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Kentrell Richardson
James Madison University

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Understanding context dependent responses to climate change in Arizona Tiger Salamanders (*Ambystoma mavortium nebulosum*)

Kentrell Richardson

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FACULTY COMMITTEE:

Committee Chair:

Dr. Kelsey Reider

Committee Members/Readers:

Dr. Christine May

Dr. Chris Rose

Dr. David Marsh

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Abstract

Future emissions scenarios project climate change to increase average global temperatures by at least two ℃ in the next 50 years resulting in changes in local climate and causing increased variability within microclimates. Ectotherms are especially sensitive to climate change due to their dependence on environmental temperatures to regulate physiological functions. Changes in temperature are likely to impact thermally cued processes within amphibians and result in changes in variable magnitudes and directions within local populations.

Salamanders were placed in cups and partially submerged in a water bath and heated at a rate of ~0.27 ℃/ minute. Once salamanders were unable to right themselves after 5 seconds their CT_{max} was determined.

Analysis of CT_{max} by morph resulted in no statistically significant difference between morph CT_{max}. There was no apparent statistically significant difference in the CT_{max} values between sexes within or across morphs. There were statistically significant differences detected across salamander age class. Hatchling salamanders CT_{max} values were 11 ℃ lower than adult paedomorphic salamanders while larval salamander CT_{max} values were 9.4 ℃ lower than adult paedomorphic salamanders. Metamorphic salamander CT_{max} were not statistically significant along an elevational gradient CT_{max} values were similar amongst high and low elevation sites. Locally in the low elevation sites, metamorphic salamander CT_{max} were statistically significant different from year to year. Metamorphs and paedomorphs reported statistically significant differences in body temperatures in several months throughout the year. Environmental conditions in the aquatic and terrestrial environments showed remarkably similar temperatures throughout the year with slight differences in windows of opportunities within morphs. Light intensity showed ponds begin to thaw out in early may resulting in earlier activity in paedomorphic salamanders. Aquatic salamanders likely have availability to resources sooner than terrestrial salamanders that must wait on snowmelt to cue them to emerge from their burrows. During the currently defined active season, I determined differences in warming tolerance between morphs with metamorphic salamanders showing higher warming tolerances than paedomorphic salamanders.

My research provides greater insight into thermal physiology of alpine adapted ectothermic organisms and is among the first of its kind to incorporate true body temperature of salamanders that can be directly correlated to critical thermal maxima. This research also combines physiological and environmental approaches to determine a thermal profile for various morphs, sexes, and age class of alpine salamanders. Future research should go beyond collecting CT_{max} of salamanders and collect CT_{min} data to provide a complete estimate of thermal breadth of animals in these alpine environments to provide a complete picture of their context dependent responses to climate change.

Introduction

Climate change

Climate change is recognized as major threat to global biodiversity and has led to shifts in distributions and abundance for thousands of species and is likely to cause the extinction of thousands in the next 100 years (Cahill et al., 2013; Midgley & Hannah, 2004). Current projections for future climate change scenarios place anthropogenic climate change as one of the major causes of extinction within the Anthropocene due to the Earth being on average warmer than it ever has in the past 40 million years (Bestion et al., 2015; Midgley & Hannah, 2004). Scientists need to understand how climate change will influence species to successfully implement conservation practices and help species survive intense climatic events and reduce their extinction risk (Urban, 2015).

Organismal response to climate change is variable and has been a focus of ecological research (Winterová & Gvoždík, 2021). Ectotherms are especially sensitive to climate change, as they depend on ambient temperatures in the environment to perform physiological tasks and maintain homeostasis (Angilletta et al., 2002; Feder et al., 1992; Hillman et al., 2008). Reliance on environmental conditions, such as air and water temperature, can cause an increased sensitivity to increases in extreme temperatures as seen with climate change (Goodwin et al., 2019). In response to climate change we see amphibians shifting their ranges to stay within optimal thermal conditions as well altering their reproductive window to prevent missing peak environmental productivity (Blaustein et al., 2010).

The sensitivity of a species to climate change is a key metric to assess a species' ability to persist in the face of changes in temperature and other environmental variable. While climate change poses a threat to the viability of many species regardless of current conservation status, the degree and magnitude of its threat is assessed through known life history traits, dispersal ability, and thermal resilience (DuBose et al., 2023). Ectotherms with permeable skin are sensitive to changes in environmental temperature and moisture making maintenance of body temperature and water balance critical for survival in their environments (Rozen-Rechels et al., 2019). Local environments are often resistant to climatic fluctuations but the magnitude and rate of climate change has the potential to contend with this resistance and result in changes in population demographics. Current knowledge on ectotherm response to climate change is largely based on single population datasets and can make determining uniform changes in demographic trends difficult (Muths et al., 2017). The heterogeneity of the impacts of climate change pose challenges to ecologists and require innovative approaches that bridge physiological and environmental metrics to understand amphibian response in the wake of climate change.

Known Influence of Temperature on Ectotherm Physiology

Temperature acts as a key driver of performance for ectotherms controlling their ability to regulate nearly all biological processes from reproduction to locomotion, as well as determining important physiological tolerance limits such thermal limits (Angilletta et al., 2002; Bodensteiner et al., 2021). In ectotherms, the circuitous effect of temperature on behavioral and phenotypic plasticity makes determining exact influence of temperature on physiology increasingly complex (Lutterschmidt & Hutchison, 1997). Body temperature influences all aspects of ectotherms including immune function, foraging ability, growth rates, and locomotion (Angilletta et al., 2002). Ectotherms exist in a wide range of habitats from mountaintops to barren deserts and must be able to respond to fluctuations in environmental temperatures (Taylor et al., 2020). Often to respond to the variations in temperatures, ectotherms will display various behaviors to navigate their environment and best optimize their overall fitness. Understanding the impact of temperature on various organisms might provide insight into how well organisms will be able to evade thermal stress pushing them past thermal limits for extended periods of time through behavior as well as acclimation (Buckley & Huey, 2016).

Ectotherms display thermoregulatory behaviors, such as basking or seeking refuge under logs, to respond to fluctuations in temperature within their environments (Hutchison & Maness, 1979). Typically, performance, a measure of some physiological function such as nutrient digestion or homeostatic maintenance, will increase in efficiency as temperature does, up until organisms approach their thermal limits which results in damage or loss of function to organisms. When exposed for a short period of time to conditions within ~2℃ of critical thermal maximum heat shock protein production is triggered and works to protect amphibians from temporary exposure to life-threatening conditions, known as heat hardening (Easton et al., 1987; Feder et al., 1992; von May et al., 2019). Heat hardening acts as a temporary measure to allow organisms to avoid reaching their $CT_{max,}$ the upper body temperature at which animals lose the ability to function, and ultimately prevent a reduction in organismal fitness. Current climatic projection models estimate global average surface air temperature to rise between 1.0 ℃ and 3.8 ℃ by 2100, with somewhere around 2 ℃ being the most likely scenario (Cavallo et al., 2015). Increased temperatures will continue to push organisms closer to or past their CT_{max} points and places increased importance on understanding how temperature affects species to assist in improving amphibian conservation efforts.

Thermoregulatory behavior is the primary means by which ectothermic organisms control variation in their body temperatures by adjusting microhabitat usage, posture and position, or active periods (Bodensteiner et al., 2021; Shoo et al., 2011). Climate change is reducing the effectiveness of amphibian thermoregulatory behavioral adaptations to historical temperature fluctuations which could push animals to face more thermal stress events, especially organisms that undergo metamorphosis (Lowe et al., 2021). Thermal stress events, periods of exposure to temperatures above CT_{max}, can lead to physiological damage, reduced reproductive success, increased infection rates, or possibly death if pushed to their critical limits (Buckley & Huey, 2016). While water loss that leads to desiccation may be a more restrictive physiological limit than temperature, temperature plays a large role in governing environmental usage criteria especially in changing environments (Lertzman-Lepofsky et al., 2020).

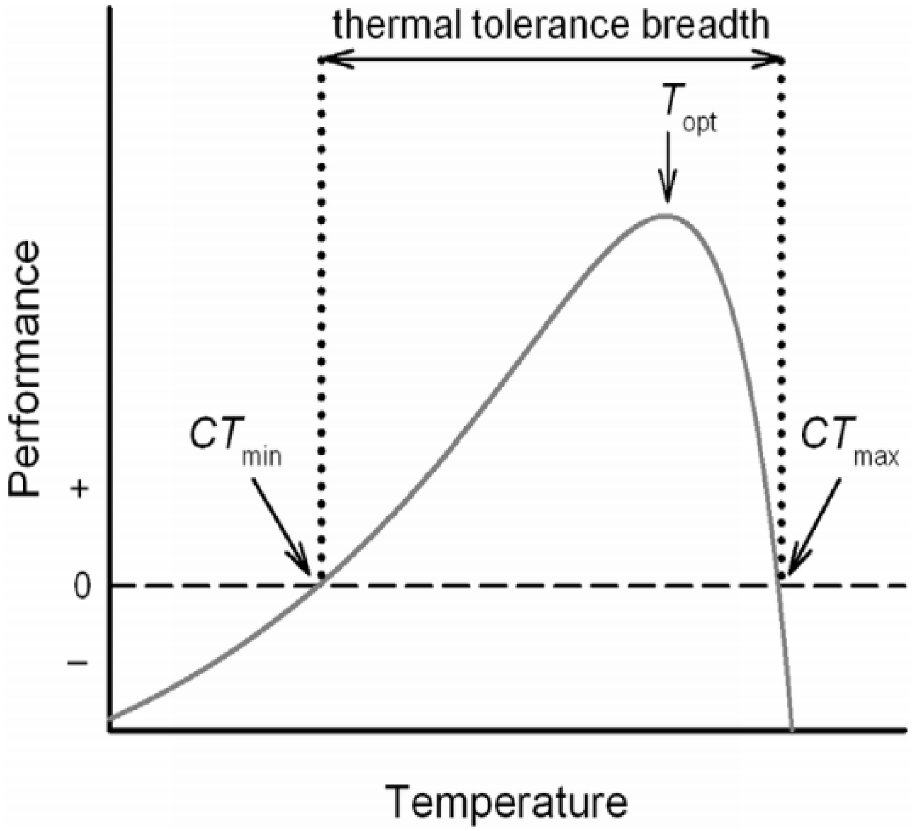

Figure 1. General thermal performance curve. Depicts the relationship between environmental temperature and some physiological rate of an ectotherm. The optimum temperature (T_{opt}), critical thermal maximum (CT_{max}), critical thermal minimum (CT_{min}), and thermal tolerance breadth are labeled on figure. Figure adapted from (Krenek et al., 2012)

Thermal performance curves measure changes in performance as a function of body temperature exhibited by an organism (Angilletta et al., 2002; Goodwin et al., 2019). The most common points along this curve include critical thermal minimum, thermal optimum, and critical thermal maximum. The thermal optimum is the body temperature that maximizes the performance metric being tested (Angilletta et al., 2002).

Critical thermal maximum (CT_{max}) refers to the highest temperature at which an organism becomes unable to escape conditions that could lead to death or produces a loss of righting response (Hutchison, 1961; von May et al., 2019). Critical thermal minimum (CT_{min}) is similar to the CT_{max} but refers to the lowest temperature an organism can experience before losing neuromuscular function in the form of loss of coordination or death (Andersen et al., 2015).

The effects of climate change will likely vary based on environmental context and influence the way organisms navigate their environments. Locomotion in aquatic environments require substantially more applied force than in terrestrial environments because of the high viscosity and density of water compared to air (Feder et al., 1992; Schmidt-Nielsen, 1997). Applying force to navigate through aquatic and terrestrial environments without obstruction when organisms approach near optimal temperatures results in differences in oxygen consumption, with each 10 °C increase in temperature resulting in a two-fold increase in oxygen consumption (Schmidt-Nielsen, 1997). Organisms that face increased oxygen consumption in various tissues throughout their bodies would have trouble providing enough oxygen to meet bodily demands required for optimal locomotion through their environment (Pörtner et al., 2017). With climate change causing likely causing asymmetric effects depending on environmental context, organisms will more frequently experience conditions outside of their normal range which could result in alterations in their ability to navigate local environments effectively.

Variability in Amphibian Response to Climate change

Changes in temperature are likely to impact numerous thermally cued processes within amphibians and result in changes of all magnitudes and directions within populations. If we look at the influence of temperature on developmental rates in amphibians we see that rates of development increase with temperature until some population specific threshold where high temperatures begin to be detrimental to the population (Blaustein et al., 2010). Within red spotted newts, *Notophthalmus viridescens*, we already see that increases in temperature and reduced humidity result in reductions in species activity to avoid conditions leading to possible desiccation (Roe & Grayson, 2008).

Amphibians have a complex life cycle that is dependent on land and water which increases their vulnerability to modifications in habitats due to climate change (Longhini et al., 2021). Time of breeding and embryo survival are often dependent on temperatures cues within specific ranges for a species (Corn & Muths, 2002). Increases in average spring temperatures in Europe have resulted in earlier growing seasons, increased foraging availability, and consistent earlier breeding of amphibian populations (Forchhammer et al., 1998). This change in breeding habits is not consistent in all amphibian populations, late spring breeders in North America like the American bullfrogs, *Lithobates catesbiana*, show no observable change in breeding habits with increases in temperature (Green, 2017). Amphibian demographics are influenced by climate-related variables like temperature and the increased occurrence of extreme temperatures on local populations is likely to become increasingly important to understand the magnitude and direction in which climate change will impact the breeding phenology of various species (Corn & Muths, 2002; Muths et al., 2017).

For amphibians to be successful as adult organisms they must be able to adapt well in an ever-changing environment at all points throughout their life history stages (Telemeco & Gangloff, 2021). At certain points in amphibian life cycles, a shift in utilized habitat occurs that in turn shifts the thermal environment experienced (Ruthsatz et al., 2022). If organisms in a particular life stage are exposed to temperatures past optimal temperatures for their current life stage this might result in a reduction in organism viability and fitness. Research has shown that age class has an effect on upper thermal tolerance of a species with tadpoles having higher upper thermal limits than hatchlings (Turriago et al., 2015). In the African clawed frog, *Xenopus laevis,* adults and tadpoles show similar performance responses for running/swimming despite differences in body size and morphology (Telemeco & Gangloff, 2021). As organisms shift from aquatic environments to terrestrial ones, they will be exposed to large fluctuations in temperature and are forced to adapt to their thermal limits (Ruthsatz et al., 2022).

Abiotic factors such as precipitation, latitude, and climate are some of the many factors limiting a species geographic range. Climate is often thought of as the dominant abiotic force shaping distribution of many amphibian species due to their reliance on temperature and moisture (Cunningham et al., 2016). Many organisms are already shifting their geographic distribution to follow suitable local climate conditions (Enriquez-Urzelai et al., 2019). In 168 studies of ectotherm latitudinal range movement due to climate change, 93.3% of ectotherms reported some form of movement to expand their latitudinal range to maintain optimal climatic conditions (Ramalho et al., 2023).

Climate Variability Hypothesis

The climate variability hypothesis (CVH) states that a relationship exists between the breadth of thermal tolerance range and the amount of climatic variability within the ecosystem as you increase latitude (Gutiérrez-Pesquera et al., 2016; Rohr et al., 2018). The CVH suggests that animals in thermal environments with little temperature variation have correspondingly narrow thermal tolerance ranges, and animals in highly variable environments have wider thermal tolerance ranges (Wang et al., 2020). Some species thermal response may be influenced by factors that vary across elevational and latitudinal gradients, such as precipitation or temperature, requiring them to display variable thermal breadths across geographical range with variable climates producing wider thermal breadths in the organisms living in these areas (Gutiérrez-Pesquera et al., 2016; Marsh et al., 2019). Amphibians with their environmental dependence to regulate their internal functions make them excellent bioindicators of environmental quality and provide important opportunities to understand physiological impacts of climate alteration scenarios (Taylor et al., 2020). Von May et al. (2019), conducted critical thermal limits experiments on tropical lowland amphibians and discovered that within and among families there was considerable variation in critical thermal traits. Eight pairs of close relatives had non-overlapping CT_{max} values even existing in a similar ecosystem experiencing similar thermal conditions (von May et al., 2019). Directionality and strength of amphibian response to climate change is variable and is reflected in differences in CT_{max} as well as warming tolerance among amphibians with similar geographic ranges and environmental niche. Warming tolerance refers to the difference between CT_{max} and maximum environmental temperature (Nouailhetas et al., 2015).

Amphibian species with higher CT_{max} values and those able to reduce exposure to extreme temperatures events showed less sensitivity to changes in their environment (Nowakowski et al., 2018). Terrestrial ectotherms were found to display a lower acclimation potential than aquatic organisms to respond rapidly to extreme high temperatures which could pose increased risk on metamorphic organisms over aquatic individuals (von May et al., 2017). Thus, we might expect aquatic and terrestrial organisms living in some place to differ in both their exposure to extreme environmental temperatures and their tolerance to temperature extremes. If there are differential thermal stressors experienced by animals based on where they live, this places urgency on understanding the interaction of life history and temperature in polyphenic populations.

Thermal Physiology

Several physiological metrics of ectotherms are used to determine their susceptibility to climate change because relationships between the environment and fitness can be drawn so readily understanding common metrics used to understand a species provide insight into numerous areas of the species development (Gilbert & Miles, 2017). Thermal limits are commonly used to predict organismal response to climate change but estimates of thermal limits vary based on experimental design and measurement conditions when collection occurs (Kingsolver & Umbanhowar, 2018). The complexities and variation in experimental protocol within thermal biology make defining the context to which metrics are applied increasingly important. Methodological factors and species-specific traits, like morph due to phenotypic plasticity, can interact and influence the measured thermal response of ectotherms (Rohr et al., 2018). In salamanders that were exposed to CT_{max} temperatures then returned to acclimation temperatures and CT_{max} tested again 1.5 hours later, the second test yielded values 0.6 ℃ higher than initial values (Easton et al., 1987). The relationship between phenotypic plasticity and environmental thermal differences might provide insight understanding variability in amphibian response to climate change.

