

Exposure to glucocorticoids alters life history strategies in a facultatively paedomorphic salamander

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Abstract

Polyphenisms, where two or more alternative, environmentally-cued phenotypes are produced from the same genotype, arise through variability in the developmental rate and timing of phenotypic traits. Many of these developmental processes are controlled or influenced by endogenous hormones, such as glucocorticoids, which are known to regulate a wide array of vertebrate ontogenetic transitions. Using the mole salamander, *Ambystoma talpoideum*, as a model, we investigated the role of glucocorticoids in regulating facultative paedomorphosis, an ontogenetic polyphenism where individuals may delay metamorphosis into terrestrial adults. Instead, individuals reproduce as aquatic paedomorphic adults. Paedomorphosis often occurs when aquatic conditions remain favorable, while metamorphosis typically occurs in response to deteriorating or “stressful” aquatic conditions. Since glucocorticoids are central to the vertebrate stress response and are known to play a central role in regulating obligate metamorphosis in amphibians, we hypothesized that they are key regulators of paedomorphic life history strategies. To test this hypothesis, we compared development of larvae in outdoor mesocosms exposed to Low, Medium, and High exogenous doses of corticosterone (CORT). Results revealed that body size and the proportion of paedomorphs were both inversely proportional to exogenous CORT doses and whole-body CORT content. Consistent with known effects of CORT on obligate metamorphosis in amphibians, our results link glucocorticoids to ontogenetic transitions in facultatively paedomorphic salamanders. We discuss our results in the context of theoretical models and the suite of environmental cues known to influence facultative paedomorphosis.

KEYWORDS

adrenal, *Ambystoma*, corticosterone, heterochrony, phenotypic plasticity, polyphenism, stress

1 | INTRODUCTION

Phenotypic plasticity, where one genotype can express different phenotypic patterns under different environmental conditions, is an important facilitator of evolutionary change that typically arises from differential developmental rates of phenotypic traits (Nijhout, 2003; Pfennig et al., 2010; West-Eberhard, 2003). While it frequently appears as continuous (i.e. unimodal) variation in quantitative traits, phenotypic plasticity can also manifest as polyphenisms, where traits

show discontinuous (i.e. bi- or polymodal) variation with two or more discrete, alternative phenotypes (Pfennig et al., 2010; West-Eberhard, 2003). These polyphenisms can arise due to environmentally-induced adjustments in developmental timing (i.e. heterokairy; Rundle & Spicer, 2016). For example, facultative paedomorphosis, when juvenile characteristics are retained at adulthood under some environmental circumstances, is one heterokairy in particular that adjusts the relative timing of development (Gould, 1977). This polyphenism is seen in many hemimetabolous insects

(Hemiptera, Orthoptera) with winged and wingless phenotypes. The wingless, stationary phenotypes are developmentally paedomorphic, but when environmental conditions change and dispersal is a favorable strategy, such as when food is depleted or density increases, macropterous wings develop (Denno & Roderick, 1992; Denno et al., 1991; Harrison, 1980; Iwanaga & Tojo, 1986). Similarly, caudate amphibians across five families including Ambystomatidae, Salamandridae, Dicamptodontidae, Hynobiidae, and Plethodontidae are facultatively paedomorphic (Denoël et al., 2005). In these salamanders, when aquatic conditions are favorable to growth and survival, adults may retain a fully paedomorphic and aquatic larval-like phenotype complete with gills by delaying the onset of metamorphosis. However, when conditions deteriorate, adults may develop a terrestrial phenotype via metamorphosis during which aquatic traits are lost (Denoël et al., 2005; Whiteman, 1994).

Three hypotheses have been proposed to explain facultative paedomorphosis (Whiteman, 1994; Wilbur & Collins, 1973). (1) The Paedomorphic Advantage hypothesis predicts that paedomorphosis occurs when growth rates are high due to favorable aquatic conditions (biotic or abiotic) and metamorphosis occurs when poor or deteriorating aquatic conditions limit growth or increase the risk of mortality. For example, faster growing individuals can be competitively dominant, and thus become paedomorphic to continue exploiting monopolized resources, while smaller, slower growing individuals metamorphose to escape competition (or even cannibalism) from larger individuals (Denoël & Ficetola, 2014; Denoël & Poncin, 2001; Harris, 1987; Jackson & Semlitsch, 1993; Semlitsch, 1987a). (2) The Best of a Bad Lot hypothesis predicts that paedomorphosis occurs when smaller larvae with slower growth rates do not reach the minimum size necessary for metamorphosis and thus fitness is maximized by early maturation replacing metamorphosis (Whiteman et al., 2012). (3) The Dimorphic Paedomorph hypothesis predicts that both the smaller and larger individuals become paedomorphs (for the same reasons found in the Paedomorph Advantage and Best of a Bad Lot), but that the intermediate size classes metamorphose to escape competition with larger, dominant paedomorphs (Whiteman, 1994). Numerous studies have provided evidence that indicators of deteriorating environmental conditions such as short hydroperiod (Semlitsch, 1987a), increased conspecific density (Harris, 1987; Semlitsch, 1987a), low food availability (Denoël & Poncin, 2001; Ryan & Semlitsch, 2003) and high predation risk (Jackson & Semlitsch, 1993) reduce the occurrence of paedomorphosis, providing support for the Paedomorph Advantage hypothesis. Interestingly, these environmental conditions are linked by a common thread—that is, they represent environmental stressors known to stimulate the production of adrenal/interrenal glucocorticoids (Crespi & Denver, 2005; Denver, 2017; Smith & Vale, 2006), implicating glucocorticoids as a primary factor regulating this polyphenism.

