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# THE EFFECTS OF SUBSTRATE pH ON GROWTH AND SURVIVAL OF RECENTLY METAMORPHOSED MARBLED SALAMANDERS (*AMBYSTOMA OPACUM*)

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**Abstract.**—In terrestrial habitats with a history of mining activity, understanding the effects of soil acidification on the ecology of amphibians is an important part of the restoration process and of the conservation of local amphibian populations. After metamorphosis, pond-breeding amphibians spend much of their life in direct contact with the soil in upland habitat adjacent to aquatic breeding sites. Given the thin, permeable skin of amphibians, abiotic soil characteristics likely affect their physiology, behavior, and fitness. We reared recently metamorphosed Marbled Salamanders (*Ambystoma opacum*) in the laboratory at four pH levels (pH 4, 5, 6 and 7) to evaluate the effect of soil acidity on determinants of fitness, such as growth and survival. A pH of 4 was lethal to recent metamorphs, but there were no significant differences in survival among the three higher pH treatments. Among animals surviving the higher pH treatments, individuals reared on substrates of pH 5 were shorter in both total length and snout-vent-length by the end of the experiment than individuals reared on substrates of pH 6 and pH 7. We suggest that substrate acidity can affect the fitness of *A. opacum* through direct mortality and through a decrease in the growth rate of salamanders subsequent to metamorphosis. Thus, negative effects of post-mining acidification likely extend into terrestrial habitats for pond breeding amphibians.

**Key Words.**—environmental contamination; juvenile amphibian; habitat destruction and modification; strip mining reclamation; sulfuric acid

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## INTRODUCTION

The importance of salamanders in forested ecosystems has been underestimated historically (Burton and Likens 1975; Davic and Welsh 2004). However, as we learn more about their ecology, the importance of salamander populations to ecosystem function is becoming more apparent. For example, salamanders form a large proportion of forest biomass (Welsh and Droege 2001) and drive important forest ecosystem processes such as leaf litter retention and carbon sequestration as predators on the small invertebrates that break down forest litter (Best and Welsh Jr. 2014; Semlitsch et al. 2014; Hickerson et al. 2017). In addition, the importance of studies aimed at understanding the ecology of salamanders is exacerbated by evidence of the widespread decline of amphibian species globally (Stuart et al. 2004; Boone et al. 2007; Rovito et al. 2009; Adams et al. 2013; Caruso and Lips 2013).

Declines in amphibian species have been linked to a variety of factors, but greatly attributed to habitat loss, fragmentation, disease, and anthropogenic disturbance (Collins and Storfer 2003; Cushman 2006; Catenazzi 2015). Habitat loss is especially harmful to migrating amphibians, which have breeding sites separated from terrestrial foraging areas or overwintering sites (Gibbs and Shriver 2005). The removal of tree cover is a

major contributor to habitat loss and has been shown to significantly reduce population size of some amphibian species, with post-removal recovery to original population levels taking up to 50 y (Petranka et al. 1993; Ash 1997; Semlitsch et al. 2009). However, the degree to which different amphibian species are affected by activities such as logging varies (Felix et al. 2010). In addition to habitat loss caused by changes in land use, a variety of other human activities affect amphibian diversity by degrading existing habitat. For example, chemical runoff from fertilizers and pesticides decrease larval growth and survival rates in amphibians (Boone et al. 2004, 2007).

Strip mining is a land-use activity that has the combined negative effects of habitat loss due to deforestation and habitat degradation due to leaching of chemical contaminants. Strip mining in the USA is largely focused in Appalachia, a region ranging from southern New York to northern Alabama along the Appalachian Mountains. There is no clear metric on how much land is affected by mining in the U.S.; however, a 2009 study using vector data estimated that approximately 1.2 million acres of central Appalachia, approximately 12% of the total land area, have been mined (Ross Geredient, unpubl. report). The process of mining consists of the removal of vegetation, followed by the removal and homogenization of soil (Indorante

et al. 1981). The negative effects of strip-mining on terrestrial habitats have long been recognized (Lawrey 1977a; 1977b; Wali 1999), and as such, reclamation efforts are required after mining activities have been completed. Soil from reclaimed mine sites is commonly acidic (Plass and Vogel 1973; Thurman and Sencindiver 1986), has low organic material and nutrient content (Palmer et al. 2010), and frequently is contaminated with heavy metals (Yao et al. 2010). The problems caused by mining are especially apparent in Appalachia, which is notable for frequent mining activity because of large shallow deposits of coal (Guernsey 1960).

