienced decreased innate immunocompetence and reduced wound healing respectively. Thus, increased reproductive investment can have an immunosuppressive effect (Folstad and Karter 1992). Therefore, in addressing questions pertaining to endocrine physiology, it is important to measure immune and reproductive function in addition to circulating corticosterone levels to address the physiological effects due to stress.

For this study we used the side-blotched lizard (*Uta stansburiana*) because they are territorial and abundant (Lucas & French 2012), and live in relatively higher population density than lizards from similar studies, with male territories overlapping with multiple females' territories (Parker 1972; Parker 1974). This allowed for us to examine sociality as well as physiology. Our study aimed to determine whether *U. stansburiana* would have higher circulating corticosterone concentrations when housed with conspecifics, and if this would trigger changes in reproductive physiology or innate immune function. We used two experiments, the first study focusing on males and the second focusing on females. We expected that lizards housed with conspecifics would have higher corticosterone, and would show higher reproductive investment (males having higher testosterone and females having greater follicle growth), similar to a previous study done on *Cnemidophorus inornatus* (Lindzey and Crews 1988). Additionally, we expected a trade-off between reproduction and immunocompetence resulting in reduced immune function for group-housed lizards.

Methods

Collection

We captured 42 male lizards in May of 2013, and 33 females and 10 males in May of 2014 from established field sites in Washington County, Utah, USA. Animals were transported to laboratory housing and allowed to acclimatize individually for five days prior to the start of both experiments. For the male study, we used 24 females already housed in the laboratory for a different experiment.

Housing

Lizards were housed in a rack system with 30x45x15 cm translucent plastic containers. The lizards were housed on newspaper substrate and each container had one shelter. The containers maintained an ambient temperature of 23°C and a heat strip and ultraviolet light source was provided to create a thermal gradient for behavioral thermoregulation. The room was set to a photoperiod cycle of 14L:10D and a constant relative humidity of 20%. Lizards were fed a maintenance diet of crickets (Flukers Farms, Port Allen, Louisiana, USA) three times a week and water was provided *ad libitum*. All procedures were approved by the Utah State University Institutional Animal Care and Use Committee (protocol #2068).

For the experiment focusing on the males, 24 of the males were randomly paired with a female and the remaining 18 males were individually housed. For the female experiment, females were randomly split into three treatment groups: individually housed females (n = 11), females housed with one female conspecific (n = 12), and females housed with one male conspecific (n = 10).

Data Collection

Prior to the experiment, we measured each lizard's mass and snout-to-vent length (SVL) to determine body condition and found no significant difference in body condition between treatment groups ($p = 0.3945$ for males; $F_{32,2} = 2.5684$, $p = 0.0934$ for females). Lizards were housed together for eight days in the male experiment, and nine days for the female experiment, and blood samples were collected on the ninth day and tenth days, respectively, via the retro-orbital sinus using a heparinized capillary tube. All samples were collected within three minutes of capture (Romero and Reed 2005). Whole blood was centrifuged for 1 m at 2200 rpm and the plasma was separated from the red blood cells and stored at -20°C for subsequent analysis. With the plasma we measured the innate immune system because it acts as a first line of defense; innate immune responses take effect quickly while adaptive immune responses may not take effect for days or weeks, particularly in reptiles (Zimmerman, et al. 2010). We measured male and female reproductive function by measuring circulating testosterone and follicular growth.

Reproductive Investment in Females

Three days prior to the start of the study, we palpated and ultrasounded females to determine reproductive stage and total follicle length (*i.e.* the sum of all follicle lengths for an individual). There was no difference in initial average follicle length among treatment groups at the start of the experiment ($F_{2,30} = 2.6789$, $p = 0.0862$). Follicle measurements were repeated at the conclusion of the experiment on the day the lizards were bled to determine total follicular growth. Reproductive investment in females was measured as total follicular growth, the sum of all the follicles, from the beginning of the experiment to the end. Because some females laid eggs during the experiment, the initial follicle length was subtracted from an average follicle size at laying calculated for female *U. stansburiana*, and this value was added to the follicle length measured at the end of the experiment. The average follicle length at laying was approximated from 14 clutches of 2-5 eggs each, for a total of 51 eggs (mean = 114.4 mm, $SE = 0.51$ mm).