Glucocorticoids are known to play a pivotal role in amphibian metamorphosis (Bonett et al., 2010; Niki et al., 1981, reviewed in Denver, 2017). In amphibians with obligate metamorphosis, activation of the hypothalamic-pituitary-interrenal (HPI) axis stimulates thyroid stimulating hormone (TSH) via actions of corticotropin-releasing factor (CRF) on pituitary thyrotropes (Denver, 2009). Corticosterone (CORT;

the primary glucocorticoid in most amphibians) upregulates 5'-deiodinase activity that converts thyroxine (T_4) to triiodothyronine (T_3), which is the more potent thyroid hormone (Bonett et al., 2010; Darras et al., 2002; Galton, 1990; Hayes & Wu, 1995; Kühn et al., 2005). CORT also increases maximal T_3 nuclear binding capacity (Kikuyama et al., 1993; Niki et al., 1981; Suzuki & Kikuyama, 1983) and increases tissue sensitivity to thyroid hormones by initiating transcription of thyroid hormone receptors in target tissues (Bonett et al., 2010). It is unclear whether glucocorticoids play a role in regulating facultative paedomorphosis because the role of glucocorticoids in amphibian metamorphosis is based on work in anurans, all of which are obligate metamorphs (but see Boorse & Denver, 2002). However, in the obligately paedomorphic axolotl (*Ambystoma mexicanum*), metamorphosis can be induced by treatment with a combination of CORT and thyroid hormones (Darras et al., 2002; Kühn et al., 2004; Kühn et al., 2005) suggesting that these hormones may also play a role in regulating facultative paedomorphosis.

Given that a variety of environmental stressors can affect the expression of facultative paedomorphosis, and the clear links between glucocorticoids and obligate metamorphosis in anurans (Denver et al., 1998; Denver, 1997a; 1997b; Marino et al., 2014), we performed a glucocorticoid manipulation study in outdoor mesocosms containing developing larvae of the mole salamander *Ambystoma talpoideum* (Ambystomatidae) to examine the impact of glucocorticoids on facultative paedomorphosis. We used an experimental approach, since observing potentially transient elevations of circulating glucocorticoids during such a long larval period (6–12 months) would be challenging, especially given that glucocorticoids may have organizational effects (Phoenix et al., 1959) such as those documented with 5'-deiodinase activity and T_3 nuclear binding capacity (Bonett et al., 2010; Darras et al., 2002; Galton, 1990; Hayes & Wu, 1995; Kikuyama et al., 1993; Kühn et al., 2005; Niki et al., 1981; Suzuki & Kikuyama, 1983). Guided by the Paedomorph Advantage hypothesis, we predicted that CORT treatment would negatively affect body size, but increase the frequency of metamorphosis.

2 | METHODS

2.1 | Study organism

The geographic range of the facultatively paedomorphic mole salamander *Ambystoma talpoideum* extends along the coastal plains of the Southeastern United States and north into the Central United States (Southern Illinois, Western Kentucky, Southeastern Missouri; Shoop, 1964). We studied populations of *A. talpoideum* at the University of Mississippi Field Station (UMFS), a 318-hectare facility located in the Eocene hills of the interior Gulf Coastal Plain in Lafayette County, MS (34.4274°N, 89.3881°W). At UMFS, adults migrate from terrestrial hibernacula to breed in ponds from late November to early February. Aquatic larvae feed and develop until late spring, when individuals first start metamorphosing. Metamorphosis has not been observed during the peak of summer, but it

resumes in late summer/early autumn. If larvae do not metamorphose in autumn, larvae may remain aquatic, overwinter in ponds and metamorphose in following seasons (Petranka, 1998; Semlitsch, 1985). Individuals may forego metamorphosis and breed as paedomorphic adults, which retain larval characteristics and aquatic lifestyles. Paedomorphism can be advantageous as paedomorphs breed, on average, 6 weeks earlier than metamorphs, which provide their larvae with significant size advantages over offspring of metamorphosed conspecifics (Scott, 1993). Additionally, paedomorphs retain the ability to metamorphose should aquatic conditions deteriorate (Whiteman, 1994).

2.2 | Mesocosm experiment

Thirty-two 1200 L mesocosms (1.83 m diameter \times 0.61 m depth) were constructed on December 12, 2015 in an 8 row \times 4 column arrangement in an open field. Initially, each mesocosm received 2 kg of dry leaf litter (mixed hardwoods) and 50 ml inoculum of concentrated zooplankton from a fishless pond (UMFS pond #61). Mesocosms were filled with well-water, covered with fiberglass screening (1.3 \times 1.13 mm opening) and allowed to age until January 14, 2016 when the screens were sunk below the water surface to allow chironomid and mosquito oviposition. An additional 2.5 L of fishless pond water and filtrate obtained by running 175 L of fishless pond water through 80 μ m mesh was added to each mesocosm on January 30.

A. talpoideum egg masses were collected from ponds and placed in small outdoor wading pools from 4 to 11 January. Upon hatching and before yolk sacs were absorbed, larvae were haphazardly separated into groups of four. Each group of four was then randomly assigned to a mesocosm so that each mesocosm received a total of 12 larvae derived from at least three different egg masses, well within the natural range of density (10/m³) for this species (Semlitsch, 1987b). This procedure was carried out for twelve mesocosms on February 3 and 20 mesocosms on February 6. Larvae failed to establish in two blocks and those mesocosms were removed from the experiment. All mesocosms were treated identically at this stage of the experiment and salamanders were allowed to grow and develop freely. Since tree frogs (Hylidae) frequently oviposit on top of the mesh screens in field mesocosm experiments, tree frog eggs were removed daily.

