# The effects of exogeneous corticosterone on caudal regeneration in the Allegheny Mountain dusky salamander (*Desmognathus ochrophaeus*)

by
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# The effects of exogeneous corticosterone on caudal regeneration in the Allegheny Mountain dusky salamander (*Desmognathus ochrophaeus*)

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**Abstract:** Vertebrate animals are exposed to a wide range of abiotic and biotic stressors, which induce changes to behavior, physiology, or morphology via the hypothalamo-pituitary-adrenocortical (HPA) axis. Many of these changes occur through the production and release of glucocorticoids in an effort to help organisms cope with, and mitigate, stressful situations. However, chronic stress and long-term activation of the HPA axis induces costs in the form of decreased reproduction, delayed wound healing, suppressed immune responses, and inhibited growth. This study attempts to understand the effect of elevated corticosterone (CORT), the primary glucocorticoid produced by amphibians, on rates of regeneration following caudal autotomy in the Allegheny Mountain dusky salamander (*Desmognathus ochrophaeus*). We monitored caudal regeneration in individual *D. ochrophaeus* assigned to one of four treatment regimens: control (no patch), patch control (sesame oil and patch, but no CORT), low dose CORT (0.25 mg/ml) patch, or high dose CORT (0.50 mg/ml) patch. Individuals exposed to CORT supplements over the course of the 13-week study recovered significantly less of their autotomized tails in terms of both volume and length (as percentages of the autotomized tail). Although regeneration did not show a dose-dependent effect, these data support the hypothesis that individuals with elevated stress hormones exhibit lower rates of regrowth.

**Keywords:** autotomy, regeneration, corticosterone, stress, amphibian, *Desmognathus*

## Introduction

Animals are exposed to a wide range of abiotic and biotic stressors while foraging, engaging in territorial defense, and searching for mates (Watson et al. 2004; Wack et al. 2010). These stressors can range from habitat or environmental changes (Denver 1997; Novarro 2018), to interactions with parasites, competitors, and predators (Clinchy et al. 2013; Relyea 2018). Stress responses may manifest as alterations to behavior (Carr 2002; Watson et al. 2004), physiology (Wack et al. 2012; Thomas & Woodley 2015), morphology (Denver 1997), or some combination depending on the duration of exposure to stressors (i.e. acute or chronic stress) (Woodley & Lacey 2010).

Central to the stress response in vertebrates is the hypothalamo-pituitary-adrenocortical (HPA) axis, which coordinates the physiological and behavioral responses of individuals to cope with and allay stressors (Thomas & Woodley 2015). Glucocorticoids, a key component of the products of the HPA axis, are ultimately released into the blood from the adrenal cortex and precipitate a range of allostatic responses in individuals (Sapolsky 2002; Sheriff et al. 2011; Thomas & Woodley 2015). These allostatic responses to stress maintain homeostatic stability through change in physiology or behavior (McEwen & Wingfield 2003), and can result in adjustments to available metabolic fuels, cardiac output, patterns of blood perfusion, arousal, and escape behaviors (Woodley & Lacy 2010). The changes effected by glucocorticoids (e.g., mobilization of energy) are accompanied by long-term costs such as decreased reproduction (Wingfield & Sapolsky 2003; Carr 2010; MacLeod 2018), delayed wound healing (Padgett et al. 1998; Romana-Souza et al. 2014), suppressed immune function (Webster-Marketon & Glaser 2008), and inhibited growth (Boonstra et al. 1998).

Although the effects of glucocorticoids are relatively well studied in mammals (e.g., Hails 1978; Kroll 2010; Manuja et al. 2011; Hing et al. 2014; Gebregeziabhear & Ameha 2015) and birds (e.g., Silverin 1997; Angelier & Chastel 2009), an understanding of hormone-mediate stress responses in other taxa is somewhat limited, especially among amphibians (Woodley 2017). As ectotherms, amphibians tend to show different responses to stress when compared to many endotherms. For example, corticosterone (hereafter CORT) is the primary glucocorticoid released upon HPA axis stimulation in many amphibians, whereas most mammals (excluding rodents) rely on cortisol (Rollins-Smith 2001). In addition, endocrine changes and elevated CORT levels (interpreted as allostatic responses), do not necessarily suppress nonessential behaviors (i.e. reproduction) in amphibians, a phenomenon uncommon in other vertebrates. For instance, male and female Ocoee salamanders (*Desmognathus ocoee*) with elevated CORT levels, due to acute handling by experimenters, did not exhibit suppressed courtship and mating behavior, but did show decreases in locomotor activity and sex hormone production (i.e. testosterone in males and estradiol in females) (Woodley & Lacey 2010). Elevated levels of CORT in amphibians have also been linked to decreased locomotor activity (Ricciardella et al. 2010; Woodley & Lacy 2010), increased metabolic rate and oxygen consumption (Wack et al. 2012), slowed cutaneous wound healing (French et al. 2006; Thomas & Woodley 2015), reduced body weight (Bliley & Woodley 2012), and accelerated larval growth (Hayes and Wu 1995; Denver 1997). Similarly, Dahl et al. (2012) reported that artificially elevating CORT in the common frog (*Rana temporaria*) results in slower growth rates, longer larval periods, and lower body mass. Narayan and Hero (2014) showed that in the cane toad (*Rhinella marina*), acute thermal stressors elevate plasma CORT levels and result in lower testosterone, which has been implicated in decreased in reproductive output.

