THE ROLES OF AGGRESSION AND WATER LOSS IN THE ELEVATIONAL DISTRIBUTION OF CHEOAH BALD SALAMANDERS (*PLETHODON CHEOAH*) AND SOUTHERN APPALACHIAN SALAMANDERS (*PLETHODON TEYAHALEE*)

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July 2024

ACKNOWLEDGEMENTS

Thank you to Highlands Biological Station for providing a grant-in-aid of research in 2019 and allowing me to stay on site and use their facilities for my field and lab work during the summer. Thank you to Western Carolina University for a summer research assistantship in 2019. Thank you to the folks at the Cheoah Ranger District for providing access to my field sites.

I would like to thank every person who helped with this project, from late nights in the field, to long periods in the lab, to statistical assistance, and beyond. To my advisor, Joe Pechmann; thank you for never giving up on me and guiding me. To my friends and family; thank you for your unyielding supporting, especially my parents. None of this would have been possible without all of you.

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ABSTRACT

In the southern Appalachian Mountains, multiple species of large salamanders in the genus *Plethodon* occur within the same niche, with different species dominating high and low elevations and engaging in interspecific competition where they overlap. Some high-elevation species are more aggressive than their low-elevation competitors, which may prevent the latter from ascending the mountain and potentially eradicating the high-elevation species over time. In the Cheoah Mountains, this interaction may occur between the Cheoah Bald salamander (*Plethodon cheoah*) as the high-elevation species and the southern Appalachian salamander (*P. teyahalee*) as the low-elevation species. *Plethodon teyahalee* is not limited to low elevations by physiological restrictions, so I wanted to examine if interspecific aggression may be contributing to the dominance of *P. cheoah* at high elevations. I ran aggression trials with *P. cheoah* and *P. teyahalee* in a lab setting to compare both species' interspecific and intraspecific aggression. Aggression was defined as acts of a salamander looking towards, approaching, snapping, lunging, chasing, or biting the other salamander. *Plethodon cheoah* was found to be more aggressive than *P. teyahalee* in both intraspecific and interspecific trials, and larger individuals of both species committed more aggressive acts. However, smaller *P. cheoah* did show aggression toward larger *P. teyahalee*.

Another factor that may contribute to the elevational distribution of these salamanders is their evaporative water loss rates through their skin. As *P. cheoah* is restricted to high elevations, I hypothesized that they cannot retain water as well as *P. teyahalee* and do not live at lower, drier elevations due to risk of desiccation. I measured the evaporative water loss rate of salamanders of both species using a LiCor gas analyzer. Both species had similar mass-adjusted water loss rates per individual and per cm^2 of skin area. However, *P. cheoah* did have higher average water loss rates per cm^2 of skin area and per gram of salamander than did *P. teyahalee*. This was because *P. cheoah* was smaller on average than *P. teyahalee* and had a higher surface-to-volume ratio. These results suggest that aggressive interactions and physiological constraints play roles in the elevational distributions of *P. teyahalee* and *P. cheoah* as in other montane *Plethodon*.

Introduction

A species can only occupy certain habitats due to a number of environmental factors, including temperature, moisture, food supply, and available shelter. These specific places in the ecosystem are known as the niche of a species (Cain et al. 2018). Many species have niches that are found at certain elevations, because the physical and biotic habitat varies with elevation. In the southern Appalachian Mountains, some salamanders in the genus *Plethodon,* the woodland salamanders, require high elevation habitats where moisture is high and temperatures are cool. At low elevations, they cannot forage for food enough to grow and survive, due to the risk of desiccation. (Gifford and Kozak 2012, Lyons et al. 2020).

In some areas, multiple species can occur in the same niche and engage in interspecific competition for resources, including food, shelter, and territory. Sometimes the competition can become physical when individuals of these species meet, leading to acts of aggression to deter their competitors. Some high-elevation *Plethodon* of the southern Appalachians are very aggressive toward their low-elevation competitors (Nishikawa 1985, Hairston 1987). This aggression apparently serves to prevent the interlopers from ascending the mountain and taking their territory, possibly pushing the high-elevation species to the top of the mountain and eradicating the population over time (Hairston 1987, Gifford and Kozak 2012). Acts of aggression between competing salamanders can include chasing, looking toward, snapping, biting, lunging, and more (Drummond 2015, Nishikawa 1985).

