PROJECT TITLE

Title:	Road Salt in Soils and its Effects on Overwintering Amphibians
Project Number:	
Start Date:	9/1/2023
End Date:	8/31/2024
Funding Source:	104B
Congressional District:	CT-003
Research Category:	Watershed and Ecosystem Function
Focus Category:	ST, GW, ECL
Principal Investigators:	Steven Brady Associate Professor Southern CT State University Email: brady.steven@gmail.com Phone: 802-272-3247 Gaboury Benoit Vice President Mill River Watershed Association Email: gabouryb@gmail.com Phone: 203-401-1556

Summary

This project set out to investigate the prevalence and causes of edema in wood frogs by capturing frogs in the fall, tracking them over winter, and assessing their condition in the spring. We also aimed to measure soil salt levels to assess correlations with edema prevalence. Due to unforeseen delays, including illness, we were unable to locate frogs in the fall. However, we successfully pivoted to two new objectives: (1) conducting a controlled salt toxicity study on wood frog embryos and testing how edema affects swim performance and (2) analyzing water quality data to assess road salt concentrations across vernal pools in the northeastern U.S. These pivots resulted in two impactful research products, both of which have been submitted as manuscripts for publication. Our findings reveal critical insights into the ecological consequences of road salt pollution, with implications for conservation and management of seasonal pools. This work not only contributes to our understanding of environmental stressors but also highlights adaptive and maladaptive responses in amphibian populations.

Introduction

Freshwater salinization caused by road salt runoff has emerged as a critical environmental issue, threatening freshwater ecosystems across North America. Seasonal vernal pools are particularly vulnerable to salinization due to their small water volumes and proximity to impervious surfaces like roads. These pools serve as essential breeding habitats for amphibians, including the wood frog (*Rana sylvatica*), whose early life stages depend on high-quality aquatic environments.

One prominent but understudied consequence of salinization in amphibians is the development of edema, a physiological condition in which frogs accumulate fluid, leading to bloating and reduced locomotor performance. While the fitness costs of edema are suspected to be high, few studies have quantified these impacts in ecologically relevant contexts. Similarly, the early life stages of amphibians, such as embryos and larvae, are particularly sensitive to environmental stressors, including salinity. Previous studies suggest that road salt pollution can reduce survival, impair growth, and disrupt development, but the mechanistic underpinnings remain unclear.

This project was originally designed to evaluate the prevalence of edema in frogs overwintering in roadside environments and correlate its occurrence with salt concentrations in soils. However, unforeseen logistical challenges required us to adapt our approach. Instead, we pivoted to investigate two critical aspects of road salt pollution: (1) its physiological impacts on amphibians, including salt-induced edema and its fitness consequences, and (2) the spatial extent and intensity of salinization in vernal pools across the northeastern United States. By combining controlled laboratory experiments with field surveys, this project has advanced our understanding of how road salt pollution impacts amphibian populations and their habitats.

Objectives

- To assess the toxicity of road salt on wood frog embryos under controlled conditions.
- To evaluate the impacts of salt-induced edema on wood frog swim performance as a measure of fitness.
- To quantify the concentration of road salt in vernal pools throughout the Northeast and its ecological implications.

Results/Discussion

1. Salt Toxicity on Embryos

Laboratory experiments revealed that elevated salinity levels severely impaired embryonic growth and development. Specifically, salt exposure reduced vitelline membrane expansion and restricted water uptake in embryos, likely imposing spatial constraints that limit development (Fig. 1). These effects were observed at salinity levels well below current regulatory thresholds, underscoring the vulnerability of amphibian embryos to road salt pollution. These findings are now under review in *Integrative and Comparative Biology* and suggest that current chloride standards for aquatic life may be insufficient to protect sensitive amphibian species.