Phenotypic Plasticity

In order to respond to climate change, populations can evolve adaptations to respond to the changes in the environment over time, emigrate to new habitat with favorable conditions, acclimate, or die (Smith et al., 2014). Phenotypic plasticity, the ability of individual genotypes to produce different phenotypes under different environmental conditions, would allow organisms to respond to changes in the environment and allow them to maximize their relative fitness in the environment (Matsunami et al., 2015). Polyphenism is a special case of phenotypic plasticity where multiple discrete phenotypes arise from differing environmental conditions and allows organisms flexibility to respond to a wider range of environmental and biotic factors (Denoël et al., 2012). One type of polyphenism, facultative polyphenism, is expressed by many salamander species in the genus Ambystoma. Environmental conditions create heterogeneous pressures for salamanders in terms of food availability, predation events, and habitat usage creating differences in selection pressures that result in differential physical characteristics being expressed via salamander polyphenism (Storfer & White, 2004; Thurman & Garcia, 2017; Yang & Pospisilik, 2019). In alpine environments, as the sun rises shallow water warms at a more rapid pace than deeper water as well as soil and would create thermal differences within the environment confirming environmental differences between environments (Heath, 1975). Facultatively paedomorphic salamanders are thus an ideal organism to understand the potentially asymmetric impacts of climate change in both aquatic and terrestrial contexts. Amphibians with phenotypically variable traits may be able to differentially utilize microclimates and possibly persist in environments undergoing significant changes (Thurman & Garcia, 2017). Metamorphs and paedomorphs exist within the same population which eliminates initial concerns regarding genetic influence impacting the results of the study. Paedomorphic salamanders are those that do not undergo metamorphosis and instead acquire sexual maturity while retaining larval characteristics such as external gills and remain in the water (Denöel, 2017). Metamorphic individuals have resorbed their external gills to take on characteristics such as external nostrils and well-developed limbs to allow for a more terrestrial lifestyle. Phenotypic plasticity within populations, changing climatic conditions, and other compounding factors create variable contexts with potentially contrasting responses to climate change and place increased importance on understanding environmental context on amphibian responses to climate change (Taylor et al., 2020). I propose to investigate this phenomenon through understanding the impact of localized environmental context as a predictor of variation in upper thermal limits of high elevation polyphenic Arizona Tiger Salamanders.

Research Questions and Hypotheses

The overarching goal of my research was to describe and compare the relationship between environmental context and physiology of amphibians to understand context-dependent responses to climate change. I hypothesize thermal environments and body temperature of metamorphic and paedomorphic salamanders will differ providing environmental context for all associated thermal physiology. I address two research questions.

Research Question 1: How do variation in life history traits (morph, sex, and age class), elevation, and seasonal variation influence the critical thermal maxima? Research Question 2: How does environmental context influence Arizona Tiger Salamander body temperature, warming tolerance, and activity periods? By addressing both questions, my study will investigate how variation in environmental temperatures in different habitat types utilized by salamander morphs can influence the strength and direction of climate change responses.

Q1 Hypothesis 1: I hypothesized that paedomorphic salamanders will have a lower CT_{max} than metamorphic salamanders. Environmental temperature is important to amphibian life history and physiological fitness should change with alterations in temperature (Heath, 1975). We might expect that environmental variation creates variations within microclimates utilized differentially by morphs influencing strength and direction of morph response to climate change. Water reduces energy expenditures and stress associated with exposure to extreme temperatures (Lertzman-Lepofsky et al., 2020). Paedomorphic salamanders remain in water year-round and might experience environmental temperatures much lower than their CT_{max} which would make them better able to respond to warming conditions.

Q1 Hypothesis 2: I hypothesized that there will be sex-specific differences between upper thermal limits with differences between morphs. Long term population studies suggest there are important life history variation in breeding opportunities and lifetime fitness of each morph and sex combination (Lackey et al., 2019; Whiteman & Wissinger, 2005).

Male paedomorphs tended to have more reproductive opportunities than metamorphic counterparts, while female metamorphs had overall higher estimated egg production than paedomorphs (Lackey et al., 2019). Given observed differences in lifetime fitness through a sex by morph interaction, we might expect a sex by morph difference in CT_{max} with male metamorphs displaying higher CT_{max} values than all other counterparts.

Q1 Hypothesis 3: I hypothesized that larval salamanders would be more susceptible to exposure to extreme temperatures and display a lower upper thermal limit than adult salamanders. Research conducted in American Toads (*Anaxyrus americanus*) shows that adult toads displayed a stronger heat hardening response to extreme changes in their environment and this trend might extend into salamander ontogeny (Heath, 1975; Keen & Schroeder, 1975; Lowe et al., 2021). In this way we might expect stage-specific thermal sensitivities and tolerances which would promote stage specific sensitivity to climate change (Ruthsatz et al., 2022).

Q1 Hypothesis 4: I hypothesized a difference in CT_{max} values attributed to elevational differences between high and low elevation sites. Prior research conducted on Arizona tiger salamanders in the 1970s detected differences in salamander temperature selection as well as maximum environmental temperatures within ponds along an elevational gradient in the Rocky Mountains (Heath, 1975). Early research supports thermal differences in environments along an elevational gradient in mountainous regions and presents support for local environmental context influencing CT_{max} values. Lower elevation populations typically experience warmer and more variable environmental temperatures than populations in higher elevations, following ideas presented in the CVH we might expect organisms lower in latitude to exhibit higher CT_{max} values within terrestrial ecosystems.

Q2 Hypothesis 1: Salamanders are ectothermic organisms and will likely experience body temperatures, T_b, similar to environmental temperatures. I hypothesize that the winter conditions of salamanders will be different in terrestrial and aquatic environments. Environmental conditions outside of the Mexican Cut active season (June, July, and August) provide differential buffering effects to alterations in environmental temperatures. Metamorphic salamanders move to underground burrows outside of the active season and utilize snowpack as a buffer to large daily fluctuations in air temperature (Muths et al., 2020). Mexican Cut populations fluctuate yearly from numerous biotic and abiotic factors, including winter conditions, terrestrial conditions, and drought cycles, and overall survival (Whiteman & Wissinger, 2005). These conditions could influence aquatic and terrestrial environments outside of the active season and result in differential impacts on morphs as well as differences in body temperature during seasonal transition periods when temperatures in each environment would be most different.

Q2 Hypothesis 2: I hypothesized that metamorphic salamanders would have a smaller warming tolerance than paedomorphic salamanders. Metamorphic salamanders within Mexican Cut tend to be larger larvae within ponds with better body condition that utilize their fast growth rate to metamorphose early and take advantage of resources in the terrestrial environments. Larvae that cannot reach this critical size to metamorphose will become sexually mature aquatic paedomorphs (Denoël et al., 2012). Smaller-bodied organisms tend to warm and cool at faster rates than larger-bodied organisms resulting in possible correlation between body mass and CT_{max} (Rohr et al., 2018). The effect of body size on the heating rate of organisms would result in smaller organisms reaching CT_{max} sooner than larger individuals within a population (Claunch et al., 2021). This effect of body mass on CT_{max} might result in metamorphs that tend to be larger at time of metamorphosis expressing warming tolerances lower than paedomorphic salamanders.

Q2 Hypothesis 3: I hypothesized that as salamanders face changing conditions that their activity periods would change from natural rhythms to an altered state in response to environmental changes. When species face suboptimal climatic conditions they tend to modify their behavior to attempt to respond to these suboptimal conditions (Delgado-Suazo & Burrowes, 2022). With global air temperatures and high elevation lake temperatures increasing we might expect both paedomorphic and metamorphic salamander's activity periods to shift to allow for maximal environmental usage and nutrition acquisition. Metamorphic salamanders in mountainous environments utilize snowpack to insulate them from daily air and soil thermal variation (Decker et al., 2003; Muths et al., 2020). Warming temperatures can cause earlier thawing of ice layers on ponds and allow paedomorphic salamanders to become active sooner and consume prey sooner than metamorphic salamanders, causing differences in annual activity periods for each salamander morph (Fassnacht et al., 2018).

Methods

Study Sites

The primary study site was the Mexican Cut Nature Preserve (MCNP) located at 39.02 °N, -107.06 °W, a 960 acre preserve owned by the Nature Conservancy and managed by the Rocky Mountain Biological Laboratory right outside of Gothic, Colorado (Figure 2). The majority of the ~24 subalpine wetland ponds at MCNP are located between 3400 – 3500 m elevation with the highest ponds occurring at 3800 m. Snow acts as the main hydrological input into all ponds, through melting snowpack (Wissinger et al., 1999). The ponds of MCNP are home to a diverse assemblage of invertebrates, containing 26 species of benthic taxa like fairy shrimp (*Branchinecta coloradensis*) and various species of caddisflies, and Arizona Tiger Salamanders (*Ambystoma mavortium nebulosum*) that act as keystone predators for the aquatic invertebrate community within these wetlands (Wissinger et al., 1999). Ponds at Mexican Cut were visited during the months of June, July, and August in 2022, with a subsequent visit in the September in 2022 to collect late season measurements.

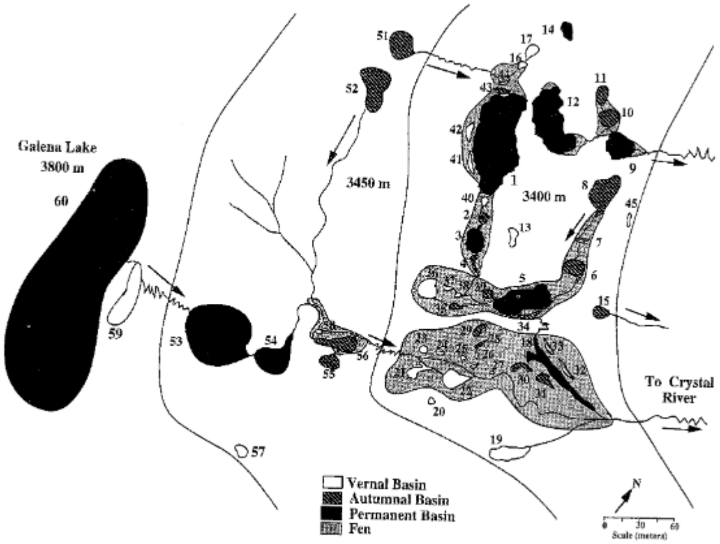

Figure 2. Map of ponds at Mexican Cut Nature Preserve within Galena Mountain Reprinted from Wissinger et al., 1999.

Kettle pond 6 is a temporary pond fed by runoff from snowmelt and lies in an open field at approximately 2865 m above sea level (Sexton & Bizer, 1978). The pond is located at 38.94 °N, -106.97 °W amongst a collection of ephemeral wetland ponds known as the Kettle Ponds located ~2.5 km south of the Rocky Mountain Biological Laboratory (Dodson & Dodson, 1971). Kettle Pond 6 is quite shallow and is rapidly warmed by solar radiation. The salamanders must metamorphose in this pond because of its rapid warming nature resulting in drying out of the pond in late summer (Sexton & Bizer, 1978). Herds of cattle roam around this pond and use it as a source of drinking water while simultaneously defecating in the pond which provides the pond with excess nutrients that result in cycles of rapid vegetative and organismal growth and development until it dries out. Kettle Pond 6 was visited during the months of June, July, and August in 2021 and 2022.

Rainbow Lake is a permanent lake located at 38.64 °N, -107.18 °W within the Collegiate Peaks Mountain range at approximately 3320 m above sea level. Rainbow Lake is ~13 km west of Buena Vista, Colorado within the San Isabel National Forest. With access to permanent sources of water Rainbow Lake is home to various fish species, including brook and rainbow trout, salamanders are forced to gather large amounts of nutrients very quickly and congregate within the shallow portions of the lake to metamorphose and escape the pressure from fish populations. Rainbow Lake was visited in July and August in 2021 and was only visited in subsequent years to monitor salamander populations.

Flat Tire 2 is a shallow temporary pond within in an open plain with scattered trees that provide shade from the intense light conditions that impact sources of water and result in rapid warming due to solar radiation. Flat Tire 2 is located at 38.61 °N, -106.77 °W approximately 2980 m above sea level, ~19 km northeast of Gunnison, Colorado. Salamanders are forced to metamorphose in these shallow conditions resulting in the populations found here being comprised of entirely metamorphic salamanders. Flat Tire 2 was visited in July and August in 2021.

Study Species

The Arizona tiger salamander, *Ambystoma mavortium nebulosum,* is an amphibian with a geographically widespread distribution found in a variety of habitats and elevations from western Colorado and Utah to south-central New Mexico and central Arizona (Collins, 1981; Whiteman et al., 1994). This is a cold-tolerant species that is prone to snow covering the terrestrial environment for eight months out of the year and ponds being frozen over for a similar duration of time (Wissinger et al., 1999).

The Arizona tiger salamander population within MCNP isolated on two bedrock benches on Galena Mountain that have 20 wetlands that vary in hydroperiod and have been censused annually since 1990 (Whiteman & Wissinger, 2005; Wissinger et al., 1999). A large percentage of the adult population are marked and individuals are recaptured yearly. Larval salamanders shift their diet from plankton to benthic fauna and are known to cannibalize smaller larvae during development as density increases (Denoël et al., 2006; Lackey et al., 2019; Wissinger et al., 1999). Cannibals act as predators for smaller larvae and act to reduce larval populations to improve overall resource availability and increase the amount of nutrients salamanders are able to gather nutrients and reach sexual maturity (Denoël et al., 2006, 2012; Wissinger et al., 1999).

The cold temperatures and short productive summer season prolong development often resulting in larvae taking two to three (and up to five) summer periods to reach sexual maturity (Whiteman & Wissinger, 2005; Wissinger et al., 1999). Upon reaching sexual maturity, salamanders can display one of two morphs, a metamorphic form (partially aquatic and terrestrial) and a gilled, paedomorphic form (fully aquatic). Salamanders that can gather enough nutrients within three summers of larval growth will typically undergo metamorphosis and become terrestrial adult metamorphs (Denoël et al., 2012). The metamorphic salamanders overwinter underground and typically emigrate in spring to the wetland ponds located around MCNP to breed and feed for 6 – 10 weeks then return to their overwintering sites (Whiteman & Wissinger, 2005). If a salamander is unable to gather enough nutrients to metamorphose within three summers, they will typically mature into paedomorphic adults (Denoël et al., 2012).

Capture and Housing Methods

The Rocky Mountain Biological Laboratory (RMBL) has a federally approved IACUC approval committee that all projects conducting research at RMBL must meet. This project was conducted with RMBL Animal Care and Use committee approval. The first field season Animal Care and Use memo was conducted under Howard H. Whiteman effective 5/24/2021. The second field season Animal Care and Use memo was conducted under Kelsey E. Reider effective 5/25/2022.

All salamanders were captured using dipnets. Salamanders captured in MCNP ponds were housed within a Weatherport located between pond 12 and 8 (Figure 1). Salamanders were captured, held in containers filled with water from the ponds where they were captured (for example ponds 1, 3, 5, 9, and 12), and then released after collecting CT_{max} and CT_{min} measurements. Kettle Pond 6, Rainbow Lake, and Flat Tire 2 salamanders were housed at the RMBL laboratory space in shoe box containers filled with 50% of their pond water and 50% dechlorinated tap water. Salamanders collected at different locations were not held or moved into MCNP and no animals were removed from Mexican Cut to prevent spread of zoonotic diseases such as chytrid fungus.

Metamorphs collected from the summer of 2022 did not include any of the previously captured animals from the summer of 2021. Salamanders collected in 2021 from the Kettle Ponds had their second toe of their front left foot (from left to right) snipped to prevent repeat sampling for that season.

Experimental Procedures

Critical Thermal Minima

Critical thermal minima (CT_{min}) were tested to determine lower thermal limits of salamanders. To determine CT_{min} salamanders (N = 7) from Kettle Pond 6 were placed in plastic cups and submerged in water from their capture location. Cups were then partially submerged in a water bath and cooled using aluminum blocks pumping an alcohol mixture from an insulated cooler in a closed loop through the water bath. The alcohol bath mixture contained 32 oz of 70% isopropyl alcohol and dry ice was added every 5 mins in tennis ball sized chunks to reduce alcohol bath temperature. Water pumps were connected to aluminum blocks via aquarium tubing to ensure water flowed at a consistent rate between the alcohol bath. Temperatures within the ethanol bath reached temperatures as low as -37.1℃, while salamander cloacal T_b reached as low as 0.2℃. At 0.2℃, the salamanders (N = 5) were able to continue to right itself within 5 seconds. Because the internal T_b might have lagged behind the cloacal T_b we also tested righting response on a longer time period. We held the individuals at 0.5 – 0.2℃ and tested the righting response every five minutes and even after 30 minutes there was no loss of righting response. The LRR as an endpoint provides ecologically relevant reduction in salamander fitness where they exhibit a temperature-induced state of poor locomotory performance that limits the ability of salamanders to respond to external stimuli (i.e., predation) (von May et al., 2019). Due to inability to induce LRR at temperatures that would not induce freezing of body tissue, we were unable to determine CT_{min} by these methods and CT_{min} will not be used in any further analyses.

Q1. Upper Critical Thermal Limits

I compared CT_{max} of metamorphs (N = 18) and paedomorphs (N = 60) taken from multiple ponds at MCNP and the CT_{max} of metamorphs (N = 32) taken from Kettle Pond

6. Salamanders were selected in pairs randomly with one paedomorph and one metamorph or larvae being tested at the same time to limit variation in CT_{max} attributed to variation in methodology such as initial water temperature. Individuals were placed in plastic cups and submerged in aerated water from their capture location. In each trial, two cups were submerged to the same depth in a water bath and heated from ~16.5℃ to CT_{max} at a rate of ~0.27℃ ± 0.08℃/minute. Heating rate was monitored using an Onset Intemp CX402 temperature logger with the probe inserted into the water of an individual's cup throughout the duration of experimentation. The water bath was heated using a Norpro instant immersion heater connected to a Jackery Explorer 1000 portable power station. Animals had their temperatures taken by inserting a Type-K thermocouple probe from a Leaton 2-channel digital thermometer (Accuracy ±1.5%) into their cloaca. Insulated gloves were sterilized and used to prevent temperature readings causing changes in salamander body temperature and their subsequent CT_{max}.

To ensure the CT_{max} method described above produces an LRR result caused by changes in temperature and not from salamanders exhibiting an acute stress response, an experimental control was included with the CT_{max} experiment. Experimental control salamanders (N =13) were held in aerated, water-filled cups and flipped simultaneously with experimental animals without the manipulation of temperature.

For CT_{max} experiments, loss of righting response (LRR) was used as a non-lethal endpoint for salamander upper critical thermal limits. Upon completion of thermal limits experiments, salamanders were placed in cool water to recover from LRR state. Recovery in these salamanders was monitored as return of locomotory function and ability to respond to external stimuli.