The Cheoah Bald salamander (*Plethodon cheoah*) is a species of woodland salamander endemic to the Cheoah Mountains of Graham and Swain counties in North Carolina. They occur at high elevations in deciduous woodlands, where they typically remain under cover or in burrows during the day. On warm nights (usually above 10 degrees C) that are moist or humid, they emerge and walk the forest floor, foraging for insects to eat and looking for mates to breed with. They can grow up to 12.5 centimeters in total length, have a dark-gray body with light-gray cheeks, and have a varying amount of red, pink, or orange coloration on their legs ranging from no color to completely colored (Powell et al. 2016). They are very similar in appearance to the red-legged salamander (*Plethodon shermani*), and since the ranges of these species are adjacent, separated by the Nantahala River gorge, they were considered the same species until genetic analyses differentiated them (Highton and Peabody 2000). *Plethodon cheoah* is considered a member of the *P. jordani* complex, as it and many other high-elevation salamanders of the southern Appalachians within the genus that have similar morphology, behaviors and niches were once all considered to be *P. jordani* (Highton and Peabody 2000). Like other members of the genus, *P. cheoah* lay their eggs on land underground in a moist environment. The females guard their eggs until they hatch. Unlike many other salamanders, in the genus *Plethodon* the eggs do not hatch into aquatic larva with gills. Instead, they hatch into small versions of the adults that are completely terrestrial and undergo no metamorphosis during their life (Beane et al. 2010).

The Southern Appalachian salamander (*Plethodon teyahalee*) occurs in the majority of North Carolina west of the French Broad River, northwestern South Carolina, and small portions of eastern Tennessee and north Georgia (Beane et al. 2010). *Plethodon teyahalee* is considered by some as a part of the *Plethodon glutinosis,* or slimy salamander, complex, as all members of the complex were considered *P. glutinosis* until genetic analysis determined that the species consisted of multiple cryptic species (Highton 1987). They occur at low elevations in moist deciduous woodlands and are considered habitat “generalists” among woodland salamanders for their ability to live in many environments of differing moisture and temperature levels, even showing a resilience to logging and other habitat disturbances (Lannoo 2005). They are the low-elevation competitors of many of the high-elevation species within their range, as they have no elevational limits unless they are kept from the tops of the mountains by aggressive high-elevation species (Gifford and Kozak 2012, Drummond 2015). They are often less aggressive than their high-elevation competitors, and as a result, they often back down when interacting with their interspecific competitors, such as *Plethodon shermani* and *P. jordani*. (Drummond 2015, Nishikawa 1985). *Plethodon teyahalee* often forage from or near their burrows or cover objects, perhaps to avoid contact with other species or hybrids when their ranges overlap (Drummond 2015). They frequently hybridize with some high-elevation species such as *P. shermani* but rarely hybridize with *P. cheoah* (Highton and Peabody 2000).

Plethodon cheoah and *P. teyahalee* are reportedly sympatric throughout the entire range of *P. cheoah*, including its elevational range (Lannoo 2005, Highton and Peabody 2000, David Beamer, personal communication). I did not conduct a formal study of the elevational ranges of these species but I made anecdotal observations of them during the course of my work. On the south side of Cheoah Bald, I observed *P. teyahalee* only at locations less than approximately 1304.5 meters (4,280 feet) in elevation, while I observed *P. cheoah* at locations above approximately1115.6 meters (3,660 feet) in elevation, with an observed overlap of approximately 189.0 meters (620 feet) in elevation. On the north side of Cheoah Bald, I observed *P. cheoah* above approximately 887.0 meters (2910 feet) in elevation. I observed *P. teyahalee* at all areas of the northern site that I could readily access by road, which was up to 1149.1 meters (3,770 feet) in elevation. Both species overlapped across at least 262d meters of elevation on the north side.the elevational range of overlap appeared to vary with topography as well as with aspect. My lowest observations of *P. cheoah* at both field sites were near streams or in gullies, which I hypothesize was due to the cooler, wetter conditions in these areas. I observed *P. cheoah* but not *P. teyahalee* on and near the top of the ridgeline of Cheoah Bald at around 1542.3 meters (5,060 feet).

