CORT Comparisons Between Allopatric and Sympatric Habitats in *Plethodon hubrichti*

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Abstract and/or Background

The Peaks of Otter salamander (*Plethodon hubrichti*) is an endemic species found in the Blue Ridge Mountains of Virginia. This species of special concern has several factors that make it susceptible to habitat loss. Some of those factors include anthropogenic disturbance, competition with the Eastern Red-backed salamander (*P. cinereus*), and natural causes that can result in a decline of physiological health and an inability to adapt to stressors. The main glucocorticoid in amphibians is corticosterone (CORT) and is released by the adrenal cortex as a stress response. When in excess, it has negative effects including reduced health, reduced immune response, and losing the ability to adequately respond to stressors. The goal of our research is to compare CORT concentrations for populations in both allopatric (where only *P. hubrichti* is found) and sympatric (where both *P. hubrichti* and *P. cinereus* are found) zones within the Peaks of Otter range. We will collect baseline and acute agitation-induced waterborne hormone samples as a bioindicator of their physiological heath. We also use the scaled mass index (SMI) to quantify body condition as an additional health measurement. The first part in processing the hormone samples is solid phase extraction followed by concentrating the samples using the Speedvac, then determining CORT levels using EIA assay. We predict that the salamanders living in allopatric sites will have higher SMI values in comparison to the sympatric dues to the salamanders not being in competition. We also predict that the salamanders at the allopatric sites will have lower baseline CORT levels and an ability to respond well to the agitation stressor in comparison to the sympatric sites which overall reflects the effect of competition on their physiological health.

Introduction

The Peaks of Otter salamander, *P. hubrichti* (Fig. 1), is an endemic species is geographically restricted to high elevation areas greater than 442m in the Peaks of Otter region of the Blue Ridge Mountains (Fig. 3). The competitor of the Peaks of Otter salamander is the Eastern Red-backed salamander, *Plethodon cinereus*, which is not as restricted geographically. They prefer environments with mature deciduous forests as it allows for a more humid and cooler climate as opposed to spots with less or no canopy cover. This species is highly susceptible to population decline from a reduction in habitable areas due to climate changes, anthropogenic factors, and competition with *P. cinereus*.

The scaled mass index (SMI) is calculated from the mass in grams and length in millimeters of each salamander. This value is used to quantify body condition which gives us a health measure on the salamanders. We predict that salamanders living in an allopatric zone (where only *P. hubrichti* is found) will have better body condition in comparison to those in a sympatric zone (where both *P. hubrichti* and *P. cinereus* are found). This would be due to the fact they are not in competition with another species.

The main glucocorticoid in amphibians is corticosterone (CORT) (Denver, 2009). The stress response of these creatures elicits a release of CORT by the hypothalamus-pituitary-interrenal axis in the brain (Dantzer, 2014). Physiological health can be assessed by the analysis of CORT concentrations along with the SMI values. It is predicted that the Peaks of Otter salamander will have higher CORT concentrations in sympatric sites (indicating stress) than in allopatric which results in the allopatric sites having better physiological health.

Chronic stress in amphibians leads to a decrease in physiological health. This results in lower reproductive success and a decrease in population density, immunosuppression (Kaiser et al. 2015) and an increase in standard metabolic rate (Wack et al. 2012). As a result, increased stress may be one factor that has led to the Peaks of Otter salamander being concentrated in such a small area.

Materials and Methods

In the fall of 2022, we visited 3 sites to collect up to 40 surface active salamanders from each site. Two of these sites were allopatric where only the Peaks of Otter salamander is found, and the third site was sympatric where both the Peaks of Otter salamander and the Eastern Red-backed salamander are found. Each salamander collected is placed into a clean petri dish with 20ml of spring water for 60 minutes to let the hormones secrete into the water from their skin, urine, and feces. After the full hour, the salamander is weighed, and length is measured to calculate an SMI value that quantifies a health measure (Peig & Green, 2009). Then, the water from the petri dish is transferred to a falcon tube and transferred back to the lab. The hormone samples are stored at -20°C until further analysis is done. The total amount of salamanders collected at each site are split in half into baseline and agitated groups to use a non-invasive waterborne sampling technique (Fig. 2) (Gabor et al. 2016; Novarro, 2018). The agitation group is subject to mild agitation by way of gently shaking the salamanders in the petri dish for 1 minute every 3 minutes for an hour. This will give us data on their response to an acute stressor and if they are able to respond well or not. This will show effect of competition and chronic stress on their physiological health.