To determine if the CT_{max} differ between hatchling, larval, and adult salamanders, I used the CT_{max} methods previously described to compare the CT_{max} of hatchlings (N = 30), 2nd and 3rd year larvae (N = 23) and adult paedomorphic salamanders (N = 60) from MCNP. Salamanders were assigned to age classes (Hatchling, Larvae, Adult) based on size and developmental differences among the classes such as gonadal development status with more developed gonadal structures indicating sexual maturity in salamanders (Denoël et al., 2012). Hatchlings are salamanders born from eggs in the current year i.e., 1st year salamanders. Larvae are salamanders that have overwintered and have entered their second or third year but have not reached sexual maturity. Upon completion of CT_{max} experiments animals were monitored for recovery of locomotor function and then released in the ponds where they were captured.

Hatchling salamanders have partially developed cloacas and therefore had to have adjusted methods to test CT_{max}. For hatchling salamanders cloacal thermometer readings were not taken due to their immature cloacas being incapable of thermocouple probe insertion. Infrared readings were taken for hatchlings (N = 30) using the Fluke 62 Max Infrared thermometer (Accuracy ±1.5%). To validate that the no-contact infrared thermometer temperature measurements were comparable to thermocouple cloacal temperature measurements, larval salamanders (N = 13) had temperature readings taken with both metrics and compared using linear regression.

We placed hatchling salamanders into an individual container and warmed in a CT_{max} experimental setup identical to the method used for adult salamanders. However, a loss of responsiveness to external stimuli was used to determine the CT_{max} endpoint instead of loss of righting response (Fernández-Loras et al., 2019). When a hatchling salamander failed to respond to 10 consecutive tactile stimuli from a probe in 2 s intervals, CT_{max} was determined as being met. Hatchling recovery methods were identical to those used for adult and larval salamanders.

Q2. Environmental Temperatures

I used environmental data (air temperature, soil temperature, water temperature, and light intensity) collected with Onset HOBO 64K pendant waterproof temperature light data loggers recording hourly to characterize the environmental temperatures for salamanders at Mexican Cut Nature Preserve and develop an environmental context. Air temperature data loggers (N = 6) were set between 2 – 2.5 m off the ground on tree branches, near locations of interest (Weather station, Weatherport, Pond 5, Upper cut pond 1, and Upper cut pond 6), and set to record temperature in hour intervals. Air temperature data loggers were fit with double-sided ventilated radiation shields to prevent biased temperature estimates of air temperatures from exposed data loggers (Holden et al., 2013). Soil temperature was collected at 10 to 15 cm intervals from surface down to 80 cm in the ground at six sites within MCNP. Soil data were analyzed to determine the range of temperatures in the terrestrial environment and if information about overwintering metamorph activity periods could be determined. Water temperature data were collected at two points within the water column (near surface and deep end of ponds). Deep water temperature loggers and surface soil loggers we also used a light intensity logger to collect light (in lux/m^2) to measure the duration of ice and snow cover for the aquatic and terrestrial environments to estimate activity periods for paedomorphic and metamorphic salamanders.

Body Temperature

Body temperature (T_b) readings were taken from adult metamorphic (N = 4) and paedomorphic salamanders (N = 7) using surgically implanted, miniature, internal temperature loggers (Star-ODDI DST nano-T; L = 17 mm, W = 6 mm, mass = 1.25 g, resolution = 0.032 ± 0.2). Surgical implantation and datalogger recovery techniques were adapted from a passive integrated transponder (PIT) identification tag and radio-transmitter implantation methods (Reider et al., 2022). All surgical equipment was sterilized to minimize infection from initial capture and housing and throughout surgery and recovery. Animals used had to be at least 25 g in mass before implantation to limit logger weight to 5% of body mass or less (Reider et al., 2022). Recovery period for animals with implant included immediate monitoring until the return of normal breathing patterns indicated by buccal pumping of animals as well as a 2-day holding period where they were fed fairy shrimp until we observed normal digestive function and found no redness/swelling at surgery site. Dataloggers recorded body temperature hourly for one to two years and produced detailed temperature profiles for each individual. Biologging allowed comparisons of T_b differences between morphs and estimates of annual activity patterns to be determined.

Body temperature was used to build comparisons between morph T_b in each month to determine if there were statistically significant differences in their experienced temperatures. Following morph by month T_b comparisons, body temperature was used to calculate warming tolerance for salamander morphs. Warming tolerance is the difference between CT_{max} and field active body temperatures (Taylor et al., 2020). Warming tolerances represents an organisms likeliness to be extirpated due to climate change with high warming tolerance representing a low likelihood of being extirpated and low warming tolerance representing a high likelihood of being extirpated due to climate change.

Statistical Analyses

All statistical analyses were performed in R Statistical Software (v. 4.2.2; R Core Team, 2022). Salamander critical thermal limits were analyzed using a linear mixed effects model. Using collected T_b and CT_{max} data, linear mixed models were created to determine warming tolerance differences between morphs with the lme4 package v. 1.1.29 (Bates et al., 2015).Body temperatures were also compared to environmental temperatures to determine relationship between salamander temperature relative to external environment. Within linear mixed models I used body condition (mass/snout-vent length), snout-vent length (SVL), total length (TL), morph, sex, year, site, and age class as fixed effects while using, salamander individual identity, heating rate, initial water temperature, and Julian day for random effects.

Body condition is a measure used to determine the amount of relative energy of an animal. Mass divided by snout vent length (measurement taken from tip of snout to beginning of the cloaca or vent), is a ratio body condition index that allows comparisons to be made between individuals from different populations. The other metric for body condition uses a regression approach to compare all individuals relative to the entire subset with positive values indicating fatter animals and negative values indicating skinnier animals (Falk et al., 2017). Each approach has benefits and were included to determine the best index to compare individuals across time and space.

Models were compared using the Akaike information criterion, AIC, values to determine the best model to explain any observed variation in CT_{max}. The AIC provides an estimate of prediction error and model quality using the data set used within the model. The AIC value can be used to compare the quality of developed models to one another. I created an array of models and chose the model with the lowest AIC value as my candidate model that was used to compare the rest of the models to. If a model is within 2 units of another in terms of AIC it indicates that the model is significantly better than the other (Fabozzi et al., 2014). I determined the best model to report for each analysis by choosing the model with the lowest ΔAIC value (< 2) from the candidate model, highest R^2 value, and lowest amount of covariates included as the best model.

Results

Year to Year Variation in CT_{max}

A linear mixed effect model was used to determine if CT_{max} differed from year to year in metamorphic salamanders. The most supported model included sex, body condition, heating rate, and year as fixed effects with Julian day as a random effect (Table 1). The reported AIC was 125.1. The CT_{max} of metamorphs captured in 2021 and 2022 at the Kettle ponds were statistically significant (p-value = 0.03378) with metamorphs tested in 2022 having CT_{max} values 1.68 °C higher than metamorphs in 2021 from the same pond during the months of June through August (Figure 3). As we saw significant differences between CT_{max} values between metamorphs between years, all analyses will be conducted within the same year rather than between years.

Table 1. Model Output for comparison between metamorphs collected in 2022 and 2021 from Kettle Pond 6

	Candidate Model			**Best model**		
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	31.83	28.71 – 34.96	**<0.001**	34.28	23.55 – 45.01	**<0.001**
Sex [M]	0.41	-0.86 – 1.67	0.517	0.47	-0.85 – 1.78	0.472
Condition	0.11	-5.99 – 6.22	0.970	-0.14	-6.43 – 6.15	0.964
Year [2022]	1.74	0.43 – 3.05	**0.011**	1.79	0.44 – 3.14	**0.011**
Initial Water				-0.14	-0.71 – 0.44	0.628
Random Effects						
σ^2	2.15			2.21		
τ_{00}	0.00 $_{\text{Julian}}$			0.00 $_{\text{Julian}}$		
N	4 $_{\text{Julian}}$			4 $_{\text{Julian}}$		
Observations	32			32		
Marginal R^2 / Conditional R^2	0.274 / NA			0.273 / NA		

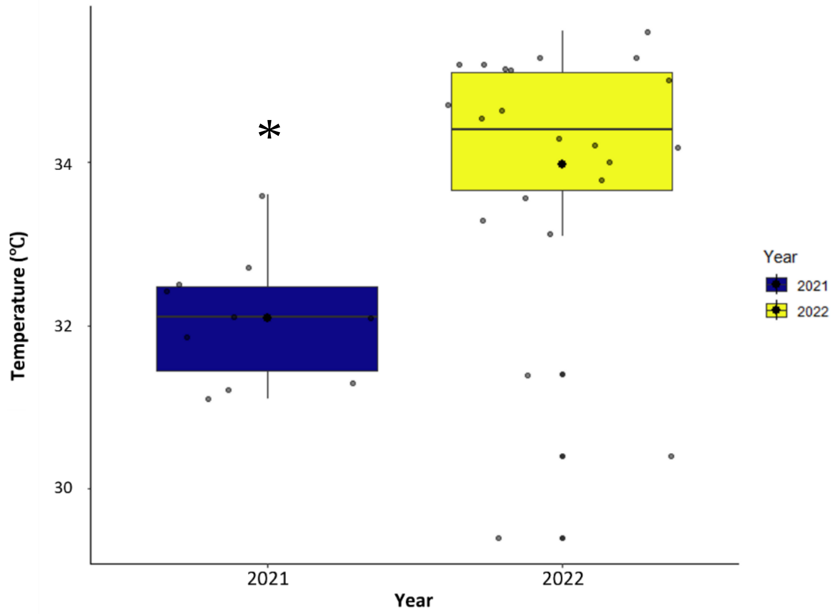

Figure 3. Annual Differences in Metamorph CTmax. Box plot depicting CTmax values for metamorphic salamanders collected from Kettle Pond 6 in 2021 and 2022 with data points representing individual salamander CTmax. The boxplot shows the mean (black circle), median (black bar), interquartile range (box), and 1.5 times the interquartile range (lines). 2021 (N = 10); 2022 (N = 22). Asterisks indicate significance.

$\mathbf{CT_{max}}$ Differences Among Sites

The most supported model reported an AIC value of 40.0 with sex by body condition, site, heating rate, and initial water temperature as fixed effects and Julian day as the random effect (Table 2). Throughout the model selection process site or elevation consistently held no statistically significant effect and no observable difference in CT_{max} was able to be determined. Metamorphic salamanders collected in 2021 from various sites that fell along an elevational gradient (455 m) showed no significant differences (p-value = 0.12237) in their CT_{max} values (Table 3).

Table 2. Comparison of metamorph CT_{max} values among 2021 sites (Rainbow Lake, Kettle Pond 6, and Flat Tire 2)

	Candidate Model			**Best Model**		
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	28.14	24.96 – 31.33	**<0.001**	25.35	17.65 – 33.05	**<0.001**
BodyCond	4.00	1.05 – 6.94	**0.013**	4.60	1.23 – 7.97	**0.013**
Sex [Male]	2.36	0.21 – 4.50	**0.035**	2.35	0.15 – 4.54	**0.039**
Site [KettlePond6]	-0.90	-1.80 – 0.01	0.052	-0.80	-1.76 – 0.16	0.093
Site [RainbowLake]	-0.15	-1.28 – 0.97	0.767	-0.17	-1.32 – 0.98	0.742
Heating Rate	10.62	2.70 – 18.54	**0.014**	9.49	0.90 – 18.07	**0.034**
BodyCond × Sex [Male]	-6.44	-11.32 – -1.56	**0.015**	-6.49	-11.48 – -1.50	**0.016**
InitialWaterTemp				0.16	-0.24 – 0.56	0.388
Random Effects						
σ^2	0.29			0.29		
τ_{00}	0.00 Julian			0.00 Julian		
N	6 Julian			6 Julian		
Observations	19			19		
Marginal R^2 / Conditional R^2	0.626 / NA			0.629 / NA		

Table 3. Metamorphic salamander critical thermal maxima ± standard deviation collected from 4 sites in Gunnison County, CO in 2021 and 2022

Site	**Year**	**Coordinates**	**Elevation**	**Sample Size**	**CT_{max}**

Mexican Cut	2022	(39.02 °N, -107.06 °W)	3400 m	18	33.35 ± 2.04 ℃
Rainbow Lake	2021	(38.63 °N, -107.18 °W)	3320 m	10	32.03 ± 0.66 ℃
Flat Tire 2	2021	(38.61 °N, -106.77 °W)	2980 m	3	33.20 ± 0.78 ℃
Kettle Pond 6	2021	(38.94 °N, -106.97 °W)	2865 m	6	32.09 ± 0.77 ℃
Kettle Pond 6	2022	(38.94 °N, -106.97 °W)	2865 m	22	33.97 ± 1.63 ℃

The most supported linear mixed effect model reported an AIC of 173.2 and included location, body condition, and sex as fixed effects with Julian day as a random effect (Table 4). Pond was removed as random effect for this analysis due to effect size based on pond being essentially zero. The conditional R^2 reported was 0.475. Metamorphic salamanders showed no statistical difference (p-value = 0.7796) between capture location whether from Mexican Cut or Kettle Pond 6 (Figure 4).

Table 4. Comparison of metamorph CT_{max} values among 2022 sites (Mexican Cut and Kettle Pond 6)

	Candidate Model			**Best Model**		
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	32.85	26.28 – 39.41	**<0.001**	33.05	26.46 – 39.65	**<0.001**
Location [MCNP]	-0.61	-4.89 – 3.68	0.775	-0.63	-5.05 – 3.80	0.775
BodyCond	2.11	-9.07 – 13.29	0.704	0.39	-10.97 – 11.75	0.944
Sex [M]				0.87	-0.50 – 2.23	0.205
Random Effects						
σ^2	2.95			2.84		

τ_{00}	1.77_{Julian}	2.09_{Julian}
ICC	0.38	0.42
N	8_{Julian}	8_{Julian}
Observations	40	40
Marginal R^2 / Conditional R^2	0.070 / 0.419	0.089 / 0.475

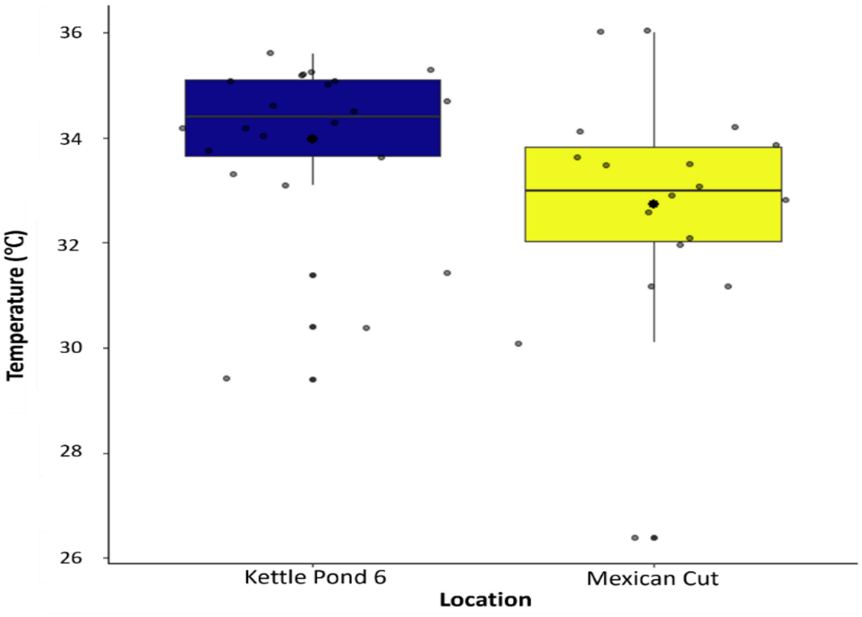

Figure 4. Differences in Metamorph CT_{max} by Location. Box plot depicting CT_{max} values for metamorphic salamanders collected from Kettle Pond 6 and Mexican Cut in 2022 with data points representing individual salamander CT_{max}. The boxplot shows the mean (black circle), median (black bar), interquartile range (box), and 1.5 times the interquartile range (lines). Kettle Pond 6 (N = 22); Mexican Cut Ponds (N = 18).

Seasonal variation in MCNP Paedomorphs

In order to determine if seasonal variation exists within CT_{max} values, a linear mixed effects model was created to determine if paedomorphs in Mexican Cut expressed statistically significant different CT_{max} values at different points in the year. Paedomorphs were the only morph used in this analysis due to metamorphic salamanders returning to their burrows in late July/August so they were unable to be collected later in the season unlike paedomorphic salamanders which can be consistently found in the ponds as long as they are accessible. The most supported model used month, sex, and body condition as fixed effects with pond and individual ID (to account for repeated measures) as random effects (Table 5). There were statistically significant differences detected (p-value = 0.001447) in CT_{max} of paedomorphs with paedomorphs in June showing lower CT_{max} values than paedomorphs in the July and September (Figure 5).