Prior studies have explored the influence of elevated levels of CORT on the behavior, physiology, and morphology of amphibians, but a clear understanding of the effects of experimentally elevated CORT on rates of regeneration following autotomy is lacking. Caudal autotomy is an antipredator response utilized by some lizards and salamanders to distract and deter predation when primary defenses (e.g., immobility, biting, fleeing, and noxious secretions) fail (Arnold 1982; Labanick 1984; Whiteman and Wissinger 1991; Maginnis 2006; Marvin 2010). Although autotomy may provide an immediate benefit in terms of escape and survival, individuals may incur significant costs; the lost appendage, specifically tail tissue, stores quantities of fat and protein, assists in locomotion, and confers higher social status (Maginnis 2006; Gillis & Higham 2016). Lowered social status in tailless individuals often results in reduced fecundity and clutch size (Fox & McCoy 2000; Bateman & Fleming 2009). In addition, by changing the overall mass and center of gravity of an individual, caudal autotomy can alter balance and stability (Gillis & Higham 2016). As such, some species of lizard, such as *Niveoscincus metallicus*, show significant decreases in locomotor performance after caudal autotomy, with reduced stamina in females and lower sprint speed in males, both of which leads to increased vulnerability to predation (Chapple & Swain 2002). Similarly, among salamanders, tailless individuals are less likely to escape from subsequent predator attacks (Arnold 1982; Ducey and Brodie 1983). Due to the significant trade-offs associated with caudal autotomy, the ability to efficiently regenerate a tail is important for long-term survival and fitness of an individual.

Though a model in behavioral ecology, chemical communication, community ecology, physiological ecology, life-history evolution, and phylogenetics (e.g., Keen & Orr 1980; Labanick 1984; Marvin 2010; Marvin & Lewis 2013; Johnson et al. 2014; Gildemeister et al. 2017; Payette & Sullivan 2019), plethodontid stress physiology and endocrinology is less well understood. Plethodontid salamanders, as specialized low-energy ectotherms (Pough 1980), can serve as models for studying stress-induced behavioral, physiological, and morphological responses (Wack et al. 2010; Woodley 2017) because they serve as a contrast to other high-energy, well studied taxa (i.e. birds and mammals). In fact, as environmental stressors become more prevalent and are implicated in questions regarding amphibian declines and extinctions, plethodontid salamanders are becoming increasingly studied (e.g., Rollins-Smith 2001; Narayan & Hero 2014; Thomas & Woodley 2015; Novarro et al. 2018). This study was designed to use Allegheny Mountain dusky salamanders (*Desmognathus ochrophaeus*) to assess the effects of elevated stress on the regeneration of tail tissue following the induction of caudal autotomy. The elevated stress occurred through an exogenous source of CORT applied to individual salamanders via a cutaneous patch, rather than exposure to a physical stressor. Salamanders were assigned to one of four separate treatment groups: control (no patch), patch control (sesame oil patch, but no CORT), low dose CORT (0.25 mg/ml) patch, or high dose CORT (0.50 mg/ml) patch. Two concentrations of CORT were used as previous research suggests that developmental, physiological, and behavioral response may vary according to dose (Blas et al. 2007; Wack et al. 2010;

McCormick et al. 2015). Physical stressors to elevate CORT were eliminated in exchange for dermal patches as previous research has shown confounding effects due to invasive or disruptive methods (reviewed by Woodley 2017). We hypothesize that individuals exposed to exogenous CORT would recover less of their tail over the 13-week study period due to elevated stress impeding wound healing and growth in some species (e.g., French et al. 2006; Thomas & Woodley 2015). We predict that individuals treated with a high dose of CORT would have lower rates of regeneration than those receiving the low dose, which would still have significantly lower rates of regeneration than a control.