Visual monitoring for metamorphs began on April 6 and was conducted every three nights. When metamorphs were detected, they were removed from the experiment, measured (see below), and released into ponds at UMFS. Individuals were only measured once they were removed from the experiment to minimize stress. The first metamorph was collected on May 27. At this time, the pools were assigned CORT treatments in a randomized complete block design. Crystalline CORT was obtained from Steraloids, Newport, RI (Cat: #Q1550-000) and dissolved in ethanol before being added to mesocosms. Pools within each of the eight rows (row = block) of four mesocosms (8 \times 4 = 32 mesocosms) were randomly assigned one of four treatments: Control (20 ml ethanol only), Low CORT (0.006 g CORT in 20 ml ethanol, for a 14.43 nM mesocosm water concentration), Medium

CORT (0.03 g in 20 ml ethanol, for a 72.16 nM concentration) and High CORT (0.06 g in 20 ml ethanol, for a 144.31 nM concentration). CORT is readily absorbed via porous branchial and dermal tissue in amphibians (Clay et al., 2019; Glennemeier & Denver, 2002a; Krain & Denver, 2004; Middlemis Maher et al., 2013; Wack et al., 2010). A concentration of 125 nM has been shown to raise whole-body CORT content of *Rana pipiens* tadpoles by approximately 35% (Glennemeier & Denver, 2002b), which corresponds to larval salamander CORT content that falls within the natural range of circulating CORT for *Ambystoma* (Cooperman et al., 2004; Homan et al., 2003a; 2003b; Houck et al., 1996).

Beginning on May 31, 2016, mesocosms received their assigned treatment at night once every 2 weeks until September 18, when the dosing interval was increased to every 3 days. This design purposefully biased CORT exposure to larger larvae in later stages in development because CORT exposure in early development stages (when circulating thyroid hormones are low) is known to slow developmental progress and delay metamorphosis in anurans (Kulkarni & Buchholz, 2014; Wright et al., 1994). The design was also intended to reflect acute “stress” events such as antagonistic encounters with congeners or conspecifics, predator attacks or disturbance, hunger, or diurnal heat stress, wherein larval CORT levels increase but then return to basal levels through negative feedback of the HPI axis (Romero, 2004). Since CORT is known to have organizational effects on tissues, these acute events potentially had long-term effects (Bonett et al., 2010; Niki et al., 1981; Suzuki & Kikuyama, 1983). CORT degrades in water after 48 h, suggesting that treatments were not compounding (Krain & Denver, 2004). The final treatment was administered on October 25 as metamorphosis became infrequent, which is a seasonal pattern observed in past studies conducted at UMFS (Pintar & Resetarits, 2018).

The 368-day experiment concluded on December 14, 2016 when all remaining aquatic (i.e., nonmetamorphosed) individuals were collected from mesocosms. Upon collection, individuals were examined for the expression of secondary sexual characteristics—gravid body condition for females and swollen cloacae for males, which are conspicuous indicators of sexual maturity in *A. talpoideum* (Semlitsch et al., 1988; Whiteman & Semlitsch, 2005; Figure 1). Once removed from the experiment, all individuals were weighed and photographed on a 1 \times 1 mm grid background. Snout-vent length (SVL) was measured using photographs in ImageJ v1.49 (Schneider et al., 2012). Growth rates were determined by dividing SVL by the number of days in the experiment. This study conformed to protocol guidelines (#14-028) of the Institutional Animal Care and Use Committee and the Mississippi Department of Wildlife, Fisheries, and Parks (permit #0521162).

2.3 | Radioimmunoassay

A subset of 19 individuals were sampled 24 h after the first treatment dose and were used to measure whole-body CORT content ($N = 19$, $n = 5$ for Control, Medium and High CORT treatments, $n = 4$ for Low CORT treatment). We sampled individuals 24 h after

