

# Natural history of the Peaks of Otter salamander (*Plethodon hubrichti*) along an elevational gradient

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**ABSTRACT** - The Peaks of Otter salamander, *Plethodon hubrichti*, is a montane species found at elevations above 442 m within a 117 km<sup>2</sup> area of the Blue Ridge Mountains in central Virginia, USA. In areas of this species' range where the Eastern Red-backed salamander *Plethodon cinereus*, a potential competitor, is absent, surface-active salamander density was hypothesised to decrease at lower elevations due to increased temperatures and lower humidities, which may adversely affect salamander reproductive output and survival rates. Eggs/female, % gravid females, surface-active salamander density, temperature and relative humidity were recorded from nine sites ranging in elevation from 488 to 1143 m. Survival rates and growth rates were estimated at three of these sites. Surface-active salamander densities, survival rates, growth rates, eggs/female and reproductive output decreased with elevation. Decreases were correlated with increases in temperature and a decrease in relative humidity associated with decline in elevation. Other habitat factors such as % canopy closure did not decrease with elevation at the study sites. Peaks of Otter salamanders had greater dehydration rates and lower critical thermal maxima than the wider-ranging Eastern red-backed salamanders, which reflects their adaptation to montane environments. These results support the importance of conserving mature hardwood forests, particularly at lower elevations, which represent marginal environments for montane species of salamanders.

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## INTRODUCTION

The Peaks of Otter salamander (*Plethodon hubrichti*) is one of several endemic salamander species found in the state of Virginia, USA (Kramer et al., 1993; Petranka, 1998). It is a montane species with a very limited distribution, being found only in mature, deciduous forests at elevations greater than 442 m within a 117 km<sup>2</sup> area of the Blue Ridge Mountains in central Virginia (Pague & Mitchell, 1990). Elevation may limit the distribution of *P. hubrichti* in areas where it is allopatric with a potential competitor, the Eastern Red-backed salamander (*P. cinereus*), due to physiological factors such as intolerance to the higher temperatures and lower humidities associated with lower elevations. Such intolerance was suggested by Thurow (1957), who observed that *P. hubrichti* had a lower tolerance for increased temperatures and evaporation rates than did *P. cinereus*, a species with a broad distribution over a wide range of elevations (Petranka, 1998). Arif et al. (2007) considered the entire distribution of *P. hubrichti* to be limited primarily by abiotic factors. Using 19 bioclimatic variables such as maximum temperature of the warmest month and precipitation of wettest month, they were able to correctly predict 80% of known localities for this species.

Amphibians living at high elevations tend to produce fewer clutches per year, larger absolute clutches and larger eggs relative to those living at lower elevations (Morrison & Hero, 2003). Resource availability is a critical factor in regulating reproductive frequency as was experimentally demonstrated with the four-toed salamander, *Hemidactylium scutatum*, under various food resource levels (Harris & Ludwig, 2004). For *P. cinereus*, resource constraints at high

elevations (over 1220 m) were correlated with smaller body size, less frequent reproduction, but greater tail fat storage relative to body size when compared to conspecifics at low elevations (Takahashi & Pauley, 2010). Resource allocation to growth and reproduction, in this case, may be restricted at high elevations because of the shorter growing season and greater allocation of energy to storage.

For montane-adapted *Plethodon* species, low elevations may be the most important constraint upon their distributions (Hairston, 1951, 1981; Kozak & Wiens, 2006). Kozak & Wiens (2006) considered that many montane sister *Plethodon* and *Desmognathus* taxa in the Appalachian Mountains are isolated from each other because they cannot tolerate abiotic conditions in the intervening lowlands. Abiotic conditions in these lowland areas may exceed the physiological tolerances of montane salamanders. Bernardo & Spotila (2006) demonstrated that limited physiological tolerance to warm temperatures of two montane species of *Desmognathus* (*D. carolinensis* and *D. ocoee*) restricted their ability to disperse through warmer, drier valleys. Understanding the factors that limit the range of *P. hubrichti* will help conservation efforts by enabling us to avoid forest management practices that contract this species' distribution.

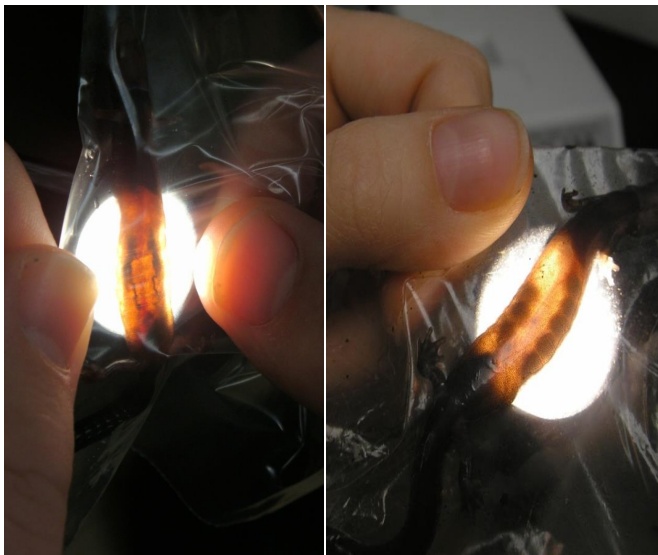
Here the effects of decreasing elevations, along with corresponding increases in temperature and decreases in relative humidity, were examined on the natural history of *P. hubrichti* in allopatry with its potential competitor *P. cinereus*. *P. hubrichti* surface-active (SA) salamander density, eggs/female, % gravid females, and growth and survival rates were hypothesised to decrease with declining elevations because of physiological limitations associated with their montane existence. In addition, this montane

species was hypothesised to have lower critical thermal maxima (CTM) and greater dehydration rates than the wider-ranging *P. cinereus*.