Table 5. Comparison of paedomorph CT_{max} values across various months at Mexican Cut

	Candidate Model			**Best Model**		
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	31.45	26.59 – 36.30	**<0.001**	31.51	25.54 – 37.48	**<0.001**
Month [7]	2.72	1.26 – 4.18	**<0.001**	2.72	1.25 – 4.19	**0.001**
Month [8]	1.41	-0.89 – 3.71	0.225	1.42	-0.91 – 3.75	0.226
Month [9]	2.77	1.19 – 4.35	**0.001**	2.76	1.12 – 4.41	**0.001**
BodyCond	-0.11	-15.97 – 15.76	0.989	-0.05	-16.12 – 16.01	0.995
Sex [M]	-0.09	-1.04 – 0.86	0.849	-0.09	-1.06 – 0.87	0.845
Heating Rate				-0.34	-12.50 – 11.82	0.955
Random Effects						
σ^2	3.13			3.19		
τ_{00}	0.02 PIT.Clip			0.03 PIT.Clip		
	0.88 Pond			0.87 Pond		
ICC	0.22			0.22		

N	4 Pond 6 PIT.Clip	4 Pond 6 PIT.Clip
Observations	59	59
Marginal R^2 / Conditional R^2	0.192 / 0.373	0.189 / 0.368

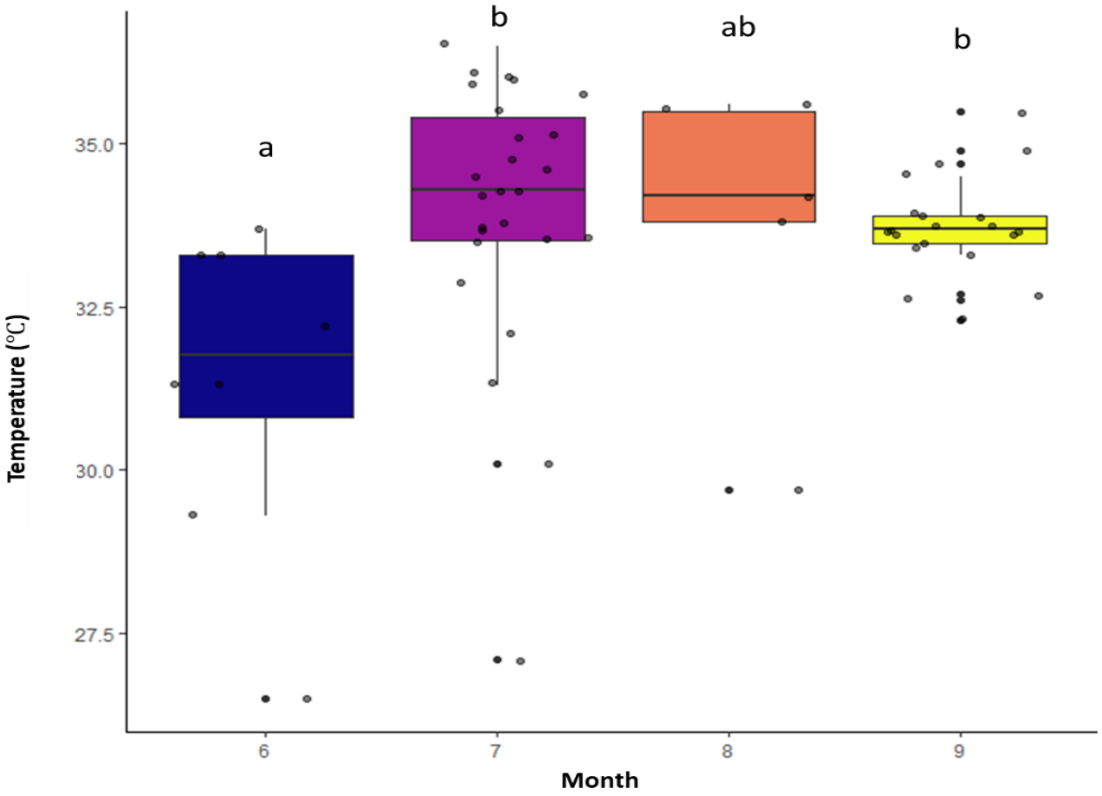

Figure 5. Seasonal variation in MCNP Paedomorph CT$_{max}$. Box plot depicting CT$_{max}$ values for paedomorphic salamanders separated by month of CT$_{max}$ experiment. The boxplot shows the median (black bar), interquartile range (box), and 1.5 times the interquartile range (lines). Months: June (N = 8); July (N = 26); August 8 (N = 5); September (N = 20)

Thermometer Validation

The linear regression comparing thermocouple temperature for larval salamanders reported a p-value <0.001 with an adjusted R^2 of 0. 9664. Thermocouple and infrared thermometer readings produced an almost 1:1 relationship (Figure 6). The near 1:1 relationship gives us confidence that the methods used to determine CT_{max} using the two methods are statistically similar to one another.

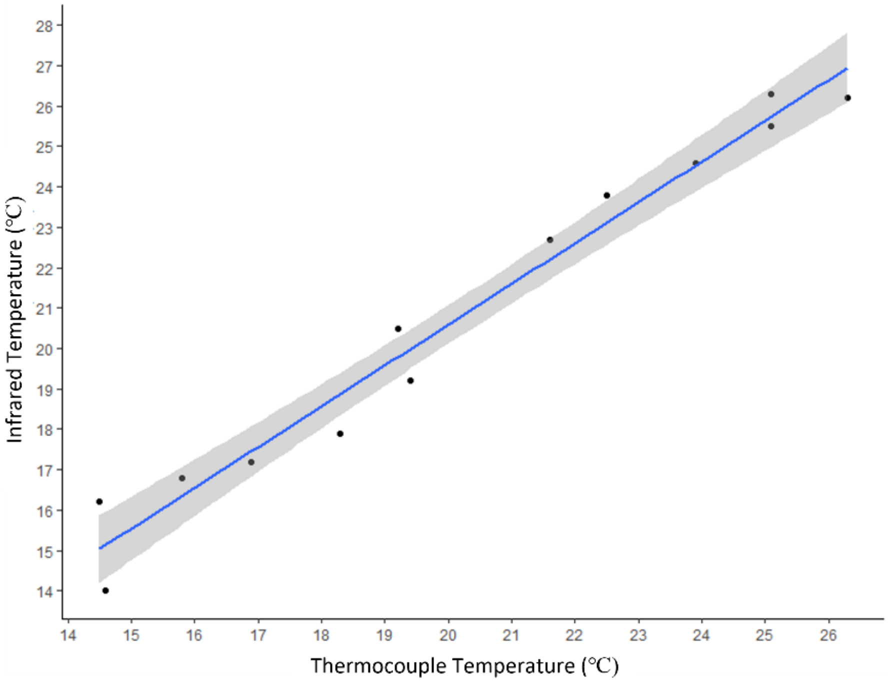

Figure 6. Thermometer validation. Linear regression between infrared thermometer and thermocouple cloacal CT_{max}. The shaded area indicates 95% confidence interval. Equation: $y = 0.96034x + 0.23407$, $R2 = 0.9664$, p-value = <0.001.

Salamander CT_{max} ranged between 26.4°C and 36.5°C and showed large areas of overlap between paedomorphic and metamorphic salamanders (Table 1). Larval salamanders showed similar CT_{max} values as salamanders, while hatchling salamanders showed the largest difference in CT_{max} values. Preliminary data indicates salamanders recover from LRR state occurs in < 1 minute.

Table 6. Summary of CT_{max} values separated by Age class for Mexican Cut 2022

Life history Stage	**N**	**CT_{max} ± SD**	**Maximum**	**Minimum**
All Adults	78	33.35 ± 2.04 °C	36.5	26.4
Paedomorph	60	33.53 ± 1.98 °C	36.5	26.5
Metamorph	18	32.73 ± 2.19 °C	36	26.4
2^{nd} /3^{rd} year Larvae	23	32.83 ± 1.48 °C	35	29.4
Hatchling	30	23.34 ± 1.46 °C	26	20.2

Morph by Sex Interaction on CT_{max}

The overlap between morph CT_{max} extended when morphs were subdivided by sex and tested to see if there was any sort of interactive effect between salamander morph and sex. The most supported linear mixed effect model reported an AIC of 450.4 and used the interaction of salamander morph and sex, body condition, and heating rate as fixed effects with pond and Julian day as random effects (Table 7). The conditional R^2 reported was 0.38. The model determined there were no statistical differences (p-value = 0.5091) of CT_{max} between the interaction of morph and sex (Figure 7).

Table 7. Comparison of CTmax values across morph by sex interaction at Mexican Cut

	Candidate Model			**Best Model**		
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	32.42	30.45 – 34.39	**<0.001**	33.13	30.92 – 35.33	**<0.001**

Sex [M]	0.49	-0.54 – 1.51	0.348	0.52	-0.51 – 1.56	0.317
Morph [Paedo]	0.75	-0.62 – 2.12	0.281	0.66	-0.58 – 1.90	0.293
BodyCond	0.96	-4.02 – 5.95	0.702	1.51	-3.61 – 6.62	0.560
Sex [M] × Morph [Paedo]	-0.40	-1.75 – 0.94	0.553	-0.45	-1.81 – 0.90	0.511
Heating Rate				-3.26	-9.39 – 2.87	0.294
Random Effects						
σ^2	2.51			2.57		
τ_{00}	1.39_{Julian}			1.36_{Julian}		
	0.30_{Pond}			0.09_{Pond}		
ICC	0.40			0.36		
N	9_{Pond}			9_{Pond}		
	22_{Julian}			22_{Julian}		
Observations	109			109		
Marginal R^2 / Conditional R^2	0.019 / 0.413			0.032 / 0.380		

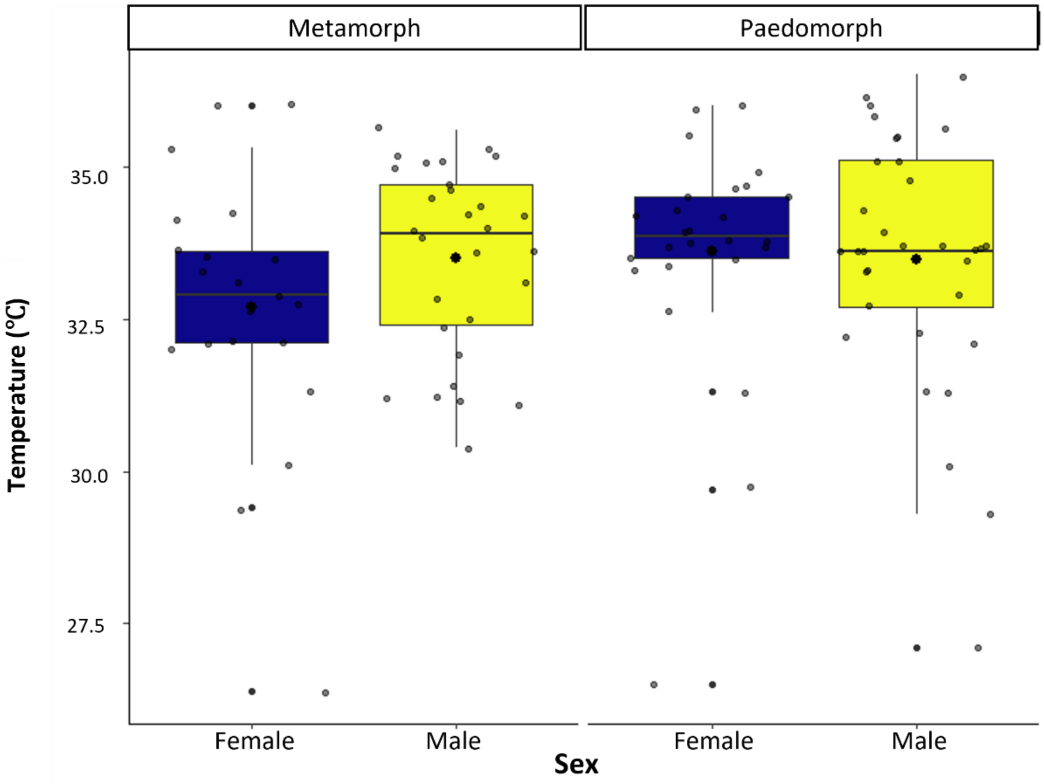

Figure 7. Morph by Sex Differences in CTmax. Box plot depicting CTmax values for adult salamanders separated by morph and faceted by sex with data points representing individual salamander CTmax. The boxplot shows the mean (black circle), median (black bar), interquartile range (box), and 1.5 times the interquartile range (lines). Metamorphs: Females (N = 21), Males (N = 29); Paedomorphs: Females (N = 26), Males (N = 33)

Influence of Age Class on CT_{max}

For the analysis of age class I used two approaches to determining if salamadners in different age classes have statistically significant differences in CT_{max}. The first approach uses hatchling salamanders but does not incorporate body condition due to mass, SVL, and TL being unavailable for body condition calculations for hatchling salamanders. Following this I will do a subsequent analysis using just larvae and adult salamanders for which I have body condition calculated.

For the age class comparison including hatchlings, the most supported linear mixed effect model reported an AIC of 441.3 with age class, heating rate, and initial water temperature as fixed effects and Julian day as a random effect (Table 8). Pond was not included as a random effect in this analysis due to hatchling salamanders coming from one pond meaning no detectable significance can be determined from including pond as an effect. The conditional R^2 reported was 0.934. The MCNP salamanders showed significant statistical differences (p -value < 0.0001) in CT_{max} between salamanders in different age class with hatchling salamanders included (Figure 8). There was a statistically significant difference in CT_{max} between age classes (p-value = 0.0011). A post hoc analysis using an emmeans test was performed determine which groups contained statistically significant different CT_{max} values. Hatchling salamander CT_{max} was determined to be statistically different from adult salamanders (p-value < 0.0001) and larval salamanders (p-value < 0.0001). The hatchling CT_{max} averaged 23.34 ± 1.46℃, and 11℃ lower than adult salamanders (paedomorphs) and 9.4℃ lower than larval salamanders (Table 6). Larval salamander CT_{max} was determined to be statistically different (p-value = 0.0021) than adult salamanders (paedomorphs) by 1.4℃.

Table 8. Comparison of CTmax values across age class (with hatchlings) at Mexican Cut. Models with hatchlings exclude body condition due to not having data needed to create body condition metrics

Candidate Model	**Best Model**

Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	33.91	32.67 – 35.14	**<0.001**	34.10	31.86 – 36.34	**<0.001**
Ontogeny [Hatchling]	-11.41	-12.42 – -10.39	**<0.001**	-11.39	-12.42 – -10.36	**<0.001**
Ontogeny [Larvae]	-1.40	-2.19 – -0.62	**0.001**	-1.40	-2.19 – -0.61	**0.001**
Heating Rate	-1.52	-5.75 – 2.71	0.478	-1.60	-5.91 – 2.71	0.464
Initial Water				-0.01	-0.12 – 0.10	0.836
Random Effects						
σ^2	1.92			1.94		
τ_{00}	2.31_{Julian}			2.33_{Julian}		
ICC	0.55			0.55		
N	19_{Julian}			19_{Julian}		
Observations	113			113		
Marginal R^2 / Conditional R^2	0.855 / 0.934			0.854 / 0.934		

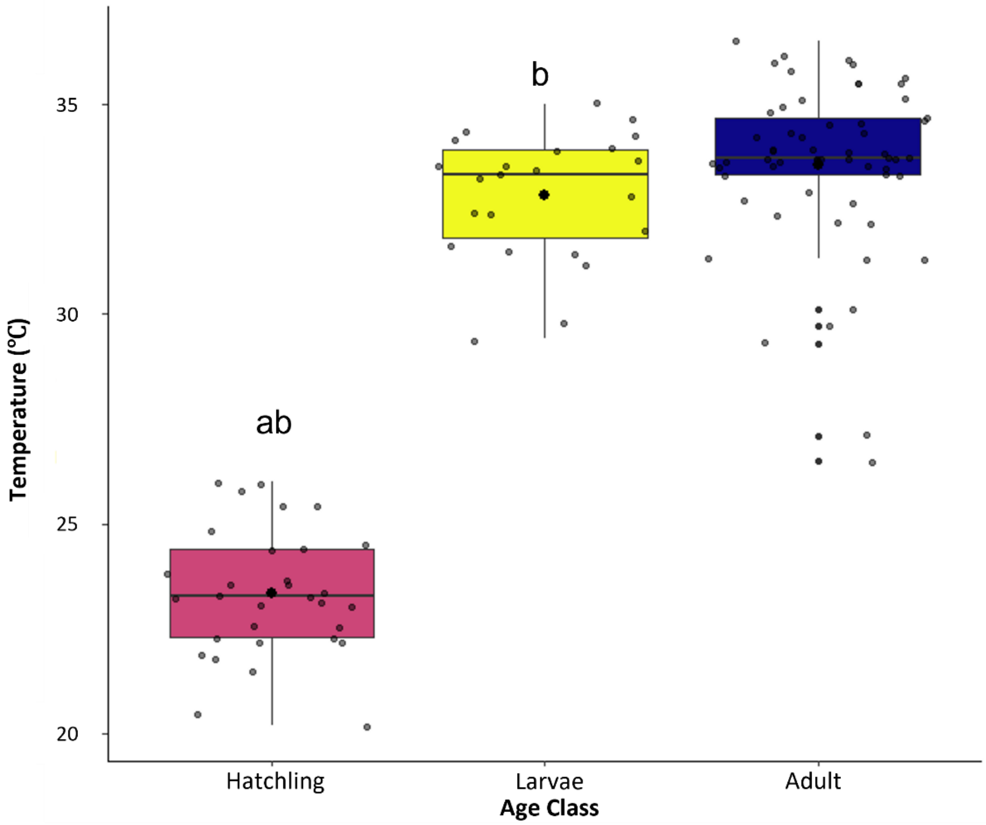

Figure 8. Influence of Age class on CTmax. Box plot depicting CTmax values for salamanders separated by age class with hatchling salamanders being young born withing the current year and larval salamanders being two- to three-year-old salamanders with adult age class consisting of only paedomorph salamanders. The boxplot shows the mean (black circle), median (black bar), interquartile range (box), and 1.5 times the interquartile range (lines). Letters indicate statistical significance (a indicates statistically significant different from Larvae; b indicates statistically significant different from Adults). Hatchlings (N = 31); Larvae (N = 23); Adults (N = 59)

For the age class comparison without hatchlings, the most supported linear mixed effect model reported an AIC of 322.4 and included salamander age class, body condition, and initial water as fixed effects with pond (capture location) and Julian day as random effects (Table 9). The conditional R^2 reported was 0.559. The significance we previously saw in the analysis excluding body condition between adult paedomorphs and larval salamanders is no longer statistically significant.

Table 9. Comparison of CTmax values across age class (without hatchlings) at Mexican Cut.

	Candidate Model			Best Model		
Predictors	*Estimates*	*CI*	*p*	*Estimates*	*CI*	*p*
(Intercept)	34.39	30.55 – 38.23	**<0.001**	33.56	29.16 – 37.96	**<0.001**
Ontogeny [Larvae]	-1.67	-3.65 – 0.32	0.098	-1.67	-3.66 – 0.32	0.099
BodyCond	-2.93	-16.32 – 10.47	0.665	-2.97	-16.40 – 10.46	0.661
Initial Water				0.05	-0.09 – 0.19	0.446
Random Effects						
σ^2	2.19			2.22		
τ_{00}	1.93 $_{\text{Julian}}$			1.85 $_{\text{Julian}}$		
	0.65 $_{\text{Pond}}$			0.63 $_{\text{Pond}}$		
ICC	0.54			0.53		
N	4 $_{\text{Pond}}$			4 $_{\text{Pond}}$		
	18 $_{\text{Julian}}$			18 $_{\text{Julian}}$		
Observations	78			78		
Marginal R^2 / Conditional R^2	0.068 / 0.572			0.067 / 0.559		

Body Temperature Differences Between Morphs

Body temperature data from the StarOddi temp loggers were used to determine if morph body temperatures experienced throughout the year were statistically significantly different (Figure 10). A linear mixed model used the morph by month interaction as a fixed effect with individual identity as a random effect to account for repeated measures for body temperature of individuals. There was a statistically significant difference in body temperatures between morphs in January, February, March, June, July, August, September, and November (p-value < 0.0001) (Figure 11). A post-hoc analysis determined metamorphs had significantly higher body temperature than paedomorphs in January, February, and March while paedomorphs had higher body temperatures than metamorphs in June, July, August, September, and November (Table 11).