## Methods and Materials

### *Collection and maintenance of study animals*

The 140 individual *D. ochrophaeus* used in this study to determine the effects of exogenous CORT on caudal regeneration were caught by hand in a wooded area of the campus of Houghton College (Houghton, New York, USA) over a span of three days in August 2018. Care was taken to exclude individuals as test subjects that showed signs of recent caudal autotomy (i.e. obvious caudal discoloration or distal bluntness). Collected salamanders were returned to the laboratory and housed individually in 15-cm-diameter Petri dishes with a moist acid-free paper towel substrate and stored in a climate-controlled chamber on a 12.5 L : 11.5 D cycle (lights on at 0700 h EST) at 15.5° C (day) and 12.8° C (night). In an effort to standardize prey intake, each individual was provided with 5 – 8 wingless *Drosophila* sp. one day prior to the induction of caudal autotomy (6 Sept 2018), and 3 – 4 additional prey twice during the course of the study on 12 October and 16 November 2018. To minimize the likelihood that differences in caudal regeneration among treatment groups were due to variation in food intake, relatively few feeding opportunities were provided because previous studies show that exposure to stressors can reduce foraging (e.g., Carr 2002; Watson et al. 2004; Johnson & Sullivan 2014; Gildemeister et al. 2017).

### *Preparation of cutaneous CORT patches*

We implemented the dermal patch design and methodology to apply exogenous CORT in test subjects as described by Wack et al. (2010). In brief, patches were made from low protein binding filter paper (Cat. No. 1820-070, Whatman) cut into 1.5 × 3.0 mm rectangles. An aliquot of one of two stock solutions of CORT was ultimately applied to each patch. In order to prepare these stock solutions, 25 mg of crystallin CORT (Cat. No. C2505, Sigma-Aldrich) were added to 100% ethanol in a 1.5 ml microcentrifuge tube (Cat. No. 05-408-129, Fisher Scientific) which was subsequently vortexed to ensure dissolution. The ethanol solution was then added to a carrier oil (sesame oil) and stirred overnight. This period of stirring provide opportunity for the ethanol to evaporate leaving behind the stock solutions with the appropriate amount of CORT per volume of carrier oil. The ‘high’ dose contained 0.50 mg CORT/ml of carrier oil, whereas the ‘low’ dose contained 0.25 mg CORT/ml of carrier. These CORT doses elevated plasma CORT levels in *D. ochrophaeus* to physiologically relevant levels (i.e. similar to the CORT levels in salamanders that experienced the stressor of handling) according to Wack et al.

(2010). Doses of the CORT solution applied to individual salamanders were not adjusted for the body size of the individual as data from Wack et al. (2010) also indicate no relationship between body weight and plasma CORT concentrations in animals that received exogenous CORT via cutaneous patches.

### *Application of cutaneous CORT patches*

In order to evaluate the effects of an experimentally elevated stress hormone on caudal regeneration, salamanders were arbitrarily assigned to one of four treatment groups: control (no patch nor CORT), patch control (cutaneous patch with sesame oil, but no CORT), low dose CORT (0.25 mg/ml) patch, or high dose CORT (0.50 mg/ml) patch. Caudal autotomy was induced in each individual on 7 Sept 2018 through a simulated predation event where each salamander tail was firmly and quickly 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 September – 10 December 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 of the three treatment groups (patch control, low CORT, or high CORT). Forceps were used to apply patches to salamanders along the dorsal midline slightly anterior to the forelegs. When patches were securely affixed to salamanders, 2.5 μl of low CORT, high CORT solution, or the sesame oil blank were applied to the patch using a micropipette. Patches were removed via clean 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, salamanders were moved to a clean home dish and returned to the climate-controlled chamber. This process was repeated weekly.

### *Morphometric and statistical analysis*

During the study, a weekly photograph was taken of each individual salamander using an Olympus stylus TG-4 digital camera permanently mounted on a tripod at a fixed distance below a plexiglass stage facing the underside of the transparent home dishes. Prior to induction of caudal autotomy photographs were taken of salamanders in order to measure the length and width of the original, or 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 session began with a photograph of a Fowler digital caliper 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. Length and radius of the base of the regenerated tail were identified from the discolored 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 volume of the autotomized tail was calculated by subtracting the volume of the remaining tail, according to the formula for a truncated cone $V = \frac{\pi}{3}(r_1^2 + r_1 r_2 + r_2^2)h$, 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-analyzing software ImageJ (Schneider et al. 2012; Payette & Sullivan 2019). The ‘freehand’ line tool in ImageJ was used 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.

As a way to account for the effect of the point at which autotomy occurred, we used both volume and length metrics of the regenerated tail as percentages of volume and length of the tail that was autotomized. Additionally, we performed correlation analyses for both the length of tail regenerated and the initial autotomized tail length, as well as the volume of tail regenerated and the initial autotomized tail volume at the conclusion of the 13-wk study.

We analyzed the effect of CORT doses on caudal regeneration using volume (as a percentage of the volume of the autotomized tail) and length (as a percentage of the length of the autotomized tail) by performing a generalized linear model one-way ANOVA using Statistica (Statsoft, Inc. 2001, version 6.0). Separate one-way ANOVAs were conducted on regenerated tail tissues at each time interval. When main effects were significant, we conducted post-hoc pairwise multiple comparisons while adjusting alpha according to Bonferroni. The generalized linear model one-way ANOVA was utilized as our data violated the assumptions of normality and homoscedasticity. Over the course of the study, three individuals were removed from the study due to injury or death, so that the final sample sizes were: control ($n = 33$), patch control ($n = 34$), low CORT ($n = 35$), and high CORT ($n = 35$).