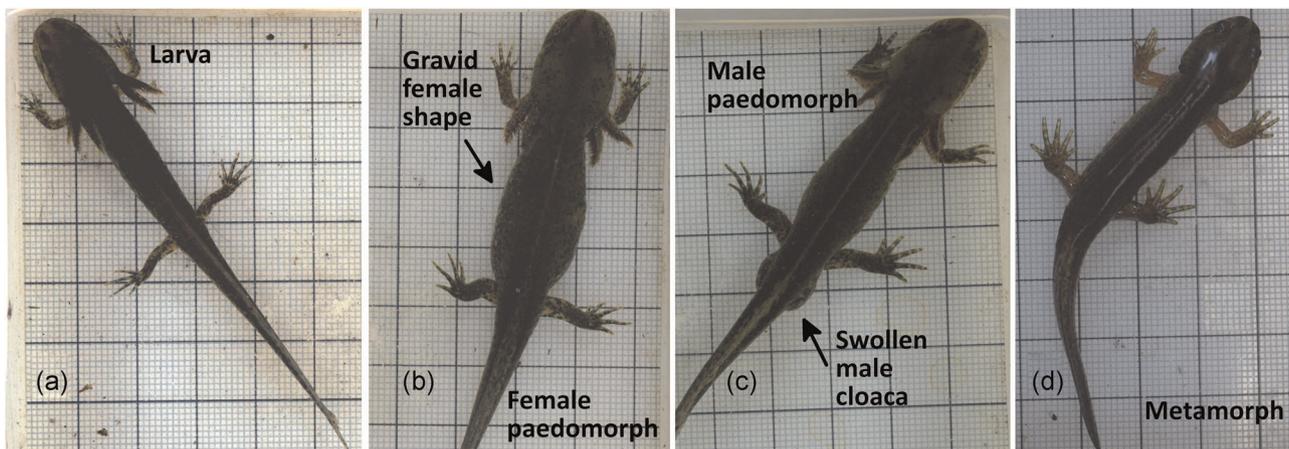


FIGURE 1 The different phenotypes of *Ambystoma talpoideum*. (a) Larva are obligately aquatic and have compressed tail fins, gills, and gill slits, but they lack conspicuous secondary sexual characteristics. (b) Female paedomorphs are also obligately aquatic and have compressed tail fins, gills, and gill slits, but additionally display a gravid body shape and slightly swollen cloaca (when compared to immature individuals). (c) Likewise, male paedomorphs share the same aquatic requirements and traits, but additionally display conspicuously swollen glands around the cloaca (Semlitsch et al., 1988; Whiteman & Semlitsch, 2005). (d) Metamorphs have resorbed or resorbing gills, dry skin, absent or shrinking gill slits, and no swollen cloaca [Color figure can be viewed at wileyonlinelibrary.com]

treatment at night, which is when they emerge from leaf litter refugia and are normally active. This approach was utilized for multiple reasons: (1) to allow the dose to diffuse through the aquatic environment, (2) to allow sufficient time for absorption, (3) to attempt to capture peak whole-body CORT content, and (4) to maintain water quality, which is dramatically reduced if the decomposing leaf litter refugia is searched. This approach was adapted from Krain and Denver (2004), whom showed that a similar dose of CORT (100 nM) to aquarium water elevated whole-body CORT to near peak levels of approximately 22 ng/g body mass in *Xenopus laevis* after 24 h. Individuals were quickly captured from mesocosm tanks with a net and immediately frozen in liquid nitrogen (<2 min). Since established densities used in our salamander mesocosm study were relatively low, we restricted sampling to only four to five individuals per treatment.

Individuals were stored at -20°C until assayed for whole-body CORT. At that time, frozen individuals were weighed, rinsed, measured (SVL), and homogenized while on ice. Each homogenized sample was diluted with purified water (5 ml water per gram biomass), vortexed vigorously for 5 min, and centrifuged at 2200 rpm for 10 min at 4°C and immediately placed back on ice. We then removed 1 ml of the liquid homogenate fraction for radioimmunoassay. We also created “pooled” homogenate samples by combining aliquots from all individuals, which were used as controls in the assay. Controls consisted of water blanks, whole-body pooled samples that were stripped with dextran-coated charcoal, and unstripped samples. The pooled stripped and unstripped samples received four different volumes of standard CORT (0, 10, 50, and 100 μl), each in duplicate.

Samples were then incubated overnight with radiolabeled CORT (PerkinElmer, Inc.) for determination of recoveries. CORT was then extracted from samples using dichloromethane, dried under nitrogen gas at

40°C , resuspended in 10% ethyl acetate in iso-octane, and loaded on to diatomaceous earth columns containing a diatomaceous earth:distilled water “glycol trap” and a propanediol:ethylene glycol mixture for hormone separation (see Leary & Crocker-Buta, 2018). The CORT fraction was collected using stepwise elution with increasingly polar mixtures of ethyl acetate in iso-octane. The CORT fraction was dried under nitrogen and resuspended in phosphate-buffered saline. The CORT antibody was purchased from MP Biomedicals, LLC (Cat: #07120016; Solon). All samples were assayed in duplicate. Samples were analyzed in a single assay. The mean intra-assay coefficient of variation for CORT based on four standards was 4.48% (10 μl of standard CORT), 3.59% (50 μl standard CORT) and 10.61% (100 μl CORT). All samples fell within the sigmoid standard curve and were above the lower limits of detection (~ 10.0 pg/g). The assay was validated by examining parallelism of the pooled stripped and unstripped samples. There was no evidence of heterogeneity in slopes across CORT standards ($F_{2, 18} = 2.26$; $p = .133$).

2.4 | Statistical analyses

Morphometrics and life history variables were analyzed using linear mixed effects models (LMMs). For variables that followed binomial distributions (survival and phenotype) we used generalized linear mixed effects models (GLMMs). Significance for LMMs was tested with approximate F tests (Type III Satterthwaite), while significance for binomial GLMMs was tested with likelihood ratio tests of nested models (Bolker et al., 2009; Warton & Hui, 2011).

Survival and phenotype were modeled as binomial logistic GLMMs with treatment and block as fixed and random effects, respectively, for both models, and survival was used as a fixed covariate for the phenotype model. However, including treatment as a predictor in the phenotype model caused complete separation, which is a common issue

in logistic regression where model estimates cannot be generated due to one treatment having all the same outcome (Allison, 2004). Thus, phenotype data were converted to proportions (aggregated by mesocosm) and were analyzed with a LMM with treatment and survival as fixed effects and block as a random effect. Since the proportion of metamorphs essentially determines the proportion of paedomorphs, it is inappropriate to analyze both individually; thus, we only analyzed the proportion of metamorphs. Larval period, body mass, SVL, and growth rate were analyzed using LMMs where treatment and survival were included as fixed effects and mesocosm nested within block was included as a random effect to assure statistical independence. Larval period was analyzed in two ways: (1) only metamorphs: number of days since mesocosm introduction until metamorphosis and (2) metamorphs + paedomorphs: number of days since mesocosm introduction until metamorphosis or until the end of the experiment (since all paedomorphs at the end of the experiment are non-larval). Body condition (size corrected mass) was analyzed with a LMM with mass as a response variable, treatment, SVL, and survival as fixed effects (Garcia-Berthou, 2001), and mesocosm nested within block as a random effect. Last, we examined the effect of body mass on phenotype by fitting a logistic GLMM with phenotype (paedomorph or metamorph) as the response variable, body mass as a fixed effect, and mesocosm nested within block as a random effect.