## MATERIALS AND METHODS

### Demographics and environmental variables vs. elevation field methods

On 28 April 2007, two teams of six to eight people collected SA *P. hubrichti* during the daytime, on a cool day following rain so that all sites were moist and cool throughout the day, by carefully turning over rocks and logs at five allopatric sites along an elevational gradient (518, 655, 762, 991, and 1052 m; slope directions were west for all sites except 1052 m site, which faced south). Two teams were used so that all sites could be collected on the same day. The sites searched varied from 577 to 1445 m<sup>2</sup> and depended upon the overall number of salamanders found at each location since the goal was to collect at least five adult females per site. *P. hubrichti* were placed in zip-lock bags along with moistened paper towels for reproductive output measurements. The bagged salamanders were then placed in a cooler with ice and transported to the lab where they were weighed ( $\pm 0.01$  g; Scout Pro SP202, Ohaus Corp., Pine Brook, New Jersey, USA); measured (snout-vent length, SVL) using calipers (CD-6"CSX, Mitutoyo Corp., Aurora, Illinois, USA) and the salamander-stick method (Walston & Mullin, 2005); and examined for sex determination (Fig. 1) and the number of large, yolked eggs (Sayler, 1966) using a non-destructive, visual method to examine the body cavity with a fiber optic light (Fig. 1; Gillette & Peterson, 2001). Animals were returned to their collection locations the following week.



**Figure 1.** Candling method for determining salamander sex and number of eggs per female. The picture on the left shows a male *P. hubrichti* (testes and vas deferens visible). On the right is a female *P. hubrichti*, with eight large yolked eggs (three in one oviduct and five in the other). Photograph by A. Fredrickson.

In 2008, additional allopatric areas were surveyed for high and low elevation sites (488, 579, 1128 and 1143 m; slope directions for sites were north, west, summit and

summit, respectively) along with the original sites surveyed in 2007, thus raising the total to nine sites ranging from 488 to 1143 m. Surface-active salamanders were counted on standardised plot sizes of 20 x 50 m at all sites except the lowest elevation site (488 m) where, because of the topography and expected low SA salamander density, a 25 x 100 m plot was established. Surface-active salamander density (SA salamander count/size of area surveyed) was used as a surrogate measure of population size since SA salamander counts correlated well with population estimates in previous mark-recapture studies, including one involving *P. hubrichti* (Smith & Petranka, 2000; Reichenbach & Sattler, 2007; Gifford & Kozak, 2012). At all sites, metal rods were hammered into the ground at the four corners of each plot, and flags were used to visibly mark the corners and sides of the plot. Salamanders were counted and collected on three dates (12, 26 April and 20 Sept 2008) by two teams of eight to ten people on cool days following rain so that all sites were moist and cool throughout the day. Two teams were used so that all sites could be collected on the same day since the fraction of the salamander population that is surface-active is known to vary significantly with time since it last rained (Kramer et al., 1993; Reichenbach & Sattler, 2007; O'Donnell et al., 2014). The 488 m site was the only site that was not surveyed on 26 April 2008 due to time constraints, so data for only two collection periods were available for this site (12 April and 20 Sept 2008). Collection, counting and reproductive output methods followed those used in 2007. In 2008, only salamanders collected on 12 April 2008 were used for reproductive output measurements so that the same salamander was not inadvertently used twice in the yearly assessment.

A regression model was used to characterise the relationship between mean surface active salamander density and elevation.  $\log_{10}$  transformation was used whenever data needed to be normalised and variances stabilised as determined by normality and residual plots associated with regression models. All regression models were significant at  $\alpha = 0.05$ . Number of eggs per female against elevation, female mass, and SVL were characterised using a multiple regression model. Data from 116 gravid female *P. hubrichti* (range of 2 to 19 gravid females per site with a mean of 10) were used to fit this model. A regression model was also used to relate % gravid females to elevation. For the low elevation sites (518 and 655 m in 2007 and 488, 518 and 579 m in 2008), where low numbers of SA salamanders were found, data were combined across sites in order to estimate % gravid females and then the associated elevations were averaged. The size of the smallest female with eggs was considered to be the minimum size for female sexual maturity. Of the 200 total mature female *P. hubrichti* found, the number per site used to determine % gravid females ranged from 6 to 34 with a mean of 18. Chi-square analysis was used to see if ratios of male to female salamanders captured at each site deviated with elevation from an expected value of 1:1. Pearson's correlation was used to determine if there was a significant relationship between gravid salamander mass and elevation. Analyses were done using either SYSTAT (SPSS Inc., Chicago, Illinois, USA) or EXCEL (Microsoft

Corp., Redmond, WA, USA) using  $\alpha = 0.05$  for all tests. Relative egg production was calculated using regression models for SA salamander density, % gravid females, and number of eggs per female for elevations within the range of this study. Estimates were calculated for number of eggs produced per 1000 m<sup>2</sup> (area of the study sites in 2008 except for the 488 m site) by multiplying the output from these models. These are relative estimates of egg production that can be used to compare sites. They would be underestimates of what the salamander population actually produces since SA salamanders represent only a fraction of the salamanders in a given population (Kramer et al., 1993; O'Donnell & Semlitsch, 2015).