## Results

Our data indicated significant correlations between the total length of the regenerated tail and the length of the autotomized tail ($r = 0.4596$; $p < 0.001$; Fig. 1), and volume of the regenerated tail and the volume of the autotomized tail ($r = 0.5391$; $p < 0.001$; Fig. 2) by the conclusion of the 13-wk study.

The generalized linear model 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 starting at week three, as soon as there was visible and measurable regrowth (Fig. 3). Furthermore, there was a significant effect of treatment on regeneration at each measured time interval (Table 1). We conducted post-hoc pairwise multiple comparisons for each time interval with a significant overall effect of treatment and adjusted alpha according to Bonferroni (adjusted significance at $p = 0.0083$). After the fifth week, stressed individuals (i.e. treated with high or low CORT) had significant differences in caudal regeneration in terms of length compared to the patch control (LD: $p < 0.001$; HD: $p = 0.004$) with this trend continuing and becoming more pronounced by the thirteenth week (LD: $p < 0.001$; HD: $p < 0.001$). Moreover, there were no significant differences, in terms of regenerative length, between the concentrations of the CORT doses, with $p$-values ranging from 0.416 during week nine to 0.986 during week five. Likewise, there were no significant differences between the controls, with $p$-values ranging from 0.021 during week thirteen to 0.998 during week four.

Similarly, the generalized linear model 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 as soon as there was visible and measurable regrowth (Fig. 4). Furthermore, there was a significant effect of treatment on the volume (as a percentage of the volume of the autotomized tail) regenerated starting at week three and continuing to week thirteen with the exception of week nine (Table 1). Subsequent pairwise multiple comparisons indicate that that CORT has a significant effect on post-autotomy caudal regeneration as individuals treated with CORT had significantly less volumetric caudal regeneration comparted to the patch control from week three (LD: $p = 0.002$; HD: $p = 0.004$) to week thirteen (LD: $p = 0.001$; HD: $p = 0.001$), excluding week nine (LD: $p = 0.012$; HD: $p = 0.041$). Furthermore, throughout our study there was no significant difference between individuals treated with low dose

CORT and high dose CORT, with *p*-values ranging from 0.149 during week eleven and twelve to 0.820 during week ten. The controls, correspondingly, showed no difference, with *p*-values ranging from 0.009 during week eight to 0.880 during week eleven.

## Discussion

Our results suggest that individual *D. ochrophaeus* repeatedly exposed to exogenous CORT regenerated significantly less of their autotomized tail using both volume and length measurements. However, the rates of regeneration were not dose dependent, as there were no significant differences in caudal regeneration between the low dose (0.25 mg/ml) and high dose CORT (0.50 mg/ml) treatment groups. Although the regrowth did not show a scaler effect due to dose as expected, these data support the hypothesis that individuals experiencing elevated levels of stress would exhibit lower rates of caudal regeneration.

The doses of CORT delivered via cutaneous patch in this study were selected to achieve elevated levels of plasma CORT that were physiologically relevant, while excluding actual stressors that could additionally impact caudal regeneration. This was crucial to our study because prior research suggests that more invasive or disruptive methods to increase CORT may generate confounding effects and result in inaccurate assessment of stress. For example, when CORT levels were elevated in male and female Ocoee salamanders (*Desmognathus ocoee*) through acute handling, Woodley and Lacey (2010) noted decreased locomotor activity by individuals. However, when cutaneous patches were used to increase of CORT levels without handling or predation-related stressors, locomotor activity did not decrease (reviewed by Woodley 2017). By using an exogenous source of CORT applied via a cutaneous patch, we attempted to limit our evaluation of stress on regeneration to the effects of CORT specifically. In our study, the low dose of CORT applied to individuals corresponded to plasma CORT levels that were similar to those measured in *D. ochrophaeus* after capture and handling in the field (Ricciardella 2008; Schubert et al. 2009; Ricciardella et al. 2010). This dose was sufficient to significantly slow post-autotomy caudal regeneration. This is consistent with the findings of Thomas and Woodley (2015) who show that this same dose delayed wound healing in *D. ochrophaeus* and is consistent with similar studies using rodents and reptiles (Horan et al. 2005; French et al. 2006). Thomas and Woodley (2015) also indicate that CORT has an impact early in the healing process by affecting inflammation. Likewise, within the first week of measurable regrowth, our study showed evidence of differentiated wound healing among treatment groups in both longitudinal and volumetric regeneration, driven in large part by delayed regeneration in the low and high dose CORT groups. Moreover, although Wack et al. (2010) indicated that plasma CORT levels increased in correlation with CORT patch dosage such that the high dose (0.50 mg/ml) resulted in significantly higher plasma concentrations than the low dose (0.25 mg/ml), there was no difference between low and high dose in terms of caudal regeneration in our study. Although unexpected, one possible explanation for the lack of regenerative differentiation between high and low doses of CORT is that our low dose reached the maximum effect CORT can have on regenerative growth, such that subsequently larger doses (i.e. the high dose) had no added effect. In such a case the two CORT concentrations may be functionally equivalent in terms of post-autotomy caudal regeneration.