Hormone Assays

Circulating testosterone and corticosterone hormone levels were determined using a radioimmunassay adapted from Moore (1986). Samples were extracted with a 30% ethyl acetate / isooctane phase and flash-frozen to decant the nonpolar solvent from the aqueous phase. The ethyl acetate phase was then dried and resuspended in a phosphate buffer solution. Samples were assayed in duplicate, and separate assays were used to measure CORT and testosterone. The intra-assay coefficient of variation was 0.277 for male corticosterone, 0.159 for male testosterone, and 0.147 for female corticosterone.

Bacterial Killing Assay

Each lizard's bactericidal ability was determined through a bacterial killing assay as described in French and Neuman-Lee (French and Neuman-Lee 2012). We combined the plasma with a phosphate buffer solution in a 96 well microplate; each sample was measured in triplicate. *E. coli* was added and the plates were incubated for 30 min at 37°C and read at 300 nm (BioRad xMarkTM Microplate Absorbance Spectrophotometer) to determine background absorbance. The plates were incubated a final 12 hours and then read to determine the sample's bactericidal ability.

Data Analysis

To meet assumptions of normality, corticosterone and testosterone values were $\log_{10}$ transformed. These data were analyzed using Student's t tests for the experiment focusing on male physiology, and an ANOVA for the corticosterone data in the experiment focusing on the females. Bactericidal ability values could not be transformed to meet assumptions of normality and thus were analyzed using a non-parametric Wilcoxon/Mann-Whitney test for the males and Wilcoxon/Kruskal-Wallis test for the females. Reproductive investment data were normal in

females and were analyzed using an ANOVA. Statistical analyses were performed using JMP (8.0.1, Cary, NC, USA) statistical software, and an α-value of $p = 0.05$.

Results

Individually housed male lizards had significantly higher circulating corticosterone concentration ($p = 0.0498$, Fig 1), lower circulating testosterone concentration ($p = 0.0275$, Fig 2), and greater bactericidal ability ($p = 0.0486$, Figs 3) than paired males. Three males were found with exceptionally high CORT, suggesting they did not show a typical, comparable reaction, and were excluded for any statistical tests involving CORT. When included, the difference was no longer significant ($p = 0.1586$). The interaction between body condition and treatment had a significant positive correlation with corticosterone concentration ($p = 0.0251$; $p = 0.0173$ with included CORT values), and this accounted for 26% of the variation (22% with included CORT values). The interaction between testosterone and treatment had a significant negative correlation with bactericidal ability ($p = 0.0338$), and accounted for 27% of the variation.

No physiological parameters differed significantly among treatment groups for females; there was no significant difference for bactericidal ability ($H = 1.7141$, $p = 0.4244$, Fig 4), nor corticosterone concentrations ($F_{2,31} = 0.7285$, $p = 0.4912$, Fig 5).

Discussion

Male *U. stansburiana* housed with a female conspecific had different physiological responses than individually-housed males, while female *U. stansburiana* had no physiological differences among treatment groups. Individually housed male *U. stansburiana* experienced higher levels of corticosterone than male *U. stansburiana* housed with female conspecifics.

Group housed males had higher testosterone concentrations, suggestive of higher reproductive investment, but had reduced immunocompetence. A significant correlation between testosterone and bactericidal ability suggests immunosuppression by testosterone (Folstad and Karter 1992). Finally, there was no significant correlation between corticosterone and testosterone, suggesting that the increase in testosterone was not mediated by corticosterone; rather, because males experience increased testosterone when in the presence of a female, as reviewed in Harding (1981). While there are no significant differences among treatment groups, females housed with no conspecifics had negative reproductive investment (*i.e.* they absorbed their follicles and the total follicle length shrunk over time), while females housed with conspecifics had a positive reproductive investment (Fig 6). This trend is not statistically significant; however, it could be biologically significant, suggesting that females reduce reproductive investment in the absence of a social environment.