I did two studies to examine differences between *P. cheoah* and *P. teyahalee*: aggression trials and physiological comparisons. I hypothesized that *P. cheoah* was more aggressive than *P. teyahalee*, and that *P. cheoah* had higher water loss rates than *P. teyahalee*.

The rationale behind the aggression hypothesis was that other high-elevation *Plethodon* species, such as *P. jordani* and *P. shermani*, display aggression toward their competitors and exclude other large *Plethodon* species from high elevations (Drummond 2015, Nishikawa 1985, Hairston 1987). Aggression between *P. cheoah* and *P. teyahalee* had never been investigated, however.

For my hypothesis on the respiration and water loss rates, I rationalized that *Plethodon cheoah* are absent from low elevations due to a lesser tolerance toward desiccation (Riddell and Sears 2020). Respiration data were collected as an indicator of energy requirements. Fulfilling higher energy requirements would require longer foraging times and therefore higher desiccation resistance.

Methods

Aggression Trials

I collected 60 *Plethodon cheoah* and 60 *Plethodon teyahalee* from two field sites, one on the north side of Cheoah Bald and one on the south side, in Graham County, NC over multiple nights from May 2019 to April 2020. Both field sites were within the elevational overlap zone of the two salamander species (Highton and Peabody 2000). Adult salamanders were collected on moist nights when the temperature was above 50℉, which is when they are typically out on the forest floor. Salamanders were not collected based on body size or sex. Only juveniles were excluded from collection.

I kept the salamanders in a windowless lab at Western Carolina University. The room temperature was maintained between 15 to 24℃, which is within the range at which these salamanders have been observed active on the forest floor during the spring and summer. I placed each salamander into an individual clear plastic container with a secured lid, 30 x 15 x 15 cm in size. Moist, unbleached paper towels lined the enclosure. I folded another moist paper towel into a shelter for the salamander to use. A timer maintained the room's lights on a 12:12 photoperiod. I slowly shifted the photoperiod an hour at a time over a few days so that darkness began at 4:00 pm for the salamanders. This allowed aggression trials to be done at a more convenient time. The photoperiod was not shifted with the season. I placed the plastic containers on shelves and put piece of black construction paper between adjacent containers to prevent the salamanders from seeing each other and possibly influencing aggression levels. Each salamander was fed fruit flies or a mealworm every two weeks with the portions depending on the salamander's size.

I housed salamanders in the containers for two-to-four weeks before conducting aggression trials, so that the salamanders considered their box to be their territory. Trials were done during the night, when the salamanders would naturally encounter each other, under complete darkness except for red light, as salamanders cannot see red light. I selected salamanders randomly for each trial, with each salamander only going through one trial. I conducted 30 *Plethodon cheoah* x *Plethodon teyahalee* trials, along with 15 *P. cheoah* x *P.*

cheoah and 15 *P. teyahalee* x *P. teyahalee* trials, in order to compare the level of intraspecific and interspecific aggression in each species. I alternated which species was the intruder and which was the resident in the interspecific trials. I picked up the resident and placed it back down in the container so each salamander experienced being picked up. The intruder would then be picked up and placed on the opposite end of the resident's container. The lid was placed back on the resident's container to start a five-minute adjustment period where no observations were made, to allow the salamanders to calm down after being picked up and allow the intruder to acclimate to its new surroundings. Once the five minutes passed, the 30-minute aggression trial began. I recorded each act of aggression between the salamanders during the trial. After the trial ended, the salamanders were placed into individual tared plastic bags and weighed using a Pesola^{TM} spring scale, measured from snout-to-vent (SVL) with a ruler, and placed back into their respective containers.