In 2008, at the nine sites where SA salamander density was measured, the following habitat variables were recorded: (1) Percent canopy closure was recorded based on measurements taken at 2-m intervals along two 50-m transects within each plot. A densitometer was used (Geographic Resource Solutions, Arcata, California) at 25 ground-level points per transect to determine whether the canopy was open or closed at each point. Percent canopy closure was then determined for each plot; (2) Percent ground cover was evaluated with a 0.5 m quadrant with 36 hazard points that was laid on the ground (tall herbaceous vegetation projected through the grid of the quadrant and was not counted) at three locations along the same two transects (5 m from each end and in the middle of each transect giving us a total of six locations for percent ground cover per site). At each hazard point, ground cover was recorded as leaves, rock, bare soil, branches, or vegetation. Percentages were then calculated for each category; (3) Leaf litter depth in cm was recorded within each 0.5 m quadrant used to measure percent ground cover by using a ruler to measure from the top of the thickest layer of leaves to the mineral soil. Median leaf depth was then calculated for each site. Correlations were conducted between the median values for the habitat variables and median SA salamander density at each site to determine if these variables, as well as elevation, were significantly related to SA salamander density. Medians were used because of the skewed distributions for the habitat variables.

Temperature and RH were measured during the 2010 field season using temperature and RH buttons (Model DS1923, Maxim Integrated, San Jose, California) placed at the nine study sites as well as other sites used in additional surveys for *P. hubrichti* ( $n = 18$ ). A 3-h time interval was set for readings, and temperature and RH were recorded from 30 May 2010 to 9 September 2010. Mean night temperatures and RH (from 2000 to 0600 h) were calculated and then the relationship between mean night temperature and night RH with elevation was characterised using regression models. The RH values at two lower elevation sites (518 and 579 m), which averaged 97% RH, were within 45 m of a stream and had lots of understory vegetation. These conditions apparently created high RH at these two low elevation sites because values recorded here were greater than those recorded at the highest elevations. In addition, they did not follow the linear decrease of RH and elevation decline seen at the 16 other sites. For these reasons they were considered outliers and were dropped from the data used to fit the

regression model of RH and elevation. Dropping these two low elevation sites restricted the RH model's predictive capability to elevations greater than 655 m. For a limited number of sites ( $n = 8$ ), where SA salamander densities were available along with temperature and RH data, a multiple regression model was used to estimate SA salamander density using mean night temperature and RH.

### Survival vs. elevation field methods

In 2010, salamander survival was recorded in the field at three different elevations (518, 655 and 991 m). For this study 30 large plastics tubs (80 x 45 x 43 cm) were modified by drilling 6.4 cm diameter holes in the sides and top to allow for ventilation and 1 cm holes in the bottom for water drainage, so that tubs would mimic field moisture and temperature conditions. Holes were covered inside the tubs with fiberglass window screen to prevent salamanders from escaping and outside with a sturdier plastic screen (1 cm mesh size) to prevent other animals from damaging the more delicate interior window screens. Fiberglass window screen was held in place with cable ties inserted through holes drilled in the plastic tubs and silicone was used to seal the edge of the screen against the wall of the tubs. The sturdier plastic screen was also fastened to the outside by cable ties. Ports made of 13 mm diameter polyvinyl chloride (PVC threaded cap on a male threaded connector) were added to the side of each tub so that food could be added to the tubs without removing the lids (Fig. 2).



**Figure 2.** A) Tub for *P. hubrichti*, survival study showing screening which covered the 6.4 cm diameter holes drilled in the sides and top so the tub environment would mimic field moisture and temperature conditions. B) Tub being set up in the field. Tub was placed in a hole dug into the forest floor. PVC tube visible by student's hand is one of two artificial burrows provided for the salamanders. C) Tub ready for salamanders showing rocks and leaves placed in tub as surface cover for salamanders. PVC threaded port through which food was introduced into the tub once the lid was sealed in place visible at left corner. Photographs by N. Reichenbach.

On 17 April 2010, 10 tubs were buried to a depth of 30 cm in the field at three different elevations. The soil dug out of each hole was placed back into the tub. As the soil was being added back, two 13 mm diameter PVC pipes were

placed at an angle in the tubs. These tubes were intended to function like burrows in the soil that were disrupted by the digging activities. These artificial burrows gave salamanders access to areas below the soil surface. Five “handfuls” of dead leaves were added along with five flat rocks to the soil surface of each tub as cover objects for the salamanders.

On 24 April 2010, salamanders were collected near the locations where the tubs were buried and taken to the lab to be weighed. On 1 May 2010, two *P. hubrichti* were placed in each tub along with four large and four small worms and about six small crickets. The smallest available salamanders (mean mass 0.34 g, range 0.11–0.93 g) were used since density in the tubs (2 salamanders/0.36 m<sup>2</sup> or 5.5 salamanders/m<sup>2</sup>) was slightly greater than the recorded density of 4.5 salamanders/m<sup>2</sup> for *P. hubrichti* from a nearby location (Kramer et al., 1993). Salamanders were not marked but since individual growth rates were going to be assessed over the course of the study, the two animals placed in each tub were either of similar mass (e.g., 0.36 and 0.37 g) or had easily-distinguishable masses (e.g. 0.17 and 0.38 g). When salamanders were weighed at the end of the experiment, the larger of the two final masses was simply paired with whichever animal had the larger initial mass. If only one salamander survived and the initial masses were not similar, those data were excluded from growth rate calculations.

