# No effect of liming on the Eastern Red-backed Salamander after 5 years

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#### Abstract

Over the last decades, the application of calcitic materials to soil to restore the vigor of Sugar Maple (*Acer saccharum* Marsh) trees has increased in northeastern North America. However, few studies have evaluated how this treatment affects other ecosystem components, especially over several years. In Sugar Maple stands, the Eastern Red-backed Salamander (*Plethodon cinereus* Green, 1818) is one of the most abundant vertebrates and an important terrestrial species for key ecological processes. Because the species commonly serves as an indicator of forest disturbances, it is important to know how anthropogenic disturbances, such as soil liming, might affect it. The goal of this study was to evaluate the medium-term (5-year) effects of liming on body condition in a wild population of *P. cinereus* in Quebec, Canada. Lime (CaCO3) was spread by helicopter on a declining Sugar Maple forest growing on an acidic and base-poor soil. The results of this study, combined with those of previous published works, show that liming has no short- or medium-term effects on the body condition of the Eastern Red-backed Salamander. This study adds to those on other species in Europe and northeastern North America that report that liming has no major effect on amphibians when it is used as a treatment to restore acidified forest ecosystems. This should help foresters decide whether or not liming treatments are compatible with conservation, ecological, and management objectives.

Keywords Amphibians | body condition | forest soil | Plethodon cinereus

## 1. Introduction

Forest liming has been used in Europe (Smallidge et al. 1993, Clair & Hindar 2005) and in eastern North America (Moore et al. 2015) to mitigate the effect of acid deposition on aquatic and terrestrial ecosystems. Basically, calcium and/or magnesium dissolved from the liming materials displace hydrogen ( $H^+$ ), which acidifies soil, from the surface of soil particles. Carbonate (CaCO<sub>3</sub>), also dissolved from the limestone materials, then reacts with displaced  $H^+$ , thereby reducing soil acidity. Most studies that have evaluated this treatment's effect on key ecological species, such as amphibians, were conducted over a 1- to 2-year period (Beattie & Tyler-Jones 1992, Bellemakers & van Dam 1992, Beattie

et al. 1993, Seagle & Curd 1995, Moore 2014, Cameron et al. 2016). Therefore, a knowledge gap remains regarding the longer-term effects of liming on forest amphibians.

The Eastern Red-backed Salamander (*Plethodon* cinereus Green, 1818) is one of the most widely distributed and abundant vertebrates in eastern North American forests (Burton & Likens 1975, Petranka 1998, Moore & Ouellet 2015). It lives, forages and breeds on the forest floor and in the soil, where it plays a role in several key ecological processes. For instance, it is an important predator in the detrital food web, influencing decomposition and nutrient cycling (Wyman 1998, Walton et al. 2006, Walton 2013, Hickerson et al. 2017). Other studies have shown that it could play a significant role in controlling introduced species (Maerz et al. 2005)



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and in sequestering carbon, which could affect the global carbon cycle as well as climate change (Best & Welsh 2014).

The Eastern Red-backed Salamander is a terrestrial, lungless amphibian that relies mainly on its skin for respiration and hydration. Liming, should it hinder cutaneous respiration and/or alter osmotic regulation, could thus affect body condition, individual health and, consequently, population sizes of this species.

Given the increased use of liming to restore the nutritional status and vigor of Sugar Maples (*Acer saccharum* Marshall) on acidic, base-poor soils of eastern North America (Long et al. 2011, Moore et al. 2012, Moore et al. 2015), more studies are needed to evaluate this treatment's effects on a longer term with respect to forest sustainability objectives (Moore et al. 2015).

Other studies that have evaluated the effect of liming on *P. cinereus* were either short-term (1 to 2 years; Seagle & Curd 1995, Moore 2014), conducted in microcosms (Moore 2014), or limited to a relatively small area (Seagle & Curd 1995, Cameron et al. 2016). Short-term studies reported that the Eastern Red-backed Salamander was not affected by amendments to restore acidified soils, such as lime (Seagle & Curd 1995, Moore 2014) or wood ash (Gorgolewski et al. 2015). In Ohio, Cameron et al. (2016) showed that soil liming did not affect body condition, population demographics or density of this species up to 5 years after the treatment.