I placed acts of aggression into six categories: looking towards, approaching, snapping, lunging, chasing, and biting (Nishikawa 1985, Drummond 2015). Looking towards occurred when the salamander directed its gaze toward its opponent. This often occurred in response to the opponent moving or coming into the salamander's line of sight. This was typically paired with the salamander changing its body language: raising its trunk off the ground, widening the eyes, breathing faster, and watching intently as the opponent moved. It often led to the salamander approaching the opponent unless the opponent moved toward the salamander. Approaching occurred when a salamander took at least three steps towards a competitor that was not moving. This act often had the same body language as looking towards, with the salamander approaching slowly and often waiting until the opponent was not looking at it to continue approaching. It was often a predecessor to further aggression and direct attacks. Snapping was described as a salamander opening and closing its mouth toward a competitor without making physical contact. Lunging was when a salamander made a sudden movement in response to an incoming competitor, suddenly flexing their entire body. This was often done defensively, as if to ward off the competitor. Chasing was when a salamander moved toward a competitor and the competitor retreated. Biting occurred when a salamander opened its mouth and closed it on an opponent's body, and was considered a direct attack (Drummond 2015).

The data were analyzed using R (RStudio Team 2020). The dependent variable was the number of aggressive acts performed by a salamander. The independent variables were that salamander's species, sex, and SVL, whether it was a resident or intruder, the species of the opponent, and the difference in SVL between participants. I also tested for all two- and three-way interactions among salamander species, opponent species, and resident-intruder status. Preliminary analyses indicated that the data were over-dispersed and zero-inflated. I used a negative binomial generalized linear model, which can adjust for data having these characteristics (MASS package in R; Venables and Ripley 2022). I summarized the results using Analysis of Deviance with Type III tests and produced P-values with Wald tests (Anova function from Car package in R; Fox and Weisberg 2019).

Physiological Measurements

I used a LiCor Model 6400XT infrared gas analyzer running the LiCor Insect.Rd program, with a plastic insect respiration chamber (10.16 centimeters in length, 2.54 centimeters in diameter, 35.92 centimeters in area) attached, to measure the respiration and water loss rates of the salamanders (Pincebourde and Casas 2006). I took measurements during the dark portion of the salamanders' photoperiod at a consistent room temperature of 18℃ and 65% relative humidity. I placed twenty of each species (40 altogether) one at a time into the respiration chamber with each salamander being weighed on an electronic scale before and after their trial to determine their water weight lost. Each salamander had previously been used in the aggression trials weeks prior. I set the air flow rate to 500 microliters/second and covered the chamber with a black cloth to put salamanders fully in the dark during the trial. After each use, the plastic chamber was removed and replaced with a second clean chamber, with the used chamber being disinfected in a 3% bleach solution for five minutes or longer, dried with a paper towel, and allowed to air dry until the next trial (Cruz 2018).

Before each trial, I set the reference points for the amounts of CO_2 and H_2O in the chamber as the conditions in the empty chamber before adding a salamander. Once a salamander was added, I set the machine to record CO_2 and H_2O concentrations in the chamber every second. Each trial ran for one hour. The LiCor software calculated the respiration rate and water loss rate every second based on the changes of CO_2 and H_2O concentrations as air passed through the chamber. After a trial, I plotted every reading of CO_2 and H_2O on a line graph to determine when the most stable 20 minutes of CO_2 and H_2O emission levels occurred. I assumed that data collected during these 20 minutes represented the amounts produced by the salamander when it was not stressed or active (Eric Riddell, personal correspondence). I then calculated the average respiration rate and water loss rate within those 20 minutes. I converted the respiration rate to milliliters CO_2 per hour per salamander at 25C and standard atmospheric pressure. I used the average temperature of the chamber during the 20-minute stable period when calculating this conversion. The water loss rate was converted to grams H_2O per hour per salamander. I then calculated the surface area of each salamander using the salamander mass at the start of the trial and the formula ($8.42*\text{mass(g)}^{0.694}$); Whitford and Hutchison 1967). As each salamander was in the plastic tube with their ventral side pressed against the tube, their ventral surface was not exposed to the air flow and cannot be taken into account, so the result of the equation was then multiplied by 0.66 to account for roughly two-thirds of each salamander's body being exposed to the air flow. I divided the average water loss rate for each salamander by its exposed surface area to get the water loss rate per unit of surface area.

I ran analyses of covariance (ANCOVAS) in R (RStudio Team 2020). to test for differences between species in respiration rates per individual, water loss rates per individual, and water loss rates per unit of surface area, each adjusted for the natural log of body mass taken before the trial. Preliminary analyses found no significant interaction between body mass and species for any of the rates (all $P > 0.237$), so I dropped the interaction from the models.