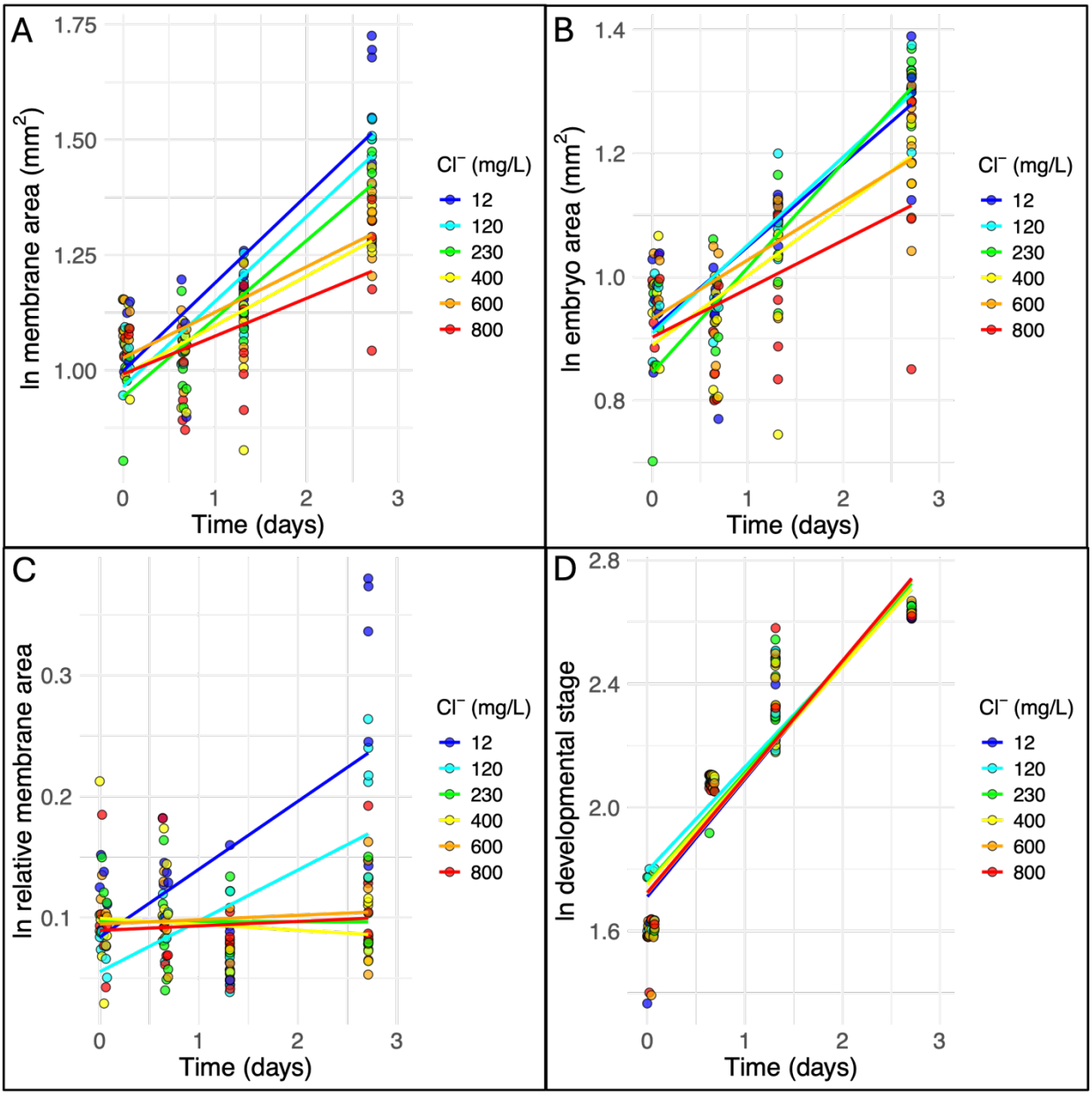

Figure 1. Salinity impacts on embryonic growth and vitelline membrane expansion. Salinity diminished the rate of A) vitellin membrane expansion and B) embryonic growth. (Measurements of membrane size and embryo size were made from two dimensional ellipses placed on photographs, thus units are in mm^2). C) Critically, salinity severely limited the expansion of the membrane relative to the size of embryo but D) had no effect on embryonic developmental rate over the observed range (from Gosner stages 6-14). In all panels, fitted lines are predicted values from linear models.

2. Edema and Swim Performance

Field trials quantified the effects of edema on adult male wood frogs, demonstrating that swim speed decreased proportionally to the severity of edema (Fig. 2). Following aspiration of edema

fluid, frogs exhibited significant improvements in swim performance, confirming that edema imposes a direct locomotor cost. These results have implications for male breeding success, as swimming is critical for accessing mates and evading predators during the explosive spring breeding period. This work is now under review in *Integrative and Comparative Biology* and contributes to a growing body of research on the sublethal impacts of salinization on amphibian fitness.