For one tub at each elevation, temperature and RH Ibuttons (Model DS1923) were placed inside and outside the tub using structures that looked like three-legged stools. Each “stool” was made of a plastic cap with three short pieces of PVC pipe for legs. The Ibutton was velcroed to the underside of the cap so that it could be easily removed to download data and to protect it from direct contact with water. Temperature-only Ibuttons (Model DS1921, Maxim Integrated, San Jose, California) were also placed under one rock on the soil surface and below the surface at the base of one of the artificial burrows in each tub. Temperature and RH data were recorded every 3-h and mean night air temperature and RH were calculated (2000 to 0600 h), as well as mean temperature beneath the rock and at the base of the artificial burrow for the entire day. Night air temperatures and RH were calculated since this is the period when *P. hubrichti* is normally active and exposed to surface conditions (Kramer et al., 1993). Temperatures were averaged across the entire day for the rock and base of artificial burrow since salamanders could be in or under those structures for the entire day.

To seal the salamanders in the tub, silicone was applied to the top edge and then a sheet of fiberglass window screen was laid over the entire open top of the tub. The plastic lid was then snapped in place and cable ties threaded through small holes, drilled through the lids and top edge of the tub, were used to secure the lid.

Six small worms were added through the side ports of each tub on a monthly basis. During one of these trips, 29 May 2010, one tub lid at the 991 m site was damaged due to apparent black bear (*Ursus americanus*) activity. The tub was examined and no salamanders were found at the surface. Two new *P. hubrichti* were then captured and placed in the repaired tub. Masses were not recorded for these

animals, but the general SVLs were larger than the original salamanders and hence would be distinguishable should the originals be found at the end of the experiment when the entire tub contents were examined.

On 10 September 2010, all tubs were opened and examined for salamanders. The soil surface was examined first followed by removal of the rocks. The soil was then removed, one handful at a time, and finally the PVC pipes (i.e. artificial burrows) were examined for salamanders. The position of live salamanders was noted as follows: (1) out on the surface; (2) under a rock; (3) in an artificial burrow; or (4) in the soil. Each salamander found was also weighed. Number of salamanders found alive versus not found (i.e., presumably dead) was compared across elevations using chi-square analyses while growth rates (g/d) were correlated with elevation.

### Physiology lab methods

In spring 2007, CTMs were recorded during the day using the method described by Hutchison (1961) for 20 adult male *P. hubrichti* and 20 adult male *P. cinereus* from three locations (allopatric *P. hubrichti* site – 655 m elevation; allopatric *P. cinereus* site – 762 m; and one sympatric site – 1268 m) that had been acclimated to 20 °C for one week. Individual animals were placed in a 2 L round-bottom flask with 300 ml of continuously aerated water, at their acclimation temperature, and then heated with an electric heating mantle at a rate of 1 °C per minute until the signs of thermal distress were noted (i.e., onset of spasms which marked the inability of the salamander to escape the thermal conditions as described by Hutchinson (1961)). Water temperatures were measured continuously using a mercury thermometer graduated to 0.1 °C. Deep body temperatures were not measured because the heating rates used prevented any measurable lag between water and salamander temperatures (Spotila, 1972). Once visual signs of distress were observed, water temperature was recorded and this was considered to be the CTM. Salamanders were revived in water at lower temperatures and returned to their original collection sites within a week of recording CTMs. Analysis of Covariance (ANCOVA) was used to compare CTMs for the two species using elevation as the covariate.

In 2008, dehydration rates were determined during the day using the method described by Spotila (1972) for 29 male and nongravid female *P. hubrichti* (0.49 to 1.85 g) and 29 male and nongravid female *P. cinereus* (0.47 to 1.85 g), all collected from one location (1183 m elevation) in the fall and acclimated to 20 °C for one week. Salamanders were weighed (Scout Pro SP202) after blotting them dry with paper towels and voiding their bladders by gently pressing on the dorsal side of the pelvic girdle. A wet spot on the paper towel was evident when the bladder was voided. Salamanders were then placed individually in small nylon screen enclosures that minimised coiling. Screen enclosures were secured using clips and placed in desiccators with anhydrous CaSO<sub>4</sub>, and then the entire apparatus was placed in an incubator at the acclimation temperature. Salamander masses were checked periodically, so they would not lose more than 15% of their initial mass, and they were weighed

again after one full hour in the desiccator. At the end of the experiment, salamanders were rehydrated in containers partially filled with water and then returned to their original collection site within one week. Respiratory surface area was determined using the formula for plethodontids from Whitford and Hutchinson (1967), and dehydration rates were expressed as mass loss per respiratory surface area per hour ( $\text{mg}/\text{cm}^2 \cdot \text{h}$ ). Dehydration rates for the two species of salamanders were compared using ANCOVA with initial mass as the covariate.