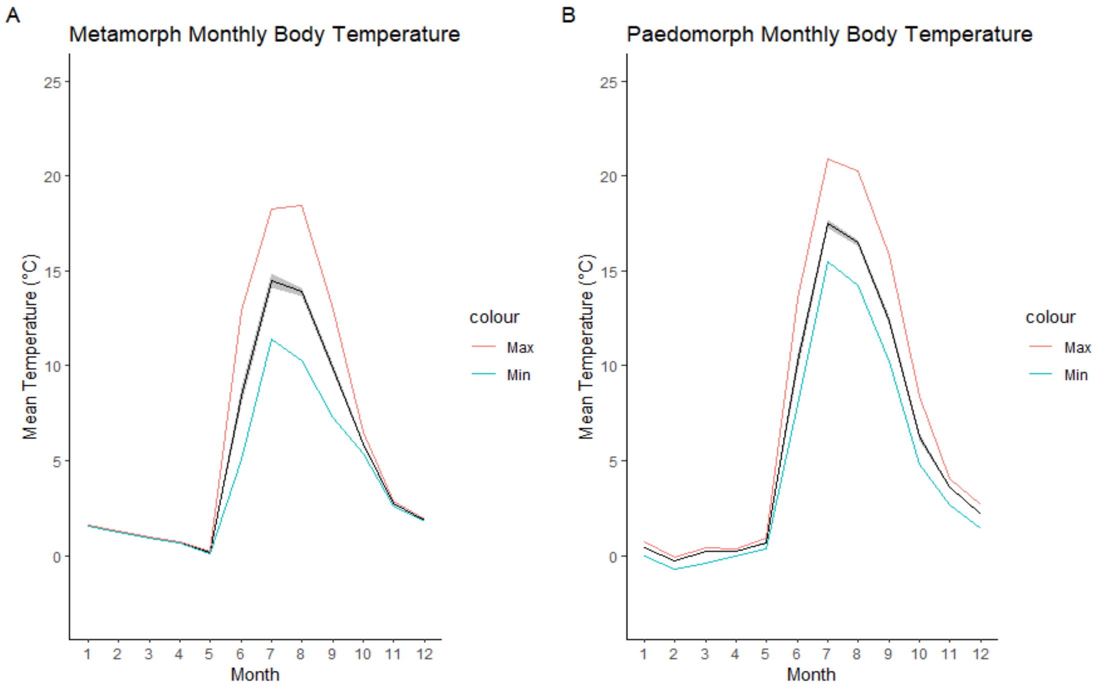

Figure 9. Average monthly body temperatures by morph. A). Metamorph (N = 4) average monthly body temperature with average maximum and minimum with shaded area being 95% confidence interval. B). Paedomorph (N = 7) body temperatures with associated average maximum and minimum with shaded area being 95% confidence

interval. Maximum (Red) and minimum temperatures (Blue) shown on graph are averaged monthly maximum temperature for each salamander morph.

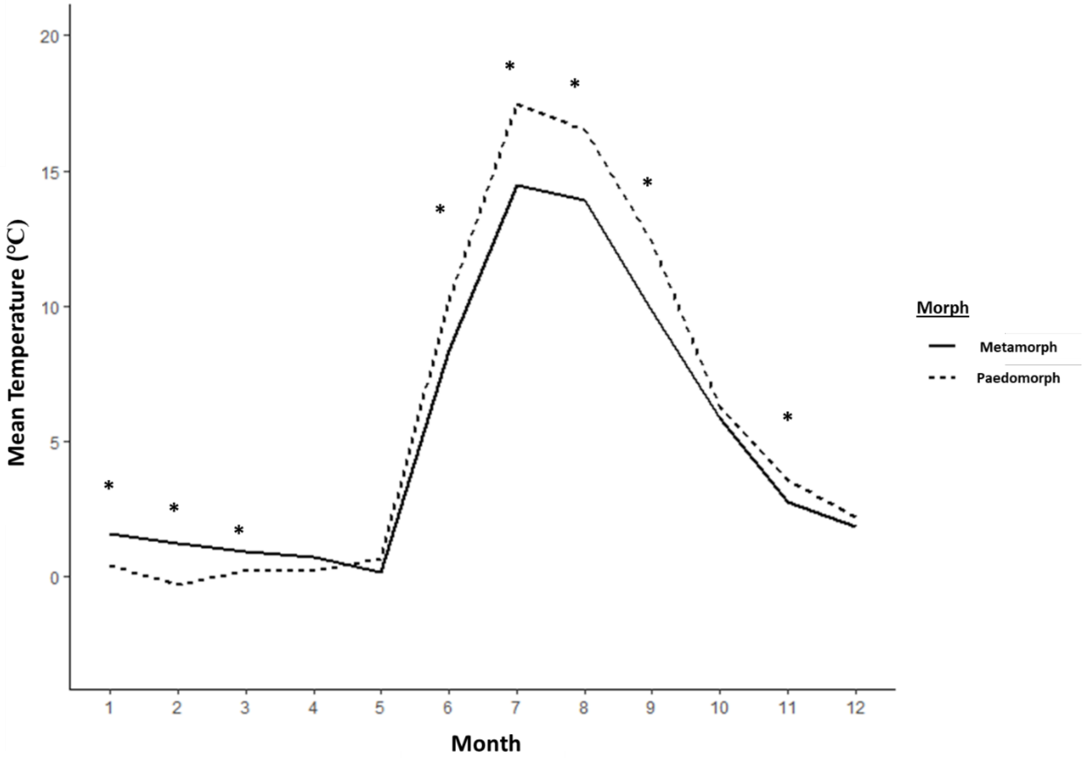

Figure 10. Summary of monthly temperature for metamorphs and paedomorphs. Body temperatures with associated average maximum and minimum with shaded area being 95% confidence interval. Asterisk indicates month of statistically significant difference in morph body temperatures.

Environmental Temperature

Soil temperatures decrease in variability with each 10 to 15 cm incremental increase in depth. Soil temperature for MCNP sites reach similar temperature values and reach extreme temperature less frequently than the surface after going 15 cm below the surface (Figure 12).

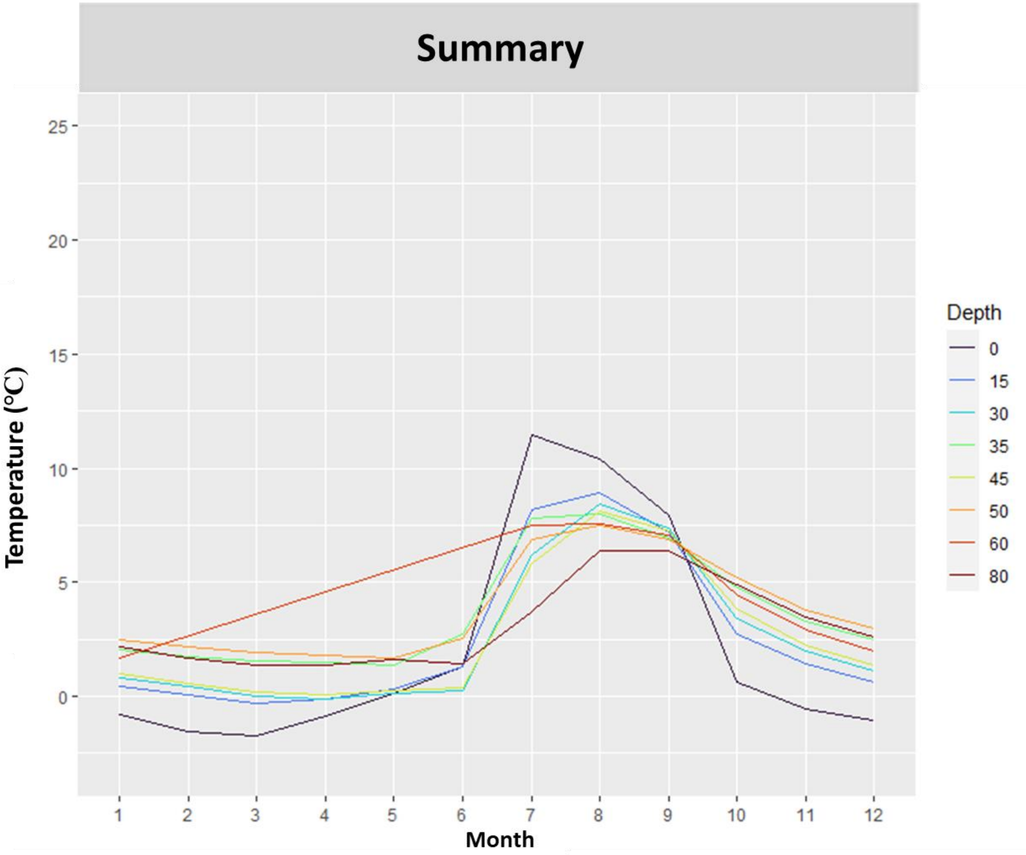

Figure 11. MCNP Monthly Soil Temperature. Average monthly soil temperature at Mexican Cut Nature Preserve labeled with corresponding depth in cm.

Water temperatures tends to be warmest in the summer months and much lower in the winter months leading up to freezing events (Figure 13). Temperature ranges were similar amongst all ponds where dataloggers were deployed with the hottest reported temperature reported in any given pond being 27.5℃.

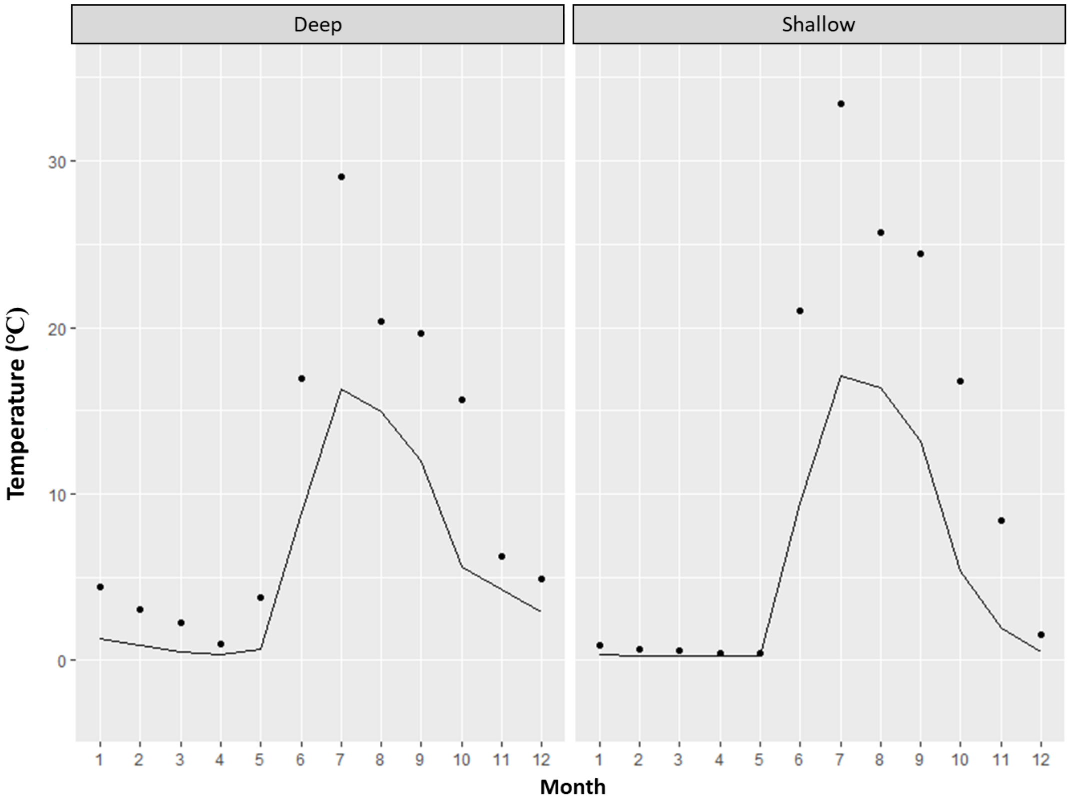

Figure 12. Average monthly temperature in aquatic salamander habitat. Average monthly temperature is displayed with the black line, monthly maximum temperatures are displayed with black dots.

Warming Tolerance Differences

To examine patterns in physiological responses to climate change, I estimated warming tolerance for each adult salamander morph using the difference in their CT_{max} and maximum experienced T_b. Metamorphic salamanders express higher warming tolerance values than paedomorphic salamanders during the active season (Figure 14).

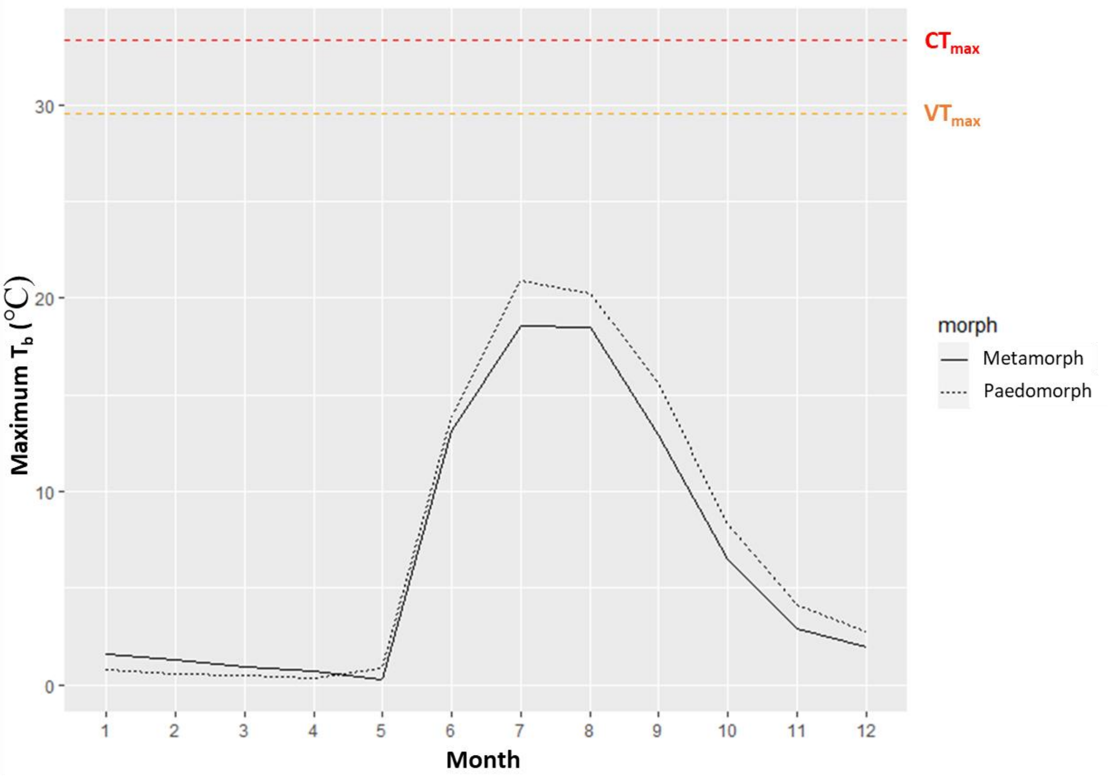

Figure 13. Maximum body temperature (T_b) experienced by metamorphic (solid line) and paedomorphic salamanders (dashed line) within MCNP. Dashed orange line refers to VT_{max} of salamanders, dashed red line refers to salamander CT_{max}

Differences in Activity Patterns

Pond light intensity can be thought of as pond thawing progress as HOBO dataloggers measure light intensity and are only set to log in the deepest parts of the ponds. When data loggers begin to receive light, they will have also started thawing out. Ponds with a higher average light intensity will subsequently have a larger number of days thawed out and receiving light. Pond 12 has the highest average light intensity compared to other ponds at MCNP (Figure 15). In May the number of active days for the two environments begins to emerge with the two-day difference increasing to a 14-day difference in June (Table 10).

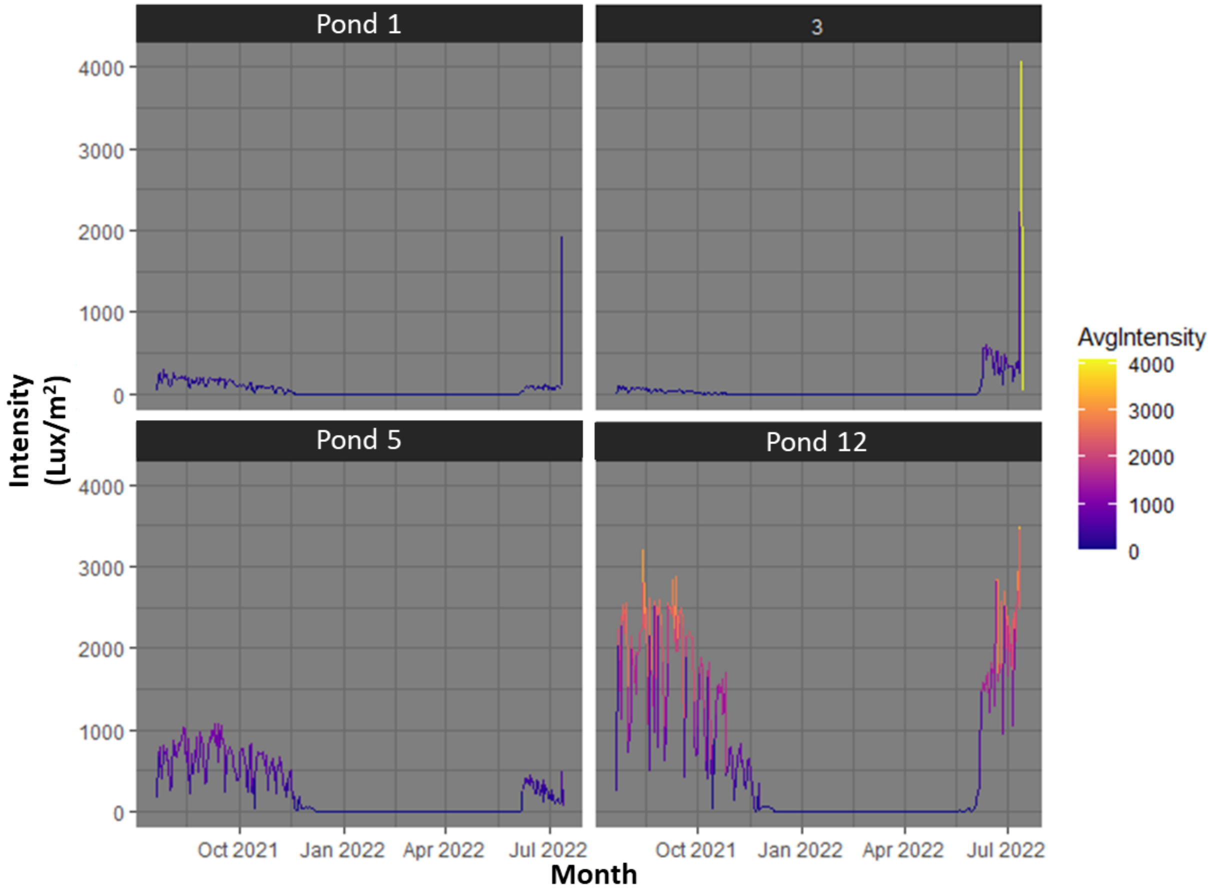

Figure 14. Average light intensity for MCNP ponds.