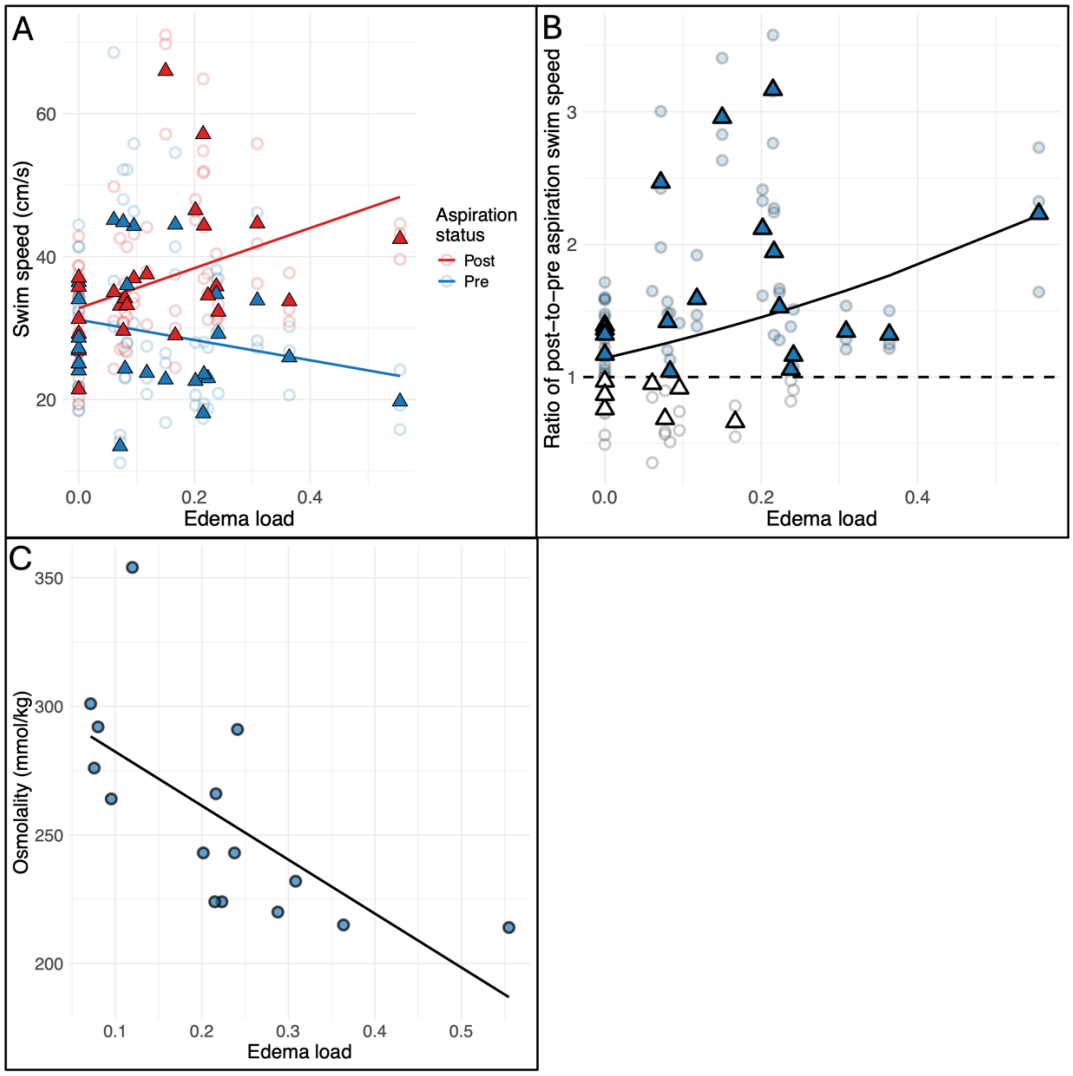

Figure 2. Edema reduces swim performance in adult male wood frogs. A) Swim speed was negatively associated with edema load (the proportion of excess body mass carried as edema) prior to aspiration (status = 'pre aspiration'). Following aspiration (status = 'post aspiration'), swim speed was positively correlated with edema load. Pre and post swim trials for each frog are shown as open circles while means are overlain as filled triangles. Lines represent fixed effects from the linear mixed model. B) The relative change in speed following aspiration is shown as the ratio of swim speed from pre-to-post aspiration. Circles correspond to individual trials with means overlain as filled triangles. Values greater than 1 (shaded blue) indicate faster swim speed following aspiration. Values of 1 or less (shaded white) indicate no change or reduced swim speed following aspiration. C) Osmolality of edema fluid decreased with edema load at a rate of 21 mmol/kg per 0.1 units edema load.

3. Road Salt Concentrations in Vernal Pools

Water quality surveys across the northeastern United States revealed alarming levels of salinization in vernal pools (Brady and Benoit, in review), with conductivity values in some pools exceeding 3,000 µS/cm—comparable to estuarine environments (Fig. 2). These findings, currently in review at *PNAS*, confirm that road salt pollution disproportionately affects small, isolated water bodies, creating potential ecological traps for amphibians that rely on these habitats. The spatial extent and intensity of salinization emphasize the need for targeted mitigation strategies, such as improved stormwater management and the use of alternative deicing agents.