Once we are ready to analyze the hormones, we will defrost them and run the sample through C18 solid phase extraction (SPE) tubes to extract the water, then run methanol through the filter to extract the hormone from the filter. The samples will then be concentrated to a powder using the SpeedVac, then resuspended in a buffer. The hormones will then be plated in a 96 well enzyme immuno-assay (EIA) plate and concentration levels read using an absorbance spectrophotometer plate reader.

We compared SMI values from fall 2022 with the SMI values from summer 2022 between allopatric and sympatric locations using f-tests and t-tests to determine statistical significance. This is preliminary research that will continue in the coming years to find the effect of competition on their CORT levels and physiological condition.

Figure 1. Peaks of Otter salamander, *Plethodon hubrichti*. Photo by Cheyenne Brooks

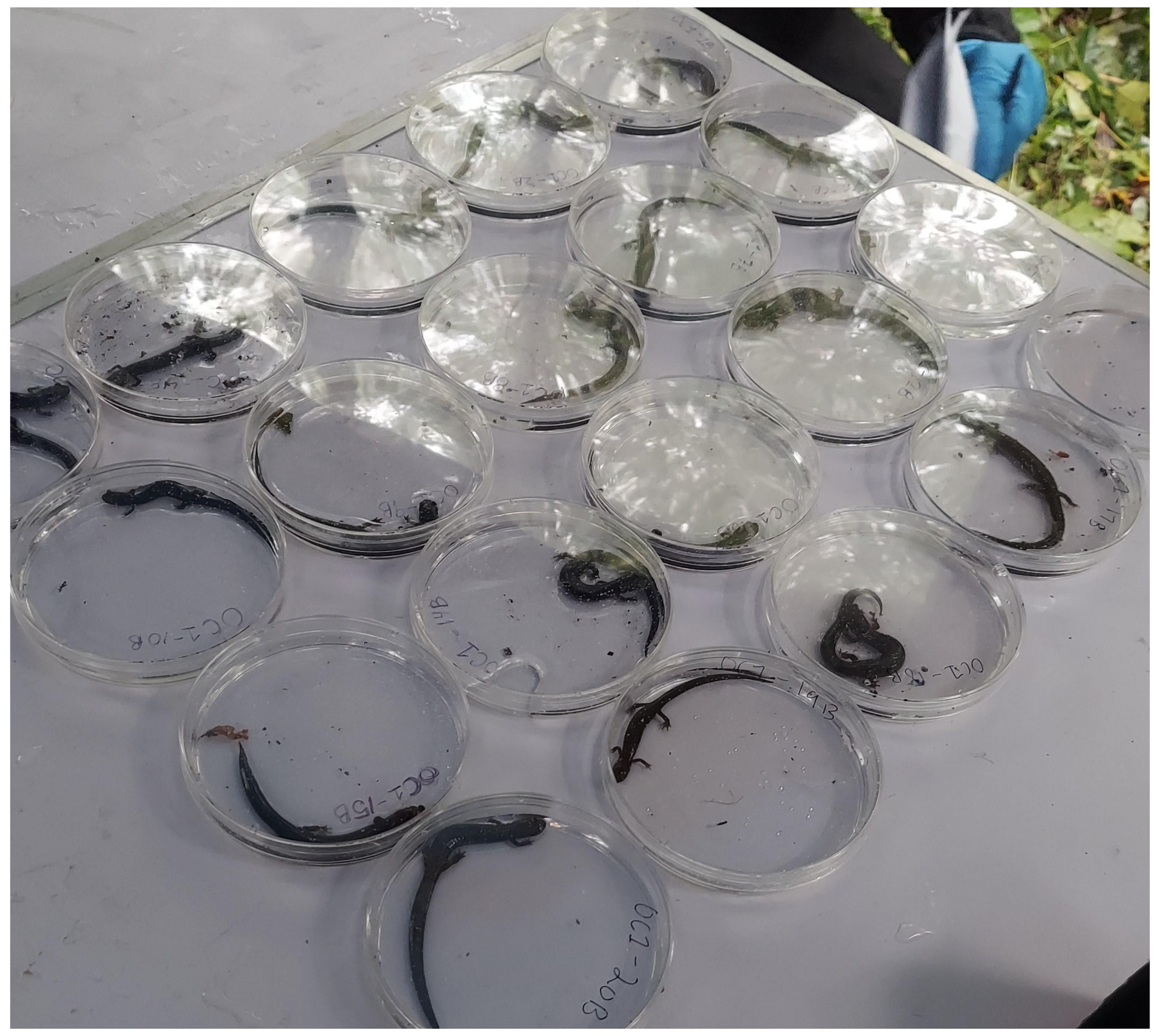

Figure 2. Non-invasive waterborne hormone collection method. Photo by Cheyenne Brooks