Current reclamation efforts in Appalachia are increasingly coordinated by the Appalachian Regional Reforestation Initiative (ARRI), which is a coalition of groups consisting of industry, private citizens, and the government. The ARRI promotes the Forestry Reclamation Approach (FRA) as the basis of their reclamation efforts, which emphasizes five primary steps in a successful reclamation process (Angel et al. 2009). The first step involves the selection of a suitable rooting medium (i.e., soil) at least 1.22 m (4 ft) deep. The suggested medium is one with low to moderate levels of salts, low sulfur, appropriate texture for drainage, and with a pH between five and seven, which is considered ideal for native hardwood diversity.

Whereas current reclamation processes focus on the organic and inorganic soil conditions necessary for the growth of tree species, explicit attention is not paid to the animal components of the forest ecosystem, such as amphibians. Ambystomatid salamanders are forest-dwelling and pond-breeding amphibians that have been found to select among alternative microhabitats for various soil properties, including pH (Mushinsky 1975) and moisture (Sugalski and Claussen 1997), and between soils collected from grassland and forest (Rittenhouse et al. 2004). Their ability to discriminate among microhabitats suggests that selected sites provide fitness advantages to ambystomatid salamanders.

Although water acidity has been shown to affect oviposition site selection in various pond breeding salamanders (Fairman et al. 2013), and growth and survival of both larval frogs (Tyler-Jones et al. 1989; Beattie and Tyler-Jones 1992; Rowe et al. 1992) and salamanders (Pough and Wilson 1976; Cook 1983; Ling et al. 1986; Ireland 1991; Brodman 1993), research on the effects of substrate acidity on post-metamorphic amphibians is limited. The fully terrestrial, direct-developing Red Backed Salamander (*Plethodon cinereus*) shows reduced levels of growth and oxygen consumption in chronic acidic conditions (Wyman and Hawksley-Lescault 1987), but sensitivity to acidic substrates has been shown to vary across species (Wyman and Hawksley-Lescault 1987; Wyman 1988). Best and Welsh (2014) suggest that levels of acidity that

reduce the growth and distribution of forest salamanders have the potential to change the makeup of the forest floor ecosystem, of which salamanders play an essential role as one of the top predators. Furthermore, both the Marbled Salamander (*Ambystoma opacum*) and the Spotted Salamander (*Ambystoma maculatum*) prefer higher substrate pH when given a choice between pH 5.5 and 7.7 (Mushinsky 1975), potentially affecting the distribution of adults. For example, the Spotted Salamanders in New York occur only at sites with soil pH of 4 or higher (Wyman 1988).

Further study is necessary to determine how salamander life-history characteristics are being affected by environmental degradation. We performed a laboratory study to determine the effect of pH on the growth and survival of recently metamorphosed Marbled Salamanders. We hypothesize that substrate acidity affects growth and survival of Marbled Salamanders. We predict that as substrate acidity increases, salamander growth and survival will decline. These declines potentially indicate a future reduction in reproductive success (i.e., fitness). The results of this work would indicate the importance of soil pH as an abiotic characteristic when addressing the recovery of mined sites.

## MATERIALS AND METHODS

**Study species.**—The Marbled Salamander is found throughout the southeastern USA, including much of Appalachia (Petranka 1998). Breeding occurs in the fall and winter, and males typically court females prior to or during migration to breeding sites (Krenz and Scott 1994). Adults migrate to breeding sites between late summer and winter; eggs are laid in dried pond beds that eventually are inundated with water when the ponds fill (Petranka 1998). Larvae feed primarily on macro-zooplankton, and larger larvae will eat other amphibian larvae and eggs (Petranka and Petranka 1980). In Kentucky, metamorphosis typically occurs in mid to late May (Keen 1975; Petranka 1998). After metamorphosis, juveniles disperse through leaf litter and course, vegetative debris adjacent to ponds, typically up to 250 m away from the edge of wetlands (Stenhouse 1985; Semlitsch 1998). Success of the juvenile stage is especially important for pond-breeding salamanders because of the potential for large, natural fluctuations in larval survival, where some years can experience failures of the entire larval cohort due to poor aquatic habitat conditions (Pechmann et al. 1991; Taylor et al. 2006).

