

2020

## **Salamander Stress and Duress: The Relationship Between CORT, Autotomy and Regeneration, and Exploratory Behaviour**

Aaron M. Sullivan

Jacquelyn L. Lewis

Follow this and additional works at: [https://digitalcommons.georgefox.edu/bio\\_fac](https://digitalcommons.georgefox.edu/bio_fac)



Part of the [Biology Commons](#)

---

# Salamander stress and duress: the relationship between CORT, autotomy and regeneration, and exploratory behaviour

Jacquelyn L. Lewis<sup>a,b,\*</sup>, Aaron M. Sullivan<sup>a</sup>

<sup>a</sup> Department of Biology, Houghton College, Houghton, NY 14744, USA

<sup>b</sup> Department of Biological Sciences, Rensselaer Polytechnic Institute, Troy, NY 12180, USA

---

## ARTICLE INFO

**Keywords:**  
stressors  
glucocorticoid  
corticosterone  
amphibian  
*Desmognathus*

## ABSTRACT

Responses to stress are generally mediated through the production of glucocorticoids by the hypothalamic-pituitary-adrenal (or -interrenal) axis. The prolonged production of stress hormones can contribute to delayed wound healing and growth, but little is known about their influence on regeneration following tail autotomy, or exploratory behaviour in autotomized individuals. Here we examined the relationship between stress, regeneration, and exploratory behaviour in Allegheny Mountain dusky salamanders (*Desmognathus ochrophaeus*) by manipulating corticosterone (CORT) levels via cutaneous patch. First, we measured tail regeneration in salamanders with elevated CORT for 13 weeks after the induction of tail autotomy. Test subjects received a weekly patch to wear for one hour that was saturated with either a low CORT (0.25 mg/ml) or high CORT (0.50 mg/ml) solution. Individuals receiving CORT patches regenerated significantly less of their tail length and volume (versus control), but without exhibiting dose-dependent effects. Second, we used a factorial design to evaluate the effects of autotomy and elevated CORT on exploration within a test arena consisting of low barriers arrayed in concentric rings. Individuals experiencing tail autotomy exhibited significantly less exploratory behaviour indicated by an increased latency to cross first barrier and a decreased number of barriers crossed. Neither elevated CORT (0.50 mg/ml), nor the interaction between elevated CORT and tail autotomy significantly affected salamander activity within the array. Although CORT did not have a direct effect on explorative behaviour, a delay in regeneration attributed to CORT could lead to changes in patterns of movement in autotomized individuals.

---

## 1. Introduction

Obnoxious agents, or stressors, can elicit a variety of changes to animal physiology (Wack et al., 2012; Thomas and Woodley, 2015), morphology (Denver, 1997; Payette and Sullivan, 2019), and behaviour (Carr, 2002; Watson et al., 2004; Moyers et al., 2018). Stress-induced effects are mediated primarily through the production of glucocorticoids by the hypothalamic-pituitary-adrenal axis in birds and mammals, and the hypothalamic-pituitary-interrenal axis in amphibians and reptiles (Sapolsky, 2002; Reeder and Kramer, 2005; Denver, 2009; Sopinka et al., 2015). In the short term, glucocorticoids serve to mitigate the effects of stressors and contribute to homeostasis through relatively rapid changes in the availability of metabolic fuels, cardiac output, patterns of blood perfusion, arousal, and escape behaviours (Sapolsky et al., 2000; McEwen and Wingfield, 2003; Wingfield and Sapolsky, 2003; Wingfield, 2005; Sheriff et al., 2011). Whereas the immediate effects of glucocorticoid production allow for an effective response to

transient stressors, its prolonged elevation can be accompanied by substantial costs such as decreases in reproduction (Wingfield and Sapolsky, 2003; Carr, 2010; MacLeod et al., 2018), immune function (Webster-Marketon and Glaser, 2008; Costantini et al., 2011), wound healing (Padgett et al., 1998; Romana-Souza et al., 2014), and growth (Boonstra et al., 1998).

Although elevated corticosterone (CORT), the primary glucocorticoid in amphibians and reptiles, has been implicated in the suppression of wound healing (Thomas and Woodley, 2015) and growth (Dahl et al., 2012), little is known about its role in the regeneration of tissues following autotomy. Autotomy is an antipredator response wherein a portion of an appendage is lost or shed at a preformed breakage plane (Stebbins and Cohen, 1995; Johnson and Jakob, 1999; Downes and Shine, 2001; Bateman and Fleming, 2009; Marvin, 2010) which increases the probability of escape (Ducey and Brodie, 1983; Maginnis, 2006). Some lizards and salamanders rely on tail autotomy to distract and deter predators when primary defences such as biting, fleeing,

---

\* Corresponding author at: Department of Biological Sciences, Rensselaer Polytechnic Institute, Troy, NY 12180, USA.

E-mail address: [Jacquelyn.Lewis19@houghton.edu](mailto:Jacquelyn.Lewis19@houghton.edu) (J.L. Lewis).

immobility, or noxious secretions fail (Arnold, 1982; Labanick, 1984; Whiteman and Wissinger, 1991; Maginnis, 2006; Marvin, 2010). Autotomy may provide an immediate survival benefit but often with substantial future costs as the tail assists in locomotion, confers social status, and stores fat and protein (Maginnis, 2006; Bateman and Fleming, 2009; Gillis and Higham, 2016; Gerald et al., 2017). Tailless individuals have reduced fecundity and clutch size (Fox and McCoy, 2000; Bateman and Fleming, 2009), altered balance and stability (Gillis and Higham, 2016), and are less likely to escape from subsequent predator attacks (Arnold, 1982; Ducey and Brodie, 1983; Downes and Shine, 2001). Due to the significant trade-offs associated with autotomy, the ability to quickly regenerate a tail is important for the long-term survival and fitness of an individual. Rapid regeneration, however, may be delayed by prolonged periods of elevated CORT.

Autotomy and CORT can each exert an effect on activity or exploratory behaviour (how quickly and extensively an individual examines a new environment). However, unravelling the effects of tail autotomy and elevated CORT with regards to activity is difficult because stimuli contributing to autotomy likely also elevate CORT (e.g., an attack from a predator) (Maher et al., 2013; Bennett et al., 2016), and relationships between elevated CORT, autotomy and regeneration, and activity are not well documented among vertebrate taxa. In general, studies examining the impact of autotomy on behaviour highlight the adverse effect on locomotion (Arnold, 1984; Maginnis, 2006). For example, ground-dwelling lizards (*Niveoscinus metallicus*) had decreased stamina or sprint speed (Chapple and Swain, 2002), arboreal lizards (*Anolis carolinensis*) showed less stability while jumping (Gillis et al., 2009), and two desmognathine salamanders (*Desmognathus quadramaculatus*, *D. ocoee*) exhibited reduced swimming burst speed (Marvin, 2010) after the induction of tail autotomy. Moreover, tailless striped plateau lizards (*Sceloporus virgatus*), and both delicate (*Lampropholis delicata*) and garden skinks (*L. guichenoti*) are more inclined to utilize refuges or sheltered basking sites, suggesting a direct relationship between autotomy and exploratory behaviour (Cooper and Wilson, 2008; Cromie and Chapple, 2012). On the other hand, studies evaluating the role of elevated glucocorticoids on exploratory behaviour and activity in species capable of autotomy can vary according to taxon and context. For example, experimentally elevated plasma CORT concentrations in juvenile wall lizards (*Podarcis muralis*) resulted in both higher rates and duration of movement (Belluire and Clobert, 2004), and in adult common lizards (*Lacerta vivipara*) led to earlier and longer periods of activity and food intake (Cote et al., 2006). Conversely, elevated plasma CORT had no discernible influence on activity levels in *D. ochrophaeus* (Ricciardella et al., 2010) or foraging behaviour in *D. ocoee* (Bliley and Woodley, 2012).