Ordered mean change post hoc tests (*mean_chg.emmc* from *emmeans*) were used to compare treatment effects since our treatments (doses) contained an inherent ordered structure. For our data, this test produced three comparisons that evaluated whether the marginal mean was significantly changing with each increasing level of dose: (1) (Low + Medium + High)/3–Control, (2) (Medium + High)/2–(Control + Low)/2, and (3) High–(Control + Low + Medium)/3 (Lenth, 2020). All analyses used $\alpha = .05$ and were performed in R v3.4.0 (R Core Team, 2018) using the *lme4* v1.1.13 package for mixed models (Bates et al., 2015), *emmeans* v1.5.1 for post hoc tests and marginal mean estimations (Lenth, 2020), and *ImerTest* v2.0.33 (Kuznetsova et al., 2015) for approximate *F* tests. Figures were made with *ggplot2* v2.2.1 (Wickham, 2009).

3 | RESULTS

Mean whole-body CORT content in larval *A. talpoideum* increased proportionately with increasing CORT dose across treatments (Figure 2). Our highest CORT dose produced whole-body content levels of 2961.38 ± 321.25 pg/g (mean \pm SE), which were similar to whole-body content found in larval *A. jeffersonianum* exposed to confinement protocol (~3000 pg/g) by Chambers et al. (2011). Other studies on *Ambystoma* CORT content mostly considered only larger adults and only measured blood plasma CORT content, which ranged from approximately 200–45000 pg/ml (Carr & Norris, 1988; Cooperman et al., 2004; Homan et al., 2003a; 2003b; Houck et al., 1996; Millikin et al., 2019). However, Baugh et al. (2018) showed there is a fairly tight correlation ($r = .81$) between blood plasma and whole-body CORT

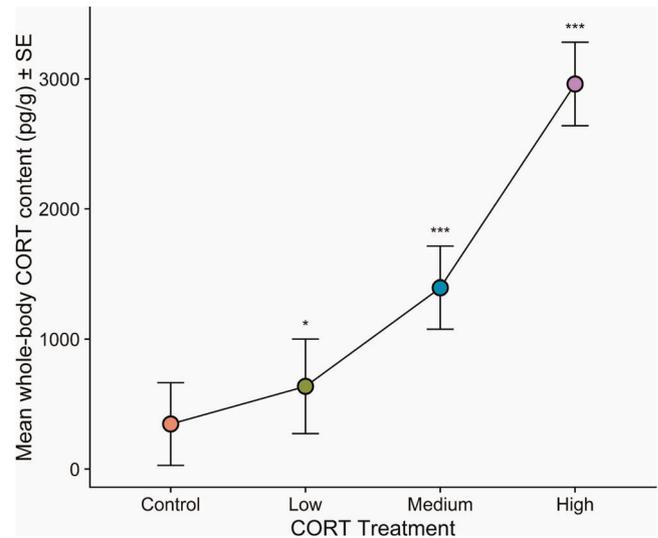


FIGURE 2 Marginal mean of whole-body CORT content (pg/g) of larval salamanders ($N = 19$) across the four treatments ($F_{3, 15} = 13.67$; $p < .001$). Individuals were removed from mesocosms 24 h after CORT administration and immediately frozen (<2 min). Post hoc significance, denoted by asterisks, is determined by ordered mean change with increasing CORT dosage. * $p \leq .05$, ** $p \leq .01$, *** $p \leq .001$. CORT, corticosterone [Color figure can be viewed at wileyonlinelibrary.com]

content (in frogs), but that whole-body CORT levels measured approximately 80%–85% lower than plasma CORT. Thus, our values appear to be within the ranges reported for *Ambystoma*, but more work needs to be done with natural populations of *A. talpoideum*.

Survival did not differ among treatments ($\chi^2 = 0.811$; $p = .847$), but the proportion of metamorphs (and thus paedomorphs) differed significantly among treatments ($F_{3, 18} = 6.21$; $p = .004$). Controls had the highest proportion of paedomorphs (39%) while the proportion of paedomorphs decreased with increasing CORT dose; treatments with High CORT dose produced no paedomorphs (Figure 3). Metamorphs had lower body mass and paedomorphs had higher body mass across all treatments ($\chi^2 = 69.78$; $p < .001$; Figure 4). There was an effect of CORT treatment on body size; higher CORT doses resulted in lower body mass ($F_{3, 77} = 7.04$; $p < .001$) and shorter SVL ($F_{3, 77} = 5.09$; $p = .003$) compared to controls (Figure 5a). There were marginally nonsignificant differences among treatments in growth rate ($F_{3, 74.83} = 2.37$; $p = .077$), and in body condition ($F_{3, 9.46} = 3.69$; $p = .057$) after controlling for SVL ($F_{1, 72.89} = 683.70$; $p < .001$; Figure 5b). Growth rate appeared to trend upwards with increasing CORT dosage (Control = 0.20 ± 0.02 [marginal mean \pm SE], Low = 0.23 ± 0.02 , Medium = 0.22 ± 0.02 , High = 0.24 ± 0.02), while body condition appeared to trend downward (Control = 4.43 ± 0.16 , Low = 4.49 ± 0.17 , Medium = 3.89 ± 0.17 , High = 4.11 ± 0.18). When considering only metamorphs, individuals did not differ in larval period among treatments (Control = 203.30 ± 21.53 , Low = 180.19 ± 20.60 , Medium = 195.35 ± 21.04 , High = 180.64 ± 19.79 ; $F_{3, 53.27} = 0.52$; $p = .672$). However, when considering all non-larval individuals (metamorphs + paedomorphs), increasing CORT dosage decreased larval period (Control = 251.10 ± 16.70 , Low = 219.58 ± 17.64 ,

