Evaluating elevational effects on CORT and body condition in *Plethodon hubrichti*

LIBERTY UNIVERSITY

Cheyenne E. Brooks, Cassie J. Fox, Cory B. Goff Ph.D

Abstract

The Peaks of Otter salamander, *Plethodon hubrichti*, is an endemic species located exclusively in a 15km range of the Blue Ridge Mountains in central Virginia. The Peaks of Otter salamander is only found at elevations between 440m up to the highest elevation in that area around 1100m. These specific parameters render *P. hubrichti* highly susceptible to habitat loss and population declines, two factors which have heavily influenced the Virginia Department of Wildlife Resources to label these salamanders a species of concern. This study focuses on corticosterone (CORT); the main glucocorticoid found in amphibians, reptiles, and birds, which plays a key role in stress response, metabolism, and immune suppression. Using a non-invasive waterborne hormone collection technique, both agitation and baseline samples of CORT were collected from up to 40 individual salamanders from two high elevation sites (991m) and two low elevation sites (665m) each season, for three seasons. In addition to hormone samples, key measurements including length and weight were recorded to assess overall body condition. These CORT samples were processed using an enzyme-linked immunosorbent assay (ELISA) and analyzed along with the body condition data to compare the physiological effects of elevation on the Peaks of Otter salamander. Our data suggests that salamanders found at higher elevations present better body condition than those found at lower elevations and is corroborated by increased corticosterone (CORT) concentrations in salamanders found at lower elevations. The data suggest this species may be experiencing chronic stress and indicates the need for further studies to better monitor factors affecting the range of this species.

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

Introduction

The Peaks of Otter salamander is endemic to high elevations exclusively above 442m with an optimal elevation being around 900-1000m (Reichenbach et.al., 2022). There is a correlation between decreased body condition and lower elevations, due to increased temperature and decreased humidity associated with lower elevations (Reichenbach et.al., 2022). CORT is the main glucocorticoid secreted by the hypothalamus-pituitary-interrenal (HPI) axis in amphibians (Dantzer et.al., 2014). This study analyzes the CORT secretions from *P. hubrichti* to understand the stress response to environmental conditions. Chronic stress can lead to suppression of the immune system and the inability to amount a response to an additional stressor (Dantzer et.al., 2014). The analysis of this data will provide associations between two physiological metrics, CORT levels and Scaled Mass Index (SMI), as they relate to habitat elevation. We hypothesize that high elevation will be associated with lower CORT concentrations and better overall body condition. While lower elevations will correlate with higher CORT concentrations and lower body condition. The objective of this study to analyze the effect of elevation as a potential stressor on the physiological health of the Peaks of Otter salamander.

Methods

The study was executed between Fall 2021 to Spring 2022. Up to 40 surface active salamanders were collected from either a high elevation (991m) or a low elevation (665m) site. This study uses a non-invasive waterborne hormone collection method modified for amphibians (Novarro et al., 2018 and Gabor et al. 2016), to collect CORT secreted from the skin of each salamander. Upon collection of each specimen, the area was marked with a flag to ensure the salamander could be returned to the same spot, then the individual was placed into a labeled petri dish with 15ml of spring water for 60 minutes. Baseline and agitation samples were collected to determine the salamanders ability to respond to an acute stressor. The agitated samples were shaken in the petri dish for 1 minute every 3 minutes for one hour. After the hormone collection period, the salamander was weighed and length measured using digital calipers. These physiological measurements were used to calculate a Scaled Mass Index (SMI) to quantify body condition (Pieg & Green, 2009). The water from the petri dish containing the CORT sample was transferred to a conical tube and stored in the lab at -20°C until extraction. The samples were then thawed and filtered through Solid Phase Extraction (SPE) columns and subsequently dried using a SpeedVac. The sample was resuspended in a buffer to be processed on an ELISA plate and read using an absorbance spectrophotometer plate reader.

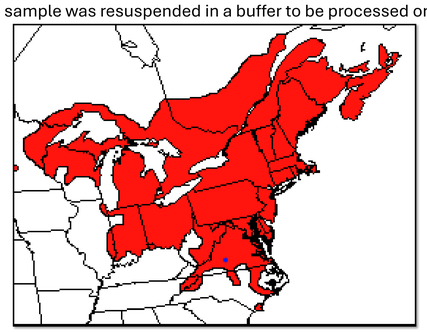

Figure 2. Range map of *P. cinereus* (shown in red) and *P. hubrichti* (blue dot)

Figure 3. Non-invasive waterborne hormone collection method. Photo by Cheyenne Brooks

Figure 4. 96-well ELISA after samples incubated overnight.

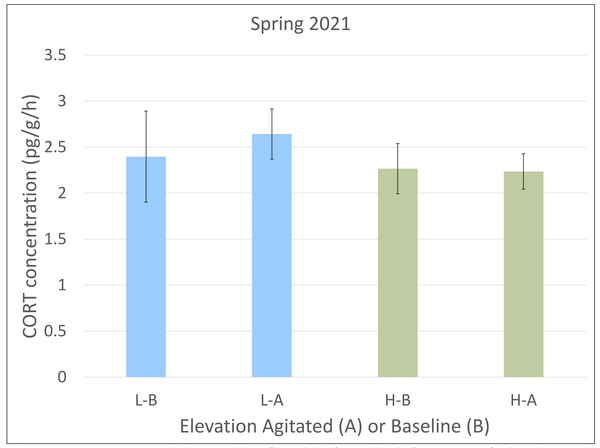

Figure 5. Comparison between low (L in blue) and high (H in green) elevation sites and baseline (B) and agitation (A) samples within each site during Spring 2021.