With this study we attempted to evaluate the relationship between experimentally elevated CORT, tail autotomy and regeneration, and exploratory behaviour in Allegheny Mountain dusky salamanders (*D. ochrophaeus*). In an effort to limit confounding effects due to invasive or disruptive methods of manipulating glucocorticoids (Sopinka et al., 2015; Woodley, 2017), we elevated stress in test subjects with an exogenous source of CORT applied to individual salamanders via cutaneous patch (Wack et al., 2010). In our first study, we examined the effects of experimentally elevated CORT on tail regeneration over a 13-week study period because the detrimental impact of CORT is linked to prolonged elevation (Sapolsky et al., 2000; Sopinka et al., 2015). We hypothesized that a) individuals exposed to exogenous CORT would regenerate less of their original tail length and volume due to the inhibitory action of glucocorticoids on wound healing and growth (French et al., 2006; Thomas and Woodley, 2015), and b) regeneration would be negatively correlated with the concentration of CORT received via cutaneous patch. In our second study, we evaluated the combined effects of experimentally elevated CORT and tail autotomy on exploratory behaviour. Previous findings suggest that under certain environmental circumstances elevated CORT may have a minimal impact on locomotion in *D. ochrophaeus* (Ricciardella et al., 2010;

Woodley and Lacy, 2010), but we hypothesize that the combination of recent tail autotomy and elevated CORT could reveal a different range of responses. For instance, the increases in exploratory behaviour observed in free-living house finches (*Haemorhous mexicanus*) (Moyers et al., 2018), and food intake among common lizards (*L. vivipara*) (Cote et al., 2006) with elevated CORT also may be exhibited by autotomized salamanders to promote the regeneration of tail tissue as quickly as possible. Alternatively, the presence of elevated CORT could intensify the inhibitory effect on behaviour seen among autotomized individuals as stressed salamanders without tails may be less likely to engage in risky behaviour.

## 2. Materials and Methods

### 2.1. Collection and maintenance of study animals

To determine the effects of exogenous CORT on tail regeneration 140 individual *D. ochrophaeus* were collected by hand at our field site in a wooded area of Houghton College (Houghton, New York, USA) over the span of three days ending 24-Aug-2018. We excluded individuals as test subjects that showed signs of recent tail autotomy (i.e., obvious tail discolouration or distal bluntness). Collected salamanders were returned to the laboratory, housed individually in 15-cm-diameter Petri dishes with an acid-free paper towel substrate moistened with spring water, and stored in a climate-controlled chamber on a 13.5 L:10.5 D cycle (lights on at 07:00 h EST) at 15.5 °C (day) and 12.8 °C (night) which reflected the natural conditions during collection period. To standardize prey intake, each individual was provided 5–8 wingless *Drosophila* sp. one day prior to the induction of tail autotomy (6-Sept-2018), and 3–4 additional prey on two occasions during the study (12-Oct-2018 and 16-Nov-2018). Few feeding opportunities were provided to salamanders in order to minimize the likelihood that differences in tail regeneration among treatments were due to variation in food intake, as previous studies show that stressors can reduce foraging (Carr, 2002; Watson et al., 2004; Johnson and Sullivan, 2014; Gildemeister et al., 2017).

To evaluate the combined effects of autotomy and CORT on exploratory behaviour, we collected an additional 180 *D. ochrophaeus* at our field site over a span of three days in May 2019. As previously described, we excluded individuals that showed signs of recent tail autotomy, and animals were housed in 15-cm-diameter Petri dishes with a paper towel substrate moistened with spring water. Collected salamanders were stored in a climate-controlled chamber on a 15 L:9 D cycle (lights on at 06:00 h EST) at 15.5 °C (day) and 12.8 °C (night) which reflected the natural conditions during the collection period. Salamanders were returned to the wild within one week of collection and were not fed while in captivity.

### 2.2. Preparation and application of cutaneous CORT patches

We used the dermal patches designed by Wack et al. (2010) to experimentally elevate CORT in salamanders. In brief 1.5 × 3.0 mm rectangular patches made from low protein binding filter paper (Cat. No. 1820-070, Whatman) were gently applied to salamanders along the dorsal midline slightly anterior to the pectoral girdle using blunt forceps. Following application, 1.5-µl of one of two possible CORT solutions were added to the patch. CORT solutions were prepared by adding, 25.5 mg of crystallin CORT (Cat. No. C2505, Sigma-Aldrich) to 1.5 ml of 100% ethanol in a microcentrifuge tube (Cat. No. 05-408-129, Fisher Scientific) and subsequently vortexed for one minute to ensure dissolution. Next, the ethanol solution was combined with 50 ml of carrier oil (sesame oil) and stirred overnight. This period of stirring provided time for the ethanol to evaporate leaving a stock solution with 0.51 mg CORT/ml carrier to serve as the 'high CORT' treatment. Half of this solution (25 ml) was diluted with an additional, 25 ml of carrier oil to achieve a solution with 0.26 mg CORT/ml carrier to serve as our 'low

CORT' treatment. These CORT doses elevated plasma CORT levels in *D. ochrophaeus* to approximately 200 ng/ml (high CORT) and 50 ng/ml (the CORT) in a validation study by Wack et al. (2010). This low dose of CORT resulted in plasma CORT levels similar to that of salamanders experiencing handling stress (Wack et al., 2010). Two concentrations of CORT were used as previous research suggests that developmental and physiological responses may vary according to dose (Blas et al., 2007; Wack et al., 2010; Maher et al., 2013; McCormick et al., 2015). To evaluate the combined effects of autotomy and CORT on exploration, we applied only the high CORT solution to stimulus patches in order to maximize the likelihood of detecting an effect. CORT doses were not adjusted for individual body size as Wack et al. (2010) indicated no significant relationship between body weight and plasma CORT concentrations in *D. ochrophaeus* that received exogenous CORT via cutaneous patches.

### 2.3. Effects of CORT on tail regeneration

To evaluate the effects of an experimentally elevated stress hormone on tail regeneration, salamanders were arbitrarily assigned to one of four treatment groups: control (no patch, no CORT), patch control (cutaneous patch with sesame oil, no CORT), low CORT (0.26 mg/ml) patch, or high CORT (0.51 mg/ml) patch. Tail autotomy was induced in each individual on 7-Sept-2018 through a simulated predation event where each salamander tail was quickly and firmly grasped approximately 1 cm posterior to the cloacal vent using a pair of forceps. Autotomized tails were immediately removed from home dishes and individuals were returned to the climate-controlled chamber.

Treatments were administered weekly in conjunction with the changing of paper towel substrates every Monday from 10-Sept-2018–10-Dec-2018 between 13:00–17:00 h. All salamanders were moved to clean experimental 15-cm-diameter Petri dishes with a single layer of acid-free paper towel moistened with 3 ml of spring water. After transfer, patches were administered to individuals in three treatment groups (patch control, low CORT, or high CORT). Patches were removed via clean blunt forceps 1 h after application. In an attempt to standardize handling stressors, similar disturbances were replicated and administered to the control group as well, but without the application of patch and solution. After the cutaneous patches were removed, each salamander was transferred to a clean home dish and returned to the climate-controlled chamber. This process was repeated weekly so that treatments were administered on thirteen separate occasions.

In order to measure tail regeneration during the study, a weekly photograph was taken of each individual using an Olympus Stylus TG-4 digital camera permanently mounted on a tripod at a fixed distance below a clear plexiglass stage facing the underside of the transparent home dishes. Prior to induction of tail autotomy, photographs were taken of salamanders in order to measure the length and width of the original intact tail. Total tail length was measured from the distal margin of the cloacal vent to the tip of the tail and diameter of original tail was measured across the distal margin of the cloacal vent. Following autotomy, each weekly measurement began with a photograph of a Fowler digital calliper to serve as a reference length. Next, salamander home dishes were placed on the plexiglass stage and a picture of their underside was taken in order to measure length of regenerated tail from point of autotomy and diameter of regenerated tail at its base. We measured the length and calculated the volume of regenerated tail tissue because selection pressures on these two metrics are likely different. Prioritizing the length of the regenerated tail may confer a survival benefit to the individual through improved locomotor ability and the ability to use autotomy in subsequent predator encounters, whereas the volume of the regenerated tail may be linked to less immediate contributions such energy storage (Jamison and Harris, 1992), although changes in the distribution of mass can alter the mechanics of locomotion of individuals (Jagnandan et al., 2014). Length

and radius of the base of the regenerated tail were identified from the discoloured or severed section of the tail, ending at the tip of the regenerated portion. The length of the autotomized tail was determined by subtracting the length of the remaining tail segment from the original tail. While the volume of the autotomized tail was calculated by subtracting the volume of the remaining tail, according to the formula for a truncated cone  $V = \frac{\pi}{3}(r_1^2 + r_1r_2 + r_2^2)h$ , from the original tail volume, calculated using the formula for a cone  $V = \pi(r)^2\frac{h}{3}$  where  $r$  is the radius of the regenerating tail at its base, and  $h$  is the length measured from the site of autotomy to the tip of the tail. A concerted effort was made to ensure that salamanders were lying flat with no portion distorted by contact with the side of the dish.