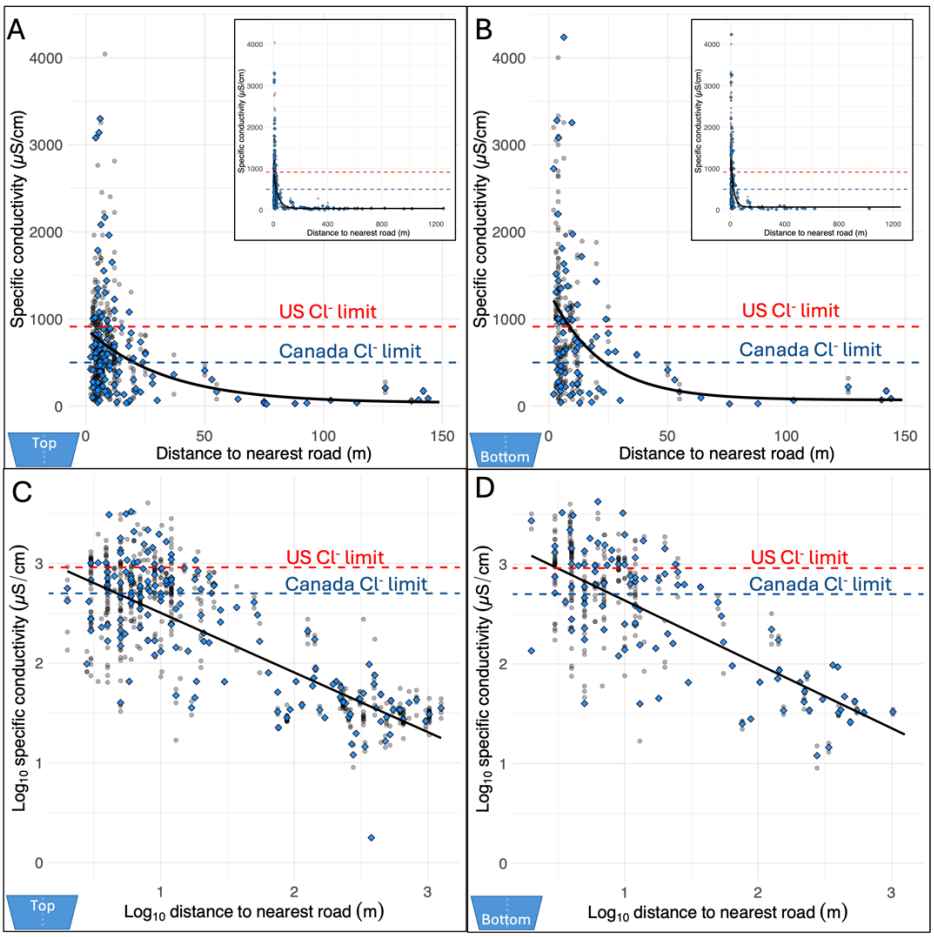

Figure 3. Pond conductivity as a function of distance from the road. In each panel, all conductivity observations are shown as gray circles, mean conductivity for each pond (N=167) is shown as blue diamonds, and model predictions are shown as fitted lines. Left panels (A, C) show conductivity from the surface waters at a depth of 10 cm while right panels (B, D) show conductivity values from the bottom of each pond. Panels A and B show the results of an exponential decay model zoomed in to show variation while the insets show the full dataset and model results. Panels C and D show the results of the linear-mixed effects models on $\log_{10}$ transformed conductivity and $\log_{10}$ transformed distance to the nearest road. Intercepts for upslope versus downslope are indicated as blue and black lines, respectively. Dashed lines indicate water quality criteria for US (red) and Canada (blue).

Conclusions

This project yielded critical insights into the ecological impacts of road salt on amphibians and their habitats. Laboratory and field studies revealed that salt pollution imposes significant physiological and developmental costs on wood frogs, from impaired embryonic growth to reduced adult locomotion caused by edema. Field surveys further highlighted the alarming extent of freshwater salinization in vernal pools, emphasizing the urgency of addressing this issue through policy and management interventions.

Moving forward, we recommend expanding research to investigate transgenerational effects of salinity and scaling up field surveys to include additional sites and seasons. Continued research in this area will inform conservation strategies and help mitigate the impacts of road salt pollution on freshwater ecosystems.

Acknowledgements

We thank the Connecticut Institute of Water Resources for funding this project and supporting its redirection to address emerging research priorities. Special thanks to collaborators and field assistants for their invaluable support and to the private landowners who granted access to study sites.

References

Brady, S. P., **Kang, S., **Wang, Z, and R. Calsbeek. Submitted to *Integrative and Comparative Biology*. Freshwater salinization leads to sluggish, bloated frogs and small, cramped embryos but adaptive counter-gradient variation in eggs.

Brady, S. P. and G. Benoit. Submitted to *PNAS*. Freshwater salinization of small ponds: High salinity and stratification threaten critical, overlooked habitats.