Monitoring physiological health and density of the Peaks of Otter salamander along an elevational gradient

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Abstract

The Peaks of Otter salamander (*Plethodon hubrichti*) is considered a special concern species by the Virginia Department of Wildlife Resources. It has a limited range of 16 km in the Blue Ridge mountains and is highly susceptible to habitat loss. These salamanders prefer higher elevations, showing higher densities, body condition, and reproductive output than those found at lower elevations. Those salamanders at lower elevations may be experiencing more stress due to the reduced habitat quality. Stress may affect their ability to adapt to any additional stressors like timbering or drought. The hormone corticosterone is released by amphibians in response to stressors and may suppress immune responses, growth and reproduction when consistently released due to constant environmental stressors, such as reduced habitat quality. To better understand the effect of elevation on Peaks of Otter salamander health, we plan to compare hormone concentrations from salamanders at two sites along an elevational gradient, one at 655m (low) and one at 991m (high/optimum). We collected mass and total length data to measure body condition, as well as water-borne hormone samples to measure baseline corticosterone levels. We used a scaled mass index (SMI) to quantify body condition of the collected salamanders. We collected hormone samples from 25 salamanders per site over one hour to measure physiological health. We compared body condition and densities of salamanders at the two locations. Though there were no significant differences, body condition and density tended to increase at higher elevations. Hormone samples will be analyzed to quantify corticosterone and identify physiological health of individuals from both sites to compare across an elevational gradient. Both sites will be resampled in Spring 2021 to assess whether chronic stress is associated with lower elevations. This will benefit future studies and the continued monitoring of population health between different parts of the salamander's limited range.

Introduction

Plethodon hubrichti is a high elevation species restricted to altitudes above 442m (Fig. 1). It has lower tolerance to reduced humidity, and higher temperatures than neighboring salamander species like *P. cinereus*, whose range far exceeds that of *P. hubrichti* (Fig. 2). The species has been found to prefer an elevation around 1000m. They prefer mature deciduous forests and populations may decline due to habitat loss, such as clear-cutting. At lower elevations, body condition decreases with increasing temperature and decreasing humidity (Reichenbach & Brophy, 2017). This may be evidence of reduced physiological health due to stress.

Corticosterone is one of the main glucocorticoid hormones involved in the stress response in reptiles and amphibians. By analyzing the concentration of this hormone and other health metrics, researchers can relate environmental conditions to overall physiological health (Dantzer *et al.,* 2014). For a montane endemic species such as the Peaks of Otter salamander, we predicted that individuals found at higher elevations should be less stressed resulting in better physiological health. Previous studies have shown higher densities, more gravid females, and more eggs per female at higher, optimal elevations versus those at lower elevations (Reichenbach & Brophy, 2017). This research aims to measure physiological health of these salamanders across an elevational gradient by measuring baseline corticosterone levels and density of POS populations at two sites. We predict that there will be fewer salamanders/m^2 at the lower site and that those salamanders at the lower site will have higher corticosterone levels reflecting increased stress. We will use the physiological metrics from this study to compare and further analyze the overall health of the population of Peaks of Otter salamanders.

Materials & Methods

A team of 10 researchers collected surface-active salamanders in a 20 x 50-meter plot at two different elevations: 655m and 991m (Fig. 3). Each plot was searched completely, and all salamanders were recorded to estimate surface-active densities. Additionally, the first 25 salamanders collected at the two elevation sites were used to collect hormones following a non-invasive, water-borne hormone collection method (modified from Novarro *et al.,* 2018). In brief, we captured salamanders and placed them individually into a sterile petri dish that contained 25 mL of fresh spring water. We left the salamanders in the dishes for 60 minutes to allow for the secretion of hormones into the water through urine, feces, and skin (Figs. 7). After 60 minutes, the salamanders were taken out of the dish and each salamander was weighed, and the total length (TL) was measured. The water left in the petri dish was poured into a falcon tube and returned to the lab where it was stored at -20°C.