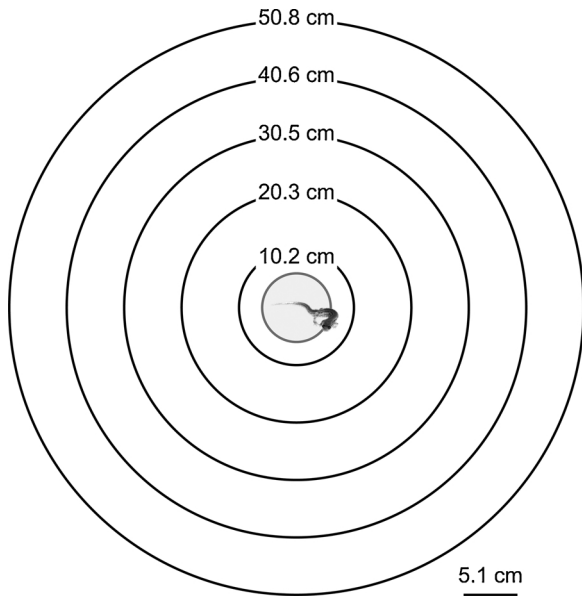
Morphometric data for each salamander were obtained from photographs using the open-source photo-analysing software ImageJ (Schneider et al., 2012; Payette and Sullivan, 2019). The 'freehand' line tool in ImageJ was used by an investigator blind to the treatments to measure length and diameter of the regenerating tail at the breakage plane. In order to minimize measurement error, dimensions for each individual were taken three times by the same observer and an average was calculated to be used as the final measurement. The regenerating tail tissues were conceptualized as a cone allowing us to estimate the volume of tail regenerated over the period of study.

### 2.4. Combined effects of CORT and autotomy on exploratory behaviour

In order to characterize the combined effects of autotomy and elevated CORT on exploratory behaviour, the 180 test salamanders collected from our field site were randomly assigned to one of six treatment groups based on the state of their tail (autotomized or intact) and treatment (CORT, patch control, or control). Individuals assigned to one of the three treatment groups requiring autotomy were quickly and firmly grasped about 1 cm posterior to the cloacal vent using a pair of forceps to prompt the release of the tail approximately 12 h prior to the start of behavioural trials. Salamanders selected to retain their tail went through a sham procedure to provide comparable levels of disturbance but without physical contact to the tail. At the conclusion of these procedures, test subjects were transferred to individual polystyrene (6-cm-diameter × 1.0-cm-height) dishes with acid-free paper towelling moistened with spring water, and returned to the climate-controlled chamber until the start of trials at 21:00 h. The application of patches (and CORT when necessary) followed the process described above and was staggered so that patches remained in contact with the salamander for 60-90 min prior to transfer to the test arena.

The apparatus used to evaluate the exploratory behaviour of *D. ochrophaeus* consisted of a circular arena with a series of surmountable barriers and was based on a design used by Lynn et al. (2019) and Reeder (2013) to assess the behaviour of other plethodontid salamander species. Our version of the circular array consists of five 1.5-cm-high rings of PVC (9101 Duct Pipe, Harrington Industrial Plastics LLC) ranging from 10.2–50.8 cm in diameter and arranged in a concentric manner on a substrate of acid-free paper towels moistened with spring water. This arrangement provided a central space for the placement of the 6-cm-diameter dish containing the test subject, and an approximately 5.1-cm gap between each PVC ring (Fig. 1).

Behavioural assays were conducted over the course of three nights (13-May-2019–15-May-2019) beginning at 21:00 h in a laboratory with red LED lights installed overhead to provide enough light to observe the movement of salamanders. Test subjects within 6-cm-diameter Petri dishes were placed in the centre of the circular array and provided 2 min to adjust to disturbance from the transfer. After this period, the lids of the dishes were removed and salamanders were free to move throughout the arena and over barriers for a period of 5 min. Between each trial, paper towel substrates were replaced and the rings of PVC were wiped with 95% EtOH to minimize contamination of CORT and salamander odours which could influence behaviour (Lynn et al.,



**Fig. 1.** Circular arena designed to evaluate the effect of autotomy and experimentally elevated CORT on exploratory behavior in *D. ochrophaeus*. The arena was comprised of five PVC rings (1.5 cm tall and ranging from 10.2–50.8 cm in diameter), resting on a substrate of paper towels moistened with spring water, and surrounding a central polystyrene dish (6.0 cm diameter × 1.0 cm height) that served as the point of salamander release.

2019). Treatments were stratified so that an equivalent number of individuals in each group were tested each night. After trials, individuals were returned to home dishes, measured for SVL, and placed in the climate control chamber until their release at the point of capture following the conclusion of testing. During each trial, we recorded the a) latency to scale the first barrier (defined as the amount of time required for an individual's pectoral girdle to pass over the vertical wall of the test dish), b) total number of barriers scaled, and c) time required to scale the outermost barrier (i.e., escape the array).

### 2.5. Statistical analysis

In order to evaluate the effect of CORT on tail regeneration, we

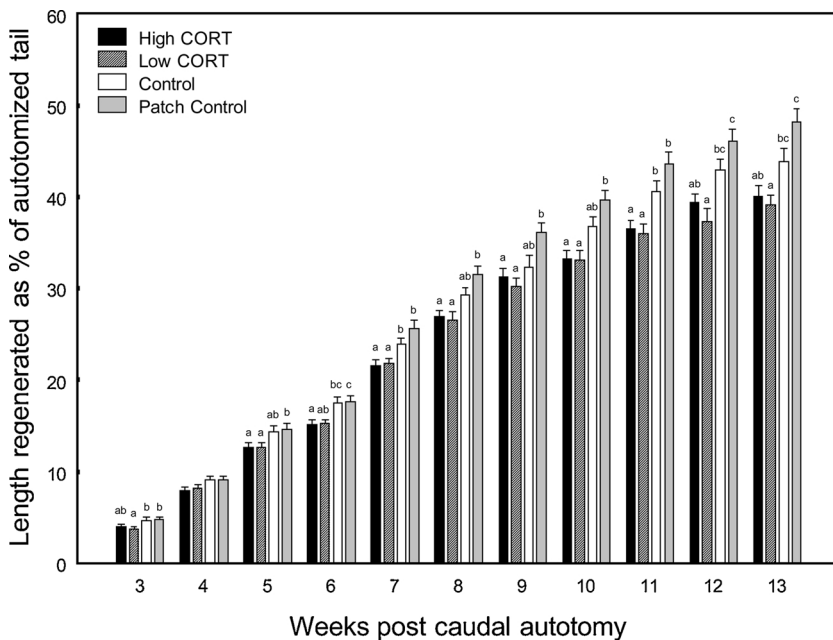
performed a Spearman's rank correlation analyses for the a) initial length of autotomized tail versus length of tail regenerated, and b) initial volume of autotomized tail versus volume of tail regenerated at the conclusion of the 13-wk study. The length of the regenerated tail was positively correlated with the length of the autotomized tail ( $r = 0.460$ ;  $p < 0.001$ ), and the volume of the regenerated tail was positively correlated with the volume of the autotomized tail ( $r = 0.539$ ;  $p < 0.001$ ). Consequently, we evaluated both length and volume as percentages of the tail tissue lost via autotomy. Because our data failed to meet the assumptions of normality and homoscedasticity, we performed a generalized linear model (GLZ) one-way ANOVA using Statistica (Statsoft, Inc., 2001, version 6.0), with separate one-way ANOVAs conducted on regenerated tail tissue for each week. When main effects were significant, we conducted post-hoc pairwise multiple comparisons within each time interval while using the Bonferroni adjustment ( $\alpha = 0.008$ ). Three individuals were removed from the study due to death, so that the final sample sizes were: control ( $n = 33$ ), patch control ( $n = 34$ ), low CORT ( $n = 35$ ), and high CORT ( $n = 35$ ).

