Morph- and sex-specific differences in corticosterone of Arizona tiger salamanders (*Ambystoma mavortium nebulosum*)

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Abstract

Chronic stress can have detrimental effects on long-term health of amphibians. Sex and morph may cause variation in stress within salamanders such as the Arizona tiger salamaders (*Ambystoma mavortium nebulosum*) at the Mexican Cut Nature Preserve due to differences in biotic or abiotic conditions (e.g., resource availability and reproductive opportunities). Established methods of sampling for stress hormones, or glucocorticoids, are often not suitable for working with threatened or endangered species, or for field use. A newly developed non-invasive sampling method of using swabs to collect dermal secretions is a rapid and reliable alternative to sample hormones from amphibian species in the field. We swabbed Arizona tiger salamanders dorsally to collect resting and elevated stress samples. Samples will be assayed using ELISA to detect variation in stress. This study will provide a better understanding of population health and body condition. Future research utilizing this method could clarify the effects of climatic variation and population density on this population.

Introduction

Glucocorticoids are steroids released by vertebrates in response to a stressor to increase chances of short-term survival (Sapolsky et al., 2000). Prolonged glucocorticoid activity, also known as chronic stress, has adverse effects on long-term individual health that can result in increased mortality (Wingfield and Romero, 2010). Environmental factors such as temperature (Rollins-Smith, 2017; Waterhouse et. al, 2017), intraspecific density (Millikin et al., 2019; Cooperman et al., 2004), and resource availability (French et al., 2007) create detectable variation in stress levels within a population. Stress and subsequent survival and reproduction of a population is often significantly influenced by habitat alteration (Wingfield and Romero, 2010). This is especially true in amphibians, as they are often dependent on both terrestrial and aquatic habitats (Semlitsch, 2000) and are extremely sensitive to changes due to physiological and behavioral attributes (Blaustein et al., 2001).

The Mexican Cut Nature Preserve (MCNP) in the Elk Mountains of Colorado is home to a polyphenic population of Arizona tiger salamanders (*Ambystoma mavortium nebulosum*). Individuals of this species may metamorphose and become terrestrial, or forgo metamorphosis to sexually mature and remain aquatic, depending on both genetics and characteristics of the environment - a trait known as facultative paedomorphosis (Whiteman et al., 1996). Stress may play a pivotal role in the life history of these polyphenic animals (Carr and Norris, 1988; Denver, 1997) as well as their lifetime fitness (Houck et al., 1996; Homan et al., 2002b; Lacket et al., 2019). The high elevation environment at MCNP is greatly impacted by increased temperatures and precipitation fluctuations caused by climate change (Randin et al., 2009; Beniston et al., 1997; Pauchard et al., 2016). The unpredictable environmental stressors of climate

change, such as reduced prey availability (Forcada and Hoffman, 2014), elevated water temperatures (Weerathunga and Rajapaksa, 2020), and altered breeding seasons (Blaustein et al., 2001), may be detrimental for long-term amphibian population health (Rollins-Smith, 2017). The sensitivity of the local environment, paired with the facultatively paedomorphic nature of the focal species, makes Arizona tiger salamanders of conservation interest (Lackey et al., 2019; Denoel et al., 2005). Stress hormones have been previously studied in congeners such as eastern tiger salamanders (*Ambystoma tigrinum*; Carr and Norris, 1988) and spotted salamanders (*Ambystoma maculatum*; Davis et al., 2009), but not in polyphenic populations such as at MCNP.

Corticosterone is the primary glucocorticoid released by amphibians in response to stress (Jungreis et al., 1970). Sex differences can create significant corticosterone variation within Ambystomatid species (Davis et al., 2019; Houck et al., 1996; Homan et al., 2002a; Homan et al., 2002b). The energy expense of reproduction differs in males and female salamanders; males typically have lower metabolisms that allow them to migrate to breeding grounds earlier in the year, risking freezing temperatures but increasing chances of locating a mate (Minton, 2001). Alternatively, gravid females that postpone migration for warmer temperatures have increased energy expenditure associated with higher metabolic rates (Morbey and Ydenberg, 2001). Other physiological and behavioral differences of the sexes may create corticosterone variation in response to similar conditions (Homan et al., 2002a). Morph may further influence stress levels due to differential pressure of predation and resource availability (Davis and Milanovich, 2010; Wissinger et al., 2010). Consequences of increased stress within a morph may include decreased fitness, as same-sex tiger salamander paedomorphs and metamorphs were found to have significantly different lifetime fitness (Lackey et al., 2019).