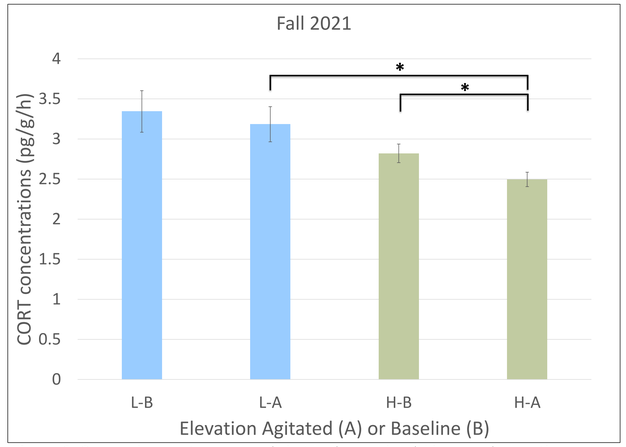

Figure 6. Comparison between low (L in blue) and high (H in green) elevation sites and baseline (B) and agitation (A) samples within each site in Fall 2021.

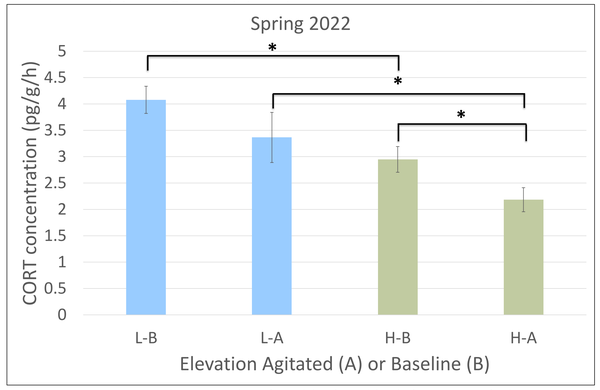

Figure 7. Comparison between low (L in blue) and high (H in green) elevation sites and baseline (B) and agitation (A) samples within each site in Spring 2022.

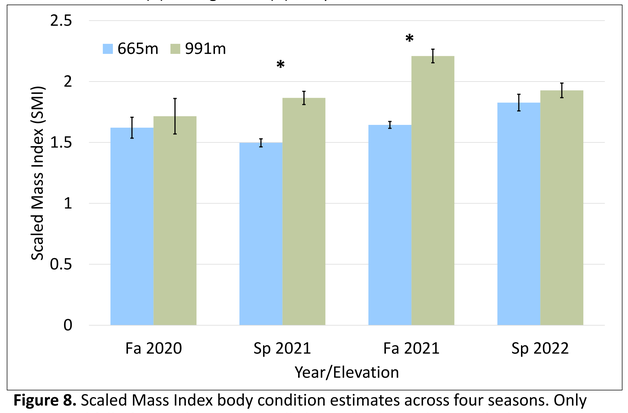

Figure 8. Scaled Mass Index body condition estimates across four seasons. Only spring and fall of 2021 show statistically better body condition at high elevation.

Results

- **Spring 2021** (Fig. 5): No significant differences in CORT concentrations between either baseline and agitation samples or between high and low elevation.
- **Fall 2021** (Fig. 6): Unexpectedly, baseline samples at high elevation are significantly higher than agitation samples (t=2.27, df= 38, p=0.02). CORT concentrations are also significantly higher in low elevation sites than high elevation sites as seen in the agitation samples (t=2.92, df=23, p=0.008).
- **Spring 2022** (Fig. 7): Baseline concentrations are significantly higher than agitation at high elevation (t=-2.28, df=28, p=0.03) but not at low elevation (t=-1.32, df=22, p=0.2). But, at low elevation baseline and agitation samples were significantly higher than at high elevation (Baseline: t=-3.18, df=28, p=0.004; Agitation: t=-2.24, df=20, p=0.03).
- **SMI** (Fig. 8): Spring 2021 and Fall 2021 show significantly better body condition at high elevation (t=4.99, df=67, p=4.6^-6; t=2.00, df=58, p=<0.001 respectively). Although insignificant, the other seasons follow the same trend.

Discussion

Our results support the hypothesis that high elevation is associated with lower CORT concentrations. In addition, the SMI showed consistently better body condition at high elevation in comparison to low elevation. High elevation areas are considered optimal due to the low temperature and high humidity allowing for optimal foraging conditions that prevent the risk of desiccation (Reichenbach et. al., 2022). The trend seen between baseline and agitation samples is opposite from our expectation that the addition of an acute stressor would result in an increase in CORT release. These data could point towards the salamander's inability to amount to a stressor indicating chronic stress (Dantzer et.al., 2014). Chronic stress, defined by the sustained increase in CORT, can lead to immunosuppression, making them more vulnerable to disease and having a worse body condition (Dantzer et.al., 2014). Alternatively, the lower CORT release after agitation could suggest adaptive suppression of the HPI response, modulating their response to additional stressors.

Future Work

The focus of this research has shifted to analyze the effects of competition as a stressor to *P. hubrichti.* However, reassessment of elevational sites will likely be necessary to see if the trend holds and to better understand why baseline CORT release is higher than agitation..

References

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