In order to evaluate the combined effects of CORT and tail autotomy on exploration, we performed a GLZ factorial ANOVA on latency to scale first barrier and number of barriers scaled using Statistica with autotomy (intact versus autotomized) and treatment (control, patch control, or CORT) as the main effects. As described above, we relied on the generalized linear model because our data failed to meet the assumptions of normality and homoscedasticity. To test for the effect of size on exploratory behaviour we performed a Spearman's rank correlation analysis on a) SVL and latency to scale the first barrier, and b) SVL and the total number of barriers scaled. As only 13 of the 180 individuals tested scaled the outermost barrier within the 5 min time-frame, the time to escape was not analysed.

## 3. Results

### 3.1. Effects of CORT on tail regeneration

The GLZ one-way ANOVAs to assess differences in regenerated tail length (as a percentage of the length of the autotomized tail) indicate significant differences among treatment groups beginning at Week 3 (Fig. 2). There was a significant effect of treatment on regeneration at each measured time interval (Table 1). Post-hoc pairwise multiple comparisons indicate that after Week 5 individuals with high or low



**Fig. 2.** Length of tail regeneration as a percent of autotomized tail (Mean ± SE) in *D. ochrophaeus*, calculated at weekly intervals, in one of four treatments: control (no patch, no CORT), patch control (cutaneous patch with sesame oil, no CORT), low CORT patch (0.26 mg/ml), or high CORT patch (0.51 mg/ml). Letters indicate significant differences among tail regeneration in treatment groups at each time interval using post-hoc pairwise multiple comparisons while adjusting alpha according to Bonferroni ( $\alpha = 0.008$ ). Sample sizes are as follows: high CORT ( $n = 35$ ), low CORT ( $n = 35$ ), control ( $n = 33$ ), and patch control ( $n = 34$ ).

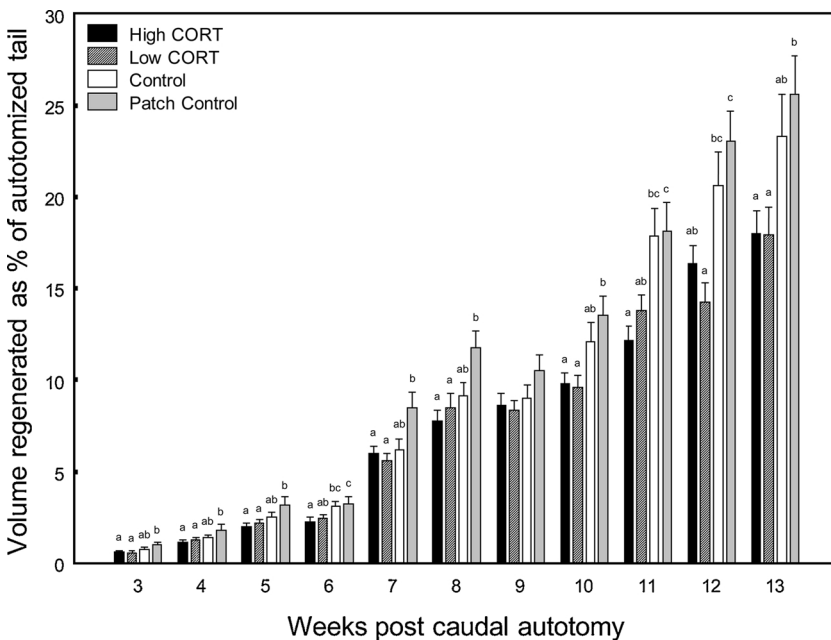
**Table 1**

Results of the generalized linear model one-way ANOVA for regenerated tail volume (as percent of the volume of the autotomized tail) and length (as a percent of the length of the autotomized tail). A separate one-way ANOVA was conducted on regenerated tail tissues at each time interval.

Time post autotomy	Volume (as % of autotomized tail)			Length (as % of autotomized tail)		
	Week	df	Wald	p	df	Wald
3	3	12.455	0.006	3	13.255	0.004
4	3	16.885	< 0.001	3	8.650	0.034
5	3	17.130	< 0.001	3	13.500	0.004
6	3	15.581	0.001	3	25.230	< 0.001
7	3	18.617	< 0.001	3	27.510	< 0.001
8	3	20.415	< 0.001	3	27.020	< 0.001
9	3	6.381	0.094	3	15.780	0.001
10	3	17.843	< 0.001	3	30.560	< 0.001
11	3	24.865	< 0.001	3	36.420	< 0.001
12	3	28.843	< 0.001	3	25.630	< 0.001
13	3	16.521	< 0.001	3	33.980	< 0.001

CORT exhibited significantly less tail regeneration in terms of length compared to the patch control (LD:  $p < 0.001$ ; HD:  $p = 0.004$ ) with this trend continuing through Week 13 (LD:  $p < 0.001$ ; HD:  $p < 0.001$ ). There were no significant differences in length between the concentrations of the CORT doses ( $p$ -values ranging from 0.416 during Week 9 to 0.986 during Week 5). Lastly, there were no significant differences between the controls ( $p$ -values ranging from 0.021 during Week 13 to 0.998 during Week 4).

Similarly, the GLZ one-way ANOVAs to assess differences in regenerated tail volume (as a percentage of the volume of the autotomized tail) indicate significant differences among treatment groups at Week 3 (Fig. 3). There was a significant effect of treatment on the volume regenerated beginning at Week 3 and continuing to Week 13 with the exception of Week 9 (Table 1). Subsequent pairwise multiple comparisons indicate that individuals treated with CORT had significantly less volumetric tail regeneration compared to the patch control from Week 3 (LD:  $p = 0.002$ ; HD:  $p = 0.004$ ) to Week 13 (LD:  $p = 0.001$ ; HD:  $p = 0.001$ ), excluding Week 9 (LD:  $p = 0.012$ ; HD:  $p = 0.041$ ). There was no significant difference between individuals treated with low and high CORT ( $p$ -values ranging from 0.149 during Week 11 and Week 12 to 0.820 during Week 10). Lastly, there was no difference



**Fig. 3.** Volume of tail regeneration as a percent of autotomized tail (Mean  $\pm$  SE) in *D. ochrophaeus*, calculated at weekly intervals, in one of four treatments: control (no patch, no CORT), patch control (cutaneous patch with sesame oil, no CORT), low CORT patch (0.26 mg/ml), or high CORT patch (0.51 mg/ml). Letters indicate significant differences among tail regeneration in treatment groups at each time interval using post-hoc pairwise multiple comparisons while adjusting alpha according to Bonferroni ( $\alpha = 0.008$ ). Sample sizes are as follows: high CORT ( $n = 35$ ), low CORT ( $n = 35$ ), control ( $n = 33$ ), and patch control ( $n = 34$ ).

**Table 2**

Result of the generalized linear model factorial ANOVA to assess the latency to scale first barrier and the number of barriers spanned in Allegheny Mountain dusky salamander (*Desmognathus ochrophaeus*) individuals exposed to autotomy, treatment (CORT patch, patch control, no patch), and the interaction of the two.

	Latency to scale first barrier			Number of barriers spanned		
	df	Wald	p	df	Wald	p
Autotomy	1	5.660	0.017	1	7.004	0.008
Treatment	2	3.087	0.214	2	1.630	0.443
Autotomy $\times$ Treatment	2	0.266	0.875	2	1.093	0.579

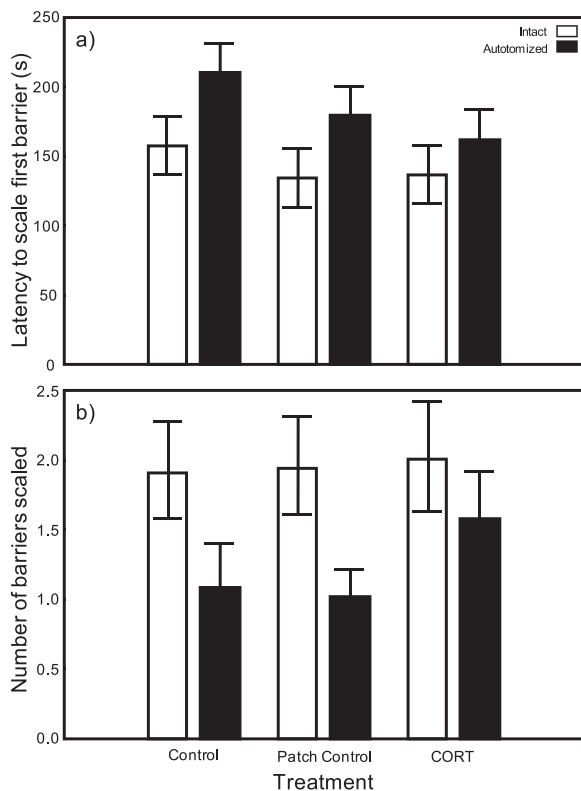
between the control treatments ( $p$ -values ranging from 0.009 during Week 8 to 0.880 during Week 11).