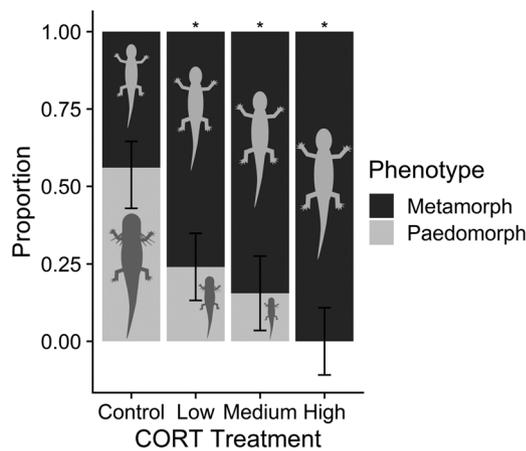


FIGURE 3 Marginal adult phenotype ratios across the four CORT treatments at the end of the experiment. The proportion of paedomorphic adults decreased with increasing dose of CORT ($F_{3, 18} = 6.21$; $p = .004$). Post hoc significance, denoted by asterisks, is determined by ordered mean change with increasing CORT dosage. * $p \leq .05$, ** $p \leq .01$, *** $p \leq .001$. CORT, corticosterone

Medium = 215.80 ± 17.94 , High = 181.66 ± 17.94 ; $F_{3, 73.58} = 3.11$; $p = .032$). Increased survival resulted in decreased SVLs ($F_{1, 77} = 6.13$; $p = .015$), which is consistent with classic density-dependent growth patterns seen with amphibians (Wilbur & Collins, 1973).

4 | DISCUSSION

Deteriorating or “stressful” aquatic conditions can stimulate production of corticotropin-releasing factor that modulates production of adrenal/interrenal glucocorticoids and thyroid hormones, and accelerates metamorphosis in amphibians (Boorse & Denver, 2002; Denver, 1997a;

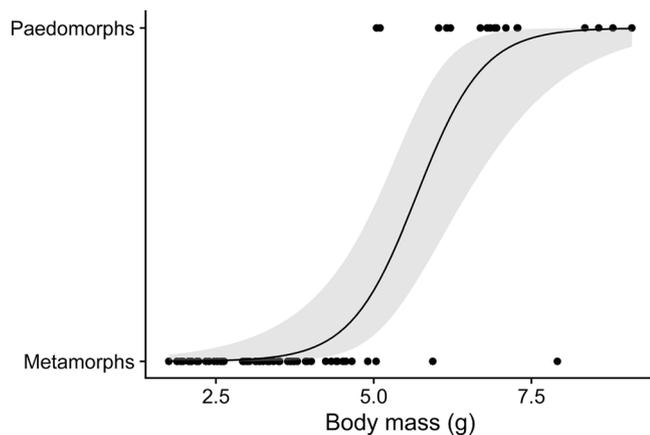


FIGURE 4 Logistic plot of final body mass (g) and adult phenotype at the end of the experiment of all individuals ($\chi^2_1 = 69.78$; $p < .001$). Fitted curve and confidence intervals are derived from fixed effects only. As predicted by the paedomorph advantage hypothesis (Whiteman, 1994; Wilbur & Collins, 1973), paedomorphs were larger in body size

Denver, 1999; Denver, 2017). Consistent with these findings, treatment with exogenous glucocorticoids increased the probability of metamorphosis and decreased the probability of paedomorphosis in *A. talpoideum*, supporting a role for glucocorticoids in the regulation of facultative paedomorphosis (Figure 6). Paedomorphs may therefore develop from larvae that experience favorable environments and low glucocorticoid levels; however, more work is required to relate these experimental findings to natural variations in CORT content of developing larvae, especially with regards to body size (Moore et al., 2020), developmental stage, and temporal variation.

Body size of all *A. talpoideum* individuals (regardless of phenotype) was inversely related to CORT dose. This effect was primarily driven by earlier metamorphosis in High CORT treatments that resulted in smaller body size than individuals from other treatments with later metamorphosis (due to additional growth acquired during a longer larval stage). Growth history and size at metamorphosis are important determinants of adult phenotype and fitness; larger body size correlates with increased fecundity and lifetime reproductive success (Alford & Harris, 1988; Beachy et al., 1999; McCormick & Hoey, 2004; Ryan & Semlitsch, 2003; Semlitsch et al., 1988; Whiteman et al., 2012; Wilbur & Collins, 1973). Common environmental stressors of amphibian larvae (e.g., pond desiccation, high conspecific density, and predation risk) can elevate glucocorticoids, decrease body size, and have deleterious long-term effects on fitness. However, the fitness costs of small body size are likely to be outweighed by the fitness benefits of survival if these individuals can escape potentially lethal stressors in the aquatic environment (e.g., predation, desiccation).