Non-invasive corticosterone sampling allows for a non-lethal, rapid evaluation of population health in species that are typically difficult to monitor, due to their small size and secretive nature (Homan et al., 2002b). A recent study has examined and validated the physiological relevance of dermally secreted corticosterone assays for aquatic Mexican axolotls (*Ambystoma mexicanum*) and mudpuppies (*Necturus maculosus*) that is appropriate for field use (Santymire et al., 2018). This procedure utilizes swabbing techniques to compare baseline, or non-stressed glucocorticoid concentrations (Malisch et al., 2007), to those of post-induced stress (Santymire et al., 2018).

This study is designed to determine if morph- and sex-specific differences occur in corticosterone of the Arizona tiger salamander (*Ambystoma mavortium nebulosum*). Physiological differences between sexes (Minton, 2001; Morbey and Ydenberg, 2001; Lackey et al., 2019) and morphs (Davis and Milanovich, 2010; Wissinger et al., 2010) in a polyphenic species may cause variation in corticosterone over time. Body condition

(Whiteman et al., 1996) and pond characteristics (Denoel et al., 2005; Whiteman et al., 1996; Wissinger et al., 2010) may influence corticosterone as well. If there is variation in adult Arizona tiger salamanders (*Ambystoma mavortium nebulosum*), then we predict that paedomorphs will ultimately have the highest corticosterone concentrations due to restricted prey availability and high population density in aquatic environments (Davis and Milanovich, 2010; Wissinger et al., 2010).

Methods

At MCNP, we used dip nets to catch 76 adult Arizona tiger salamanders (Table 1). Capture sites were of similar water depth and temperature (Figure 1). Within 3 minutes of animal capture (Romero and Reed, 2005), a baseline corticosterone sample was collected. While wearing powder-free latex gloves, the animal was held securely without touching the mid-dorsum. A 4 x 1 centimeter area of the mid-dorsum was swabbed 6 times using a cotton-tipped wooden swab precut to 4 cm. Samples were stored at ambient temperature within 2 mL sterile plastic vials, pre-prepared with 1 mL of 70% ethanol (Santymire et al., 2018).

The salamanders were then placed into plastic buckets of pond water immediately after initial sampling. Eighteen minutes after capture, stress was induced through manual restraint within water for 5 minutes. Animals were brought out of the water once a minute for 15 seconds. Elevated stress samples were collected at 0, 30, 60, 90 and 120 minutes post-stressor. Water temperature was measured directly after induced stress and 120 minutes post-stressor. After all samples were completed, animals were measured for snout to vent length (SVL; mm), total length (mm), wet mass (g), body and gill condition, and reproductive status before releasing them at their original location. Additional information such as age, life history, and individual identification (toe clips or PIT tags) was also recorded. Body condition was calculated by dividing mass by SVL (Earl and Whiteman, 2010).

Within 12 hours of collection, samples were stored at 4.4°C to prevent evaporation. Corticosterone ELISA kits (Arbor Assays) validated for axolotl dermal corticosterone secretions (Santymire et al., 2018) will be used to test samples in duplicate.

A linear mixed effects model will be used to compare samples in R. To determine variation in baseline corticosterone samples, morph, sex, pond, body condition, and water temperature will be examined as predictor variables. Animal ID and pond will be analyzed as random effects of corticosterone concentration. To examine stress responses over time, time of sample (0, 30, 60, 90, and 120 minutes post-stressor) will be examined.

Current and Expected Results

Metamorph males and paedomorphic females are predicted to have the highest corticosterone concentrations, both initially and over time (Figures 2 and 3), as these groups have fewer opportunities to find suitable mates (Lackey et al., 2019). If we find that corticosterone variation is similar within each sex, then we predict that both sexes of paedomorphs will have higher concentrations of corticosterone than metamorphs, as intraspecific competition for resources is significantly higher in permanent ponds when compared to semi-permanent ponds (Denoel et al., 2005; Whiteman et al., 1996; Wissinger et al., 2010).