### 3.2. Combined effects of CORT and autotomy on exploratory behaviour

The GLZ factorial ANOVA used to assess main effects of autotomy and CORT treatments, as well as the interactive effects, on exploratory behaviour in our test arena indicates a significant main effect of autotomy on both latency to scale the first barrier and total number of barriers scaled (Table 2). Individuals with autotomized tails showed a greater latency to scale the first barrier and a lower number of barriers scaled during the 5-min assay (Fig. 4). Neither the CORT treatment alone, nor the interaction between CORT and autotomy showed significant effects on either behavioural metric. There was no correlation between SVL and latency to scale the first barrier ( $r = 0.051$ ;  $p = 0.498$ ) nor SVL and the total number of barriers scaled ( $r = 0.030$ ;  $p = 0.694$ ).

## 4. Discussion

The results of our first study indicate that a weekly 1-hr exposure to exogenous CORT contributed to a significant reduction in tail regeneration, and the difference was detectable within three weeks from the induction of autotomy. This supports our prediction that elevated CORT would delay tail regeneration, and is consistent with studies by French et al. (2006) who showed that elevated stress had an inhibitory effect on healing in male tree lizards (*Urosaurus ornatus*), and Thomas



**Fig. 4.** The a) average latency to scale first barrier in seconds, and b) average number of barriers scaled in the 5-min behavioral assay for *D. ochrophaeus* among our six treatment groups based on experience with tail autotomy (autotomized, intact tail) and treatment (high CORT patch, patch control, control). In each group,  $n = 30$ .

and Woodley (2015) who discovered that CORT impacts wound healing in *D. ochrophaeus* through action on inflammation. The level of tail regeneration did not vary according to dosage as predicted, as there were no significant differences in tail regeneration between low (0.26 mg/ml) and high (0.51 mg/ml) CORT treatment groups. According to Wack et al. (2010), the plasma CORT levels corresponding to our high and low CORT treatments are roughly, 200 ng/ml and 50 ng/ml respectively. This suggests that although the plasma CORT levels could vary substantially between our high and low CORT groups, the two concentrations are not different in terms of their physiological effect. Alternatively, it is possible that our low CORT exceeded some minimum threshold such that subsequently larger doses had no further effect on regeneration. The results of our second study show that exploratory behaviour, in terms of both latency to scale the first barrier and number of barriers crossed, were significantly lower in salamanders with autotomized tails. However, the exploratory behaviour of individuals in the test arena was not noticeably impacted by experimentally elevated CORT. These results are consistent with studies that show a negative impact of autotomy on movement (Maginnis, 2006; Gillis and Higham, 2016). Furthermore, they reinforce previous findings that changes in activity of *D. ochrophaeus* are not mediated by elevated CORT (Wack et al., 2013).

Although the physiological mechanism underlying the differential regeneration is unclear, our results bolster the link between environmental stressors, CORT, and aspects of growth in ectotherms. For example, overcrowding can increase plasma CORT and slow the growth of individuals (Elsey et al., 1990), and predators may stimulate CORT production ultimately leading to different morphology (McCullum and Leimberger, 1997; Hossie et al., 2010) and growth rate (Collier et al., 2008). More germane to the current study, individual *D. ochrophaeus* exposed to kairomones from predatory eastern garter snakes

(*Thamnophis sirtalis*) regenerated significantly less tail tissue than control salamanders over a 12-week period (Payette and Sullivan, 2019). This parallels the effect on regeneration observed in the current study among individuals receiving CORT patches. Whether exposure to cues from predatory snakes promotes increased plasma CORT in test subjects is unknown, although Fonner and Woodley (2015) present data suggesting that kairomones from spring salamanders (*Gyrinophilus porphyriticus*) did not generate a significant increase in CORT in *D. ochrophaeus* over the span of their study. Interestingly, a partial mechanism to account for reductions in growth and regeneration in amphibians has been suggested by Novarro et al. (2018) who report that red-backed salamanders (*Plethodon cinereus*), with CORT elevated via thermal stress, showed a reduction in food conversion efficiency. A decrease in conversion efficiency coupled with a decrease in foraging activity among stressed or threatened individuals (e.g., Johnson and Sullivan, 2014) could result in a substantial suppression of regeneration of individuals. It should be noted that animals tested in our experiment may have experienced minor food stress due to the limited foraging opportunities, but this seems insufficient to explain differences among our treatment groups.

Reduced exploratory behaviour in recently autotomized individuals could result from the physical limitations stemming from the loss of the tail, or perceived increase of risk from the simulated predator attack. The detrimental impact of tail loss on various aspects of movement and activity has been documented in a number of species (reviewed by Maginnis, 2006; Gillis and Higham, 2016). Of special relevance to this study is the effect of tail loss on the ability of salamanders to scale the barriers presented in our circular array. In arboreal lizards, autotomy has an adverse on climbing because it affects balance and stability (Brown et al., 1995; Medger et al., 2008). The degree to which an intact tail is required by salamanders to climb the 1.5-cm barriers in our assay is uncertain, but in at least one case, a salamander species appears to use tails for balance while climbing (Stebbins, 1947). As such, it is plausible that the physical limitations of autotomy can account for the decrease in exploratory behaviour observed in our study. A non-mutually exclusive alternative is that autotomized salamanders are less exploratory as a carry-over from their recent experience with simulated predator attack (O'Connor et al., 2014). This is consistent with the generalized responses of plethodontid salamanders to predators, namely decreased activity (Epp and Gabor, 2008; Johnson and Sullivan, 2014) and increased shelter use (Sullivan et al., 2002, 2005). However, recent experience with autotomy may also suppress behaviour as Gildemeister et al. (2017) show that relatively small ( $\leq 3.2$  cm) *D. ochrophaeus* that experienced tail autotomy decreased their foraging behaviour, presumably due to the perception of elevated risk. Because the induction of tail autotomy in that study occurred over two weeks prior to testing, it seems unlikely that elevated risk was linked to an imminent attack from the same predator, but rather the perceived vulnerability to subsequent predation attempts. A similar effect was seen in striped plateau lizards (*S. virgatus*) that increased flight initiation distance and refuge use after autotomy due to an increased risk of predation (Cooper and Wilson, 2008).

Resolving the observation that the experimental elevation of CORT had a significant influence on regeneration but not exploratory behaviour in conjunction with autotomy is challenging. The duration and timing of the CORT exposure, as well as the temporal nature of the observed response, could account for some of our experimental results. In general, amphibians promptly release CORT in response to a stressor but some effects of the HPA/HPI axis are manifest up to several hours later (Maser et al., 1980; Mbangkollo and deRoos, 1983). Thus, it is possible that our evaluation of exploratory behaviour occurred too soon after the application of the CORT patch for behavioural changes to occur. This notion emphasizes the role of CORT in mediating prolonged stressors rather than immediate reactions in amphibians (Herman, 1992), but also questions its function in behavioural responses to stress, especially in a predator-prey context. Because previous studies suggest

that neither kairomones from a predatory salamander (Fonner and Woodley, 2015), nor body condition (Thomas et al., 2017) are linked to changes in plasma CORT, Woodley (2017) speculates that CORT is not a crucial component of the stress response in plethodontid salamanders, or at least not key to antipredator behaviour (e.g., Davis and Gabor, 2015). The case is made that the typical increase in metabolic rate due to elevated CORT observed in some taxa (Sapolsky et al., 2000; Moore and Jessop, 2003; Sopinka et al., 2015) may be costly in ectothermic species adapted to a low-energy existence. Therefore, it is possible that our results can be explained as a strategy to initially suppress CORT (and its behavioural and energetic consequences) to avoid detection by predators but increase CORT with longer or repeated exposure to stressors (Maher et al., 2013).