Results

Aggression Trials

The *Plethodon cheoah* used in the trials ranged in snout-to-vent length from 40 to 59 millimeters, with an average length of 49.78mm. The *Plethodon teyahalee* ranged in SVL from 51 to 82mm, with an average length of 67.98mm. The number of aggressive acts performed by a salamander was associated with its SVL ($p = 0.0212$) and species ($p = 0.0002$), and marginally associated with the species of its opponent ($p = 0.0596$; Fig. 1, Table 1). *Plethodon cheoah* were more aggressive than *P. teyahalee* (Fig. 1). Individuals of both species tended to be more aggressive when their opponent was a *P. cheoah* (Fig. 1). Larger individuals of both species committed more aggressive acts (Fig. 1). Whether an individual was a resident or intruder, its sex, the difference in SVL between the two individuals, and interactions between variables had no significant effect on the number of aggressive acts committed (all $p > 0.2827$; Table 1).

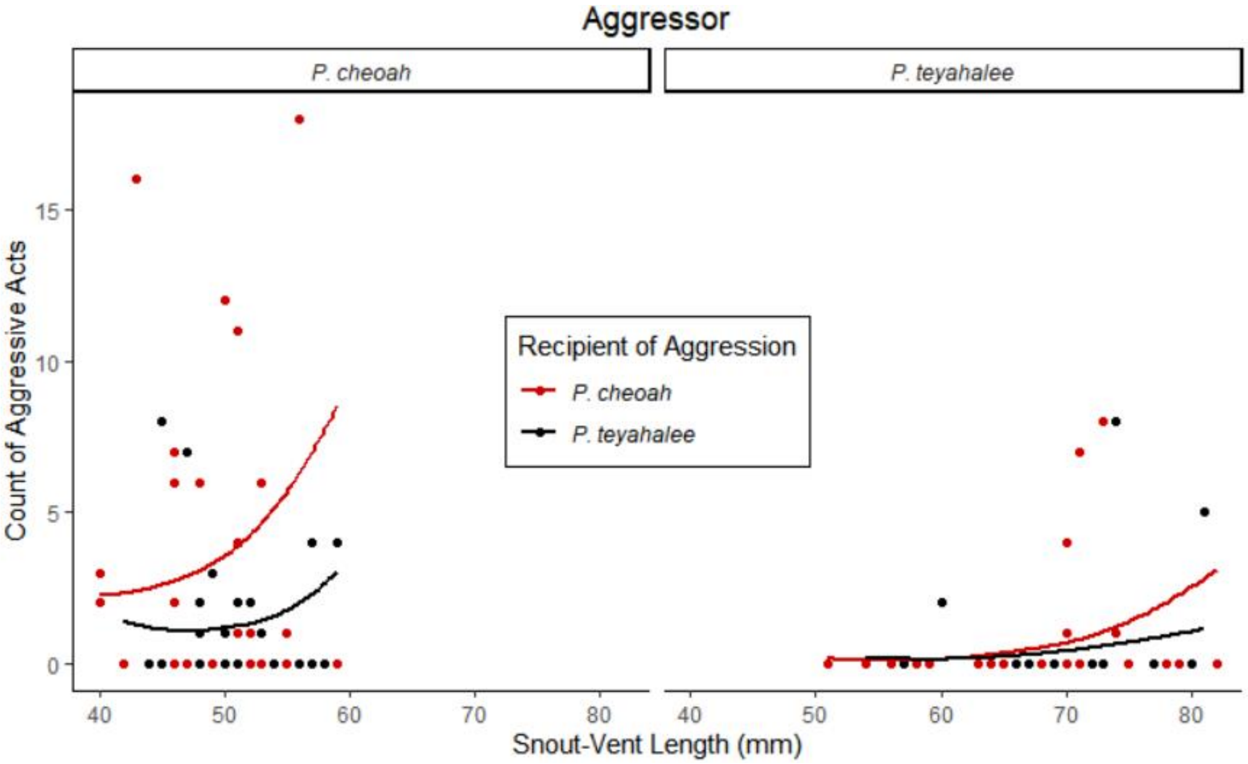

Figure 1: Counts of aggressive acts by snout-vent length for each species. The solid lines are loess smoothed predictions of the negative binomial regression model. The color of the line indicates the recipient of the aggression (red is *P. cheoah*, black is *P. teyahalee*).