We used the mass and total length data of each salamander to calculate a scaled mass index (SMI) for each individual (Pieg & Green, 2009). One-tailed t-tests were run to compare masses, total lengths, and SMI's of the two samples of salamanders. We also compared densities found at the two sites in 2020, to previously recorded densities from 2008.

Figure 1. Peaks of Otter salamander, *Plethodon hubrichti.* Photo by Kirk Ricketts, used with permission.

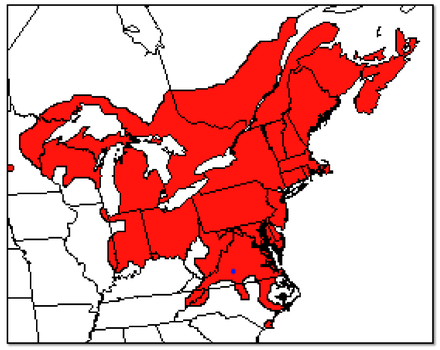

Figure 2. The distribution of the Peaks of Otter Salamander (*Plethodon hubrichti*; blue dot, approximately 15 km along the Blue Ridge Parkway) and the Eastern Redbacked Salamander (*P. cinereus*; red area).

Figure 3. Several students involved in the field collection process.

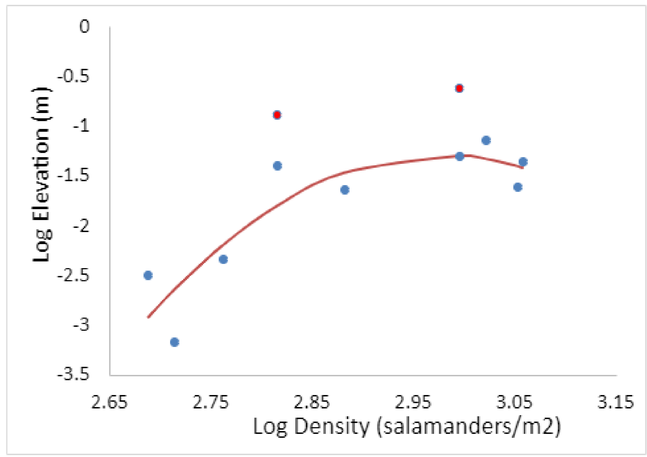

Figure 4. Historical density of salamanders at different elevations from 2008 (blue dots) compared to density of salamanders at surveyed elevations from 2020 (red dots). The red line is the predicted density from the regression.

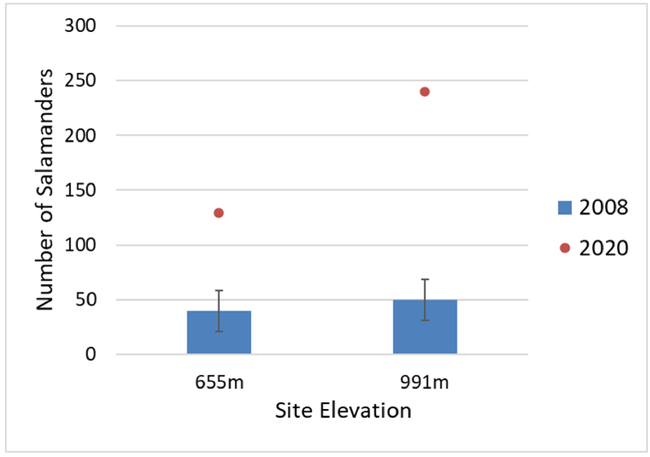

Figure 5. Average numbers of salamanders found at the two sites in 2008 over 3 surveys (bars), compared to numbers found in 2020 during one survey (dots).