Table 10. Maximum number of ice/snow free days for salamanders by habitat. Active days calculated as the number of days within a month where average light intensity (Lux/m^2) is greater than 0. The aquatic environment active days is the average across all ponds where light loggers were distributed. The terrestrial environment light logger was placed at the surface and did not need to be averaged across sites.

Month	Aquatic Environment	Terrestrial Environment
January	0	0

February	0	0
March	0	0
April	0	0
May	2	0
June	22	8
July	24	21
August	29	31
September	30	30
October	30	31
November	21	17
December	13	0
Total	171	138

Discussion

Elevational Differences

I expected that metamorphic salamanders at the higher elevation would produce CT_{max} values much lower than metamorphic salamanders from a lower elevation, but they produced no statistical difference in CT_{max}, These results are similar in earlier studies in which CT_{max} was tested along an elevational gradient from montane ecosystems (2500-3000 m) to deserts (900-1500 m) and only showed differences in CT_{max} of hatchling salamanders (Delson & Whitford, 1973). While no differences were detected, the results do confirm the hypothesis predicted by the CVH and we see that a generalist species that exists along a wide latitudinal gradient displays a reflective wide thermal breadth (Gutiérrez-Pesquera et al., 2016).

I detected a year-to-year difference in metamorph salamander upper thermal limits within a low elevation pond. The important difference might lie in the year-to-year environmental conditions rather than an elevational effect as previously thought. Lower elevations typically receive very little snow and are afforded no snowpack compared to higher elevation sites. If one site is compared year to year, conclusions can be drawn regarding sources of variation causing differences in CT_{max}. Colorado in the summer of 2021 experienced severe drought conditions but received considerable snowfall the following winter. Snowpack is known to act as an insulator for terrestrial animals to extreme fluctuations in air temperatures especially in high elevation temperate areas (Muths et al., 2020). This increased snowfall event might have acted as a buffer for the newly metamorphosed salamanders at the low elevation site and resulted in them experiencing conditions slightly different from their predecessors and this was expressed as an increase in their CT_{max}. They might have been afforded a stronger influx of heat shock proteins which allowed them to extend the range of their thermal limit far higher than the salamanders from the previous year. Heat shock protein levels increase in relation to the frequency to exposure to naturally occurring abnormal thermal conditions (Chen et al., 2018).

Environmental Differences

Paedomorphs and metamorphs utilize different landscapes in attempts to maximize individual fitness under environmental norms that influence food availability, thermal zones, and population density (Thurman & Garcia, 2017). The conditions impacting the aquatic and terrestrial environment present each phenotype with a unique set of conditions and circumstances that cause variation in energy expenditure required to acquire nutrients, maintenance, and maintain certain metabolic rates this adds a new dimension associated with phenotype expression (Currens et al., 2002). If organisms experience local differences this would create localized adaptations to their microclimate and push organisms to independent evolutionary pathways promoting differences in populations due to environmental variation (Snyder & Weathers, 1975). Instead I found that this salamander that exhibits a generalist thermal strategy utilizes similar thermal environments in both adult morphs and is exposed to similar environmental variation and showcases similar CT_{max} values as expected by the CVH. I expected that the body temperatures reported for each morph to differ significantly in all months of the year as the metamorphs can utilize the terrestrial environment and this would promote differences in environmental pressures. I detected statistically significant differences in average monthly body temperatures differences between metamorphic and paedomorphic salamanders in eight months out of the calendar year (January, February, March, June, July, August, September, and November). As metamorphs are thermally cued by snowpack melt to emerge from burrows to begin to acquire food and reproduce, differences among experienced temperatures may promote mismatches in activity periods between morphs resulting in temporal mismatch within the population. This effect is currently seen in a population of toads that breed later than similar nearby species but only differ in their cue to emerge from hibernation (Green, 2017).

I suspect that in the early months of the year metamorphic salamanders are buffered from changes in temperature in the terrestrial environment because they are underground in rodent burrows that remain relatively stable thermally due to snowpack. Snowpack acts as an insulator as far down as 30 cm and decreases the effect of air temperature on soil temperature as you go further down in the soil column (Decker et al., 2003). This is confirmed in their body temperatures showing less than 0.5°C difference in temperature ranges. While we do not know exact where in the soil column these salamanders go when they retreat into their burrow, we can estimate that they go at least ~30 cm down in the soil column, based on the buffered effect to the extremely cold air temperatures measured in the winter months. I suspect that at this depth the metamorphic salamanders experience conditions so stable that they expend relatively little energy and would express low body temperatures that reflect a low amount of metabolic activity. We also see differences in T_b in the summer months likely due to snowmelt cueing metamorphs to emerge from their burrows meaning they experience greater variation in body temperature as the effect from air temperature increases. The shift in significance we see in body temperature in November can likely be explained by metamorphs reentering their burrows following summer months and snowpack building up as winter picks up causing that increase in insulative effect from the returning snow.

In paedomorphic salamanders we see a larger range of temperatures experienced within the early winter months. Conditions under ice may be heavily influenced by hydrodynamic conditions and change over the course of the winter as external and internal temperature change. Limited hydrologic movement under the ice creates stratified water column thermal zones with temperatures directly under the ice being closer to zero and temperature increasing closer to sediment (Joung et al., 2017). These thermal zone differences would promote different conditions when compared to metamorphic salamanders in the winter months and promote higher energy expenditure within the aquatic environment in winter conditions to continuously move to optimal thermal zones within frozen ponds. In the summer months temperatures increase but retain greater stability when compared to metamorphic salamanders. Paedomorphs are trapped within their ponds and are required to stay in zones that optimize their fitness but they have access to ample food sources and reproductive opportunities when compared to metamorphs that have to actively travel to obtain both of these resources. In November we once again see temperatures switching once ponds begin to freeze over and thermal stratification zones beginning to form within ponds.

Morph Differences

Salamanders make tradeoffs in life history trajectories depending on an array of environmental conditions (food availability, population density, temperature, etc.), these tradeoffs have been known to influence overall organism fitness seen through survival and reproductive success (Denoël et al., 2012; Thurman & Garcia, 2017). Paedomorphs remain in the water after reaching sexual maturity and focus on becoming efficient aquatic foragers in turn increasing their fitness in relationship to the rest of the individuals in the populations. Metamorphs sexually mature and utilize the terrestrial environment allowing them an opportunity to maximize access to resources and allow them to select for the environmental conditions best suited for their current needs (McCarthy et al., 2017). I believed that this fitness tradeoff between paedomorphs and metamorphs seen in nature would extend into climatic vulnerability within the species and potentially promote variation in the upper thermal limits of each morph. In a changing environment, the morph that was better able to handle variation in the ecosystem would have an increased fitness under these new normal environmental conditions. Throughout the study I found that although selective pressures create differing conditions and produce phenotypic plasticity within the Arizona Tiger salamander (*Ambystoma mavortium nebulosum*), there were no statistically detectable differences between the morphs in terms of their thermal environment. This enforces an idea that morph will contribute little to adult Arizona tiger salamanders ability to respond to climate change. The results of my study follow ideas expressed by previous research conducted at MCNP finding that spatiotemporal variation contributed more to variation in fitness within morphs rather than between morphs (Lackey et al., 2019). If morphs expressed differing abilities to tolerate high temperatures reflected in their CT_{max} then we might be able to draw conclusions regarding selective pressures causing an increase in evolutionary mismatch within a single species and this would be reflected in the ratio of one morph to become more common place (Fitzpatrick, 2012; Smith et al., 2014). I suspect that currently the influence of climate change within the population is acting relatively weakly on each morph within this population of salamanders. These salamanders exhibit a generalist range strategy and experience a wide range of thermal conditions within this snowmelt-dominated wetland that is prone to extreme weather conditions and large fluctuations in temperature (Thurman & Garcia, 2017; Whiteman & Wissinger, 2005).

Sex-Specific Differences in CT_{max}

Males and females within various species can differ in a wide range of morphological, physiological, and behavioral aspects which can create varying selective pressures on individuals of a particular sex (Pottier et al., 2021). Previous research conducted at Mexican Cut Nature Preserve determined a morph by sex difference influencing lifetime fitness (Lackey et al., 2019). If sex-specific differences were determined in CT_{max} it might promote biased sex ratios to rise in the population causing differential survival and decreasing viability within the population (Pottier et al., 2021). No differences existed in the way of sex-specific differences within this species and reflected similar CT_{max} values indicating similar abilities for each sex of salamander to respond to climate change.

While I did not detect morph or sex-specific differences in CT_{max}, I argue that this does not mean that a climate change will not differentially influence subgroups within the species. New research has begun to highlight the overlap in thermal sensitivity and fertility and raises importance to understand how various performance metrics are altered as temperatures rise. Connections between thermosensitivity influencing fertility have been found in several species, fruit flies within the family *Drosophilidae* show decreased sperm motility and offspring production with increases in temperature (Iossa, 2019). The Ezo salamander, *Hynobius retardatus*, has been noted as showing differential expression of *Cyp19a1*, an gene responsible for the creation of aromatase. The differential expression of *Cyp19a1* between sexes shifts as temperature increases and influence their gonadal development causing feminization of male salamanders (Ruiz-Garciá et al., 2021). This creates future areas of research and raises the need to research more than just sex-specific differences within a population. Increases in temperature might have create unseen pressures on various groups within a population and impact developing organisms, like hatchlings, within the population and influence sex ratios within a population altering the overall fitness of the species.

Age Class

In species that exhibit complex life histories, undergoing metamorphosis can result in increased vulnerability to environmental stressors (Lowe et al., 2021). Larval organisms face an increased pressures to be able to respond and utilize variations in their environment to maximize productivity and gain enough nutrients to reach sexual maturity. Larval salamanders were shown to not be able to thermoregulate except in environments where they face thermally challenging habitats (Piasečná et al., 2015). Larval salamanders like those living in MCNP experience extreme conditions that result in a prolonged interval in a premature status before they reach the point where they become sexually mature (Denoël et al., 2012; Whiteman & Wissinger, 2005). I expected larval salamanders to be more vulnerable in these premature states and this would result in a lowered CT_{max} when compared to adults, but things were not as simple as anticipated. Temperatures experienced during early life stages can result in a buffer in later life stages and allow organisms to be more protected from thermal variation later on in life (Taylor et al., 2020). Salamanders that overwinter are experiencing conditions that result in their ability to better tolerate exposure to high temperatures beyond their preferred temperatures. This idea is reenforced by the statistical difference seen in hatchling CT_{max} and no statistical difference between larval and adult salamanders.

The hatchling salamanders supported this prevailing idea that premetamorphic organisms exhibit a CT_{max} lower than postmetamorphic organisms, but the larvae do not contradicting this idea (Delson & Whitford, 1973; Lowe et al., 2021). This is not the first instance of older individuals showcasing higher thermal limits than other life stages. In some insect species like the common fruit fly, *Drosophila melanogaster,* we see that pupae exhibit higher thermal tolerances than other life stages of development (Lowe et al., 2021). While this might explain some of the similarities we see in CT_{max}, there might also be a behavioral component that results in similarities among various life history stages. Salamanders can behaviorally utilize various zones within ponds and this can result in differences between individuals in the amount of thermal variation they might experience in their lifetime (Heath, 1975). As we see in other ectotherm species, salamanders might be increasing their thermal tolerances through behavioral exploitation of available temperatures by moving throughout the heterogeneous environment available to them and intentionally exposing themselves to a wide range of temperatures (Hutchison & Maness, 1979). Further research needs to be conducted to determine if salamanders are behaviorally exposing themselves to extreme temperatures or whether this is just a product of other behaviors such as foraging or thermoregulating.

Activity Period Differences

Salamanders experience environmental conditions remarkably similar in all months of the year but variation within each environment varies. While thermal environment varied very little between salamanders, we might expect that slight differences in pond thaw and snow melt cause variation in salamander activity periods allowing certain morphs to more readily utilize the environment. Correlating increases in environmental temperatures with salamander activity periods, paedomorphic salamanders could become active earlier and extend cohort than metamorphic salamanders following the availability of optimal conditions in their environment earlier. Paedomorphic salamanders see complete partial thawing of ponds in early May in some instances long before the snow has completely melted and metamorphs receive the cue to return to ponds to acquire food and breed. Paedomorphs are able to utilize a total of 63 hours in May throughout the month. This mismatch between organismal access to resource can create temporal mismatch between morphs and their availability to resources which can increase selective pressures influencing growth, development, and reproductive opportunities. Metamorphs are able to allocate more energy toward development and reproduction than paedomorphs (Currens et al., 2002). Earlier acquisition of nutrients might allow paedomorphs to reduce the energetic advantage metamorphic salamanders have and create a reciprocal tradeoff for morphs with paedomorphs becoming active sooner and for longer.

Previously it was discovered that sex-specific differences existed within MCNP. Male paedomorphs had more reproductive opportunities than their metamorph counterparts (Lackey et al., 2019). One reason for this sex-specific difference might lie in a slight increase in disparity between salamander active periods guided by changes in environmental cues. As ponds begin to thaw sooner, paedomorphs become able to utilize prey availability sooner than metamorphs, this would cause a more rapid increase in paedomorph body condition and more sustained foraging opportunities in late active season following overwintering events and allow them to obtain ideal body conditions to focus on reproducing and possibly removing the tradeoff in longevity associated with breeding sooner that we currently seen within the population. In the wake of climate change, species are shifting their geographic ranges and this shift might result in the introduction of a new food source into aquatic environment. Paedomorphic salamanders will have access to this food source much sooner and might be able to utilize it for an increase in development and growth rates. The aquatic environment would allow for larval salamanders to gather a larger amount of resources to be used toward their development.

Warming Tolerance

Warming tolerances was calculated using body temperature and CT_{max} data, results in estimates of their ability to respond to changes in their environment. Metamorphic salamanders presented higher warming tolerances than paedomorphic salamanders. If climate change was to negatively impact a particular morph we might expect that paedomorphs would be more likely to be extirpated in the wake of climate change. High warming tolerance values, as seen by the MCNP metamorphic salamanders, are associated with an animals being less sensitive to climate change (Taylor et al., 2020). An amphibian with a large warming tolerance may not benefit from the thermal buffer if climate change does not occur uniformly along the entirety of its geographic range (Duarte et al., 2012).

CT_{min} Inferences

While CT_{min} was unable to be determined in salamanders, it is incredibly important to discuss reasons for why these salamanders were not able to reach their CT_{min} values. Lower thermal limits experiments were able to lower salamander temperatures to as low as ~0.2 ℃ but following this body temperature began to rise back up. Originally it was believed that these salamanders were not experiencing subzero conditions but upon looking at collected body temperature data it was determined that these salamanders, both metamorphs and paedomorphs, experience conditions close to 0℃. I suggest two mechanisms for why I was unable to determine CT_{min} in salamanders, freeze tolerance and freeze avoidance. The StarODDI biologgers we would have used to ultimately contrive low winter temperature conditions salamanders experience was not extremely useful as ~4 of the implanted data loggers showed low temperature failures and made the data hard to decipher if useable at all. This hindered my ability to make inferences about what temperatures might hold their CT_{min} values.

Freeze tolerance is the ability to survive freezing of body tissues through the use of ice nucleating proteins (Bagwell & Ricker, 2019). Freeze tolerance typically develops in fauna that overwinter on sites where temperature drops to points that can freeze tissue (Storey & Storey, 2017). The use of ice nucleating proteins is already found in amphibians and reptiles and provides them protection against extreme cold weather events (Bagwell & Ricker, 2019). If organisms experience freezing temperatures and are unable to cope they would face conditions that would disrupt cellular processes and lead to high mortality rates (Bagwell & Ricker, 2019). Freeze avoidance and freeze tolerance are common strategies in species inhabiting environments where temperatures fluctuate widely, fall close to freezing frequently, and where freezing events are unpredictable (Reider et al., 2021).

Freeze avoidance, typically in the form of supercooling, is the ability of organisms to remain unfrozen even when exposed continuously to temperatures below the freezing point of tissues in their bodies (Storey & Storey, 2017). For species that survive in cold climates they must prevent the freezing of bodily fluids and tissue via depressing the freezing point of their bodily fluids to endure exposure to subzero temperatures (Storey, 1990). Freeze avoidance behavior is typically paired with the expression of antifreeze proteins that work similarly to heat shock proteins but stabilize the supercooled state required to resist freezing temperatures (Storey & Storey, 2017).

Significance in Data

Throughout my data one prevailing variable that remains important to consider when discussing context-dependent responses to climate change is body condition. I believe that a lot of the underlying significance seen within my data can be contributed to body condition. Body condition largely influences how fast an organism would warm and this might influence how these animals will respond to changes in their environment with climate change (Claunch et al., 2021).We see differences in the CT_{max} of salamanders in different age classes, likely attributed to variation in how the various salamanders ages are able to utilize their environment and their body conditions. It has been proven that seasonal climatic conditions can influence body condition in other *Ambystoma* species and this might explain the variation we see in the CT_{max} of paedomorphs throughout the season (Moldowan et al., 2021). We might have captured declining body condition of the paedomorphic organisms throughout the season as they became more active and saw this reflected in differences in their CT_{max} values.