A previous study (Lindzey and Crews 1988) also examined similar physiological parameters but found differing results in response to group-housing in whiptail lizards, (*Cnemidophorus inornata*). When male whiptail lizards were housed with another female, they experienced higher circulating corticosterone levels and lower circulating testosterone levels than those housed individually. The discrepancy between our results and these may be due to whiptails being relatively less social than other species, thus exhibiting a higher stress response when group-housed. *Uta stansburiana* are quite social, unlike whiptails, which do not spend much time with conspecifics (Crews, et al. 1983; Tinkle 1967). Furthermore, *U. stansburiana* are territorial (Tinkle 1967) while *C. inornatus* are not (Lindzey and Crews 1988). Lindzey and Crews suggest that increased contact with conspecifics in a non-territorial, less social lizard may have caused the increased corticosterone and decreased testosterone levels. Our study supports

the idea that the physiological effect of group housing is species-specific, and may depend on the relative sociality of the reptile.

Similarly, there are not any clear relationships for whether social housing conditions can create changes in circulating adrenal steroid levels indicative of stress in mammals. These relationships appear to be species-specific and contradictory (Arndt, et al. 2009; Bartolomucci, et al. 2003; Hunt and Hambly 2006). Conversely, behavioral evidence of stress in response to housing seems universal across species. For example, wild-caught brushtail possums (*Trichosurus vulpecula*) exhibit higher mortality, anorexia and weight loss, and decreased immune function when they are housed individually(McLeod, et al. 1997). Horses housed in groups of three or more were more easily trained, while horses that were housed individually were more aggressive (Søndergaard and Ladewig 2004). Shelter dogs that were housed together were less noisy and ultimately more adoptable (Mertens and Unshelm 1996). Budgerigars housed in groups of six ate significantly more food and flew more than budgerigars housed solitarily (Nicol and Pope 1993). More specific to reptiles, behavioral evidence in veiled chameleons demonstrated that those housed individually displayed more submissive behavior in contests with group housed individuals, and were less colorful (Ballen, et al. 2014).

Our results suggest that husbandry facilities should consider the costs and benefits of group housing lizards; laboratories should keep these trade-offs in mind with regards to experiment design, and zoos and refuges should consider whether their goal is to maintain a population or expand numbers at the cost of the immune health of the breeders. Because the differences in physiological parameters between lizards appears to be due to their relative sociality, future studies may wish to examine even more social reptiles such as the monogamous

blue-tongued skink (*Tiliqua rugosa*) which is involved in bi-parental care (Pianka and Vitt 2003).

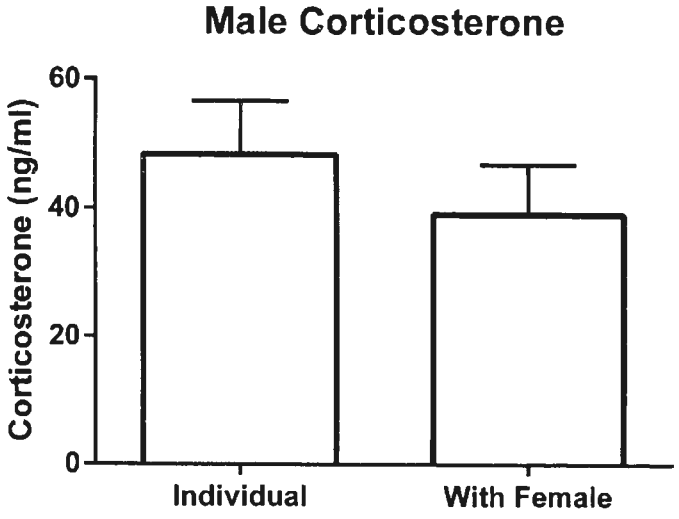

Figure 1. Untransformed circulating corticosterone concentration (ng/ml) by treatment group for male *Uta stansburiana*. Individually housed males had significantly higher plasma corticosterone concentrations than group housed males ($p = 0.0498$).