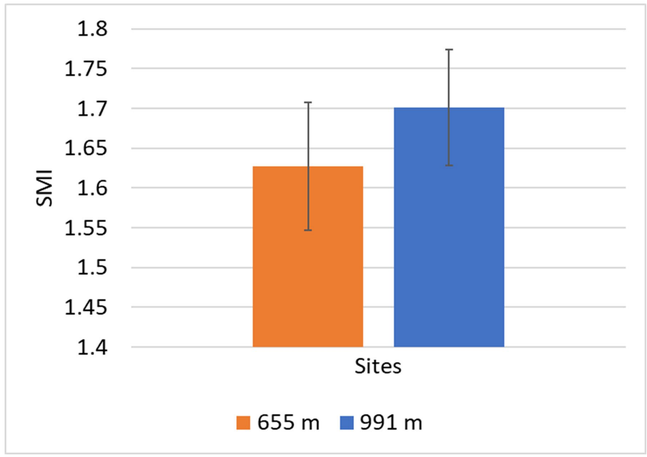

Figure 6. Comparison of scaled mass indexes (SMI) of salamanders at the two sites in 2020.

Figure 7. Water-borne hormone collection method.

Results

1. We collected 129 *P. hubrichti* at the lower elevation site (665m) and 240 at the higher elevation site (991m). These numbers were higher than historical densities collected along an elevational gradient (Fig. 4) and higher than the averages for both sites (Fig. 5).
2. The SMI's were not significantly different between the two sites (t = 0.834, df= 34, p = 0.205), though salamanders at the higher elevation tended to have higher SMI (Fig. 6). Salamanders at the lower site tended to be longer on average (M_{665} = 96.787 mm, M_{991} = 96.072 mm). Though not statistically different, salamanders at the higher site tended to weigh more than those at the lower site (t = 0.658, df = 30, p = 0.258).

Conclusions

The lower densities at lower elevations could be due to environmental factors and possibly additional stress. The collection of hormones will be able to determine if environmental factors are not only affecting their health but furthermore how it can relate to the density differences with the varying elevations. The Peaks of Otter salamanders have been found up to 1143m in elevation (Reichenbach & Brophy, 2017). It can be noted that salamander density increased at elevations between 900-1100, whereas levels over 1100 were seen to have a slight decrease in density (Reichenbach & Brophy, 2017). At elevations less than 800m there was a decrease in density to the point that no salamanders were found at the lowest elevations, potentially due to increased temperatures and lower RH which affects the reproduction of the salamanders (Reichenbach & Brophy, 2017). Additionally, higher stress levels from the environmental factors associated with lower elevations could play a role as well. In amphibians, increased stress due to environmental factors may contribute to decreased density, reproductive output, and immune suppression (Denver, 2009). Analyzing the hormones secreted by the Peaks of Otter salamanders will be conducted this Spring to assess the impacts of environmental conditions at both sites on their physiological health. All of these factors combined may be limiting this species to higher elevations. Therefore, any abrupt change in environmental conditions, such as logging operations that open the canopy, increasing temperatures and reducing humidity, may have severe impacts on this species and other montane salamanders throughout the region.

Future Work

The water-borne CORT collection method will be validated for *P. hubrichti* using a pooled sample of hormones from 10 non-experimental salamanders serially diluted (following Gabor *et al.,* 2016). This pooled sample will be created by combining 5 samples at elevation 991m and 5 samples at elevation 665m. Once defrosted, all water samples will be drawn through C18 solid phase extraction (SPE) columns to extract hormones, dried to a powder, and resuspended in a buffer. Hormone samples will then be plated on 96-well enzyme immuno-assay (EIA) plates and concentrations will be measured using an absorbance spectrophotometer plate reader.

In Spring 2021, both elevational sites will be resampled for density and hormones. In addition to collecting "baseline" hormones over an hour, an additional 15-20 salamanders will be "agitated" by shaking the petri dishes for 1 minute every third minute for an hour to assess their hormonal ability to respond to an acute agitation stressor.

Future work by the Reichenbach-Goff lab will compare the physiological health of these salamanders between allopatric and sympatric sites, and between core and disjunct populations across their range. In this way, we can monitor their health in response to multiple abiotic and biotic stressors.

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