As environmental stressors become more prevalent and questions regarding amphibian decline and extinction increase, understanding stressors and their implications is becoming increasingly important. For example, thermal stressors have been shown to increase CORT levels in a variety of ectotherms, such as alligator lizards (*Anguidae* sp.) (Telemeco & Addis 2014), water snakes (*Nerodia sipedon*) (Sykes & Klukowski 2009), New Zealand common geckos (*Hoplodactylus maculatus*) (Preest & Cree 2008), and cane toads (*Rhinella marina*) (Narayan & Hero 2014). Additionally, in *Plethodon cinereus,* a terrestrial salamander, Novarro et al. (2018) reported that elevated temperatures subsequently elevated CORT levels, which were negatively correlated with the salamander's food conversion efficiency or the amount of mass gained per unit food ingested. With a lowered food conversion efficiency, one would expect to see lower regenerative growth as shown in our study, possibly contributing to the mechanism behind the reduced caudal regeneration.

Previous studies have shown a link between glucocorticoids and the risk of predation. For instance, zebra fish (*Danio rerio*) exhibit antipredator behavior coupled with significantly higher cortisol (stress hormone in fish) levels when exposed to chemical alarm cues signifying predation (Barkhymer et al. 2018). Similarly, wood frog tadpoles (*Lithobates sylvaticus*) show elevated levels of CORT when exposed to dragonfly predator cues (Bennett et al. 2016), and corticosterone levels in *L. sylvaticus* in natural ponds were reported to be positively correlated with predator mass (Maher et al. 2013). Additionally, northern leopard frog larvae (*Rana pipiens*) were found to have lower growth rates when exposed to predators treated with metyrpone, a CORT inhibitor (Hossie et al. 2010) and McCollum and Leimberger (1997) reported elevated growth rates of larval Cope's gray tree frog (*Hyla chrysoscelis*) when reared in the presence of a dragonfly nymph predator. Furthermore, and more pertinent to our study, Payette and Sullivan (2019), showed that in the presence of garter snake predator kairomones *D. ochrophaeus* significantly reduce their post-autotomy caudal regeneration. Together, these findings suggest that predation stress may increase glucocorticoids, specifically CORT in amphibians and subsequently affect regenerative growth.

## Conclusion

Despite autotomy providing immediate survival benefits, the resulting consequences may significantly impact ensuing survival and reproductive output, making expedient and efficient post-autotomy caudal regeneration imperative (Maginnis 2006). In this study we demonstrate that elevated levels of CORT in *D. ochrophaeus* negatively impact the rate of post-autotomy caudal regeneration in terms of length and volume. Some studies suggest that elevated CORT levels in amphibians can impact food conversion efficiency, which could result in decreased growth rates and subsequently explain the variation we detected in caudal regeneration. The effects on caudal regeneration were significant beginning at measurable growth (week three) and continues to grow in significance throughout our thirteen-week study (excluding week nine). Because low levels of stress, such as through handling, had a similar effect to that of high levels of stress, our study suggests that *D. ochrophaeus* are vulnerable to experimentally elevated levels of stress, but that regeneration may not be dose-dependent. Overall, the synergistic effects between different stressors, stress, and regeneration are not fully understood in amphibians, but as various environmental stressors become more prevalent and amphibian populations continue to decline or go extinct, understanding stressors and their implications is becoming increasingly important.

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**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). Separate one-way ANOVA were conducted on regenerated tail tissues at each time interval.

| Time post autotomy | Volume (as percent of autotomized tail) | | | Length (as percent of autotomized tail) | | |
|---|---|---|---|---|---|---|
| Week | df | Wald | *P* | df | Wald | *P* |
| 3 | 3 | 12.45524 | 0.006 | 3 | 13.25500 | 0.004 |
| 4 | 3 | 16.88535 | <0.001 | 3 | 8.65000 | 0.034 |
| 5 | 3 | 17.13000 | <0.001 | 3 | 13.50000 | 0.004 |
| 6 | 3 | 15.58190 | 0.001 | 3 | 25.23000 | <0.001 |
| 7 | 3 | 18.61700 | <0.001 | 3 | 27.51000 | <0.001 |
| 8 | 3 | 20.41500 | <0.001 | 3 | 27.02000 | <0.001 |
| 9 | 3 | 6.38100 | 0.094 | 3 | 15.78000 | 0.001 |
| 10 | 3 | 17.84300 | <0.001 | 3 | 30.56000 | <0.001 |
| 11 | 3 | 24.86500 | <0.001 | 3 | 36.42000 | <0.001 |
| 12 | 3 | 28.84300 | <0.001 | 3 | 25.63000 | <0.001 |
| 13 | 3 | 16.52100 | <0.001 | 3 | 33.98000 | <0.001 |