The objective of this study was to evaluate the effect of forest liming on the body condition (length and weight) of a wild population of the Eastern Red-backed Salamander, 5 years after aerial lime spreading. We examined this in 3 limed blocks and 2 control blocks by using cover object surveys to compare the size of captured specimens. Knowing how liming affects this species in the longer term, in the wild and in other ecosystems is essential to better predict the effect of this treatment on a larger scale.

## 2. Materials and methods

**Field Site Description.** The experiment was performed in the Portneuf Wildlife Sanctuary (lat. 47.099° N, long. 72.318° W), located approximately 100 km northwest of Québec City (Quebec, Canada). In this area, elevation ranges from 250 m to 350 m, with an average slope of approximately 10%. Mean annual temperature is 2.5 °C, and annual precipitation (1971–2000) averages 1150 mm.

Forest stands in the area are mainly uneven-aged and dominated by Sugar Maple in association with Yellow Birch (*Betula alleghaniensis* Britton) and American Beech (*Fagus grandifolia* Ehrh.). The soil is a well-

drained, stony, sandy loam Orthic Humo-Ferric Podzol (Raymond et al. 1976, Soil Classification Working Group 1998). The humus is 8 cm thick and of a mor to moder type. The humus and soil are relatively acid, with a mean pH of 4.2 in the forest floor and of 5.2 in the upper 15 cm of the mineral soil (Rock Ouimet, *personal communication*). The parent material is a stony, glacial till originating from the regional bedrock consisting of granite, gneiss, and syenite.

**Methods**. The experiment was initially designed to test the effects of forest liming on Sugar Maple trees. Sugar maple stands with relatively similar overstory vegetation types and abundance were selected, and 2 treatments (limed, control) were randomly allocated among 6 blocks. These blocks are distributed in patches of 2.7 to 5.1 ha (Table 1) in an area of approximately 100 ha. Distance between blocks is at least 500 m.

For the salamander experiment, we excluded one control block because it differed too much from the 5 others in terms of understory composition. The remaining 5 blocks (2 control and 3 limed) were relatively similar in terms of humus, soil, ground cover, overstory vegetation type and abundance, as well as ground cover habitats for *P. cinereus*.

Lime was spread by helicopter in November 2011. We placed collectors on the ground to evaluate the effective dose applied, and monitored the application to verify the uniformity of spreading. Lime material consisted of sandy calcium carbonate (CaCO<sub>3</sub>, commercial grade). The overall liming rate at the soil surface was 1.8 T/ha (Standard error [SE]:  $\pm$  0.9 T/ha).

Salamanders were monitored during 3 consecutive days in August 2016, during the daytime and under similar weather conditions (sunny with cloudy intervals, no rainfall), in order to minimize local/temporal differences in survey conditions. Moreover, we alternated between control blocks and limed blocks to minimize temporal effects between both groups. Although some studies have indicated that additional sampling during the nighttime may help to provide a truer picture of the population/ demographic of terrestrial plethodontids (Crawford and Semlitsch 2007, Connette et al. 2015), this option was not possible in this remote area for security reasons. A field crew of 4 persons searched each block once, looking for salamanders under logs, bark and rocks. The total time spent to survey each entire block was recorded. As an indication, the capture frequencies of P. cinereus (number of salamanders per block/search time per block) in the limed and control blocks were 2.1 and 2.2 per hour, respectively.

Measurements included snout vent length (SVL) and total length of individuals (mm) using an electronic caliper (resolution of 0.1 mm), and weight (g) using

a digital balance (resolution of 0.1 g). For the length measurements, salamanders were handled in a transparent plastic bag that had previously been sprayed with stream water. Only 3 salamanders had incomplete tails; in 2 cases, only the tip was missing. The other was excluded from the regression analysis. After they were measured, captured salamanders were released on site.