## RESULTS

### Demographics and environmental variables vs. elevation

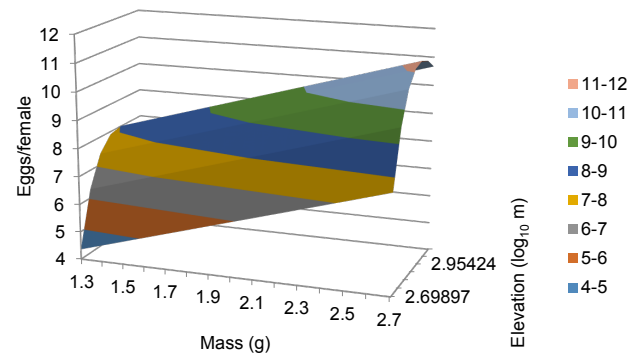
The multiple regression model comparing the number of eggs per female *P. hubrichti* to elevation and female mass was as follows (Fig. 3):  $\text{eggs}/\text{female} = 509.815 + 349.409 \text{ elevation} (\log_{10} \text{ m}) - 59.281 \text{ elevation} (\log_{10} \text{ m})^2 + 2.278 \text{ mass (g)}$  [1] ( $F = 15.0, df = 3, 112, P < 0.001, r^2 = 0.29$ ). The number of eggs per female *P. hubrichti* increased directly with mass and elevation to a maximum of 12 eggs per female at 1000 m and then decreased slightly above 1000 m. The number of eggs per female ranged from 1 to 12 with a mean of 8.5 (95% CI 8.2–8.9).

There was an inverse relationship between % gravid females and elevation (Fig. 4):  $\% \text{ gravid females} = 112.73 - 0.0571 \text{ elevation (m)}$  [2] ( $F = 21.3, df = 1, 9, P = 0.001, r^2 = 0.71$ ). Percent gravid females ranged from 62 to 83% at low elevation sites (488–762 m) to 40 to 61% at high elevation sites (991–1143 m). The minimum SVL for a gravid female (i.e. size at sexual maturity) was 45.6 mm.

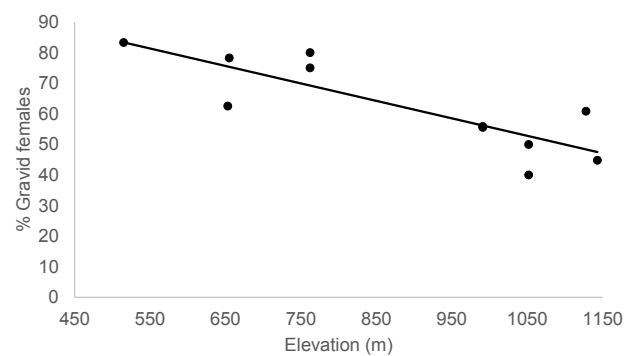
Surface-active salamander density for *P. hubrichti* in allopatry ranged from a low of 0.0007 to 0.0032 *P. hubrichti*/m<sup>2</sup> at the low elevation sites (488–518 m), then increased to a maximum of 0.072 *P. hubrichti*/m<sup>2</sup> at the 1052 m site followed by a decrease between 0.024 to 0.044 *P. hubrichti*/m<sup>2</sup> at the highest elevation sites (1128–1143 m) (Fig. 5):  $\text{SA salamander density} (\log_{10} \text{ number}/\text{m}^2) = -174.2 + 116.1 \text{ elevation} (\log_{10} \text{ m}) - 19.5 \text{ elevation} (\log_{10} \text{ m})^2$  [3] ( $F = 11.9, df = 2, 6, P = 0.008, r^2 = 0.80$ ).

Of the habitat factors measured at each site, elevation was significantly related to median SA salamander density ( $r = 0.736, n = 9, P = 0.02$ ), while all other habitat variables (% canopy closure, % rocks, % branches, % bare soil, % leaves, and leaf litter depth) were not significantly related to SA salamander density ( $P > 0.05$ ).

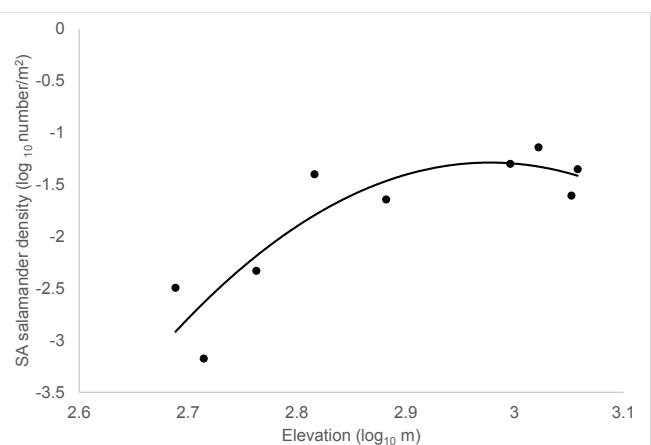
Combining regression models for number of eggs/female, % gravid and SA salamander density produced relative estimates of egg output across the study sites that ranged from a low of 5 eggs per 1000 m<sup>2</sup> at 518 m to a high of 148 eggs per 1000 m<sup>2</sup> at 991 m. Egg production decreased to 73 eggs per 1000 m<sup>2</sup> as elevation increased to 1128 m (Fig. 6). For these calculations, 50% of the SA salamander density was considered female since there was no significant deviation from a 1:1 sex ratio across the sites ( $\chi^2 = 10.8, df = 7, P = 0.15$ ). Female mass was also held constant at the mean (1.81 g) for the regression model for eggs/female since the correlation between female salamander mass and elevation did not show any significant relationship ( $r = -0.024, n = 116, P = 0.80$ ).