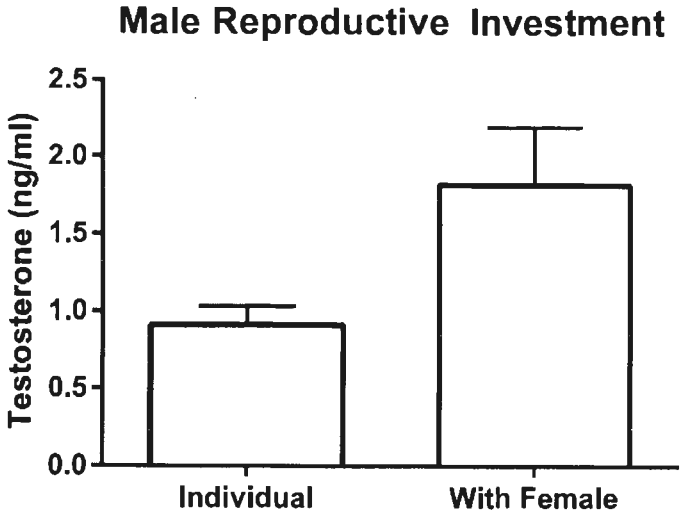

Figure 2. Untransformed circulating testosterone

concentration (ng/ml) by treatment group for male *Uta stansburiana*. Males housed with a female showed significantly higher plasma concentrations than individually housed males ($p = 0.0275$).

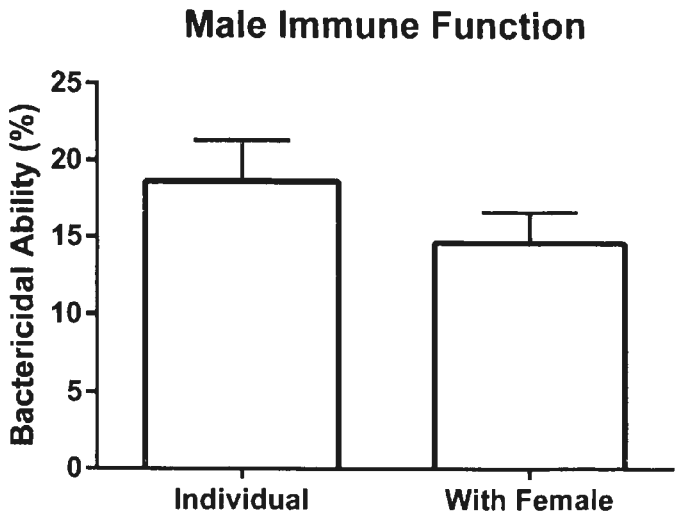

Figure 3. Percent bacteria killed by treatment group for male *Uta stansburiana*. Group-housed males had significantly decreased bactericidal ability than individually housed males ($p = 0.0486$).

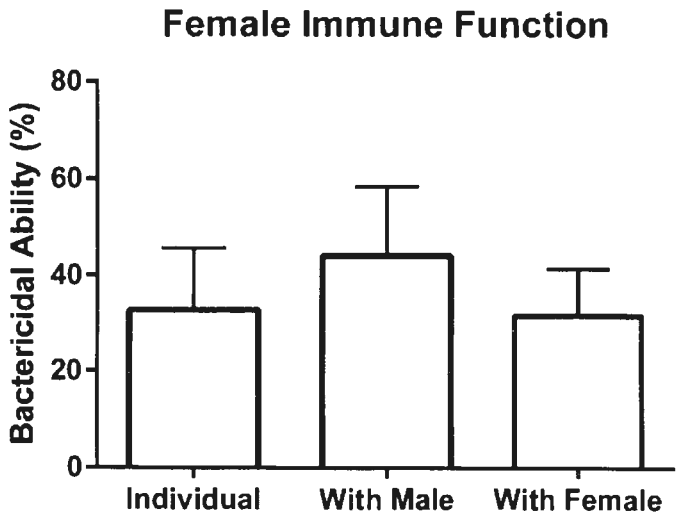

Figure 4. Bactericidal ability by treatment group for female *Uta stansburiana*. There was no significant difference among treatment groups ($H = 1.7141, p = 0.4244$).