A comparison between metamorph and paedomorph metabolic rate determined they have essentially indistinguishable rates of oxygen consumption in water. Metamorphs had substantially higher metabolic rates in water than when exposed to the air (Currens et al., 2002). We captured differences in metamorph and paedomorph body temperature in various months as well as slight differences in activity windows using the light intensity data. We might have collected data that suggests some sort of temporal variation within this population that would suggest paedomorphs should have an advantage in terms of access to food, and reproductive events, but the efficiency of metamorphs when they return to water allows them to out compete their aquatic counterparts and offset differences in their earlier access to resources.

Conclusions

Here I have shown while adult salamanders do not differ in their upper thermal limits, hatchling salamanders do express a lowered CT_{max}. Larval salamanders express statistically different CT_{max} values than paedomorphic salamanders, confirming an influence of age class on upper thermal limits of salamanders. While adult salamanders do not differ in their CT_{max} they do differ in their T_b in 8 months of the year. While salamanders do not currently have differences in their active periods, increases in temperature due to climate change are likely to result in an increase in differences in their activity periods affording differences in resource availability associated with each morph. I believe that my study raises new questions regarding the future directions of long term studies.

Future research should focus on gathering CT_{min} values and CT_{max} values to allow analysis of full thermal profile of salamanders to be determined. I also believe that study to determine the metabolic costs associated with locomotion as well as exiting in aquatic and terrestrial environments would prove beneficial to assess the full viability of this population and populations similar to it in the wake of climate change.

Appendix

I. Model Selection Tables

Sex by Morph

Model	Fixed Effects	ΔAIC	ΔBIC	R^2	RMSE
1	Sex*Morph, Body Cond	0	0	0.413	1.436
2	Sex*Morph, Body Cond, Heating Rate	0.34	3.03	0.380	1.453
3	Sex*Morph, resBody, Heating Rate	0.54	3.23	0.380	1.455
4	Sex*Morph, resBody, Initial Water	1.64	4.33	0.395	1.446
5	Sex*Morph, Body Cond, Initial Water	1.69	4.38	0.406	1.440
6	Sex*Morph, Body Cond, Heating Rate, Initial Water	2.26	7.64	0.375	1.455
7	Sex*Morph, resBody, Heating Rate, Initial Water	2.44	7.82	0.373	1.457

Elevational Gradient

Model	Fixed Effects	ΔAIC	ΔBIC	R^2	RMSE
1	Body Cond, Elevation, Initial Water	0	0	0.483	0.647
2	Body Cond, Elevation	0.886	-0.059	0.576	0.673
3	Body Cond, Elevation, Sex, Initial Water Temp	1.889	2.843	0.548	0.631

4	Body Cond, Elevation. Sex	2.897	2.897	0.613	0.665

Annual difference at KP

Model	Fixed Effects	ΔAIC	ΔBIC	R^2	RMSE
1	Sex, Condition	0	0	0.388	1.376
2	Condition, Heating Rate	0.484	0.484	0.386	1.387
3	Condition, Initial Water	0.787	0.787	0.439	1.382
4	Sex, Condition, Heating Rate	1.641	3.107	0.347	1.378
5	Sex, Condition, Initial Water	1.810	3.276	0.396	1.370
6	Condition, Heating Rate, Initial Water	2.388	3.854	0.390	1.383
7	Sex, Condition, Heating Rate, Initial Water	3.420	6.351	0.352	1.371

MCNP VS KP Metamorphs

Model	Fixed Effects	ΔAIC	ΔBIC	R^2	RMSE
1	Location, Body Cond	0	0	0.419	1.571
2	Location, Body Cond, Sex	0.498	2.187	0.475	1.513
3	Location, Body Cond, Heating Rate	1.636	3.325	0.423	1.562
4	Location, Body Cond, Sex, Heating Rate	1.937	5.315	0.479	1.501
5	Location, Body Cond, Initial Water	1.976	3.665	0.415	1.572

6	Location, Body Cond, Sex, Initial Water	2.463	5.841	0.467	1.515
7	Location, Body Cond, Initial Water, Heating Rate	3.160	6.987	0.418	1.563
8	Location, Body Cond, Sex, Heating Rate, Initial Water	3.898	8.965	0.470	1.503

Life History

Age Class No Hatchlings

Model	**Fixed Effects**	**ΔAIC**	**ΔBIC**	**R^2**	**RMSE**
1	Ontogeny, Body Cond	0	0	0.572	1.320
2	Ontogeny, Body Cond, Initial Water	1.367	3.724	0.559	1.322
3	Ontogeny, Body Cond, Heating Rate	1.780	4.137	0.562	1.323
4	Ontogeny, Body Cond, Heating Rate, Initial Water	3.204	7.918	0.550	1.325

Age Class with Hatchlings

Model	**Fixed Effects**	**ΔAIC**	**ΔBIC**	**R^2**	**RMSE**
1	Ontogeny, Heating Rate	0	0	0.934	1.267
2	Ontogeny, Initial Water	0.531	0.531	0.935	1.268
3	Ontogeny, Initial Water, Heating Rate	1.955	4.682	0.934	1.267
4	Ontogeny, Pond	2.511	7.966	0.933	1.267

5	Ontogeny, Pond, Heating Rate	4.306	12.488	0.932	1.266
6	Ontogeny, Initial Water, Pond	4.415	12.598	0.933	1.266
7	Ontogeny, Heating Rate, Initial Water, Pond	6.167	17.076	0.932	1.265

Seasonal variation of Paedomorphs

Model	**Fixed Effects**	**ΔAIC**	**ΔBIC**	**R^2**	**RMSE**
1	Month, Sex	0	0	0.144	1.839
2	Julian, Sex	0.371	0.371	0.131	1.852
3	Month, Sex, Heating Rate	2.547	4.044	0.139	1.841
4	Julian, Sex, Heating Rate	2.923	4.419	0.127	1.854

II. DOI Link to OSF Folder

https://osf.io/ryj7n/?view_only=f9312a3ae76c49f596b72a3c1361e9e8

III. Supporting Information

Table 11.Post-hoc Analysis of Body Temperature differences. Summary of monthly body temperature difference estimates of metamorph and paedomorph salamanders from MCNP.

Month	**Estimate**	**SE**	**Z-ratio**	**p-value**
1	1.094	0.259	4.220	<0.0001
2	0.856	0.261	3.285	0.0010
3	0.605	0.259	2.333	0.0196
4	0.505	0.259	1.949	0.0513
5	-0.388	0.259	-1.499	0.1338
6	-1.741	0.260	-6.705	<0.0001
7	-2.999	0.265	-11.319	<0.0001

8	-2.600	0.257	-10.128	<0.0001
9	-2.502	0.257	-9.711	<0.0001
10	-0.314	0.257	-1.224	0.2211
11	-0.862	0.257	-3.355	0.0008
12	-0.409	0.257	-1.588	0.1122

Table 12. Metamorph average T_b, average maximum T_b, and absolute maximum T_b in the months of June, July, and August

Hour	**Mean T_b ±SD**	**Mean Max**	**Absolute Max**
0	11.74 ± 4.73 ℃	17.40	18
1	11.59 ± 4.43 ℃	16.96	17.5
2	11.30 ± 4.30 ℃	16.45	16.9
3	10.98 ± 4.19 ℃	16.05	16.64
4	10.64 ± 4.09 ℃	15.56	16.39
5	10.39 ± 4.01 ℃	15.12	16.06
6	10.23 ± 3.93 ℃	15.08	16.39
7	10.07 ± 3.83 ℃	14.85	16
8	9.93 ± 3.77 ℃	14.69	15.87
9	9.89 ± 3.69 ℃	14.65	15.87
10	10.12 ± 3.66 ℃	14.68	15.87
11	10.61 ± 3.81 ℃	16.3	17.25
12	11.58 ± 4.11 ℃	19.28	20.69
13	12.96 ± 4.58 ℃	21.54	23.02
14	14.06 ± 5.01 ℃	22.18	28.14
15	14.99 ± 5.14 ℃	22.35	26.9
16	15.61 ± 5.02 ℃	22.63	26.27
17	15.71 ± 4.93 ℃	22.25	23.2
18	15.65 ± 4.83 ℃	22.42	23.17
19	15.19 ± 4.67 ℃	21.86	23.1
20	14.62 ± 4.45 ℃	20.03	21.13
21	13.95 ± 4.34 ℃	19.24	20.47
22	13.19 ± 4.35 ℃	18.69	19.6
23	12.44 ± 4.51 ℃	17.93	18.9

Table 13. Paedomorph average Tb, average maximum Tb, and absolute maximum Tb in the months of June, July, and August when extreme temperatures are experienced more frequently

Hour	**Mean T_b ± SD**	**Mean Max**	**Absolute Max**
0	14.51 ± 4.63 °C	18.99	20.25
1	14.22 ± 4.64 °C	18.71	20.11
2	14.02 ± 4.59 °C	18.43	19.84
3	13.80 ± 4.58 °C	18.14	19.63
4	13.61 ± 4.58 °C	17.97	19.5
5	13.42 ± 4.54 °C	17.73	19.25
6	13.24 ± 4.50 °C	17.44	19.13
7	13.08 ± 4.49 °C	17.32	18.91
8	12.96 ± 4.47 °C	17.17	18.87
9	12.93 ± 4.43 °C	17.04	18.78
10	13.06 ± 4.39 °C	17.20	19
11	13.45 ± 4.38 °C	17.95	19.34
12	14.25 ± 4.58 °C	20.27	23.3
13	15.26 ± 4.84 °C	22.15	26.67
14	16.25 ± 5.14 °C	23.09	25.32
15	16.87 ± 5.25 °C	23.59	25.88
16	17.20 ± 5.22 °C	24.97	29.63
17	17.24 ± 5.13 °C	23.77	26.63
18	16.97 ± 5.00 °C	23.63	26.07
19	16.56 ± 4.81 °C	22.76	25.27
20	16.09 ± 4.60 °C	21.41	22.48
21	15.67 ± 4.50 °C	20.44	21.41
22	15.24 ± 4.45 °C	19.79	20.86
23	14.92 ± 4.44 °C	19.32	20.49

Literature Cited

Andersen, J. L., Manenti, T., Sørensen, J. G., Macmillan, H. A., Loeschcke, V., & Overgaard, J. (2015). How to assess Drosophila cold tolerance: Chill coma temperature and lower lethal temperature are the best predictors of cold distribution limits. *Functional Ecology*, *29*(1), 55–65. https://doi.org/10.1111/1365-2435.12310

Angilletta, M. J., Niewiarowski, P. H., & Navas, C. A. (2002). The evolution of thermal physiology in ectotherms. In *Journal of Thermal Biology* (Vol. 27, Issue 3, pp. 249–268). https://doi.org/10.2741/e148

Bagwell, S. N., & Ricker, J. V. (2019). Antifreeze proteins: effective adaptations of organisms for low temperature survival. *BIOS*, *90*(3), 158. https://doi.org/10.1893/bios-d-17-00007

Bates, D., Mächler, M., Bolker, B. M., & Walker, S. C. (2015). Fitting linear mixed-effects models using lme4. *Journal of Statistical Software*, *67*(1). https://doi.org/10.18637/jss.v067.i01

Bestion, E., Teyssier, A., Richard, M., Clobert, J., & Cote, J. (2015). Live Fast, Die Young: Experimental Evidence of Population Extinction Risk due to Climate Change. *PLoS Biology*, *13*(10), 1–19. https://doi.org/10.1371/JOURNAL.PBIO.1002281

Blaustein, A. R., Walls, S. C., Bancroft, B. A., Lawler, J. J., Searle, C. L., & Gervasi, S. S. (2010). Direct and indirect effects of climate change on amphibian populations. *Diversity*, *2*(2), 281–313. https://doi.org/10.3390/d2020281

Bodensteiner, B. L., Agudelo-Cantero, G. A., Arietta, A. Z. A., Gunderson, A. R., Muñoz, M. M., Refsnider, J. M., & Gangloff, E. J. (2021). Thermal adaptation revisited: How conserved are thermal traits of reptiles and amphibians? *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, *335*(1), 173–194. https://doi.org/10.1002/jez.2414

Buckley, L. B., & Huey, R. B. (2016). Temperature extremes: geographic patterns, recent changes, and implications for organismal vulnerabilities. In *Global Change Biology* (Vol. 22, Issue 12, pp. 3829–3842). https://doi.org/10.1111/gcb.13313

Cahill, A. E., Aiello-Lammens, M. E., Caitlin Fisher-Reid, M., Hua, X., Karanewsky, C. J., Ryu, H. Y., Sbeglia, G. C., Spagnolo, F., Waldron, J. B., Warsi, O., & Wiens, J. J. (2013). How does climate change cause extinction? In *Proceedings of the Royal Society B: Biological Sciences* (Vol. 280, Issue 1750). Royal Society. https://doi.org/10.1098/rspb.2012.1890

Cavallo, C., Dempster, T., Kearney, M. R., Kelly, E., Booth, D., Hadden, K. M., & Jessop, T. S. (2015). Predicting climate warming effects on green turtle hatchling viability and dispersal performance. *Functional Ecology*, *29*(6), 768–778. https://doi.org/10.1111/1365-2435.12389

Chen, B., Feder, M. E., & Kang, L. (2018). Evolution of heat-shock protein expression underlying adaptive responses to environmental stress. In *Molecular Ecology* (Vol. 27, Issue 15, pp. 3040–3054). Blackwell Publishing Ltd. https://doi.org/10.1111/mec.14769

Claunch, N. M., Nix, E., Royal, A. E., Burgos, L. P., Corn, M., DuBois, P. M., Ivey, K. N., King, E. C., Rucker, K. A., Shea, T. K., Stepanek, J., Vansdadia, S., & Taylor, E. N. (2021). Body size impacts critical thermal maximum measurements in lizards. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, *335*(1), 96–107. https://doi.org/10.1002/jez.2410

Collins, J. P. (1981). Distribution, Habitats and Life History Variation in the Tiger Salamander, Ambystoma tigrinum, in East-Central and Southeast Arizona. *Copeia*, *1981*(3), 666–675.

Corn, P. S., & Muths, E. (2002). Variable Breeding Phenology Affects the Exposure of Amphibian Embryos to Ultraviolet Radiation. *Ecology*, *83*(11), 2958–2963. https://www.jstor.org/stable/307

Cunningham, H. R., Rissler, L. J., Buckley, L. B., & Urban, M. C. (2016). Abiotic and biotic constraints across reptile and amphibian ranges. *Ecography*, *39*(1), 1–8. https://doi.org/10.1111/ecog.01369

Currens, C. R., Niewiarowski, P. H., & Whiteman, H. H. (2002). Effects of Temperature and Time of Day on the Resting Metabolic Rates of Paedomorphic and Metamorphic Mole Salamanders, Ambystoma talpoideum. *Copeia*, *2002*(2), 489–495.

Decker, K. L. M., Wang, D., Waite, C., & Scherbatskoy, T. (2003). Snow Removal and Ambient Air Temperature Effects of Forest Soil Temperatures in Northern Vermont. *Soil Science Society of America Journal*, *67*(5), 1629–1629. https://doi.org/10.2136/sssaj2003.1629

Delgado-Suazo, P., & Burrowes, P. A. (2022). Response to thermal and hydric regimes point to differential inter- and intraspecific vulnerability of tropical amphibians to climate warming. *Journal of Thermal Biology*, *103*, 103148. https://doi.org/10.1016/j.jtherbio.2021.103148

Delson, J., & Whitford, W. G. (1973). Critical Thermal Maxima in Several Life History Stages in Desert and Montane Populations of Ambystoma tigrinum. *Herpetologica*, *29*(4), 352–355.

Denöel, M. (2017). On the identification of paedomorphic and overwintering larval newts based on cloacal shape: Review and guidelines. *Current Zoology*, *63*(2), 165–173. https://doi.org/10.1093/cz/zow054

Denoël, M., Whiteman, H. H., & Wissinger, S. A. (2006). Temporal shift of diet in alternative cannibalistic morphs of the tiger salamander. *Biological Journal of the Linnean Society*, *89*(2), 373–382. https://doi.org/10.1111/j.1095-8312.2006.00681.x

Denoël, M., Whiteman, H. H., & Wissinger, S. A. (2012). Larval growth in polyphenic salamanders: Making the best of a bad lot. *Oecologia*, *168*(1), 109–118. https://doi.org/10.1007/s00442-011-2076-z

Dodson, S. I., & Dodson, V. E. (1971). The Diet of Ambystoma tigrinum Larvae from Western Colorado. *Copeia*, *1971*(4), 614–624.

Duarte, H., Tejedo, M., Katzenberger, M., Marangoni, F., Baldo, D., Beltrán, J. F., Martí, D. A., Richter-Boix, A., & Gonzalez-Voyer, A. (2012). Can amphibians take the heat? Vulnerability to climate warming in subtropical and temperate larval amphibian communities. *Global Change Biology*, *18*(2), 412–421. https://doi.org/10.1111/j.1365-2486.2011.02518.x

DuBose, T. P., Moore, C. E., Silknetter, S., Benson, A. L., Alexander, T., O’Malley, G., & Mims, M. C. (2023). Mismatch between conservation status and climate change sensitivity leaves some anurans in the United States unprotected. *Biological Conservation*, *277*. https://doi.org/10.1016/j.biocon.2022.109866

Easton, D. P., Rutledge, P. S., & Spotila, J. R. (1987). RAPID COMMUNICATION Heat Shock Protein Induction and Induced Thermal Tolerance Are Independent in Adult Salamanders. In *THE JOURNAL OF EXPERIMENTAL ZOOLOGY* (Vol. 241).

Enriquez-Urzelai, U., Kearney, M. R., Nicieza, A. G., & Tingley, R. (2019). Integrating mechanistic and correlative niche models to unravel range-limiting processes in a temperate amphibian. *Global Change Biology*, *25*(8), 2633–2647. https://doi.org/10.1111/gcb.14673

Fabozzi, F. J., Focardi, S. M., Rachev, S. T., & Arshanapalli, B. G. (2014). Appendix E: Model Selection Criterion: AIC and BIC. In *The Basics of Financial Econometrics* (pp. 399–403). John Wiley & Sons, Inc. https://doi.org/10.1002/9781118856406.app5

Falk, B. G., Snow, R. W., & Reed, R. N. (2017). A validation of 11 body-condition indices in a giant snake species that exhibits positive allometry. *PLoS ONE*, *12*(7). https://doi.org/10.1371/journal.pone.0180791

Fassnacht, S. R., Venable, N. B. H., McGrath, D., & Patterson, G. G. (2018). Sub-seasonal snowpack trends in the Rocky Mountain National Park Area, Colorado, USA. *Water (Switzerland), 10*(5). https://doi.org/10.3390/w10050562

Feder, M. E., Burggren, W. W., Rome, L. C., Stevens, E. D., John-Alder, H. B., Hutchison, V. H., & Dupré, R. K. (1992). *Environmental Physiology of the Amphibians* (M. E. Feder & W. W. Burggren, Eds.; 1st ed.). University of Chicago Press.