Table 1: Analysis of Deviance Table (Type III tests) for the negative binomial generalized linear model, using Wald tests to produce P-values. Species, opponent species, and SVL (snout-vent length) were important variables in describing the number of aggressive acts performed by a salamander. "Resident or Intruder" is whether the salamander was a resident or intruder. Colons indicate interactions among variables.

	Degrees of Freedom	Chi-square	P-value
(Intercept)	1	6.0338	0.0140
Species	1	14.0984	0.0002
Opponent	1	3.5474	0.0596
Resident or Intruder	1	0.0592	0.8078
SVL	1	5.3073	0.0212
Sex	1	0.7183	0.3967
SVL Difference	1	1.1542	0.2827
Species:Opponent	1	1.0956	0.2952
Species:Resident or Intruder	1	0.2144	0.6433
Opponent:Resident or Intruder	1	1.3946	0.2376
Species:Opponent:Resident or Intruder	1	1.3510	0.2451

Physiological Measurements

Respiration rate per individual and water loss rate per individual increased significantly with salamander mass, as indicated by the positive slopes for log mass in the ANCOVAS (Table 2). Water loss rate per surface area decreased significantly with salamander mass, as indicated by the negative slope for log mass in the ANCOVA (Table 2). None of the relationships between physiological rates and mass differed significantly between the two species (Table 2). *Plethodon cheoah* was significantly smaller than *P. teyahalee* on average (*P. cheoah* = 1.691 g, *P. teyahalee* = 4.534 g), with little overlap in body masses between them (T_{38} = 9.036, p = 5.29e^{-11}; Fig 2). Thus, water loss rate per unit surface area was higher on average for *P. cheoah* than for *P.*

teyahalee (*P. cheoah* = 0.0007, *P. teyahalee* = 0.0004) (). The combination of *P. cheoah*'s higher water loss rate per surface area and lower body mass resulted in a much higher water loss rate to body mass ratio than that for *P. teyahalee* (Fig. 2). The average water loss rate of all *P. cheoah* sampled was 0.00070522 g H_20 per hour per g body mass, which was twice that of all *P. teyahalee* sampled, 0.000367703 g H_20 per hour per g of body mass ($T_{38} = 7.527$, $p = 4.82e^{-9}$).

Table 2. ANCOVA results for differences between *P. cheoah* and *P. teyahalee* in respiration rates per individual, water loss rates per individual, and water loss rates per unit of salamander surface area, adjusted for body mass.

Rate	Variable	Parameter	95% CI	P
Respiration	(Intercept)	-6.0	-6.3, -5.7	<0.001
	Species			0.212
	P. cheoah	0.12	-0.07, 0.31	
	P. teyahalee	—	—	
	log(Mass)	0.91	0.58, 1.2	<0.001
Water Loss(mol)	(Intercept)	-14	-14, -14	<0.001
	Species			0.853
	P. cheoah	-0.01	-0.10, 0.09	
	P. teyahalee	—	—	
	log(Mass)	0.26	0.09, 0.43	0.003
Water Loss/Surface Area(g/cm^2)	(Intercept)	-8.6	-8.8, -8.5	<0.001
	Species			0.877
	P. cheoah	-0.01	-0.10, 0.09	
	P. teyahalee	—	—	
	log(Mass)	-0.43	-0.60, -0.27	<0.001