**Experimental design.**—We collected late-stage, free-swimming larval Marbled Salamanders (20–25 mm, 0.5–0.8 g) from an autumnal pool in Hart County,

Kentucky, USA, near the Green River Preserve of Western Kentucky University during the spring of 2015. We housed each larva in a 473 mL (16 oz) PET plastic deli cup (Solo Cup Co., Lake Forest, Illinois, USA), and we fed them brine shrimp (*Artemia* sp.) and California Blackworms (*Lumbriculus variegatus*) every 2–3 d until metamorphosis, which occurred within two weeks of capture. We assigned newly metamorphosed salamanders to one of four treatment categories: pH 4, pH 5, pH 6, and a neutral pH control group. We randomly assigned larvae to a treatment as they metamorphosed. For every four salamanders that metamorphosed, we assigned one to each of the four treatments. This increased the sample size of each treatment by one for each set of four metamorphosed salamanders. For all treatments, we raised juvenile salamanders at 20° C on a 12L:12D cycle. For each individual, we began the experiment when metamorphosis was completed, rather than waiting for all individuals to metamorphose before initiating the experiment, to account for potential differences in fitness between individuals that metamorphosed early or late.

During the experiment, we housed each metamorphosed individual in a 473 mL (16 oz) PET plastic deli cup with a foam substrate saturated with about 80 ml modified 40% Holtfreter's solution (60 mM NaCl, 2.4 mM sodium bicarbonate, 0.8 mM calcium chloride, 0.67 mM potassium chloride). We used 1M sulfuric acid diluted with Holtfreter's solution to adjust pH to 4, 5, or 6 in the three reduced pH treatments. Although the use of buffering agents would have helped maintain a constant pH in each treatment, we did not want to confound our experimental design through the use of different buffered solutions in each treatment. Thus, we opted to replace the treatment pH solutions frequently throughout the experiment. During a trial prior to the beginning of the experiment, we found that acid solutions would maintain the desired treatment pH range for up to 3 d. Therefore, we rinsed and re-saturated the foam substrate with newly adjusted Holtfreter's solution in all treatments every 3 d. Also, we fed all salamanders an equal amount of food every 3 d. We fed salamanders flightless Common Fruit Flies (*Drosophila hydei*) during the first 60 d of the experiment, and switched to small House Crickets (*Acheta domesticus*) during the final 60 d of the experiment. For each feeding, we offered an equal number of individual fruit flies or crickets to each individual.

We placed each salamander in a clear empty deli cup to measure snout-vent length (SVL) and total length (TL) to the nearest 1 mm with a ruler, and we used a digital scale to weigh each salamander to the nearest 1 mg. We took measurements every two weeks during the experiment, and we exposed individuals to their treatment substrates for 120 d (from metamorphosis in

June 2015 until November 2015). We terminated the experiment when the ambient temperature near the natal pond collection site dropped below 0° C, when we presumed metamorphs in the wild would have moved to underground burrows for overwintering (Semlitsch 1983).

**Statistical analyses.**—We used the survival package in R (Therneau 2015) to create salamander failure time curves (i.e., survivorship curves). We used Growth Curve Analysis (Mirman et al. 2008) to analyze growth in terms of change in mass and length over time. For each measurement (SVL, TL, and mass), we constructed a series of models to simulate the growth curve, and these models were compared to observe differences in growth patterns between treatments. Before models were constructed, we transformed time data into first and second order orthogonal polynomials to avoid problems of collinearity among data points (Mirman et al. 2008).