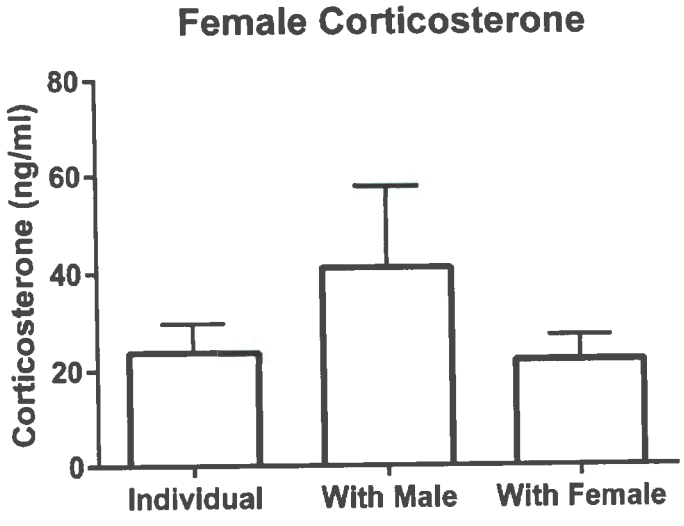

Figure 5. Circulating corticosterone concentration (ng/ml) by treatment group for female *Uta stansburiana*. There was no significant difference among treatment groups ($F_{2,31} = 0.7285$, $p = 0.4912$).

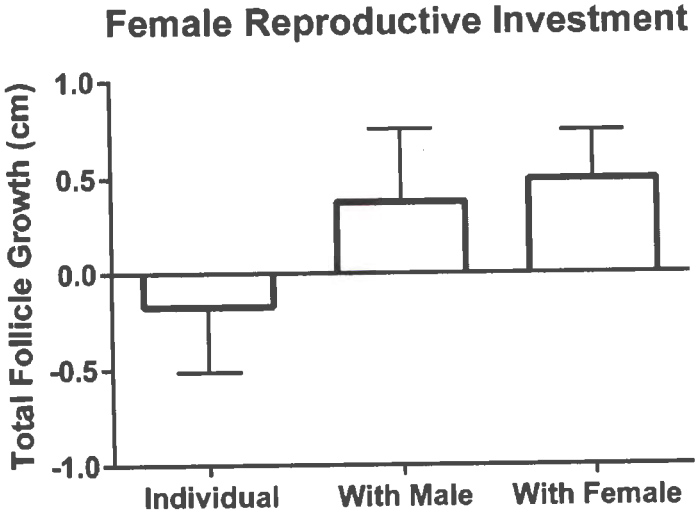

Figure 6. Reproductive investment by treatment group for female *Uta stansburiana*. There was no significant different among treatment groups.

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Reflection

My interest in this project started when I began seriously considering zoological veterinary medicine as a career. I had already worked at the lab for about a year and a half so I had a good grasp on stress physiology and all of its aspects. During a visit to the Hogle Zoo's Reptile House, I noticed that some of their lizards were housed in pairs. This was surprising because reptiles are perceived as entirely anti-social; generally one does not house reptiles in groups. Combining this observation with my background in stress physiology research, I wondered if we could test whether group housing acted as a stressor to lizards by using their plasma hormone concentrations to quantify the effects.

I did not immediately plan a project and propose funding as I was already planning a project for the summer on another topic. However, fate was on my side that month! When I returned from the field with the male lizards for this other project, we decided to pair them up with females from a study a veterinary student was conducting. Her study was looking at reproduction in the lizards and so she needed to allow the lizards to be housed together for several days so they could mate. Remembering my interest in the topic, I met with my faculty advisor and graduate student mentors and we decided to take blood samples after we separated the lizards.

Measuring the hormone concentrations is a very intense process. We do a radioimmunoassay where we take radio-labeled antibody for our hormone (corticosterone and testosterone) and allow it to competitively bind with non-labeled antibody. We can then run it through a machine that measures the fluorescence, and from that we can figure out the concentration in a lizard's plasma. The entire process takes four days, and can span from four to ten hours each day depending on how many samples we are running. While I helped with the

immense corticosterone assay, I was actually able to run the testosterone assay by myself. Over the summer I had helped with enough assays that I would be running this one solo! It went fantastically, however I came down with the stomach flu on the fourth day and was unable to finish it. Luckily the rest of the French lab members working that week were able to jump in and finish it up. (And I learned not to assume that a stomach flu would be over in 24 hours.)