The required permit was obtained from the provincial authorities (Ministère du Développement durable, de la Faune et des Parcs, permit no. 2016053103403SF).

We sampled the upper forest floor layer (humus), where the salamanders were found (one humus sample per debris for the first 10 salamanders found, for a total of 10 samples per block), by gently scraping the surface of the floor with a plastic bag. The samples were airdried and passed through a 2 mm sieve prior to chemical analyses. Soil pH was measured with water using a 1:2.5 (w:w) soil:solution ratio.

Statistical analysis. Given the small number of experimental units, we used 2 different approaches to compare the health of P. cinereus populations in the limed and control blocks. The first was at the salamander level, and used a regression technique, namely a linear mixed model with a random block effect on the intercept, to determine whether liming affected the relationship between salamander weight and SVL compared to the control. Weight-length regressions have been used previously to evaluate the physical condition of Eastern Red-backed Salamander populations (Heatwole & Heatwole 1962, Jaeger 1981). Other studies have suggested using this technique to evaluate the general health of amphibians (Heyer et al. 1994, Fellers & Freel 1995) and reptiles (Kaufman & Gibbons 1975). In this model, intercept and slope were allowed to vary among

blocks. The response variable was weight, and the fixed effects were SVL, treatment, and the interaction between SVL and treatment.

The second analysis was at the experimental unit (block) level, and involved an Anova on individual body variables (SVL, total length and weight), with block nested in treatment as a random effect and treatment (liming) as a fixed effect. The analysis was performed using the SAS MIXED procedure (SAS Software Inc. 2016) after checking for normality and heterogeneity of variance. The threshold used to determine significance was  $\alpha = 0.05$ .

## 3. Results

Forest floor pH was significantly higher (+0.45 unit) in the limed blocks (5.25) than in the control blocks (4.80) (*P* < 0.001, *F* = 12.27, DF = 45). A total of 124 *P. cinereus* were observed during the study, but due to some escapes, morphometric data were collected on only 120 individuals. Of these specimens, 78% were adults and 22% were juveniles (Fig. 1). Only one other salamander species was observed during the inventory (Eurvcea *bislineata*, n = 2). Both statistical approaches showed that liming did not influence the size of P. cinereus, 5 years after the treatment (Table 1, Fig. 1). The salamanders measured in the limed and control blocks showed no significant differences in body weight, SVL or total length ( $P \ge 0.088$ ;  $F \le 6.23$ , DF = 3, Table 1), although salamanders in the limed blocks were 7% larger (SVL) and had 15% greater body mass. Also, the intercept (P = 0.375, F = 1.08, DF = 3) and slope (P = 0.230, P = 0.230)

 Table 1. Mean body parameters of the Eastern Red-backed Salamanders captured in August 2016 in control and limed blocks in the study site located in the Portneuf Wildlife Sanctuary, approximately 100 km northwest of Québec City (Quebec, Canada).

Experimental unit	Area (ha)	Treatment	Number of salamanders observed	Weight* (g)	<b>Snout vent</b> <b>length*</b> (mm)	Total length* (mm)
2C	2.9	Control	37	$0.77\pm0.31$	$35.62\pm6.14$	$72.86 \pm 13.79$
4C	2.7	Control	25	$0.82\pm0.30$	$36.42\pm5.12$	$71.22\pm12.63$
1L	5.1	Limed	21	$1.02\pm0.38$	$37.92 \pm 6.56$	$78.91 \pm 13.49$
3L	3.7	Limed	15	$0.96\pm0.31$	$39.80 \pm 3.31$	$80.82\pm9.65$
4L	4.1	Limed	26	$0.83\pm0.31$	$38.33 \pm 5.32$	$73.02\pm11.74$
Total/Mean**		Lime	62	$0.93\pm0.05$	$38.51\pm0.72$	$77.00 \pm 1.96$
		Control	62	$0.79\pm0.06$	$35.95\pm0.73$	$72.13\pm2.12$
		Р		0.156	0.088	0.190
		Estimate of effect size		0.14	2.56	4.87
		se		0.08	1.02	2.89