Despite the immediate survival benefits conferred through tail autotomy, the resultant costs to survival and fitness of an individual make expedient and efficient tail regeneration imperative (Maginnis, 2006). However, repeated exposure to predators (Payette and Sullivan, 2019), and experimentally elevated levels of CORT (current study) compromise the recovery process. Furthermore, exploratory behaviour, which can lead to increases in foraging opportunities and energy acquisition in some species (Crino et al., 2017; Moyers et al., 2018), was suppressed in individuals recently experiencing autotomy. The combined results of our two studies, and previous research (Maher et al., 2013; Davis and Gabor, 2015; Fonner and Woodley, 2015), suggest that an increase of CORT may be suppressed initially in the presence of threatening stimuli, in order to avoid detection by predators, but may increase with longer or repeated stress. Our studies also highlight the complexity of stress and how it may affect responses as distinct as tail regeneration after autotomy and behavioural tendencies. Although the current study did not consider factors such as sex, body condition, or population differences in salamanders in an attempt to keep the design manageable, subsequent research examining sex-specific differences in rates of regeneration (e.g., Chapple and Swain, 2002), or habitat influences on CORT and activity (e.g., Crino et al., 2017) could provide valuable insight. Additionally, an attempt to understand the mechanism accounting for differential regeneration (e.g., Novarro et al., 2018) could elucidate the process by which stress tends to depress growth of individuals.

## Acknowledgements

This manuscript was submitted in partial fulfilment of the requirement for Major Honours in Biology at Houghton College, and supported by the Summer Research Institute at Houghton College. Thanks to J. Bintz and J. Potter for feedback on early drafts of the manuscript, W.I. Payette, S.K. Woodley, and A. Mathis for suggestions on methodology, A. Carl, S. Dunnett, E.A.R. Gildemeister, I. Kratzer, B. Lenart, L. Reitler, T. Sile, and M. Tyrrell for field and laboratory assistance, and two anonymous reviewers for constructive feedback. This research did not receive any specific grant from funding agencies in the public, commercial, or not-for-profit sectors. The license to collect and possess study animals was granted by the New York State Department of Environmental Conservation (#1926). Protocols were consistent with the guidelines of the American Society of Ichthyology and Herpetology and approved by the Houghton College Animal Care and Use Committee (#08-01).

## References

Arnold, S.J., 1982. A quantitative approach to antipredator performance: salamander defense against snake attack. *Copeia* 1982, 247–253. <https://doi.org/10.2307/1444602>.

Arnold, E.N., 1984. Evolutionary aspects of tail shedding in lizards and their relatives. *J. Nat. Hist.* 18, 127–169. <https://doi.org/10.1080/00222938400770131>.

Bateman, P.W., Fleming, P.A., 2009. To cut a long tail short: a review of lizard caudal autotomy studies carried out over the last 20 years. *J. Zool.* 277, 1–14. <https://doi.org/10.1111/j.1469-7998.2008.00484.x>.

Belliure, J., Clobert, J., 2004. Behavioral sensitivity to corticosterone in juveniles of the wall lizard, *Podarcis muralis*. *Physiol. Behav.* 81, 121–127. <https://doi.org/10.1016/j.physbeh.2004.01.008>.

Bennett, A.M., Longhi, J.N., Chin, E.H., Burness, G., Kerr, L.R., Murray, D.L., 2016. Acute changes in whole body corticosterone in response to perceived predation risk: A mechanism for anti-predator behavior in anurans? *Gen. Comp. Endocr.* 229, 62–66. <https://doi.org/10.1016/j.ygcen.2016.02.024>.

Blas, J., Bortolotti, G.R., Tella, J.L., Baos, R., Marchant, T.A., 2007. Stress response during development predicts fitness in a wild, long lived vertebrate. *P. Natl. Acad. Sci. Biol.* 104, 8880–8884. <https://doi.org/10.1073/pnas.0700232104>.

Bliley, J.M., Woodley, S.K., 2012. The effects of repeated handling and corticosterone treatment on behavior in an amphibian (Ocoee salamander: *Desmognathus ocoee*). *Physiol. Behav.* 105, 1132–1139. <https://doi.org/10.1016/j.physbeh.2011.12.009>.

Boonstra, R., Hik, D., Singleton, G.R., Tinnikov, A., 1998. The impact of predator stress on the snowshoe hare cycle. *Ecol. Monogr.* 68, 371–394. [https://doi.org/10.1890/0012-9615\(1998\)068\[0371:TIOPIS\]2.0.CO;2](https://doi.org/10.1890/0012-9615(1998)068[0371:TIOPIS]2.0.CO;2).

Brown, R.M., Taylor, D.H., Gist, D.H., 1995. Effect of caudal autotomy on locomotor performance of wall lizards (*Podarcis muralis*). *J. Herpetol.* 29, 98–105. <https://doi.org/10.2307/1565091>.

Carr, J.A., 2002. Stress, neuropeptides, and feeding behavior: a comparative perspective. *Integr. Comp. Biol.* 42, 582–590. <https://doi.org/10.1093/icb/42.3.582>.

Carr, J.A., 2010. Stress and reproduction in amphibians. In: Norris, D.O., Lopez, K.H. (Eds.), *Hormones and Reproduction in Vertebrates*. Academic Press, New York, pp. 99–116.

Chapple, D.G., Swain, R., 2002. Effect of caudal autotomy on locomotor performance in a viviparous skink, *Niveoscincus metallicus*. *Funct. Ecol.* 16, 817–825. <https://doi.org/10.1046/j.1365-2435.2002.00687.x>.

Collier, A., Bronk, C.C., Larson, B., Taylor, S., 2008. The impact of predation stress by largemouth bass (*Micropterus salmoides*) on the growth and development of leopard frog tadpoles (*Rana sphenoccephala*). *J. Freshwater Ecol.* 23, 281–289. <https://doi.org/10.1080/02705060.2008.9664200>.

Cooper, W.E., Wilson, D.S., 2008. How to stay alive after losing your tail. *Behaviour* 145, 1085–1099. <https://doi.org/10.1163/156853908784474515>.

Costantini, D., Marasco, V., Möller, A.P., 2011. A meta-analysis of glucocorticoids as modulators of oxidative stress in vertebrates. *J. Comp. Physiol. B.* 181, 447–456. <https://doi.org/10.1007/s00360-011-0566-2>.

Cote, J., Clobert, J., Meylan, S., Fitze, P.S., 2006. Experimental enhancement of corticosterone levels positively affects subsequent male survival. *Horm. Behav.* 49, 320–327. <https://doi.org/10.1016/j.yhbeh.2005.08.004>.

Crino, O.L., Buchanan, K.L., Trompf, L., Mainwaring, M.C., Griffith, S.C., 2017. Stress reactivity, condition, and foraging behavior in zebra finches: effects on boldness, exploration, and sociality. *Gen. Comp. Endocr.* 244, 101–107. <https://doi.org/10.1016/j.ygcen.2016.01.014>.

Cromie, G.L., Chapple, D.G., 2012. Impact of tail loss on the behaviour and locomotor performance of two sympatric *Lampropholis* skink species. *PLoS ONE* 7, e34732. <https://doi.org/10.1371/journal.pone.0034732>.

Dahl, E., Orizaola, G., Winberg, S., Laurila, A., 2012. Geographic variation in corticosterone response to chronic predator stress in tadpoles: Geographical variation in corticosterone response. *J. Evolution. Biol.* 25, 1066–1076. <https://doi.org/10.1111/j.1420-9101.2012.02493.x>.

Davis, D.R., Gabor, C.R., 2015. Behavioral and physiological antipredator responses of the San Marcos salamander, *Eurycea nana*. *Physiol. Behav.* 139, 145–149. <https://doi.org/10.1016/j.physbeh.2014.11.013>.

Denver, R.J., 1997. Environmental stress as a developmental cue: corticotropin-releasing hormone is a proximate mediator of adaptive phenotypic plasticity in amphibian metamorphosis. *Horm. Behav.* 31, 169–179. <https://doi.org/10.1006/hbeh.1997.1383>.