We constructed models using linear mixed-effect regression and evaluated them with likelihood ratio tests using the R package lme4 (Bates et al. 2015). Mixed-effect models include both fixed and random variables, which allowed us to separately evaluate the response of individuals (i.e., the random effects) and the effect of the treatments (i.e., the fixed effects). Further, mixed-effect regression was required because of the repeated measurement of individuals throughout the study. We evaluated the significance of fixed effects of the best-performing models using the R package lmerTest (Kuznetsova et al. 2017), which implements the Satterthwaite method for approximating degrees of freedom for *t*-tests. Regression models were as follows: one of three measurements of growth (SVL, TL, and mass) as the dependent variable, pH treatment levels and time elapsed as fixed effect terms, and salamanders ID as a random effect terms. We evaluated the magnitude of the effects of model parameters with analysis of variance (ANOVA) for model selection. We evaluated model effects by a log-likelihood test distributed as  $\chi^2$  values using the number of added parameters levels as degrees of freedom. We fitted and graphed growth curve models using the ggplots2 package in R (Wickham 2009). We used R version 3.2.4 for all analyses (R Core Team 2015). For all tests,  $\alpha = 0.05$ .

## RESULTS

There was no significant difference between the initial treatment groups for body mass ( $F_{2,55} = 0.402$ ,  $P = 0.671$ ), SVL ( $F_{2,55} = 0.009$ ,  $P = 0.991$ ), or total length ( $F_{2,55} = 1.28$ ,  $P = 0.286$ ). Survival times varied among treatments from 3 d until the end of the experiment (Fig. 1). The pH treatment level had a significant effect on salamander survival ( $F_{3,97} = 31.49$ ,  $P < 0.001$ ). By day 38

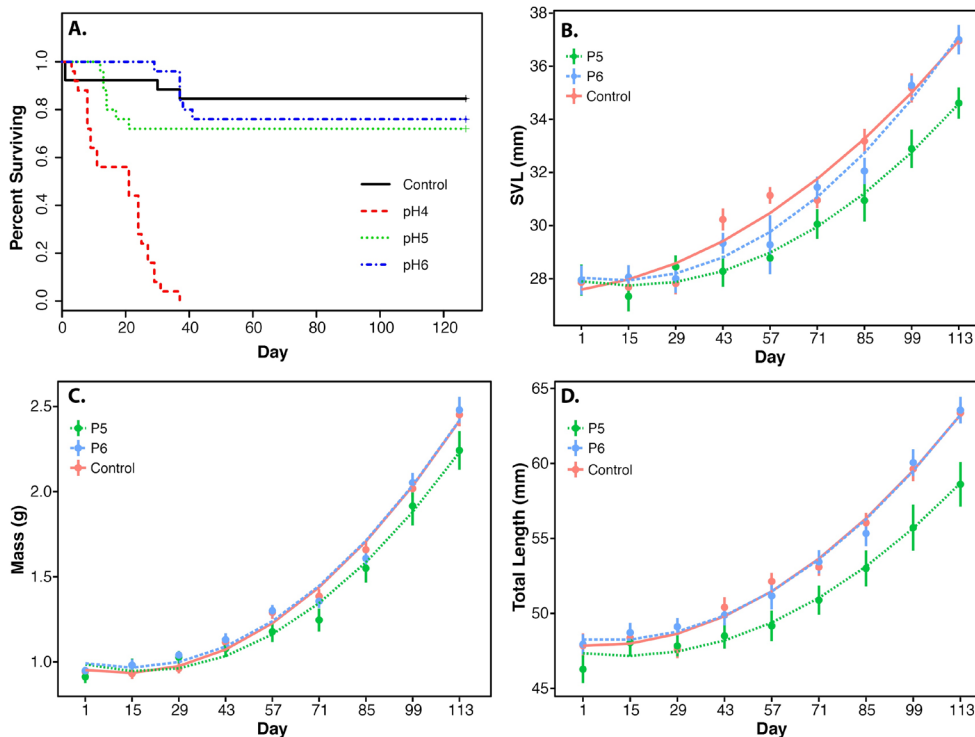


FIGURE 1. Survival and growth of recently metamorphosed Marbled Salamanders (*Ambystoma opacum*) from Hart County, Kentucky, USA. A) Failure-time curve indicating proportion of individuals surviving over the course of 113 d on substrates at pH 4, pH 5, pH 6 and an unadjusted control substrate at pH 7. (B-D) Growth data and growth curve model fits for change in B) snout vent length, C) mass, and D) total length. Symbols indicate observed mean data points, dashed lines indicate model fits of growth curves, and vertical lines indicate  $\pm$  one standard error (SE).