\*  $\pm$  SD, \*\*  $\pm$  SE

F = 1.46, DF = 111) of the regressions of weight as a function of SVL did not differ significantly between the limed and control treatment (Fig. 1). The estimate of the intercept and its SE were  $-1.17 \pm 0.14$  g for the limed and  $-0.97 \pm 0.13$  g for the control blocks. The estimate of the slope and its SE were  $0.055 \pm 0.003$  g·mm<sup>-1</sup> for the limed and  $0.049 \pm 0.003$  g·mm<sup>-1</sup> for the control blocks.

## 4. Discussion

This study aimed to evaluate the medium-term effect of liming (5 years after treatment) on the Eastern Redbacked Salamander, one of the most widely distributed and abundant vertebrate species in eastern North American forests, and an important species for key ecological processes in these ecosystems. Although Cameron et al. (2016) also evaluated the medium-term (5-year) effect of liming on P. cinereus, their study was limited to two close hardwood stands in the state of Ohio (United Sates). Knowing how liming affects this species in other ecosystems of northeastern North America is essential in order to better predict this treatment's effect on a larger scale. The results of the present study in Quebec (Canada), combined with those of others (Seagle & Curd 1995, Moore 2014, Cameron et al. 2016), suggest that liming has no short- or mediumterm effect on body condition of the Eastern Red-backed

Salamander. The fact that *P. cinereus* can be found under a wide range of pH conditions, both in very acid and basic forest floors (Wyman & Hawksley-Lescault 1987, Wyman 1988, Wyman & Jancola 1992, Moore & Wyman 2010, Bondi et al. 2016) suggests that the increase in soil pH after liming observed in this study does not affect this species.

Moreover, in another study conducted in Quebec (Moore 2014), two grades of lime were used (finely ground and sandy CaCO<sub>2</sub>) in field microcosms to verify if the finely powdered grade could clog salamander skin pores and affect its health and growth, thereby inducing mortality. Its results suggested that direct contact even with finely ground lime had no short-term effect on the Eastern Red-backed Salamander's health and survival rate. In Maryland, Seagle & Curd (1995) found that liming was not a significant factor to explain the presence or absence of the Eastern Red-backed Salamander, 2 years after its application. In Ohio, Cameron et al. (2016) reported no effect of liming on body condition, population demographics or density of P. cinereus, 5 years after liming. In Europe, positive short-term effects of liming have been reported on some amphibian species (Beattie & Tyler-Jones 1992, Bellemakers & van Dam 1992, Beattie et al. 1993). In a recent literature review, Moore et al. (2015) showed that liming usually has positive effects on components (forest and soil fauna and flora) of acidified forest ecosystems in northeastern North America.

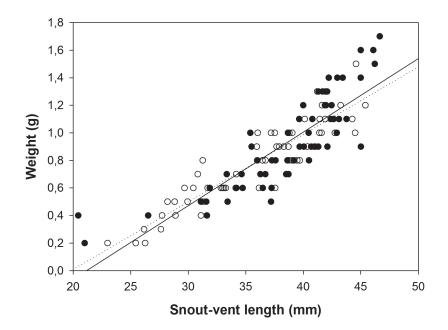


Figure 1. Comparison of the regressions of weight as a function of snout vent length of Eastern Red-backed Salamanders captured in control (open circles, dashed line) or limed (black circles, solid line) blocks in August 2016 in the Portneuf Wildlife Sanctuary, located approximately 100 km northwest of Québec City (Quebec, Canada).

## 5. Conclusion

This study, combined with those of previous published works, suggests that liming has no short- or mediumterm effects on the body condition of the Eastern Redbacked Salamander. It builds on other reports that this treatment has no significant effects on amphibians in forest ecosystems of northeastern North America and Europe. This information should help foresters decide whether or not liming treatments are compatible with conservation, ecological and management objectives.

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