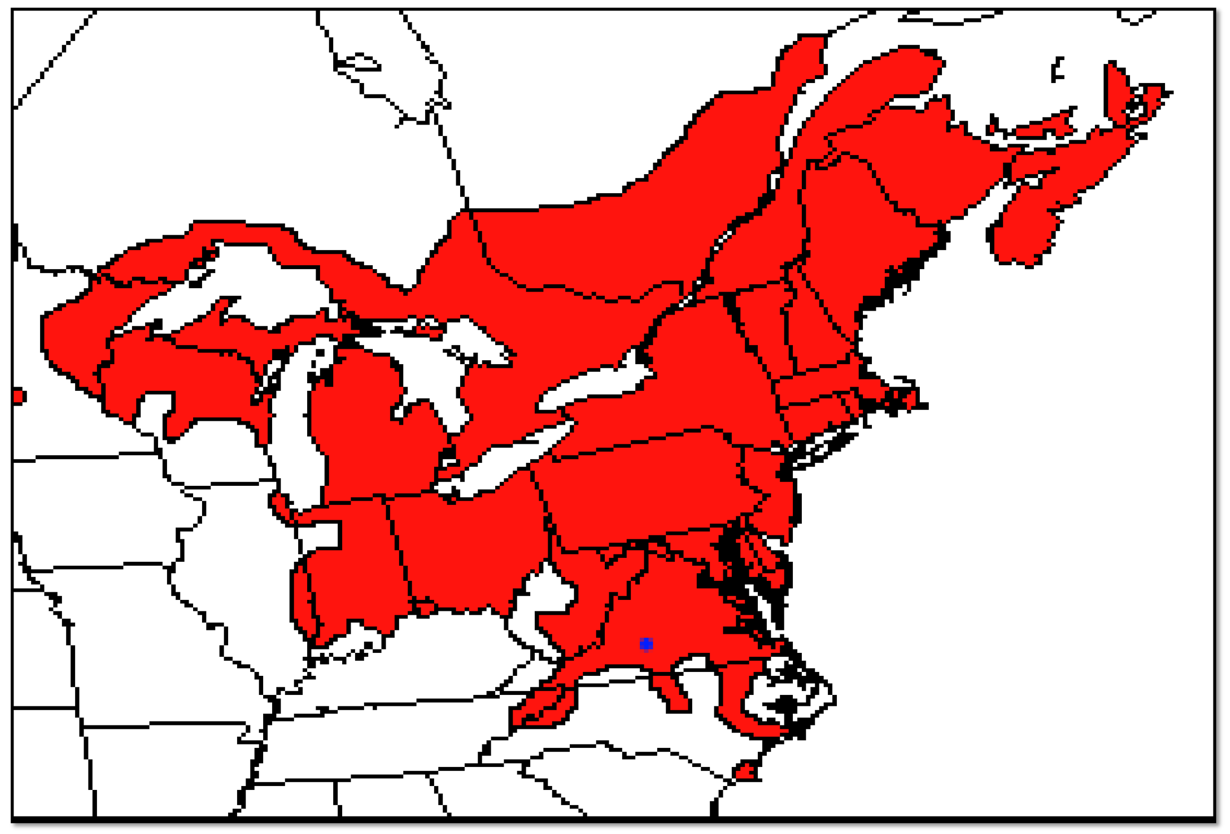

Figure 3. The distribution of the Peaks of Otter Salamander (*Plethodon hubrichti*; blue dot, approximately 15 km along the Blue Ridge Parkway) and the Eastern Redback Salamander (*P. cinereus*; red area).