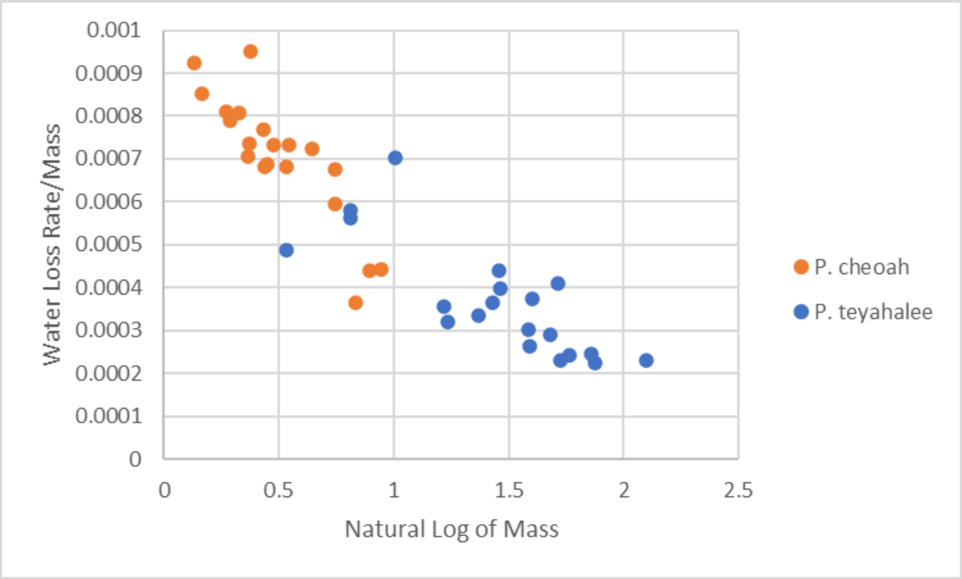

Figure 2: The water loss rate of each salamander divided by its mass as a function of the natural log of salamander mass.

Discussion

Aggression Trials

Plethodon cheoah had a higher frequency of aggression than *Plethodon teyahalee*. Large salamanders of both species were more aggressive than small salamanders. Nonetheless, there were many instances of aggression from smaller *P. cheoah* towards larger *P. teyahalee*. Salamanders committed more acts of aggression when *P. cheoah* was the opponent. These results suggest that the dominance of *P. cheoah* at higher elevations in the Cheoah Mountains is due to the higher aggression levels of *P. cheoah* compared to *P. teyahalee.*

Previous work has shown that other species of high-elevation salamanders in the genus *Plethodon*, such as *P. jordani* and *P. metcalfi* (Saad et al 2007), are aggressive toward their low-elevation heterospecific competitors, while also showing high amounts of conspecific aggression among themselves. (Nishikawa 1985; Gifford and Kozak 2012). My results indicate that *P. cheoah* is aggressive toward *P. teyahalee* and intraspecific competitors, with few instances of *P. teyahalee* showing interspecific or intraspecific aggression. *Plethodon shermani* and *P. teyahalee* interactions are more complicated than the previous examples due to the widespread hybridization between the two species, with hybrids often having aggression levels that are intermediate to those of *P. shermani* and *P. teyahalee* (Drummond 2015).

The results of this experiment suggest that competition with *P. cheoah* disincentivizes *P. teyahalee* from ascending higher in elevation and overlapping with *P. cheoah*. *Plethodon cheoah* and *P. teyahalee* do reportedly overlap for roughly 655.3 meters (2,150 feet) n elevation, however, with the highest ridgetops still having records of *P. teyahalee* (Lannoo 2005, Highton and Peabody 2000, David Beamer, personal correspondence). My observations indicate that *P. teyahalee* are far less numerous at high elevations than at low elevations, consistent with my inference that aggression from *P. cheoah* greatly reduces the number of *P. teyahalee* at high elevations. *Plethodon jordani* has a small overlap zone with *P. teyahalee* of only a few hundred feet in elevation. *Plethodon metcalfi* has a wide overlap zone with *P. teyahalee* (4,230 feet (1289.3 meters) near Heintooga Overlook in Great Smokey Mountains National Park), which could be a sign of weaker aggressive competition between *P. metcalfi* and *P. teyahalee* than between *P. jordani* and *P. teyahalee*(Hairston et al. 1992). Comparing elevational overlaps suggests that *P. cheoah* has a level of aggression intermediate between *P. jordani* and *P. metcalfi.*

Various species across different taxa display aggression towards interspecific competitors along elevational gradients as they displace one another. Songbirds in New Guinea respond aggressively toward the calls of the species that replace them at the upper and lower elevational limits of their range (Freeman et al. 2016). Chipmunks in the Sierra Nevada of California have a hierarchy of aggressive dominance, relegating less-aggressive species to the lower, more arid elevations (Chappell 1978). The less-aggressive chipmunks could feasibly live at the higher elevations, but the higher elevation species cannot live at the lower elevations due to physiological and habitat restrictions (Chappell 1978). The aggressive exclusion in both examples creates spatial niche partitioning while increasing the biodiversity of the assemblage on the mountains. While not as exclusionary as the songbirds and chipmunks, the interactions between *P. cheoah* and *P. teyahalee* mirror these examples.