Sex and body condition influence an individuals life history, as there are tradeoffs for metamorphosing (Lacket et al., 2019). These tradeoffs often lead to uneven sex:morph ratios in a polyphenic population, and are apparent in the MCNP Arizona tiger salamander population and are reflected in our sampling pool (Table 1). Body condition may play a role in corticosterone variation (Figure 4).

During this study we discovered that swab color varied between metamorphs and paedomorphs. Swabs were always white when sampling secretions of metamorphs; when sampling paedomorphs, their secretions were consistently a dark grey color, and the swabbing site would also turn grey. This may be due to differences in slime coats and skin between the morphs (Stuelpnagel and Reiss, 2005).

Revised Timeline

Data Collection	7/2/2020 - 08/01/2020
Sample Analysis at Murray State University	8/31/2020 - ?
Data Analysis	8/31/2020 - ?

Discussion

Sex and morph may cause baseline corticosterone variation in adults within the MCNP Arizona tiger salamander population, as well as causing a difference in stress responses over time. A non-invasive method of sampling secreted corticosterone could provide insight regarding the effects of stress on growth, fitness, and dispersal. Sampled over time, such data will provide a better understanding of the impacts of climate change and other environmental stressors, such as intraspecific density and predator density, on individual body condition, corticosterone concentration, morph, and overall population health.

Smaller or thinner animals, especially paedomorphs in intraspecific-dense ponds, are predicted to have higher corticosterone than larger, fatter animals due to intraspecific

competition for limited prey availability (Whiteman et al., 1996) and risk of becoming victim to cannibalism (Wissinger et al., 2010; Denoel et. al, 2006). Such conditions may cause corticosterone to be highest in small paedomorphs (Wissinger et al., 2010).

As ectothermic animals, water temperature variation could influence corticosterone. Increased water temperatures have environmental impacts such as altered pH and reduced growth rate in amphibians (Keen and Schroeder, 1975). We expect animals of the same pond and body condition to have similar corticosterone concentrations, due to environmental characteristics such as resource availability (Forcada and Hoffman, 2014) and population density (Wissinger et al., 2010). If these results are not similar, then we can show that the predictor variables of morph and sex influence corticosterone variation.

Future research into this subject would allow exploration into how individuals of the MCNP population vary in stress and body condition over time or by season, through annual sampling. Additionally, this study could provide a foundation for studying the effects of environmental variation on stress. Elevational gradient could impact stress through variation of water oxygen content and rate of snowmelt (Ding et al., 2009). Climatic variation, such as altered temperatures and hydroperiod, may increase stress (Rollins-Smith, 2017; Waterhouse et. al, 2017). Intraspecific population and correlated resource availability could cause significant stress variation in individuals of differing body conditions (Millikin et al., 2019; Cooperman et al., 2004; French et al., 2007). Finally, cannibalistic predator density could have an interesting effect on paedomorphic corticosterone concentrations (Wissinger et al., 2010) on corticosterone variation. Chronic stress could increase an individual's susceptibility to chytrid through suppression of the immune system (Davidson et al., 2003).

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Tables and Figures

Tables

Table 1. Biometrics of sampled Arizona tiger salamanders (*Ambystoma mavortium nebulosum*).

Morph	Sex	# of animals sampled	Mean SVL (mm)	Range	Mean mass (g)	Range	Mean body condition
Metamorph	Female	21	93.86	80 – 106	31.68	17.15 – 64.95	0.338
Metamorph	Male	11	92.09	84 – 100	26.77	18.83 – 38.36	0.291
Paedomorph	Female	14	89.64	73 – 117	29.71	10.64 – 78.82	0.331
Paedomorph	Male	30	85	75 – 105	19.17	10.18 – 36.06	0.226