Fernández-Loras, A., Boyero, L., Correa-Araneda, F., Tejedo, M., Hettyey, A., & Bosch, J. (2019). Infection with Batrachochytrium dendrobatidis lowers heat tolerance of tadpole hosts and cannot be cleared by brief exposure to CTmax. *PLoS ONE*, *14*(4). https://doi.org/10.1371/journal.pone.0216090

Fitzpatrick, B. M. (2012). Underappreciated consequences of phenotypic plasticity for ecological speciation. *International Journal of Ecology*, *2012*, 1–12. https://doi.org/10.1155/2012/256017

Forchhammer, M. C., Post, E., & Stenseth, N. Chr. (1998). Breeding phenology and climate. *Nature*, *391*, 29–30. http://www.cgd.ucar.edu:80/cas/climind/

Gilbert, A. L., & Miles, D. B. (2017). Natural selection on thermal preference, critical thermal maxima and locomotor performance. *Proceedings of the Royal Society B: Biological Sciences*, *284*(1860). https://doi.org/10.1098/rspb.2017.0536

Goodwin, K. J. A., Kissel, A. M., & Palen, W. J. (2019). Individual Variation in Thermal Performance of a Temperate , Montane Amphibian (Rana cascadae). *Herpetological Conservation and Biology*, *14*(August), 420–428.

Green, D. M. (2017). Amphibian breeding phenology trends under climate change: predicting the past to forecast the future. *Global Change Biology*, *23*(2), 646–656. https://doi.org/10.1111/gcb.13390

Gutiérrez-Pesquera, L. M., Tejedo, M., Olalla-Tárraga, M. A., Duarte, H., Nicieza, A., & Solé, M. (2016). Testing the climate variability hypothesis in thermal tolerance limits of tropical and temperate tadpoles. *Journal of Biogeography*, *43*(6), 1166–1178. https://doi.org/10.1111/jbi.12700

Heath, A. G. (1975). Behavioral Thermoregulation in High Altitude Tiger Salamanders, Ambystoma tigrinum. *Herpetologica*, *31*(1), 84–93. https://www.jstor.org/stable/3891993?seq=1&cid=pdf-

Hillman, S. S., Wither, P. C., Drewes, R. C., & Hillyard, S. D. (2008). *Ecological and Environmental Physiology of Amphibians* (Vol. 1). Oxford University Press.

Holden, Z. A., Klene, A. E., F. Keefe, R., & G. Moisen, G. (2013). Design and evaluation of an inexpensive radiation shield for monitoring surface air temperatures. *Agricultural and Forest Meteorology*, *180*, 281–286. https://doi.org/10.1016/j.agrformet.2013.06.011

Hutchison, V. H. (1961). Critical Thermal Maxima in Salamanders. *Physiological Zoology*, *34*(2), 92–125. https://doi.org/10.1086/physzool.34.2.30152688

Hutchison, V. H., & Maness, J. D. (1979). The Role of Behavior in Temperature Acclimation and Tolerance in Ectotherms. *American Zoology*, *19*, 367–384. https://academic.oup.com/icb/article/19/1/367/174533

Iossa, G. (2019). Sex-Specific Differences in Thermal Fertility Limits. *Trends in Ecology and Evolution*, *34*(6), 490–492. https://doi.org/10.1016/j.tree.2019.04.006

Joung, D. J., Leduc, M., Ramcharitar, B., Xu, Y., Isles, P. D. F., Stockwell, J. D., Druschel, G. K., Manley, T., & Schroth, A. W. (2017). Winter weather and lake-watershed physical configuration drive phosphorus, iron, and manganese dynamics in water and sediment of ice-covered lakes. *Limnology and Oceanography*, *62*(4), 1620–1635. https://doi.org/10.1002/lno.10521

Keen, W. H., & Schroeder, E. E. (1975). Temperature Selection and Tolerance in Three Species of Ambystoma Larvae. *Copeia*, *1975*(3), 523–530. http://www.jstor.org/stable/1443653

Kingsolver, J. G., & Umbanhowar, J. (2018). The analysis and interpretation of critical temperatures. *The Journal of Experimental Biology*, *221*. https://doi.org/10.1242/jeb.167858

Krenek, S., Petzoldt, T., & Berendonk, T. U. (2012). Coping with temperature at the warm edge - patterns of thermal adaptation in the microbial eukaryote paramecium caudatum. *PLoS ONE*, *7*(3). https://doi.org/10.1371/journal.pone.0030598

Lackey, A. C. R., Moore, M. P., Doyle, J., Gerlanc, N., Hagan, A., Geile, M., Eden, C., & Whiteman, H. H. (2019). Lifetime Fitness, Sex-Specific Life History, and the Maintenance of a Polyphenism. *The American Naturalist*, *194*(2). https://doi.org/10.5061/dryad.52kd8tg

Lertzman-Lepofsky, G. F., Kissel, A. M., Sinervo, B., & Palen, W. J. (2020). Water loss and temperature interact to compound amphibian vulnerability to climate change. *Global Change Biology*, *26*(9), 4868–4879. https://doi.org/10.1111/gcb.15231

Longhini, L. S., Zena, L. A., Polymeropoulos, E. T., Rocha, A. C. G., da Silva Leandro, G., Prado, C. P. A., Bícego, K. C., & Gargaglioni, L. H. (2021). Thermal Acclimation to the Highest Natural Ambient Temperature Compromises Physiological Performance in Tadpoles of a Stream-Breeding Savanna Tree Frog. *Frontiers in Physiology*, *12*. https://doi.org/10.3389/fphys.2021.726440

Lowe, W. H., Martin, T. E., Skelly, D. K., & Woods, H. A. (2021). Metamorphosis in an Era of Increasing Climate Variability. *Trends in Ecology and Evolution*, *36*(4), 360–375. https://doi.org/10.1016/j.tree.2020.11.012

Lutterschmidt, W. I., & Hutchison, V. H. (1997). The critical thermal maximum: History and critique. *Canadian Journal of Zoology*, *75*(10), 1561–1574. https://doi.org/10.1139/z97-783

Marsh, D. M., Townes, F. W., Cotter, K. M., Farroni, K., McCreary, K. L., Petry, R. L., & Tilghman, J. M. (2019). Thermal preference and species range in mountaintop salamanders and their widespread competitors. *Journal of Herpetology*, *53*(2), 96–103. https://doi.org/10.1670/18-110

Matsunami, M., Kitano, J., Kishida, O., Michimae, H., Miura, T., & Nishimura, K. (2015). Transcriptome analysis of predator- and prey-induced phenotypic plasticity in the Hokkaido salamander (Hynobius retardatus). *Molecular Ecology*, *24*(12), 3064–3076. https://doi.org/10.1111/mec.13228

McCarthy, M. L., Wallace, D., Whiteman, H. H., Rheingold, E. T., Dunham, A. M., Prosper, O., Chen, M., & Hu-Wang, E. (2017). Modeling the population dynamics and community impacts of Ambystoma tigrinum: A case study of phenotype plasticity. *Mathematical Biosciences*, *288*, 35–45. https://doi.org/10.1016/j.mbs.2017.02.011

Midgley, G., & Hannah, L. (2004). Extinction risk from climate change. *Biodiversity and Climate Change: Transforming the Biosphere*, *437*, 294–296. https://doi.org/10.2307/j.ctv8jnzw1.37

Moldowan, P. D., Tattersall, G. J., & Rollinson, N. (2021). Climate-associated decline of body condition in a fossorial salamander. *Global Change Biology*, *April*, 1–15. https://doi.org/10.1111/gcb.15766

Muths, E., Chambert, T., Schmidt, B. R., Miller, D. A. W., Hossack, B. R., Joly, P., Grolet, O., Green, D. M., Pilliod, D. S., Cheylan, M., Fisher, R. N., McCaffery, R. M., Adams, M. J., Palen, W. J., Arntzen, J. W., Garwood, J., Fellers, G., Thirion, J. M., Besnard, A., & Grant, E. H. C. (2017). Heterogeneous responses of temperate-zone amphibian populations to climate change complicates conservation planning. *Scientific Reports*, *7*(1). https://doi.org/10.1038/s41598-017-17105-7

Muths, E., Grant, C., Hossack, B. R., Pilliod, D. D., & Mosher, B. A. (2020). Effects of snowpack , temperature , and disease on demography in a wild population of amphibians. *Herpetologica*, *76*(2), 132–143.

Nouailhetas, M., Ribeiro, P. L., & Navas, C. A. (2015). Upper thermal tolerance plasticity in tropical amphibian species from contrasting habitats : Implications for warming impact prediction. *Journal of Thermal Biology*, *48*, 36–44. https://doi.org/10.1016/j.jtherbio.2014.12.008

Nowakowski, A. J., Watling, J. I., Thompson, M. E., Brusch, G. A., Catenazzi, A., Whitfield, S. M., Kurz, D. J., Suárez-Mayorga, Á., Aponte-Gutiérrez, A., Donnelly, M. A., & Todd, B. D. (2018). Thermal biology mediates responses of amphibians and reptiles to habitat modification. *Ecology Letters*, *21*(3), 345–355. https://doi.org/10.1111/ele.12901

Piasečná, K., Pončová, A., Tejedo, M., & Gvoždík, L. (2015). Thermoregulatory strategies in an aquatic ectotherm from thermally-constrained habitats : An evaluation of current approaches. *Journal of Thermal Biology*, *52*, 97–107. https://doi.org/10.1016/j.jtherbio.2015.06.007

Pörtner, H. O., Bock, C., & Mark, F. C. (2017). Oxygen- & capacity-limited thermal tolerance: Bridging ecology & physiology. In *Journal of Experimental Biology* (Vol. 220, Issue 15, pp. 2685–2696). Company of Biologists Ltd. https://doi.org/10.1242/jeb.134585

Pottier, P., Burke, S., Drobniak, S. M., Lagisz, M., & Nakagawa, S. (2021). Sexual (in)equality? A meta-analysis of sex differences in thermal acclimation capacity across ectotherms. *Functional Ecology*, *35*(12), 2663–2678. https://doi.org/10.1111/1365-2435.13899

R Core Team (2022). R: A language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. URL https://www.R-project.org/.

Ramalho, Q., Vale, M. M., Manes, S., Diniz, P., Malecha, A., & Prevedello, J. A. (2023). Evidence of stronger range shift response to ongoing climate change by ectotherms and high-latitude species. *Biological Conservation*, *279*. https://doi.org/10.1016/j.biocon.2023.109911

Reider, K. E., Larson, D. J., Barnes, B. M., & Donnelly, M. A. (2021). Thermal adaptations to extreme freeze–thaw cycles in the high tropical Andes. *Biotropica*, *53*(1), 296–306. https://doi.org/10.1111/btp.12875

Reider, K. E., Zerger, M., & Whiteman, H. H. (2022). Extending the biologging revolution to amphibians: Implantation, extraction, and validation of miniature temperature loggers. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, *337*(4), 403–411. https://doi.org/10.1002/jez.2575

Roe, A. W., & Grayson, K. L. (2008). Terrestrial movements and habitat use of juvenile and emigrating adult Eastern Red-Spotted Newts, Notophthalmus viridescens. *Journal of Herpetology*, *42*(1), 22–30. https://doi.org/10.1670/07-040.1

Rohr, J. R., Civitello, D. J., Cohen, J. M., Roznik, E. A., Sinervo, B., & Dell, A. I. (2018). The complex drivers of thermal acclimation and breadth in ectotherms. *Ecology Letters*, *21*(9), 1425–1439. https://doi.org/10.1111/ele.13107

Rozen-Rechels, D., Dupoué, A., Lourdais, O., Chamaillé-Jammes, S., Meylan, S., Clobert, J., & Le Galliard, J. (2019). When water interacts with temperature: Ecological and evolutionary implications of thermo-hydroregulation in terrestrial ectotherms. *Ecology and Evolution*, *9*(17), 10029–10043. https://doi.org/10.1002/ece3.5440

Ruiz-Garciá, A., Roco, Á. S., & Bullejos, M. (2021). Sex Differentiation in Amphibians: Effect of Temperature and Its Influence on Sex Reversal. In *Sexual Development* (Vol. 15, Issue 1, pp. 157–167). S. Karger AG. https://doi.org/10.1159/000515220

Ruthsatz, K., Dausmann, K. H., Peck, M. A., & Glos, J. (2022). Thermal tolerance and acclimation capacity in the European common frog (Rana temporaria) change throughout ontogeny. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*. https://doi.org/10.1002/jez.2582

Schmidt-Nielsen, K. (1997). *Animal Physiology: Adaptation and Environment* (Fifth). Cambridge University Press.

Sexton, O. J., & Bizer, J. R. (1978). Life History Patterns of Ambystoma tigrinum in Montane Colorado. *The American Midland Naturalist*, *99*(1), 101–118.

Shoo, L. P., Olson, D. H., Mcmenamin, S. K., Murray, K. A., van Sluys, M., Donnelly, M. A., Stratford, D., Terhivuo, J., Merino-Viteri, A., Herbert, S. M., Bishop, P. J., Corn, P. S., Dovey, L., Griffiths, R. A., Lowe, K., Mahony, M., Mccallum, H., Shuker, J. D., Simpkins, C., … Hero, J. M. (2011). Engineering a future for amphibians under climate change. *Journal of Applied Ecology*, *48*(2), 487–492. https://doi.org/10.1111/j.1365-2664.2010.01942.x

Smith, T. B., Kinnison, M. T., Strauss, S. Y., Fuller, T. L., & Carroll, S. P. (2014). Prescriptive evolution to conserve and manage biodiversity. *Annual Review of Ecology, Evolution, and Systematics*, *45*, 1–22. https://doi.org/10.1146/annurev-ecolsys-120213-091747

Snyder, G. K., & Weathers, W. W. (1975). Temperature adaptations in amphibians. *The American Naturalist*, *109*(965), 93–101. http://www.journals.uchicago.edu/t-and-c

Storey, K. B. (1990). Life in a frozen state: adaptive strategies for natural freeze tolerance in amphibians and reptiles. *American Journal of Physiology*, *258*(3), 599–568.

Storey, K. B., & Storey, J. M. (2017). Molecular physiology of freeze tolerance in vertebrates. *Physiological Reviews*, *97*(2), 623–665. https://doi.org/10.1152/physrev.00016.2016

Storfer, A., & White, C. (2004). Phenotypically plastic responses of larval tiger Salamanders, Ambystoma tigrinum, to different predators. *Journal of Herpetology*, *38*(4), 612–615. https://doi.org/10.1670/24-04N

Taylor, E. N., Diele-Viegas, L. M., Gangloff, E. J., Hall, J. M., Halpern, B., Massey, M. D., Rödder, D., Rollinson, N., Spears, S., Sun, B., & Telemeco, R. S. (2020). The thermal ecology and physiology of reptiles and amphibians: A user's guide. *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology*, *335*(1), 13–44. https://doi.org/10.1002/jez.2396

Telemeco, R. S., & Gangloff, E. J. (2021). Introduction to the special issue–Beyond CTMAX and CTMIN: Advances in studying the thermal limits of reptiles and amphibians. In *Journal of Experimental Zoology Part A: Ecological and Integrative Physiology* (Vol. 335, Issue 1, pp. 5–12). John Wiley and Sons Inc. https://doi.org/10.1002/jez.2447

Thurman, L. L., & Garcia, T. S. (2017). Differential plasticity in response to simulated climate warming in a high-elevation amphibian assemblage. *Journal of Herpetology*, *51*(2), 232–239. https://doi.org/10.1670/16-502

Turriago, J. L., Parra, C. A., & Bernal, M. H. (2015). Upper thermal tolerance in anuran embryos and tadpoles at constant and variable peak temperatures. *Canadian Journal of Zoology*, *93*(4), 267–272. https://doi.org/10.1139/cjz-2014-0254

Urban, M. C. (2015). Accelerating extinction risk from climate change. *Science*, *348*(6234), 571–573. https://doi.org/10.1126/science.aaa4984

von May, R., Catenazzi, A., Corl, A., Santa-Cruz, R., Carolina Carnaval, A., & Moritz, C. (2017). Divergence of thermal physiological traits in terrestrial breeding frogs along a tropical elevational gradient. *Ecology and Evolution*, *7*(9), 3257–3267. https://doi.org/10.1002/ece3.2929

von May, R., Catenazzi, A., Santa-Cruz, R., Gutierrez, A. S., Moritz, C., & Rabosky, D. L. (2019). Thermal physiological traits in tropical lowland amphibians: vulnerability to climate warming and cooling. *PLoS ONE*, *14*(8), 1–18. https://doi.org/10.1371/journal.pone.0219759

Wang, Y. J., Stoks, R., Sentis, A., & Tüzün, N. (2020). Support for the climatic variability hypothesis depends on the type of thermal plasticity: lessons from predation rates. *Oikos*, *129*(7), 1040–1050. https://doi.org/10.1111/oik.07181

Whiteman, H. H., & Wissinger, S. A. (2005). Amphibian population cycles and long-term data sets. *Amphibian Declines: The Conservation Status of United States Species*, 177–184. https://doi.org/10.1525/california/9780520235922.003.0025

Whiteman, H. H., Wissinger, S. A., & Bohonak, A. J. (1994). Seasonal movement patterns in a subalpine population of the tiger salamander, Ambystoma tigrinum nebulosum. *Canadian Journal of Zoology*, *72*(10), 1780–1787. https://doi.org/10.1139/z94-241

Winterová, B., & Gvoždík, L. (2021). Individual variation in seasonal acclimation by sympatric amphibians: A climate change perspective. *Functional Ecology*, *35*(1), 117–126. https://doi.org/10.1111/1365-2435.13705

Wissinger, S., Bohonak, A. J., Whiteman, Howard H., & Brown, W. S. (1999). Habitat Permanence, Salamander Predation, and Invertebrate Communities. In D. P. Batzer, S. A. Wissinger, & R. B. Rader (Eds.), *Invertebrates in Freshwater Wetlands of North America: Ecology and Management* (1st ed., pp. 757–790). Wiley.

Yang, C. H., & Pospisilik, J. A. (2019). Polyphenism – A window into gene-environment interactions and phenotypic plasticity. *Frontiers in Genetics*, *10*(132), 1–9. https://doi.org/10.3389/fgene.2019.00132