of the experiment, all individuals in the pH 4 treatment had died, and survival of salamanders in this treatment differed significantly from the other treatments and the control (Table 1). Survival times were not significantly different among salamanders in the pH 5, pH 6, and control (pH 7) treatments (Table 1).

For both length and SVL, the Linear model that contained the interaction of treatment and time (represented as first order orthogonal polynomials) showed a significant improvement in model fit over the Treatment model lacking the interaction term (Table 2). For mass, none of our models containing a pH treatment

TABLE 1. Tukey HSD table for analysis of time to survival across pH treatments for recently metamorphosed Marbled Salamanders (*Ambystoma opacum*). Diff is the difference between scores, lwr and upr are the lower and upper ranges of the confidence interval, and *P* adj is the adjusted *P* value for the test.

Comparisons	Diff	lwr	upr	<i>P</i> adj
pH4-Control	-92.20	-120.52	-63.87	< 0.001
pH5-Control	-14.51	-42.84	13.81	0.543
pH6-Control	-4.83	-33.16	23.49	0.974
pH5-pH4	77.68	49.08	106.28	< 0.001
pH6-pH4	87.36	58.76	115.96	< 0.001
pH6-pH5	9.68	-18.92	38.28	0.812

term showed significant improvement over the Base model (Table 2). Individuals exposed to pH 5 showed significantly reduced growth, relative to the individuals in the control group, for both length measurements (Table 3). These pH 5 treatment effects were 9.6, and 6.9 times greater than the effect of the pH 6 treatment, respectively (Table 3). Exposure to different pH treatment levels resulted in the largest differences in growth curves for SVL, followed by total length, and minimal differences for mass (Table 4; Fig. 1). Growth of recently metamorphosed salamanders based on SVL differed significantly from both salamanders in the pH 6 treatment (HSD, *P* = 0.020) and the control (HSD, *P* = 0.010). These results demonstrate that exposure to pH 5 substrates resulted in shallower growth curves when compared to the control treatment, but that growth on pH 6 substrates was very similar to the control.

## DISCUSSION

Our data demonstrate that constant exposure of newly metamorphosed Marbled Salamanders to a substrate of pH 4 is lethal, and that similar exposure to a substrate of pH 5 hinders growth. The cause of lethality at low substrate pH is not entirely clear.

**TABLE 2.** Likelihood ratio test (LRT) statistics for models of treatment effects for snout-vent length (SVL), total length (TL), and mass of Marbled Salamanders (*Ambystoma opacum*). The Base model contained only the random effect terms T and ID. T is comprised of ot1 and ot2, which are, respectively, 1<sup>st</sup> and 2<sup>nd</sup> order orthogonal polynomials created from time. ID is a unique identifier for each individual in the experiment. The Treatment model added pH treatment, the Linear model added the interaction between treatment and ot1, and the Quadratic model added the interaction between both orthogonal polynomials and treatment. The degrees of freedom (df<sub>1</sub>) and log-likelihood value (logLik) for each model are given, as well as the results of a LRT for each model with its subsequent higher-order model. The Chi-squared test statistic ( $\chi^2$ ) was used to determine the significance level (*P*) with degrees of freedom (df<sub>2</sub>) equal to the difference in model degrees of freedom.