Figures

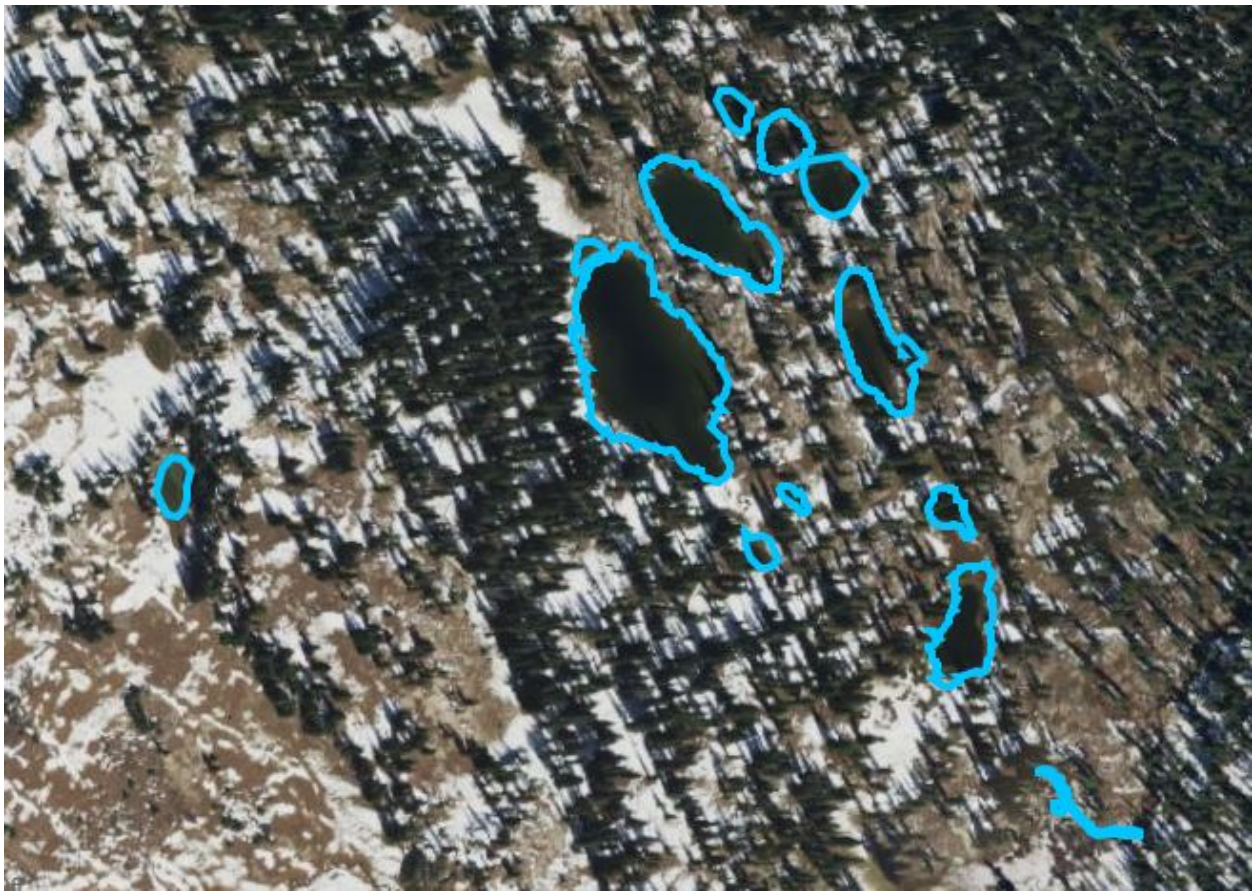

Figure 1. Ponds at the Mexican Cut Nature Preserve that Arizona tiger salamanders (*Ambystoma mavortium nebulosum*) were captured from.