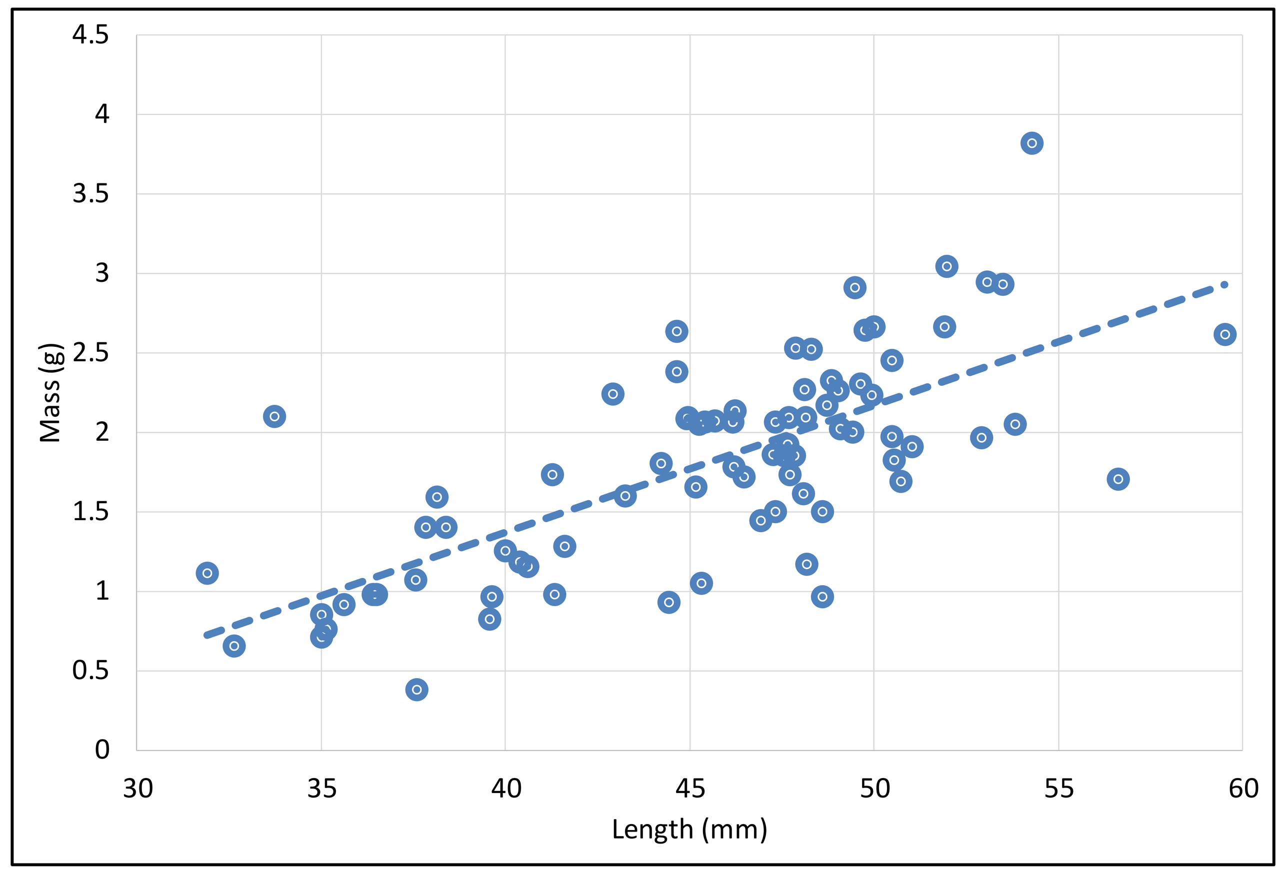

Figure 4. Comparison between mass in grams and length in millimeters of each salamander used to determine the scaled mass index.