		Model	df <sub>1</sub>	logLik	$\chi^2$	df <sub>2</sub>	<i>P</i>
SVL	Base	SVL ~ (T) + (T   ID)	10	-1097.4			
	Treatment	SVL ~ pH + (T) + (T   ID)	12	-1096.0	2.94	2	0.230
	Linear	SVL ~ pH*ot1 + (T) + (T   ID)	14	-1089.1	13.63	2	0.001
	Quadratic	SVL ~ pH*ot1*ot2 + (T) + (T   ID)	16	-1087.1	3.99	2	0.136
TL	Base	TL ~ (T) + (T   ID)	10	-1172.9			
	Treatment	TL ~ pH + (T) + (T   ID)	12	-1172.1	1.76	2	0.415
	Linear	TL ~ pH*ot1 + (T) + (T   ID)	14	-1167.7	8.63	2	0.013
	Quadratic	TL ~ pH*ot1*ot2 + (T) + (T   ID)	16	-1165.3	4.81	2	0.090
Mass	Base	Mass ~ (T) + (T   ID)	10	270.5			
	Treatment	Mass ~ pH + (T) + (T   ID)	12	270.9	0.91	2	0.634
	Linear	Mass ~ pH*ot1 + (T) + (T   ID)	14	273.0	4.21	2	0.122
	Quadratic	Mass ~ pH*ot1*ot2 + (T) + (T   ID)	16	273.7	1.30	2	0.523

Regarding growth, individuals in the pH 5 treatment were significantly shorter in terms of SVL, but did not have a greatly reduced mass, meaning that individuals in the pH 5 treatment tended to be shorter and stouter than individuals in the control and pH 6 treatments. Previous studies show an acid-mediated reduction in metabolic function in fishes (McWilliams 1983; Evans 1987), but studies of the effects of acidity on amphibians are limited. In freshwater fish, low environmental pH is linked to a reduction in the osmoregulatory ability of

fish, and is accompanied by a reduction in Na<sup>+</sup> and Cl<sup>-</sup> concentration in the blood; the magnitude of reduction in osmoregulatory ability is correlated with ambient Ca<sup>2+</sup> concentration (Evans 1987). Exposure to acid conditions leads to the loss of membrane bound Ca<sup>2+</sup> at two binding sites, one of which is involved in membrane permeability, leading to the leakage of Na<sup>+</sup> and Cl<sup>-</sup> in acid conditions (McWilliams 1983).

Similar effects to that of fish are seen in amphibians, suggesting that acid acts through the same mechanism.

**TABLE 3.** Parameter estimates for analyses of variance on growth curve models for Marbled Salamanders (*Ambystoma opacum*). Models were constructed with snout-vent length (SVL) or total length (TL) compared with the fixed effects of both linear and quadratic time points and the random effects of individual ID. *P* values and degrees of freedom for the *t*-test were calculated by the Satterthwaite approximation for denominator degrees of freedom.

	Model	Estimate	Standard. Error	<i>t</i>	df	<i>P</i>
SVL	Intercept	31.17	0.34	91.6	58.4	< 0.001
	Linear	9.07	0.48	19.0	58.0	< 0.001
	Quadratic	2.48	0.23	10.6	58.0	< 0.001
	pH5 treatment	-1.25	0.51	-2.47	58.0	0.017
	pH6 treatment	-0.17	0.51	-0.34	58.0	0.736
	pH5 treatment: Linear	-2.60	0.71	-3.66	58.0	< 0.001
	pH6 treatment: Linear	-0.27	0.71	-0.38	58.1	0.702
TL	Intercept	53.16	0.65	81.5	58.1	< 0.001
	Linear	14.86	0.82	18.0	58.6	< 0.001
	Quadratic	4.38	0.30	14.7	58.0	< 0.001
	pH5 treatment	-2.21	0.97	-2.27	58.0	0.027
	pH6 treatment	0.05	0.97	0.05	58.0	0.959
	pH5 treatment: Linear	-3.64	1.22	-2.98	58.0	0.004
	pH6 treatment: Linear	-0.52	1.22	-0.43	58.0	0.670



Anderson and Johnson.—Effect of substrate pH on Marbled Salamanders.