**Figure 3.** Multiple regression model output relating the number of eggs per female to mass (g) and elevation (m) for *P. hubrichti*. Colour bands represent ranges for eggs/female.



**Figure 4.** Relationship between percent gravid females and elevation (m) for *P. hubrichti*.



**Figure 5.** Relationship between surface-active (SA) salamander density ( $\log_{10} \text{ number}/\text{m}^2$ ) and elevation ( $\log_{10} \text{ m}$ ) for *P. hubrichti*.

Elevation and mean night temperatures during the active season were inversely related while mean night RH was directly related to elevation. Night temperatures ranged from a high of 20 °C at the low elevation sites to a low of 16.5 °C at the high elevation sites:  $\text{Mean night temperature (}^\circ\text{C)} = 23.13 - 0.0049 \text{ elevation (m)}$  [4] ( $F = 180.8, df =$

1,16,  $P < 0.001$ ,  $r^2 = 0.92$ ). Using the model, a decrease of 1 °C was calculated with increase in elevation of 204 m for elevations ranging from 518 to 1268 m. Mean night RH during the active season ranged from 88% at low elevation sites to approximately 94% at high elevation sites: Mean night RH (%) =  $81.62 + 0.0082 \text{ elevation (m)}$  [5] ( $F = 9.8$ ,  $df = 1,14$ ,  $P = 0.007$ ,  $r^2 = 0.41$ ). The model showed a 1% increase in RH with increase in elevation of 120 m for elevations ranging from 655 to 1268 m.

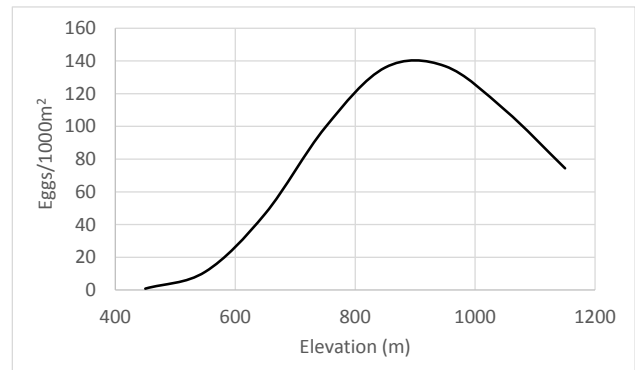
The model for predicting *P. hubrichti* SA salamander density using night temperature and RH was as follows (Fig. 7): SA salamander density (number/m<sup>2</sup>) =  $0.050138 - 0.05273 \text{ mean night temperature (°C)} + 0.000586 \text{ mean night temperature (°C)} * \text{mean night RH (%)}$  [6] ( $F = 8.14$ ,  $df = 2,5$ ,  $P = 0.027$ ,  $r^2 = 0.76$ ; model prediction valid for mean night temperatures between 16 to 20 °C and night RH between 86 to 94%). Surface-active salamander densities ranged from a low of 0.004 *P. hubrichti*/m<sup>2</sup> (86% and 20 °C for mean night RH and temperature, respectively) to a high of 0.09 *P. hubrichti*/m<sup>2</sup> (94% and 16 °C for mean night RH and temperature, respectively).

**Survival vs. elevation**

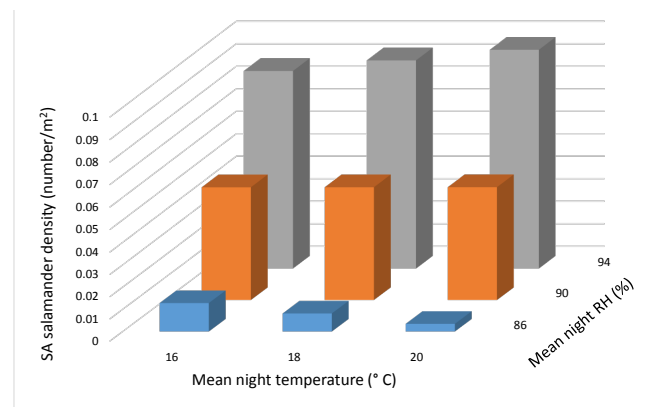
When the tubs were opened in September 2010, surviving salamanders (n = 37) were found under rocks (48.7%), in artificial burrows (32.4%), in the soil (13.5%) and on the surface (5.4%).

The number alive versus dead (i.e. not found) was not homogeneous across the three elevations tested ( $\chi^2 = 10.7$ ,  $df = 2$ ,  $P = 0.005$ ). The number alive out of the ones placed in the tubs was highest (17/20 = 0.85) at the high elevation site (991 m) and then decreased to 13/20 (0.65) at 655 m and was the lowest at the 518 m site (7/20 = 0.35). All 29 salamanders that had initial and final mass measurements that could be linked to specific salamanders showed positive growth rates ranging from 0.0009 to 0.0047 g/d, and growth rates increased with increase in elevation ( $r = 0.49$ ,  $n = 29$ ,  $P = 0.007$ ).