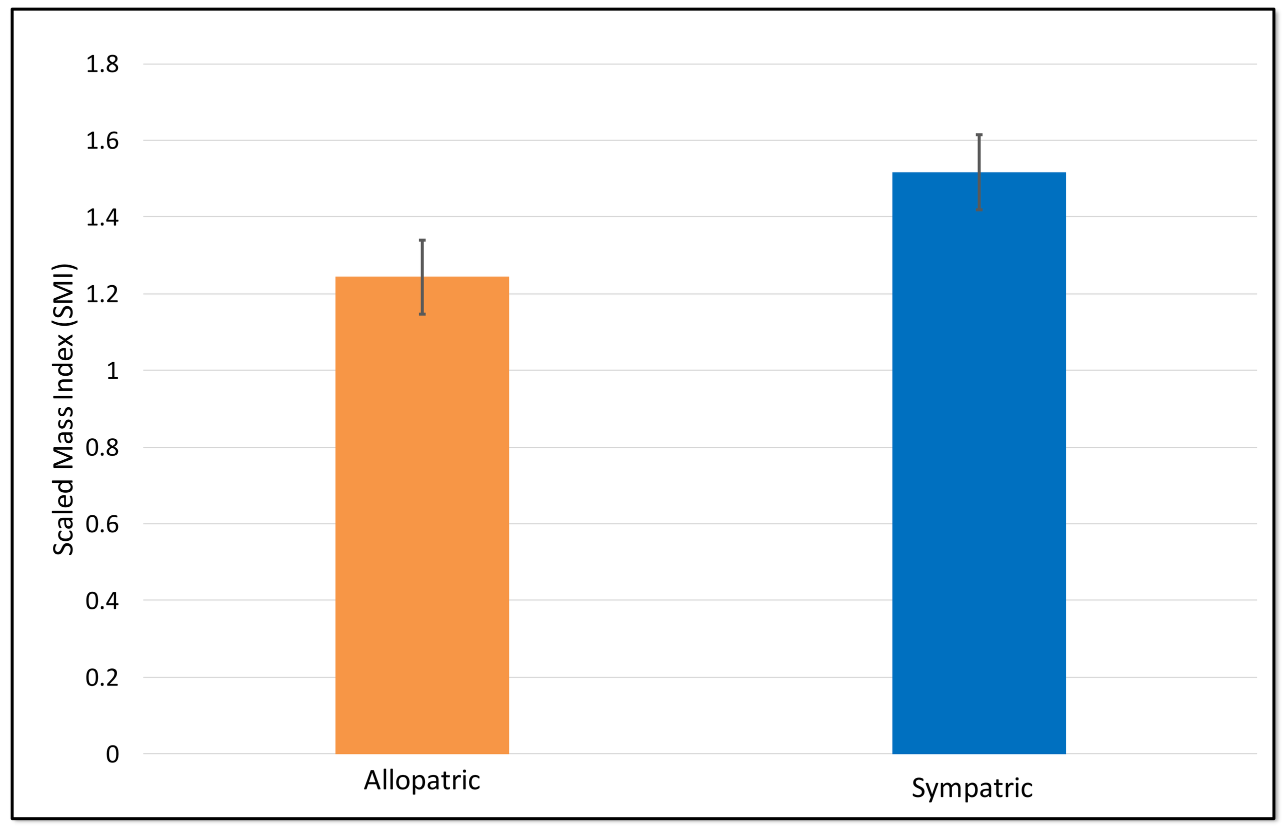

Figure 5. Comparison of Scaled Mass Index (SMI) values between the combined allopatric sites, Over street Creek 1 and 2 (OC1 & OC2), and the sympatric site, Apple Orchard 1, from salamanders collected during the fall of 2022. Error bars included to indicate standard error.