Denver, R.J., 2009. Structural and functional evolution of vertebrate neuroendocrine stress systems. *Ann. NY Acad. Sci.* 1163, 1–16. <https://doi.org/10.1111/j.1749-6632.2009.04433.x>.

Downes, S., Shine, R., 2001. Why does tail loss increase a lizard's later vulnerability to snake predators? *Ecology* 82, 1293–1303. [https://doi.org/10.1890/0012-9658\(2001\)082\[1293:WDTLIA\]2.0.CO;2](https://doi.org/10.1890/0012-9658(2001)082[1293:WDTLIA]2.0.CO;2).

Ducey, P.K., Brodie, E.D., 1983. Salamanders respond selectively to contacts with snakes: survival advantage of alternative antipredator strategies. *Copeia* 1983, 1036–1041. <https://doi.org/10.2307/1445106>.

Eelsey, R.M., Joanel, T., McNease, L., Lance, V., 1990. Growth rate and plasma corticosterone levels in juvenile alligators maintained at different stocking densities. *J. Exp. Zool.* 255, 30–36. <https://doi.org/10.1002/jez.1402550106>.

Epp, K.J., Gabor, C.R., 2008. Innate and learned predator recognition mediated by chemical signals in *Eurycea nana*. *Ethology* 114, 607–615. <https://doi.org/10.1111/j.1439-0310.2008.01494.x>.

Fonner, C.W., Woodley, S.K., 2015. Testing the predation stress hypothesis: behavioural and hormonal responses to predator cues in Allegheny Mountain dusky salamanders. *Behaviour* 152, 797–819. <https://doi.org/10.1163/1568539X-00003254>.

Fox, S.F., McCoy, J.K., 2000. The effects of tail loss on survival, growth, reproduction, and sex ratio of offspring in the lizard *Uta stansburiana* in the field. *Oecologia* 122, 327–334. <https://doi.org/10.1007/s004420050038>.

French, S.S., Matt, K.S., Moore, M.C., 2006. The effects of stress on wound healing in male tree lizards (*Urosaurus ornatus*). *Gen. Comp. Endocr.* 145, 128–132. <https://doi.org/10.1016/j.ygcen.2005.08.005>.

Gerald, G.W., Thompson, M.M., Levine, T.D., Wrinn, K.M., 2017. Interactive effects of leg autotomy and incline on locomotor performance and kinematics of the cellar spider, *Pholcus manueli*. *Ecol. Evol.* 7, 6729–6735. <https://doi.org/10.1002/ece3.3231>.

Gildemeister, E.A.R., Payette, W.I., Sullivan, A.M., 2017. Effects of size, caudal autotomy, and predator kairomones on the foraging behavior of Allegheny Mountain dusky