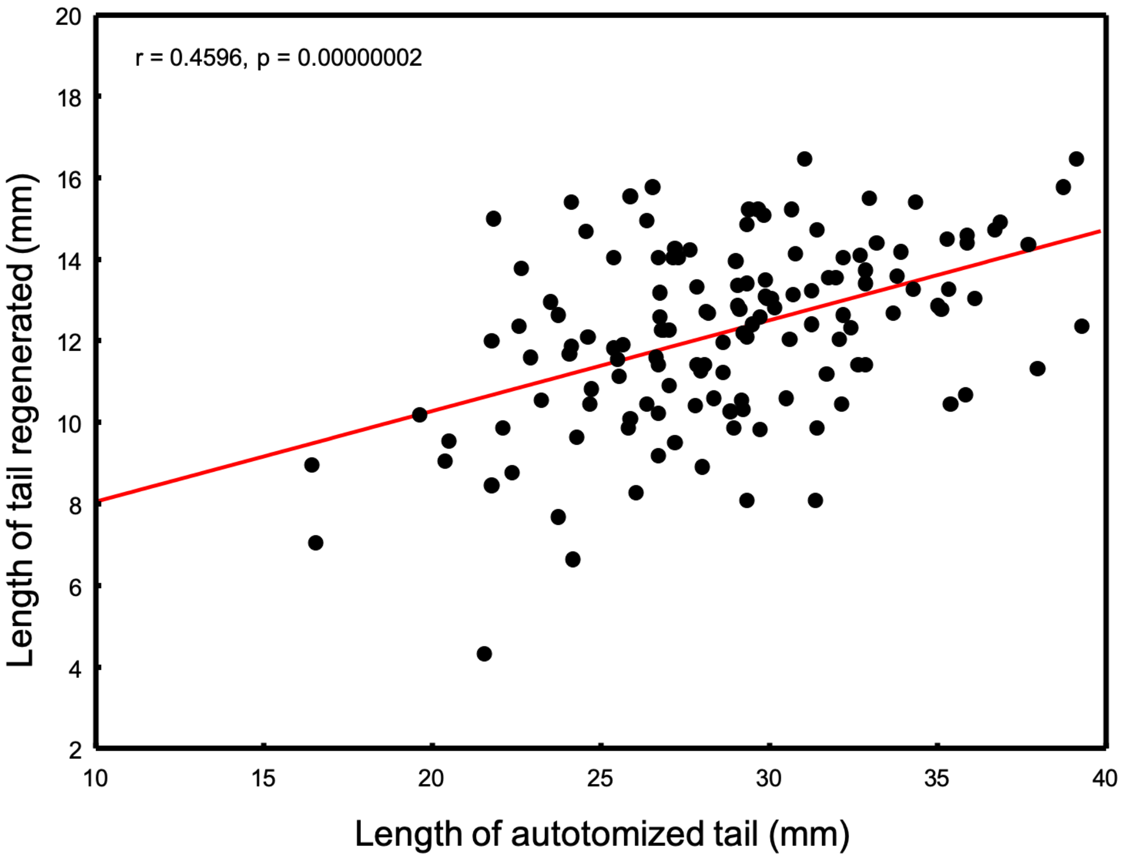

**Figure 1.** The significant positive relationship between the length of tail regenerated (mm) at the conclusion of the 13-wk study and the initial length of autotomized tail (mm) in *D.* ochrophaeus using a Spearman’s rank correlation (r = 0.4596; p < 0.001).