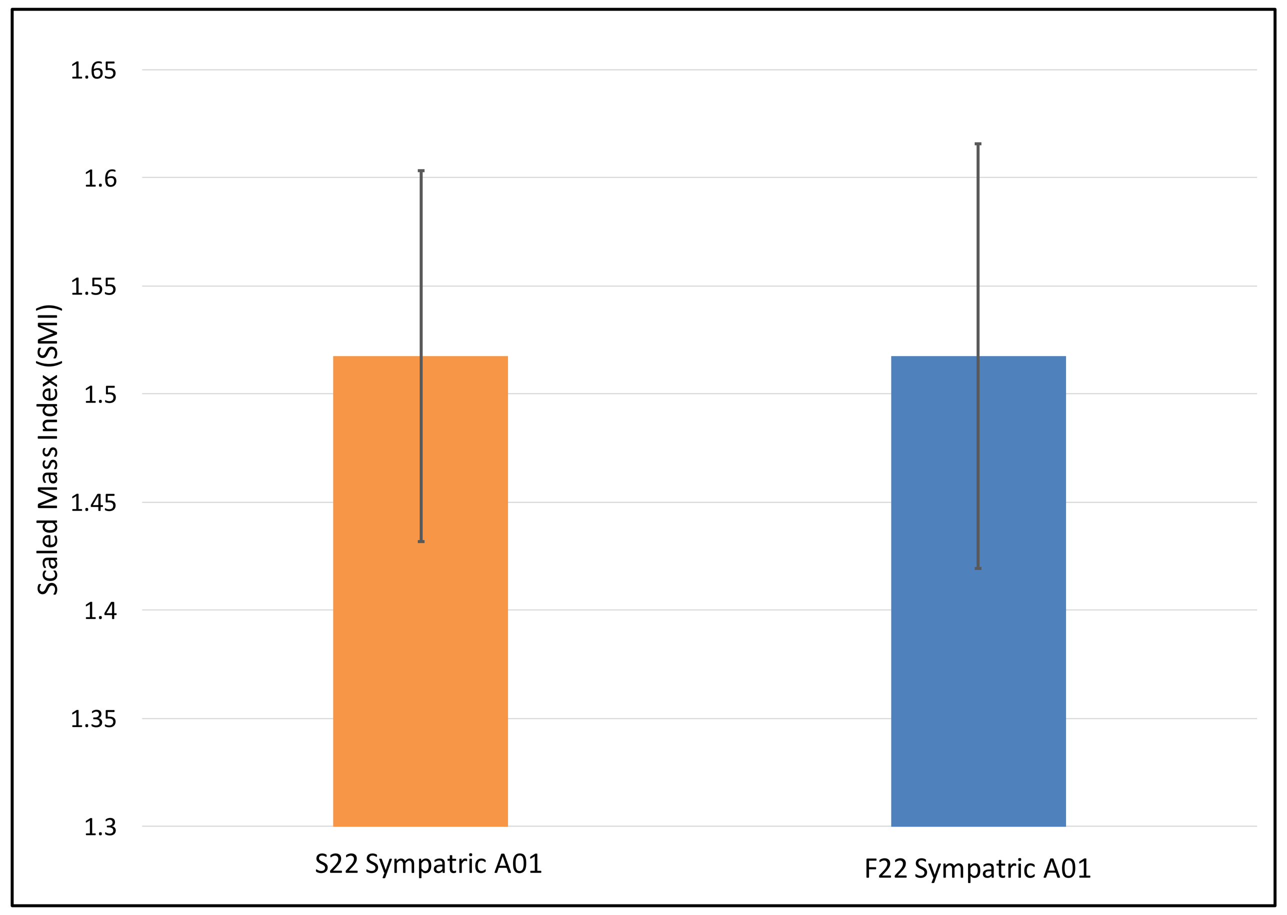

Figure 6. Comparison of the sympatric site Apple Orchard, AO1, between summer (left in orange) and fall 2022 (right in blue). Error bars included to account for standard error.

Results

- There is a positive correlation between mass and length in terms of body proportion of the salamanders (Fig. 4)
- The average SMI values are significantly different between the two allopatric sites and the sympatric site in fall 2022 (Fig. 5) (t = -1.98, df = 83, p = 0.05).
- Comparison of the sympatric site AO1 between summer 2022 and fall 2022 (Fig. 6) showed no significant differences. This location over both seasons kept very similar average SMI values (t=0.002, df=48, p = 0.99)

Conclusion

These results did not support our prediction that the Peaks of Otter salamander would have a better average SMI when living in allopatric areas in comparison to lower values when living in sympatric areas. A lower SMI value in the sympatric location would have been due to the effect of competition for food and space with *P. cinereus* but the results suggested otherwise. However, this difference only included 3 sites total (OC1, OC2, and AO1) and AO1 only had n = 30 individuals.

As predicted, the scaled mass index (SMI) had minimal change between seasons (Fig. 6) as confirmed by statistical analysis. This suggests SMI data between these seasons may be comparable in future analyses and will have marginal impact on CORT release across seasons.

Body condition in sympatric locations will be researched more in future seasons and analysis of CORT levels at these sites will be done to acquire more complete physiological data on the salamanders from these sites.

Future Work

This Spring we will be analyzing the CORT release rates from these three sites collected in the Fall. The CORT from the water samples are currently being extracted for quantification and analysis using the EIA kits.

Further research will be done in the coming seasons to collect this same data to continue the use of the Scaled Mass Index as an indication of physiological health. This will allow us to determine if the body condition of the salamanders is gradually declining in the allopatric areas where they should have better physiological health or if it was just this set of data that is an exception from the norm. Future work will show if there is a trend in body condition fluctuation between seasons.

New data will also be collected on the baseline and agitation corticosterone levels at allopatric and sympatric areas. Up to 40 salamanders from each site will be the sample population from the same sites we collected from in the fall. This analysis paired with the SMI data will give us a better idea of the effects of competition on their overall physiological health by their ability to adjust to an acute stressor.

Acknowledgements

We would like to thank Cassie Fox, Makenna Landis, Timothy Naff, Tory Roberts, and Kyro Sewall for assisting with field data collection and sample extraction in the lab.

We also thank the National Park Service, United States Forest Service, and the Virginia Department of Wildlife Resources for the access and permits to conduct our research.

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