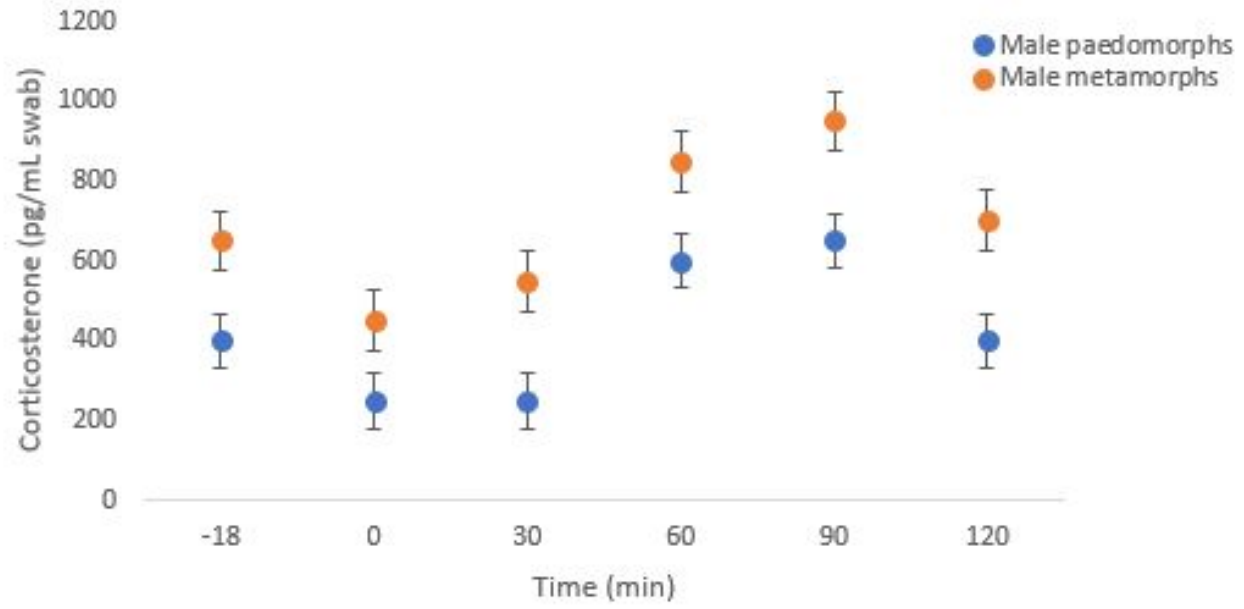

Figure 2. Expected average corticosterone concentrations of male paedomorph and metamorph Arizona tiger salamanders (*Ambystoma mavortium nebulosum*).