In anurans, the effects of environmental stressors and elevated glucocorticoids on life history traits can differ depending on larval stage (Kulkarni & Buchholz, 2014; Wright et al., 1994). CORT slows down development in premetamorphosis, but accelerates development in prometamorphosis (Belden et al., 2005; Frieden & Naile, 1955; Gray & Janssens, 1990; Hu et al., 2008; Kikuyama et al., 1983; Kobayashi, 1958). Because our experimental design focused on later stage larvae that were at or near the minimum body size required for metamorphosis (Semlitsch, 1987a), it allowed for analysis of predictions associated with the Paedomorph Advantage hypothesis (Whiteman, 1994; Wilbur & Collins, 1973). This hypothesis predicts that favorable conditions (e.g., Controls) produce large individuals that develop into paedomorphs, whereas increasingly unfavorable conditions (simulated here by CORT treatment dosage) produce smaller, slower growing individuals that are more likely to metamorphose. However, the minimum body size required for metamorphosis may represent a developmental threshold (Day & Rowe, 2002) similar to that between pre- and prometamorphosis in anuran larvae. This same process may be occurring in salamanders, where below the body size threshold is analogous to premetamorphosis and above the body size threshold is analogous to prometamorphosis.

Environmental stressors, and thus the effects of glucocorticoids, acting on a population of larval salamanders whose size distribution spans this body size threshold can potentially unify the Paedomorph Advantage, Best of a Bad Lot, and Dimorphic Paedomorph hypotheses proposed by Whiteman (1994). For example, stressors acting on

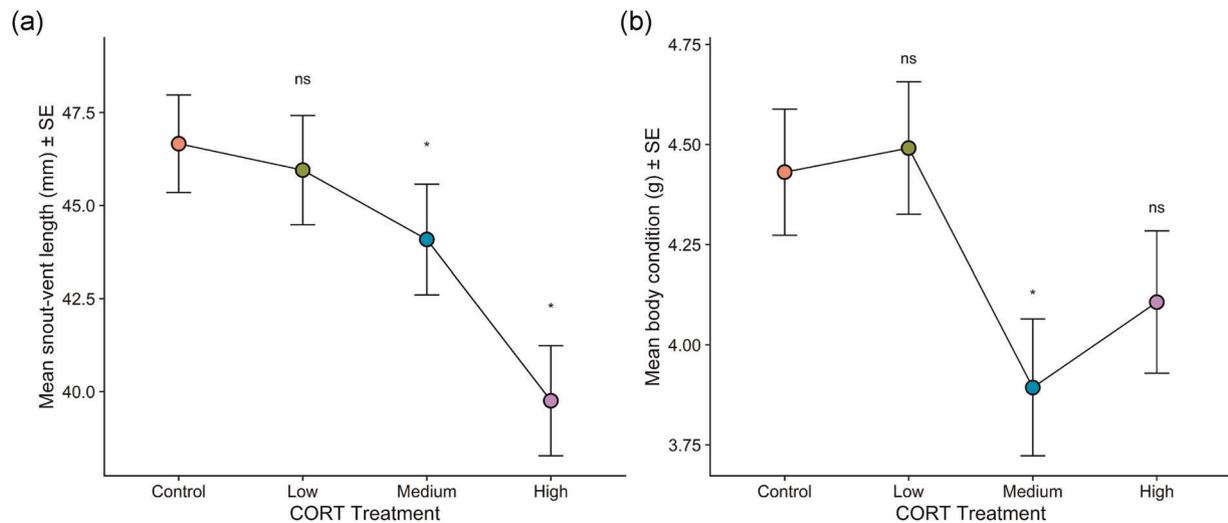


FIGURE 5 (a) Marginal mean snout-vent length (mm) of individuals across the four CORT treatments at the end of the experiment after removal from the mesocosm experiment ($F_{3, 77} = 5.09$; $p = .003$). Larger sizes at metamorphosis are positively correlated with fitness (Semlitsch et al., 1988). (b) Marginal mean body condition (SVL-adjusted grams) of individuals across the four CORT treatments ($F_{3, 9.46} = 3.69$; $p = .057$). Post hoc significance, denoted by asterisks, is determined by ordered mean change with increasing CORT dosage. * $p \leq .05$, ** $p \leq .01$, *** $p \leq .001$, nonsignificant (ns)= $p > .05$. CORT, corticosterone [Color figure can be viewed at wileyonlinelibrary.com]

individuals below the body size threshold can decrease somatic development and, if body size never crosses the size threshold during the growing season, individuals may develop into Best of a Bad Lot (i.e., small) paedomorphs during the next reproductive season. Stressors acting on individuals above the body size threshold during the growing season can elicit metamorphosis, while individuals experiencing few stressors while above the body size threshold develop into Paedomorph Advantage (i.e., large) paedomorphs during the next reproductive season. More work is required to firmly establish the growth and developmental effects of CORT above and below this threshold.