The next spring I decided to take the project one step further and look at how group housing affected females. I wrote up an URCO and a SURCO proposal to pay for the materials and my salary, and I started planning my experiment. The week after finals we headed down to St. George to catch the lizards for the experiment. I needed 30 females and 10 males, but with the help of the other lab members working in the field that summer we were able to catch all of them in one evening. To catch a lizard, you tie a noose out of floss, tape it to the end of a fishing pole, and, with a flick of your wrist, whisk up the lizard of the face of its planet. While field days are spectacular because the lizards only arise when the sun starts baking the rocks (about 10 in the morning), and they retreat once it is too warm (about 1 in the afternoon), field work can sometimes be tedious and long. After we catch the lizards, we "process" them by taking morphometric measurements such as weight, length, color, and more. The plasma must also be centrifuged (in the field), and pipetted off into another tube. Then we release them at the end of the day. It is often difficult to do work because it tends to be extremely windy in St. George during this time of year, and so obtaining measurements and keeping track of field data sheets can be frustrating. Ultimately, I love the fact that I can camp in the middle of one of the most beautiful places on earth with excellent company, all while contributing to science.

Upon returning to the lab in Logan, UT, I started the study. I required the help of the vet student in our lab to do the ultrasounds to measure female reproductive function reliably. She

put in a lot of work to ultrasound 33 lizards in one morning! After we collected all the data and blood samples, it was time to run assays. I played a major lead role on many of the days for each assay, and I was even able to improve my skills in creating the standard curve for the assay. This work is not without a certain amount of elbow grease, and a lot of the time in the lab is spent cleaning dishes and doing animal care. I continued to do animal care until we released the females a month later.

After all the data was collected, I worked on the statistical analysis. This was frustrating because not only must I learn how to use the statistical analysis software, but I must also try to learn or teach myself about all the different kinds of statistical test I could run. I was lucky to be helped by my co-authors, because I could not have moved forward from this step otherwise. I also had to repeat the statistical analyses five different times because I never write down *everything* I look at, and then, often when I repeat the tests to discover the small numerical values I am missing, I obtain different results from the tests I have already run. Of course I always find my mistake, but this would be avoided if I just wrote down all the steps and all the values I obtain from the start of the analysis.

And finally, I started writing. I had absolutely no idea, not the slightest hint!, that writing comprised approximately 70% of the work. Draft after draft after draft. I am lucky to have graduate student mentors that are so invested in helping me succeed. However, the editing is never-ending. At first I thought it was hard to get the first draft out because I didn't know where to start. I was very proud of myself upon finishing the first draft! Yet every comment I fix receives three more. Once I think I am done, I am overwhelmed by the sense that I have not even started! This was the hardest part. Not the field work, not the many statistical repetitions from assuming I will remember what I have done, but the editing.

By going through the whole process I have learned so much. I have learned what it is to be a scientist. I know what it will take to be a graduate student; ultimately, the writing is why I decided not to be. I know that when things get rough in the field, it is best to remain positive because the truth is that you really couldn't do much better than field work. There is always something beautiful, or something to be proud of. And St. George is certainly beautiful; it is a landscape that is almost a living being. It is a great honor to be able to be a part of that. I learned that even if I am sure I will remember something, I should write it down. Any future research projects I conduct will be meticulously recorded in a lab journal or diary. I have learned this lesson the hard way! I learned that writing is a bigger monster than I ever gave it credit for. Most importantly, I learned the importance of having coworkers and friends that support you in your endeavors and who are never too busy to help, though it may be a week or so before they are able to. These were the most important aspects of this project: the relationships I developed and the realization that there is no other way to do it successfully!

My experiences doing this study are what made my experience at Utah State University so fantastic. I will always remember the things that I learned, the wonderful adventures I was a part of, and my accomplishments in my time here. This capstone project will be with me for the rest of my career!

Biography

Marilize Van der Walt studied Biology at Utah State University with a minor in Chemistry. She received an Undergraduate Research Fellowship and a scholarship from the Department of Biology, and was a Presidential Scholar and honors student during her time as an undergraduate. She was a member of the French lab for all four years, and her research on stress physiology in reptiles allowed her to travel across the United States to multiple conferences to present her work. She was the primary author on two separate research projects and helped with many more. Marilize's hard work and dedication to her academic career paid off when she gained acceptance to the Washington-Idaho-Montana-Utah Regional Veterinary Program through the Utah State University School of Veterinary Medicine and the Washington State University College of Veterinary Medicine. As an ambitious student with a passion for saving the world, Marilize plans on obtaining a Doctor of Veterinary Medicine and then going on to complete internship and residency programs in zoological medicine. She then plans to become board certified in zoological veterinary medicine so she can actively participate in the conservation of wildlife through care of zoo animals and continued research with wildlife and zoo animals.