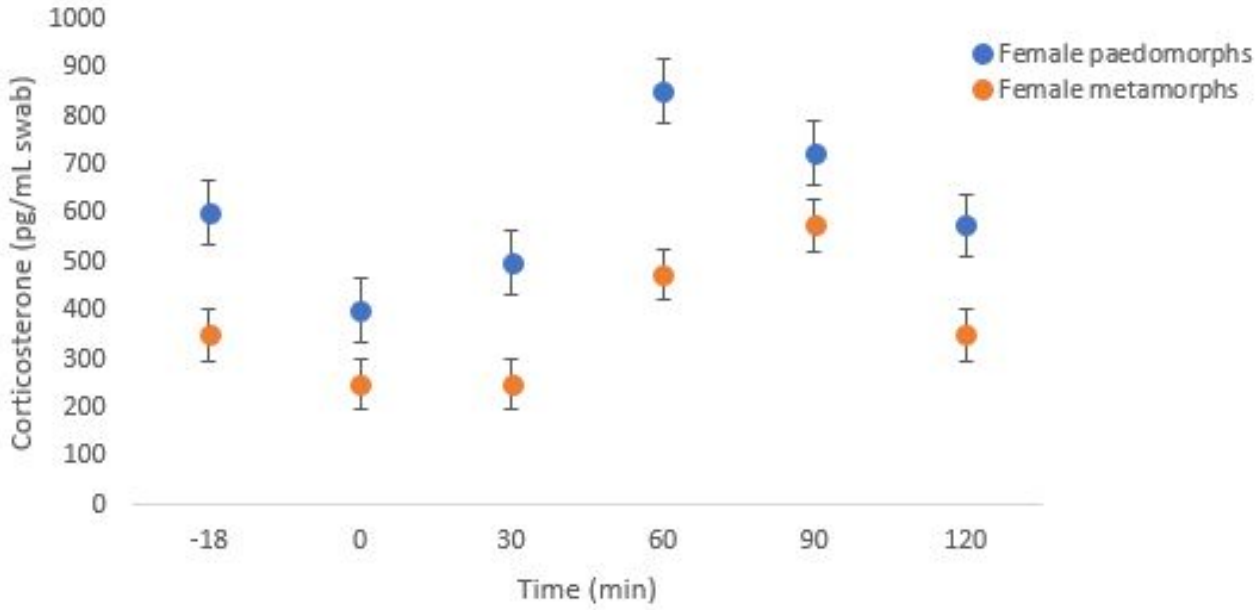

Figure 3. Expected average corticosterone concentrations of female paedomorph and metamorph Arizona tiger salamanders (*Ambystoma mavortium nebulosum*).

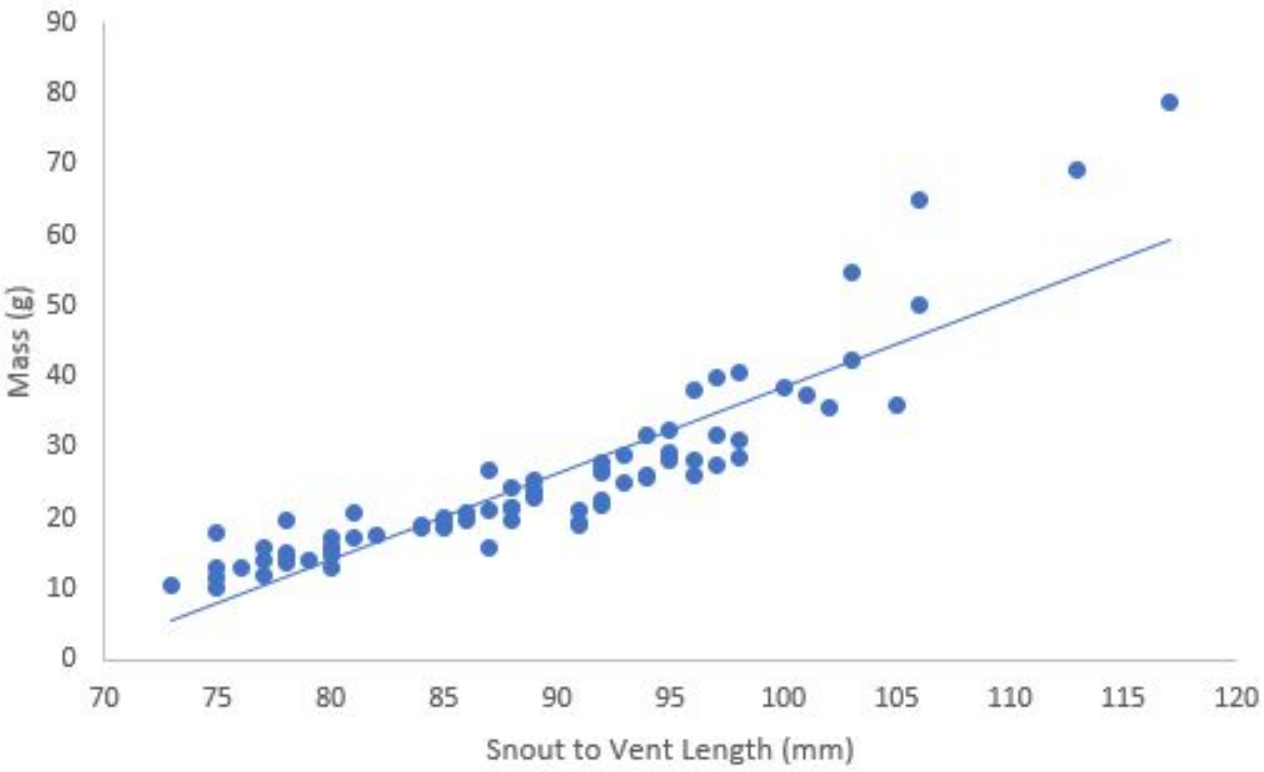

Figure 4. Distribution of body conditions of sampled Arizona tiger salamanders (*Ambystoma mavortium nebulosum*).