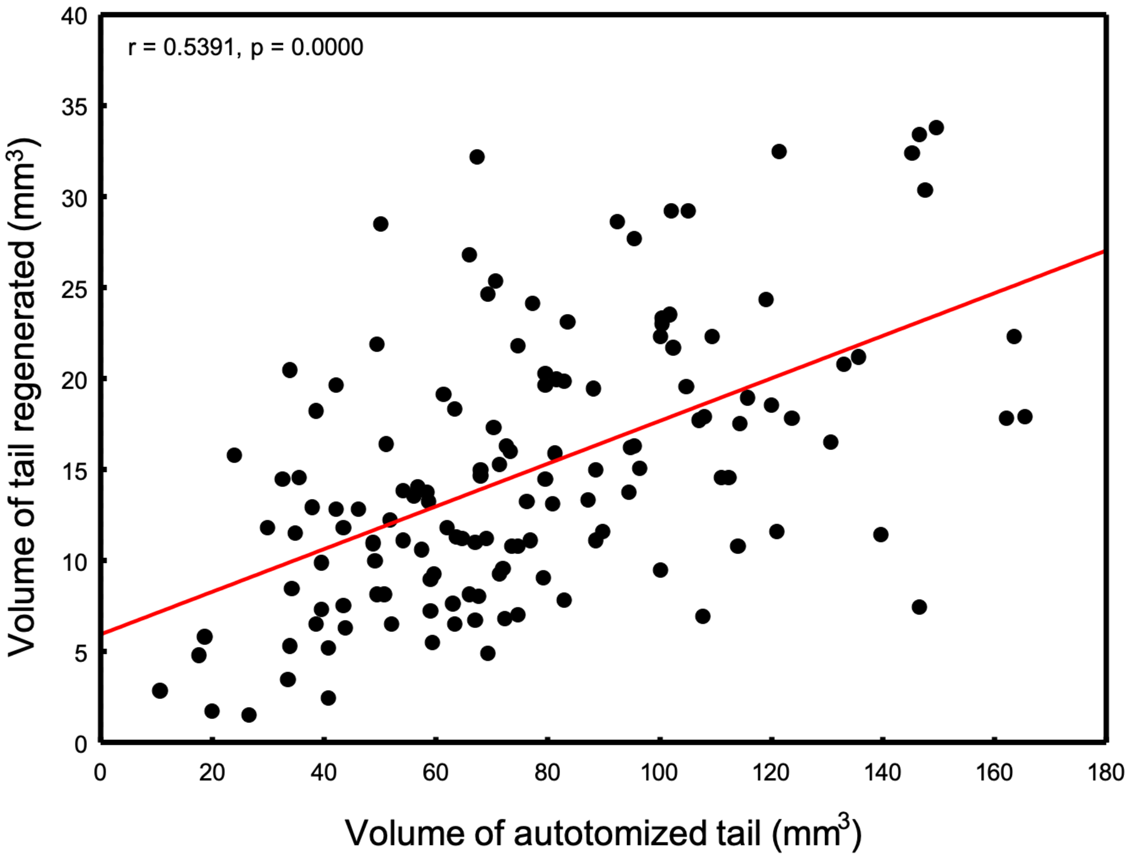

**Figure 2.** The significant positive relationship between the volume of tail regenerated ($mm^3$) at the conclusion of the 13-wk study and the initial volume of autotomized tail ($mm^3$) in *D.* ochrophaeus using a Spearman's rank correlation ($r = 0.5391$; $p < 0.001$).