Temperatures from Ibuttons at sites with tubs decreased with increase in elevation. Mean night air temperatures decreased from 20.6 to 18.1 °C for elevations ranging from 518 to 991 m, respectively. Temperatures under the rocks and at the base of the artificial burrows (measured over the entire day) in the tubs also decreased with an increase in elevation (518 to 991 m) from 20.8 to 18.5 °C and 19.6 to 17.1 °C, respectively. Mean night air temperatures inside and outside the tubs were very similar (518 m site inside and outside tub temperatures 20.7 and 20.6 °C, respectively; 655 m site the means were equivalent at 20.4 °C; comparison could not be made at the 991 m site because the Ibutton placed in the tub malfunctioned). Elevational trends, as was done with night temperatures, could not be done for RH because of the failure of the Ibutton inside the tub at the 991 m site and because the low elevation site (518 m) was near a stream and had an abundance of understory vegetation that likely contributed to RH being similar to the 991 m site. Outside night RH means were 94, 87 and 97% for the 518, 655 and 991 m sites, respectively. For the 518 and 655 m sites, the tubs averaged 5% higher RH inside the tub than outside most likely due to reduced air flow inside the tubs



**Figure 6.** Relative egg output per 1000 m<sup>2</sup> calculated for *P. hubrichti*, using models developed in this study which related elevation (m) to surface-active salamander density, eggs/female, and % gravid females.



**Figure 7.** Multiple regression model output for surface-active (SA) *P. hubrichti* (number/m<sup>2</sup>) versus mean night temperature (°C) and relative humidity (RH, %).

from the screening placed over the holes. Therefore decrease in survival rates with decline in elevation was primarily correlated with increase in temperature and not change in RH.

**Critical thermal maxima and dehydration rates**

For CTM, there was no significant interaction between elevation and species ( $F = 2.25$ ,  $df = 1,36$ ,  $P = 0.13$ ) so the interaction term was dropped from the ANCOVA model. In the reduced model, *P. hubrichti* had a significantly lower CTM relative to *P. cinereus* (CTM least square means 33.3 and 34.4 °C for *P. hubrichti* and *P. cinereus*, respectively,  $F = 52.5$ ,  $df = 1,37$ ,  $P < 0.001$ ). CTMs for both species also significantly increased with a decrease in elevation ( $F = 10.3$ ,  $df = 1,37$ ,  $P = 0.003$ ).

For dehydration rates, there was no significant interaction between mass and species ( $F = 0.1$ ,  $df = 1,54$ ,  $P = 0.73$ ) so the interaction term was dropped from the ANCOVA model. In the reduced model, *P. hubrichti* had a significantly greater dehydration rate relative to *P. cinereus* (dehydration rate for a mean salamander mass of 2.3 g was 16.3 and 15.2 mg/cm<sup>2</sup> \* h for *P. hubrichti* and *P. cinereus*, respectively,  $F = 5.1$ ,  $df = 1,55$ ,  $P = 0.027$ ), and dehydration rates increased significantly as masses decreased ( $F = 28.7$ ,  $df = 1,55$ ,  $P < 0.001$ ).

## DISCUSSION

In this study, SA salamander density for *P. hubrichti* in allopatric areas was hypothesised to decrease at lower elevations due to increased temperatures and lower RH which may adversely affect montane salamander survival and reproduction. Surface-active salamander density peaked between elevations ranging from 900 to 1100 m and then decreased rapidly with decrease in elevation. A small decrease in SA salamander density also occurred at elevations greater than 1100 m. Potential causal factors for these SA salamander density decreases, primarily with decline in elevation, included habitat (poor habitat quality), temperature (thermal stress from high temperatures), RH (water stress from dehydration when RH is too low), and potentially differing predator and prey communities with change in elevation. Differences between habitat variables (depth of leaf litter, % canopy closure, etc.) measured at sites from a range of elevations were not significantly correlated with SA salamander density and therefore did not explain the decreases with elevation. Temperature and RH were correlated with elevation and did relate to SA salamander density as seen in the multiple regression equation [6]. Along the elevational gradient in the Peaks of Otter area, with decline in elevation, it got warmer (1 °C with every decline of 204 m) and drier (1% decrease in RH with a decline in elevation of 120 m). Temperature increases with decreasing elevation were more consistent than RH decreases with decrease in elevation due to factors affecting RH such as proximity to streams and transpiration from understory vegetation.

Temperature increases and RH decreases at lower elevations might cause added physiological stress for a montane salamander like *P. hubrichti*, with its lower CTM and higher dehydration rates, relative to a widespread species like *P. cinereus* (this study). Spotila (1972) showed that these physiological parameters, as well as others he measured, indicated that some species, like *P. yonahlossee* and *P. jordani*, were physiologically adapted to life at high elevations. The kind of physiological stress mentioned above may be due to the impact that warmer and drier conditions found at lower elevations place on a salamander's foraging efficiency. Going down the side of a mountain, where it gets warmer and drier, may affect salamander foraging efficiency in a manner similar to what happens over time as the forest dries following a rainfall event. When the forest is very moist, such as immediately following a rainfall event or during light rains, some plethodontids, including *P. hubrichti*, climb vegetation (Jaeger, 1978; Kramer et al., 1993) where they forage more efficiently than conspecifics in the litter on the forest floor (Jaeger, 1978) and/or avoid ground-dwelling predators while foraging (Roberts & Liebgold, 2008; McEntire, 2016). Foraging on vegetation occurs only for short periods of time and is apparently limited by desiccation (Jaeger, 1978). After a rainfall event, as the forest floor dries, plethodontids remain increasingly under cover objects like rocks and logs and may consume even fewer prey than do salamanders in forest litter (Jaeger, 1972, 1980). And finally, between rainfall events, the forest

floor, including areas under rocks and logs, may become so dry that salamanders are forced to retreat underground where little feeding occurs (Heatwole, 1962; Fraser, 1976; Feder, 1983).