TABLE 4. ANOVA results of recently metamorphosed Marbled Salamander (*Ambystoma opacum*) in terms of total change in snout-vent length (SVL), total change in total length (TL), and total change in mass across pH treatments.

		df	SS	MS	F	P
SVL	Treatment	2	72	36.01	5.68	0.006
	Residuals	55	348.80	6.34		
TL	Treatment	2	128.60	64.28	2.99	0.058
	Residuals	55	1179.80	21.45		
Mass	Treatment	2	0.45	0.22	1.87	0.160
	Residuals	55	6.62	0.12		

In frogs of the family Ranidae, a reduction of sodium influx and an increased sodium efflux is seen at low experimental pH treatments (Freda and Dunson 1984). Terrestrial plethodontid salamanders also have shown an increase in sodium efflux when housed on acidic substrates, suggesting a disruption of membrane permeability along the integument (Frisbie and Wyman 1991). These changes in skin permeability were accompanied by a reduction in body mass and water content suggesting a disruption of osmoregulatory abilities (Frisbie and Wyman 1991). Sodium and potassium are essential in various cell functions as part of sodium/potassium ATP mediated pumps and channels, and the impairment of their function could additionally lead to increased energy expenditure for the maintenance of homeostasis leading to less energy expenditure on growth (Skou 1989). We observed a reduction in salamander length in the pH 5 treatment, but not a reduction in mass, suggesting that any changes in osmoregulation resulting from the acidic substrate did not reduce mass because of water loss, which contrasts with the previous results for plethodontids (Frisbie and Wyman 1991). Alternatively, a differential allocation of resources, in which individuals in an acidic environment allocate more resources into increasing mass than length could explain the lack of a reduction in mass (Frisbie and Wyman 1991). There are many examples of differential resource allocation in response to environmental stressors (Emerson 1986; Congdon et al. 2001; Relyea 2001; Lester et al. 2004), and under acidic conditions, we speculate that nutrients may be allocated to the storage of energy rather than to somatic growth.

Exposure to soils at pH 5 or lower during emigration of recently metamorphosed salamanders might potentially decrease the success of salamander populations, as recruitment of individuals post metamorphosis is essential. Catastrophic failure of larval cohorts has been observed as a somewhat common occurrence in natural populations of the Marbled Salamanders, but even with high variance in larval survival, populations remain stable because of the persistent annual breeding activity of the long-lived terrestrial adult salamanders (Taylor

et al. 2006). The juvenile period immediately after metamorphosis is critical in determining the probability of survival to the first reproduction (Rothermel and Semlitsch 2006). A reduced growth rate during this period is likely to lead to a reduction in reproductive success by increasing the time to reach maturity and limiting the fecundity of individuals, likely due to tradeoffs between gonadal development and somatic growth (Smith 1987; Semlitsch et al. 1988; McCoy et al. 2007). Specifically, in the Marbled Salamander, size is directly related to the number and size of eggs produced by females (Scott and Fore 1995).

Currently, the most successful reclamation approach for Appalachian forests, the Forestry Reclamation Approach (FRA), recommends soil at a pH between 5 and 7 (Angel et al. 2009). Because a soil pH of 5 may decrease growth of Marbled Salamanders, mining companies working within the guidelines of the FRA may be creating forest ecosystems with soil pH levels that are detrimental to the establishment of salamander populations. Salamanders have repeatedly been shown to be important in the function of North American forested ecosystems, providing ecosystem services as mid-level predators, distributing energy and nutrients between aquatic and terrestrial environments during migration, contributing to soil dynamics in the form of burrowing, and serving as an abundant source of high quality nutrients for higher level consumers (Davic and Welsh 2004). Additionally, forest ecosystems developed on previously mined landscapes are less efficient at carbon sequestration and specifically showed a reduction in soil carbon in mined landscapes (Amichev et al. 2008). The plethodontid salamander *Ensatina eschscholtzii* was found to increase litter retention in forests, likely through its role as a consumer of leaf-litter invertebrates as the retention of leaf litter keeps carbon in the soil rather than being released to the atmosphere (Best and Welsh 2014). Although salamanders are not the only factor that improves carbon sequestration, their presence in reclaimed forest ecosystems would be beneficial to the recovery of Appalachian coal spoils.

Our study shows that at pH 5 or lower, there was a significant reduction in the growth and survival of the Marbled Salamander. This, in turn, may lead to slower development and a lower fitness over the course of the life of an individual. Individual fitness reduction has the potential to reduce salamander populations on recovering forests and to decrease the potential for salamanders to provide beneficial ecosystem services.

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