Facultative paedomorphosis has been viewed as a specific plastic response to spatially and temporally variable hydroperiod (Semlitsch & Gibbons, 1985; Semlitsch, 1987a; Wilbur & Collins, 1973), conspecific larval densities (Harris, 1987; Semlitsch, 1987a), resource availability (Denoël & Poncin, 2001; Ryan & Semlitsch, 2003; Semlitsch, 1987a), temperature (Sprules, 1974) and other relevant environmental factors (Denoël & Ficetola, 2014). However, instead of responding directly to variation in specific environmental factors (cues), larval salamanders may be responding to the cumulative effects of one or more interchangeable environmental stressors that affect CORT production. Under this framework, polyphenic salamanders have a general response that is useful for unpredictable, heterogeneous habitats (Moran, 1992; Wilbur & Collins, 1973).

Salamanders inhabit a wide variety of ecosystems that vary both spatially and temporally in the relative quality of available habitat types (aquatic vs. terrestrial). These environments can select for either fixation of a phenotype or maintenance of a polyphenism, thereby producing a wide range of life history strategies across diverse lineages. For example, evolutionary processes may be different for populations living in habitats with harsh, surrounding terrestrial environments, like alpine lakes, where selection favors paedomorphs and metamorphosis is

infrequent (e.g., montane *Ambystoma tigrinum*) or lost (e.g., *Ambystoma mexicanum*—axolotls; Sprules, 1974; Whiteman, 1994; Wilbur & Collins, 1973). However, the maintenance of facultative paedomorphosis does not require harsh terrestrial environments to select against metamorphs, as facultative paedomorphosis is common in areas with suitable terrestrial habitats (Denoël & Ficetola, 2014). Paedomorphosis can be maintained with available suitable terrestrial habitat due to greater expected fitness of paedomorphs in certain contexts (Denoël et al., 2005). For example, paedomorphs can reach large body sizes (Rose & Armentrout, 1976; Whiteman, 1994), have reduced age at first reproduction (Ryan & Semlitsch, 1998; Semlitsch et al., 1988; Semlitsch, 1985) and earlier seasonal oviposition (Ryan & Plague, 2004), which can have large fitness benefits for paedomorphs over metamorphs (Cole, 1954; Roff, 1992; Stearns, 1992).

Our experiment shows that the dynamics of life histories and phenotypic expression of polyphenisms can be affected by glucocorticoids. The maintenance of this polyphenism may, therefore, depend not only on evolutionary fitness tradeoffs between the alternative phenotypes, but also on spatial and temporal heterogeneity of the landscape of environmental stressors (Jessop et al., 2013). Past research has investigated specific links between each individual environmental stressor and the expression of facultative paedomorphosis (Bohenek & Resetarits, 2018; Harris, 1987; Ryan & Plague, 2004; Semlitsch & Wilbur, 1988), but our data may suggest a more parsimonious, comprehensive mechanism based on the common currency of glucocorticoids. However, performing loss of function experiments (where hormone production or action is blocked), characterizing the stress response of individuals in natural ponds, and investigating the differential endocrine impacts of environmental stressors are required to completely describe the physiological mechanism. This mechanism provides a framework for the integration of multiple environmental

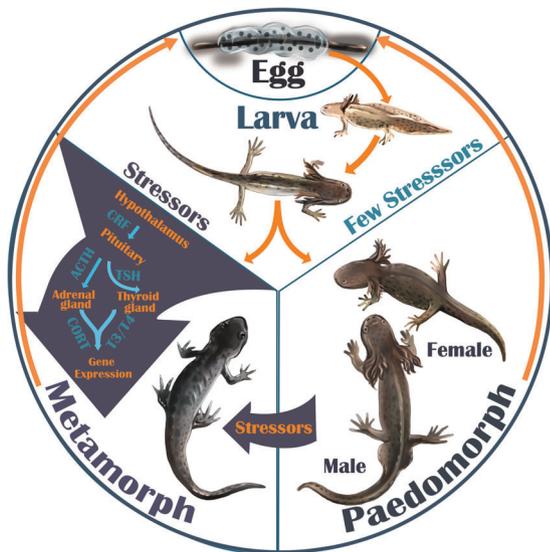


FIGURE 6 Model incorporating endocrine mechanisms regulating facultative pedomorphosis. Under the pedomorph advantage hypothesis, and above a minimum body size, larvae that are exposed to environmental stressors (e.g., crowding, pond drying, predator presence, starvation) and have at least reached the minimum body size required for metamorphosis (35 mm; Semlitsch & Wilbur, 1988) are more likely to metamorphose than become pedomorphic. Stressors activate the HPI axis (blue arrow) releasing CRF from the hypothalamus and adrenocorticotropic hormone and TSH from the anterior pituitary. Adrenocorticotropic hormone stimulates the adrenal gland to release CORT, while TSH stimulates the thyroid gland to release thyroid hormones (T_3/T_4). CORT and thyroid hormones act synergistically to affect gene expression in target tissues resulting in metamorphosis (left; reviewed in Denver, 2017). Few, or absent, stressors do not sufficiently activate the HPI axis and metamorphosis is delayed resulting in aquatic pedomorphs (right). Both metamorphs and pedomorphs attain sexual maturity, but pedomorphs reach sexual maturity earlier (Ryan & Semlitsch, 1998), potentially increasing lifetime expected fitness (Cole, 1954; Roff, 1992; Stearns, 1992). [Color figure can be viewed at wileyonlinelibrary.com]

stressors that simultaneously (or sequentially) impact life history trajectories and may be broadly applicable to other life history polyphenisms and polymorphisms in a variety of taxa.

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CONFLICT OF INTERESTS

The authors declare that there are no conflict of interests.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available in figshare at <https://doi.org/10.6084/m9.figshare.7300403>, reference number 10.6084/m9.figshare.7300403.

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