In a similar fashion, considering the effects of elevation on temperature and RH, the optimal elevation sites might have the greatest number of days where environmental conditions allow salamanders to forage optimally on vegetation and/or avoid ground-dwelling predators while foraging. Seventy-six percent of *P. hubrichti* found at an optimal elevation site (1100 m) were observed at night on vegetation (Kramer et al., 1993). At lower elevation sites, salamanders might rarely have environmental conditions that allow them to forage on vegetation and, consequently, they forage more frequently in forest litter or under rocks and logs where foraging efficiency is reduced. Eventually, at certain low elevations, varying based on local conditions such as site aspect and proximity to streams, environmental conditions at the surface might force *P. hubrichti* to remain underground most of the time, thus creating an unsustainable energy budget (Spotila, 1972; Gifford & Kozak, 2012). This physiological stress, from reduced foraging efficiency with decline in elevation, may have translated into reduced reproductive output with decline in elevation as noted in this study. If arboreal foraging is primarily related to predator avoidance, then restricted arboreal foraging at low elevations may result in decreased survival rates due to increased predation. Metabolic depression at low elevations may also be involved in decreased reproductive output and survival rates. Two montane species of *Desmognathus* had an inverse relationship between metabolic depression and elevation, and low elevation populations of these species were likely living near the limit of their physiological tolerances (Bernardo & Spotila, 2006). For elevations above the optimum, a shorter active season may have contributed to decreases in SA salamander density and relative egg output.

Reproductive output (eggs/1000 m<sup>2</sup>) was affected by SA salamander density, % gravid females, and eggs/female. Salamanders at the higher elevation sites, up to the optimum elevation, produced more eggs/female, a trend similar to that noted for other amphibians with increase in elevation (Morrison & Hero, 2003). However with increase in elevation, % gravid females decreased similar to that seen with *P. cinereus* (Takahashi & Pauley, 2010). This was different from what was hypothesised. At the higher elevation sites in this study, reproduction was primarily biennial as suggested by % gravid females ranging from 40 to 60%. At the lower elevation sites, in contrast, at least some of the salamanders produced eggs annually since % gravid females ranged from 67 to 80% (Takahashi & Pauley, 2010). No reduction in body size was noted for gravid *P. hubrichti* with elevation, similar to what was seen with many other plethodontids (Adams & Church, 2008). The overall effect with decline in elevation, even with more frequent reproduction, was a decrease in reproductive output.

Survival rates and elevation showed a similar trend to SA salamander density and elevation, with a rapid decrease in survival rates with decline in elevation. At the highest

elevation (991 m), the survival rate recorded in the caged experiment of 0.85 was comparable to those noted for free ranging *P. hubrichti* at a 1035 m elevation site (mean = 0.993, 95% CI 0.988–0.997; Kniewski & Reichenbach, 2009).

Understanding the reasons why the Peaks of Otter salamander has such a restricted distribution can contribute greatly to its conservation. Previous work has shown that, in the core of its range of, where elevations are optimal (> 650 m), *P. hubrichti* dominates the salamander community (94.8% of salamander species) and can be found at high population densities (mean = 2.7/m<sup>2</sup>; Reichenbach & Sattler, 2007). The range of *P. hubrichti* is located primarily in National Park and National Forest lands, and this helps protect the core of its range. It is along the perimeter of this species' distribution, however, that extreme care must be exercised in forest management. These lands include ones managed by the National Park and National Forest as well as private citizens. This research stresses the importance of conserving mature hardwood forests, particularly in lower elevation areas which represent more fragile salamander habitats. Reduction of forest canopy increases temperatures and decrease RH (Homyack et al., 2011), which subsequently would decrease salamander densities even more at low elevations.

This study could also be applied to timbering effects on *P. hubrichti*. Sattler & Reichenbach (1998) and Reichenbach & Sattler (2007) found that while densities of *P. hubrichti* in clearcut sites showed significant decreases, densities at shelterwood cut sites did not differ significantly from those at non-timbered reference sites. This indicated that shelterwood cuts provided an alternative to clearcuts that did not harm populations of *P. hubrichti*. These shelterwood cut sites were all located at elevations considered to be optimal for *P. hubrichti* (>650 m). Small increases in temperature and decreases in RH due to the shelterwood cuts might still be within acceptable ranges for *P. hubrichti* at optimal elevations. If these same shelterwood cuts had been conducted in lower elevation areas, however, the already sparse populations of *P. hubrichti* might have been adversely affected since the forest canopy would have been opened, likely resulting in increased temperatures and decreased RH (Homyack et al., 2011). These increased temperatures and decreased RH might exceed or be closer to the tolerable limits for *P. hubrichti*, which could either extirpate them from these low elevations or reduce densities. Therefore, if shelterwood cuts are planned for low elevation areas within the range of *P. hubrichti*, research should be done since it would be hypothesised that this form of timbering would adversely affect *P. hubrichti* populations at these elevations. A set of long-term monitoring sites should also be set up for *P. hubrichti*, particularly in the perimeter areas of its range. These could be monitored annually or every two years to look for long-term trends in population densities. Ibuttons could also be placed at these sites to measure temperature and RH. If SA salamander density changes are noted, these could be potentially correlated with changes in temperature or RH and/or any changes in land management practices.

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