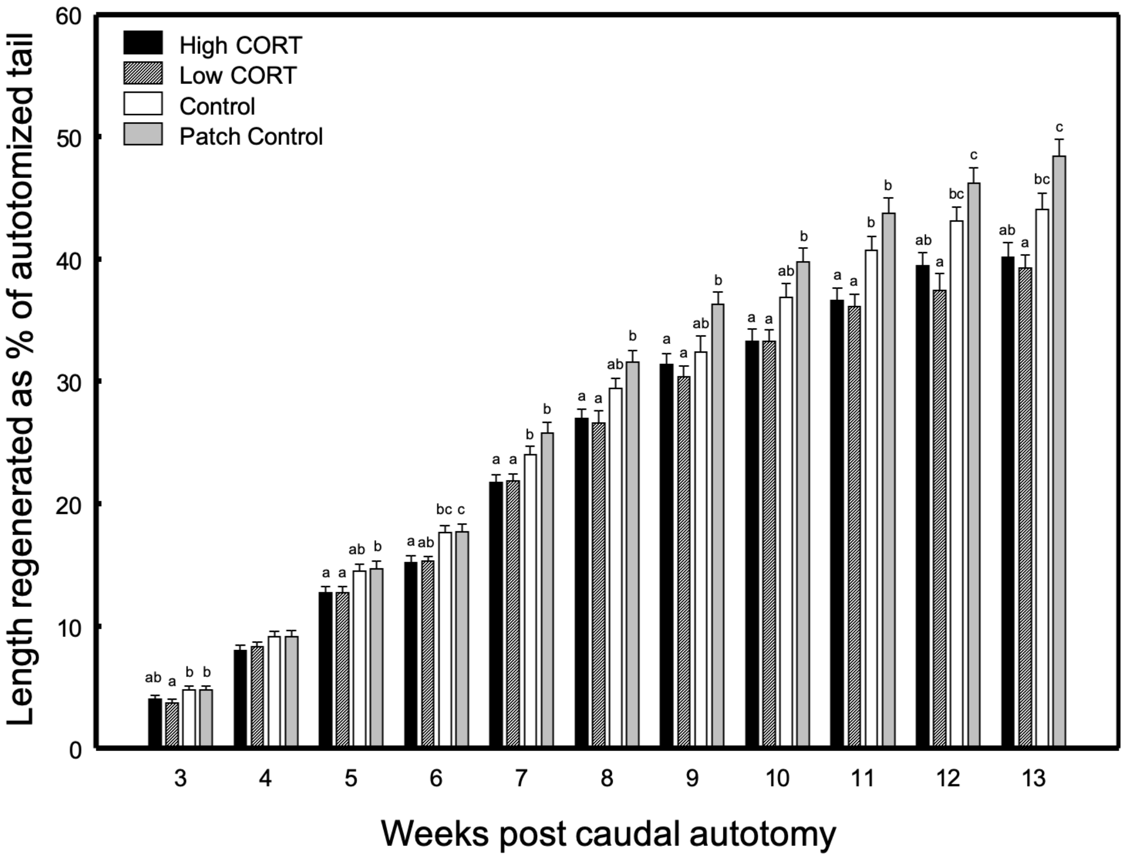

**Figure 3.** Length of caudal regeneration as a percent of autotomized tail (Mean ± SE) in *D. ochrophaeus*, calculated at weekly intervals, in one of four treatments: control (no patch nor CORT), patch control (cutaneous patch with sesame oil, but no CORT), low dose CORT (0.25 mg/ml) patch, or high dose CORT (0.50 mg/ml) patch. Letters indicate significant differences among caudal regeneration in treatment groups at each time interval using post-hoc pairwise multiple comparisons while adjusting alpha accordingly to Bonferroni.

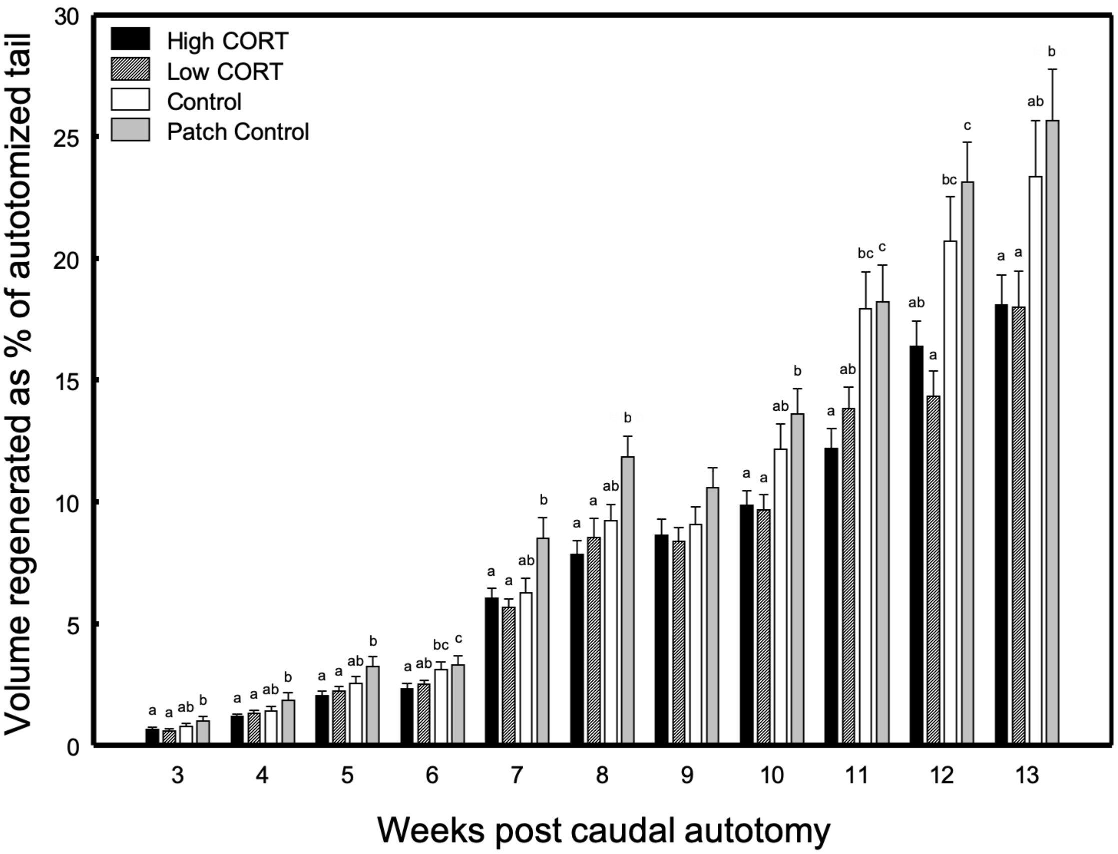

**Figure 4.** Volume of caudal regeneration as a percent of autotomized tail (Mean ± SE) in *D. ochrophaeus*, calculated at weekly intervals, in one of four treatments: control (no patch nor CORT), patch control (cutaneous patch with sesame oil, but no CORT), low dose CORT (0.25 mg/ml) patch, or high dose CORT (0.50 mg/ml) patch. Letters indicate significant differences among caudal regeneration in treatment groups at each time interval using post-hoc pairwise multiple comparisons while adjusting alpha accordingly to Bonferroni.