Physiological Measurements

Mass-adjusted respiration and water loss rates did not differ significantly between the two species. The mass of the salamanders was the only significant predictor of these physiological measurements. As a general rule, the greater the mass of an animal, the greater the surface area and metabolic rate, leading to greater evaporative water loss (Hill et al. 2012). I had hypothesized that *P. cheoah* had a higher water loss rate than *P. teyahalee* because the former is not found at low elevations despite its aggressive dominance over the latter. This suggested that *P. cheoah*'s water loss rate might limit its persistence in the warm, dry conditions found at low elevations. My prediction about water loss rates was correct, but the difference between the species was solely due to body size, not species. Compared to *P. cheoah*, *P. teyahalee* on average is larger, has a lower surface-to-volume ratio, and has a lower water loss rate per gram of salamander. These characteristics would allow *P. teyahalee* to forage longer and more often than *P. cheoah* in warm, dry habitats, likely increasing the former's fitness at low elevations (Gade et al. 2020). *Plethodon cheoah* could counteract the constraint of high water loss rates by having a lower respiration rate and consequently lower energy needs, but we found no difference between the two species in mass-specific respiration rate. *Plethodon cheoah* actually has a higher metabolic rate per gram than *P. teyahalee* because of the former's smaller body size (Hill et al 2012). The high elevation large *Plethodon* in the Great Smoky Mountains, *P. jordani*, is similarly precluded from occupying low elevation habitats by physiological constraints (Gifford and Kozak 2012). *Plethodon teyahalee*, on the other hand, is physiologically capable of persisting throughout the elevational ranges found in the Great Smoky Mountains, which is greater than that of the Cheoah Mountains (Gifford and Kozak 2012).

Some of the salamanders were kept in the lab for ten months prior to testing. They may have acclimated to the constant warmer temperature of the lab over time, reducing any physiological differences between the species. Salamanders in the genus *Plethodon* can reduce their water loss rates at the cost of their metabolic rate, using more energy to retain water as a physiological trade-off (Riddell 2018). However, this may be unlikely due to data similar to mine obtained from *P. shermani* and *P. teyahalee* collected in the field and tested the next day by John Maerz and Emily Taylor (personal communication). They found that the size of the salamander and the elevation it was found at, but not species, influenced its water loss rate (J. Maerz and E. Taylor, personal communication).

Conclusions

In accordance with my hypothesis, aggression between *P. cheoah* and *P. teyahalee* appears to play a role in the high prevalence of *P. cheoah* and the scarcity of *P. teyahalee* at higher elevations. *Plethodon cheoah*'s small body size, high surface-to-volume ratio, and high water loss rate per gram of salamander limit its ability to live at lower elevations. The altitudinal distribution of *P. jordani* and *P. teyahalee* seems to be governed by similar mechanisms (Gifford & Kozak 2012). Similar interplay between interspecific aggression and physiological limitations may also explain other altitudinal changes in the distributions of montane *Plethodon* species, e.g. *P. metcalfi* and *P. teyahalee* (Hairston 1987, Nishikawa 1985, Riddell & Sears 2015).

With its small range, *P. cheoah* is especially susceptible to any circumstances that could negatively affect its population numbers. Because it is only known to live in s mature hardwood forests, urbanization, large-scale logging operations, or intense wildfires could be disastrous to the future of the species (Gade et al. 2020). The warming trend of Earth's climate seems likely to force *P. cheoah* up the mountains into higher elevations to keep from desiccating, potentially until there is no higher to climb (Lyons et al. 2020). Understanding more about this endemic resident of North Carolina is crucial to conserving it for years to come.

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