- salamanders (*Desmognathus ochrophaeus*). *Acta Ethol* 20, 157–164. <https://doi.org/10.1007/s10211-017-0259-2>.
- Gillis, G., Higham, T.E., 2016. Consequences of lost endings: caudal autotomy as a lens for focusing attention on tail function during locomotion. *J. Exp. Biol.* 219, 2416–2422. <https://doi.org/10.1242/jeb.124024>.
- Gillis, G.B., Bonvini, L.A., Irschick, D.J., 2009. Losing stability: tail loss and jumping in the arboreal lizard *Anolis carolinensis*. *J. Exp. Biol.* 212, 604–609. <https://doi.org/10.1242/jeb.024349>.
- Herman, C.A., 1992. *Endocrinology*. In: Feder, M.E., Burggren, W.W. (Eds.), *Environmental Physiology of the Amphibians*. University of Chicago Press, Chicago, pp. 40–54.
- Hossie, T.J., Ferland-Raymond, B., Burness, G., Murray, D.L., 2010. Morphological and behavioural responses of frog tadpoles to perceived predation risk: A possible role for corticosterone mediation? *Écoscience* 17, 100–108. <https://doi.org/10.2980/17-1-3312>.
- Jagnandan, K., Russell, A.P., Higham, T.E., 2014. Tail autotomy and subsequent regeneration alter the mechanics of locomotion in lizards. *J. Exp. Biol.* 217, 3891–3897. <https://doi.org/10.1242/jeb.110916>.
- Jamison, J.A., Harris, R.N., 1992. The priority of linear over volumetric caudal regeneration in the salamander *Plethodon cinereus* (Caudata: Plethodontidae). *Copeia* 1992, 235–237. <https://doi.org/10.2307/1446558>.
- Johnson, S.A., Jakob, E.M., 1999. Leg autotomy in a spider has minimal costs in competitive ability and development. *Anim. Behav.* 57, 957–965. <https://doi.org/10.1006/anbe.1998.1058>.
- Johnson, E.C., Sullivan, A.M., 2014. Antipredator behavior in *Desmognathus ochrophaeus*: threat-specific responses to chemical stimuli in a foraging context. *Ethology* 120, 672–680. <https://doi.org/10.1111/eth.12239>.
- Labanick, G.M., 1984. Anti-predator effectiveness of autotomized tails of the salamander *Desmognathus ochrophaeus*. *Herpetologica* 40, 110–118.
- Lynn, C.S., Dalton, B., Mathis, A., 2019. Territorial behaviour in southern red-backed and Ozark zigzag salamanders: effects of sex, species, and ownership. *Behaviour* 156, 1–21. <https://doi.org/10.1163/1568539X-00003554>.
- MacLeod, K.J., Sheriff, M.J., Ensminger, D.C., Owen, D.A.S., Langkilde, T., 2018. Survival and reproductive costs of repeated acute glucocorticoid elevations in a captive, wild animal. *Gen. Comp. Endocr.* 268, 1–6. <https://doi.org/10.1016/j.ygcen.2018.07.006>.
- Maginnis, T.L., 2006. The costs of autotomy and regeneration in animals: a review and framework for future research. *Behav. Ecol.* 17, 857–872. <https://doi.org/10.1093/beheco/ari010>.
- Maher, J.M., Werner, E.E., Denver, R.J., 2013. Stress hormones mediate predator-induced phenotypic plasticity in amphibian tadpoles. *Proc. R. Soc. B* 280, 20123075. <https://doi.org/10.1098/rspb.2012.3075>.
- Marvin, G.A., 2010. Effect of caudal autotomy on aquatic and terrestrial locomotor performance in two desmognathine salamander species. *Copeia* 2010, 468–474. <https://doi.org/10.1643/CP-09-188>.
- Maser, C., Hittler, K., Hanke, W., 1980. External induction of changes of corticosterone level in Amphibia. *Gen. Comp. Endocrinol.* 40, 335.
- Mbangkollo, D., deRoos, R., 1983. Comparative effects of epinephrine, norepinephrine, and a gentle handling stress on plasma lactate, glucose, and hematocrit levels in the American bullfrog (*Rana catesbeiana*). *Gen. Comp. Endocr.* 49, 167–175. [https://doi.org/10.1016/0016-6480\(83\)90133-8](https://doi.org/10.1016/0016-6480(83)90133-8).
- McCullum, S.A., Leimberger, J.D., 1997. Predator-induced morphological changes in an amphibian: predation by dragonflies affects tadpole shape and color. *Oecologia* 109, 615–621. <https://doi.org/10.1007/s004420050124>.
- McCormick, G.L., Shea, K., Langkilde, T., 2015. How do duration, frequency, and intensity of exogenous CORT elevation affect immune outcomes of stress? *Gen. Comp. Endocr.* 222, 81–87. <https://doi.org/10.1016/j.ygcen.2015.07.008>.
- McEwen, B.S., Wingfield, J.C., 2003. The concept of allostasis in biology and biomedicine. *Horm. Behav.* 43, 2–15. [https://doi.org/10.1016/S0018-506X\(02\)00024-7](https://doi.org/10.1016/S0018-506X(02)00024-7).
- Medger, K., Verburg, L., Bateman, P.W., 2008. The influence of tail autotomy on the escape response of the Cape Dwarf Gecko, *Lygodactylus capensis*: tail autotomy in a gecko. *Ethology* 114, 42–52. <https://doi.org/10.1111/j.1439-0310.2007.01445.x>.
- Moore, I.T., Jessop, T.S., 2003. Stress, reproduction, and adrenocortical modulation in amphibians and reptiles. *Horm. Behav.* 43, 39–47. [https://doi.org/10.1016/S0018-506X\(02\)00038-7](https://doi.org/10.1016/S0018-506X(02)00038-7).
- Moyers, S.C., Adelman, J.S., Farine, D.R., Moore, I.T., Hawley, D.M., 2018. Exploratory behavior is linked to stress physiology and social network centrality in free-living house finches (*Haemorrhous mexicanus*). *Horm. Behav.* 102, 105–113. <https://doi.org/10.1016/j.yhbeh.2018.05.005>.
- Novarro, A.J., Gabor, C.R., Goff, C.B., Mezebish, T.D., Thompson, L.M., Grayson, K.L., 2018. Physiological responses to elevated temperature across the geographic range of a terrestrial salamander. *J. Exp. Biol.* 221, jeb178236. <https://doi.org/10.1242/jeb.178236>.
- O'Connor, C.M., Norris, D.R., Crossin, G.T., Cooke, S.J., 2014. Biological carryover effects: linking common concepts and mechanisms in ecology and evolution. *Ecosphere* 5, art28. <https://doi.org/10.1890/ES13-00388.1>.
- Padgett, D.A., Marucha, P.T., Sheridan, J.F., 1998. Restraint stress slows cutaneous wound healing in mice. *Brain Behav. Immun.* 12, 64–73. <https://doi.org/10.1006/brbi.1997.0512>.
- Payette, W.I., Sullivan, A.M., 2019. The effect of predator kairomones on caudal regeneration by Allegheny Mountain Dusky Salamanders (*Desmognathus ochrophaeus*). *Can. J. Zool.* 97, 502–509. <https://doi.org/10.1139/cjz-2018-0225>.
- Reeder, T.W., 2013. A behavioral syndrome in the southern red-backed salamander (*Plethodon serratus*). Thesis. Biology Department, Missouri State University, Springfield, MO.
- Reeder, D.M., Kramer, K.M., 2005. Stress in free-ranging mammals: integrating physiology, ecology, and natural history. *J. Mammal* 86, 225–235. <https://doi.org/10.1644/BHE-003.1>.
- Ricciardella, L.F., Bliley, J.M., Feth, C.C., Woodley, S.K., 2010. Acute stressors increase plasma corticosterone and decrease locomotor activity in a terrestrial salamander (*Desmognathus ochrophaeus*). *Physiol. Behav.* 101, 81–86. <https://doi.org/10.1016/j.physbeh.2010.04.022>.
- Romana-Souza, B., Assis de Brito, T.L., Pereira, G.R., Monte-Alto-Costa, A., 2014. Gonadal hormones differentially modulate cutaneous wound healing of chronically stressed mice. *Brain Behav. Immun.* 36, 101–110. <https://doi.org/10.1016/j.bbi.2013.10.015>.
- Sapolsky, R.M., 2002. *Endocrinology of the stress response*. In: Becker, J.B., Breedlove, S.M., Crews, D., McCarthy, M.M. (Eds.), *Behavioral Endocrinology*. MIT Press, Cambridge, pp. 409–450.
- Sapolsky, R.M., Romero, L.M., Munck, A.U., 2000. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocr. Rev.* 21, 55–89. <https://doi.org/10.1210/edrv.21.1.0389>.
- Schneider, C.A., Rasband, W.S., Eliceiri, K.W., 2012. NIH Image to ImageJ: 25 years of image analysis. *Nat. Methods* 9, 671–675. <https://doi.org/10.1038/nmeth.2089>.
- Sheriff, M.J., Dantzer, B., Delehanty, B., Palme, R., Boonstra, R., 2011. Measuring stress in wildlife: techniques for quantifying glucocorticoids. *Oecologia* 166, 869–887. <https://doi.org/10.1007/s00442-011-1943-y>.
- Sopinka, N.M., Patterson, L.D., Redfern, J.C., Pleizier, N.K., Belanger, C.B., Midwood, J.D., Crossin, G.T., Cooke, S.J., 2015. Manipulating glucocorticoids in wild animals: basic and applied perspectives. *Conserv Physiol* 3, cov031. <https://doi.org/10.1093/conphys/cov031>.
- Stebbins, R.C., 1947. Tail and foot action in the locomotion of *Hydromantes platycephalus*. *Copeia* 1947, 1–5.
- Stebbins, R.C., Cohen, N.W., 1995. *A Natural History of Amphibians*. Princeton University Press, Princeton, NJ.
- Sullivan, A., Maerz, J., Madison, D., 2002. Anti-predator response of red-backed salamanders (*Plethodon cinereus*) to chemical cues from garter snakes (*Thamnophis sirtalis*): laboratory and field experiments. *Behav. Ecol. Sociobiol.* 51, 227–233. <https://doi.org/10.1007/s00265-001-0434-2>.
- Sullivan, A.M., Madison, D.M., Maerz, J.C., 2005. Nocturnal shift in the antipredator response to predator-diet cues in laboratory and field trials. In: In: Mason, R.T., LeMaster, M.P., Müller-Schwarze, D. (Eds.), *Chemical Signals in Vertebrates* 10. Springer, New York, pp. 349–356. [https://doi.org/10.1007/0-387-25160-X\\_40](https://doi.org/10.1007/0-387-25160-X_40).
- Thomas, J.R., Woodley, S.K., 2015. Treatment with corticosterone delays cutaneous wound healing in male and female salamanders. *Gen. Comp. Endocr.* 216, 33–38. <https://doi.org/10.1016/j.ygcen.2015.04.013>.
- Thomas, J.R., Magyan, A.J., Freeman, P.E., Woodley, S.K., 2017. Testing hypotheses about individual variation in plasma corticosterone in free-living salamanders. *J. Exp. Biol.* 220, 1210–1221. <https://doi.org/10.1242/jeb.149765>.
- Wack, C.L., Lovern, M.B., Woodley, S.K., 2010. Transdermal delivery of corticosterone in terrestrial amphibians. *Gen. Comp. Endocr.* 169, 269–275. <https://doi.org/10.1016/j.ygcen.2010.09.004>.
- Wack, C.L., DuRant, S.E., Hopkins, W.A., Lovern, M.B., Feldhoff, R.C., Woodley, S.K., 2012. Elevated plasma corticosterone increases metabolic rate in a terrestrial salamander. *Comp. Biochem. Phys. A* 161, 153–158. <https://doi.org/10.1016/j.cbpa.2011.10.017>.
- Wack, C.L., Ratay, M.K., Woodley, S.K., 2013. Effects of corticosterone on locomotor activity in red-legged salamanders. *Herpetologica* 69, 118–126. <https://doi.org/10.1655/HERPETOLOGICA-D-12-00040R2>.
- Watson, R.T., Mathis, A., Thompson, R., 2004. Influence of physical stress, distress cues, and predator kairomones on the foraging behavior of Ozark zigzag salamanders, *Plethodon angusticlavius*. *Behav. Process.* 65, 201–209. <https://doi.org/10.1016/j.beproc.2003.09.007>.
- Webster-Marketon, J.I., Glaser, R., 2008. Stress hormones and immune function. *Cell. Immunol.* 252, 16–26. <https://doi.org/10.1016/j.cellimm.2007.09.006>.
- Whiteman, H.H., Wissinger, S.A., 1991. Differences in the antipredator behavior of three plethodontid salamanders to snake attack. *J. Herpetol.* 25, 352–355. <https://doi.org/10.2307/1564596>.
- Wingfield, J.C., 2005. The concept of allostasis: coping with a capricious environment. *J. Mammal.* 86, 248–254. <https://doi.org/10.1644/BHE-004.1>.
- Wingfield, J.C., Sapolsky, R.M., 2003. Reproduction and resistance to stress: when and how. *J. Neuroendocrinol.* 15, 711–724. <https://doi.org/10.1046/j.1365-2826.2003.01033.x>.
- Woodley, S.K., 2017. Life in the slow lane: stress responses in plethodontid salamanders. *Herpetologica* 73, 259–268. <https://doi.org/10.1655/HERPETOLOGICA-D-16-00072.1>.
- Woodley, S.K., Lacy, E.L., 2010. An acute stressor alters steroid hormone levels and activity but not sexual behavior in male and female Ocoee salamanders (*Desmognathus ocoee*). *Horm. Behav.* 58, 427–432. <https://doi.org/10.1016/j.yhbeh.2010.05.011>.