



# EFFECT OF SALT POLLUTION AND PREDATION ON HATCHING AND EARLY DEVELOPMENT OF THE SPOTTED SALAMANDER (*AMBYSTOMA MACULATUM*)

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## Introduction

Water pollution affects the survivorship, development, and growth of pond-breeding amphibians, and is one of the causes of the global amphibian decline.

- Salt (NaCl) pollution from salting roads during the winter has been disruptive in freshwater ecosystems
- Some amphibian species have been known to hatch prematurely in response to threats such as the presence of predators<sup>3</sup>
- Pollution that comes from road de-icing salts affects the spotted salamander (*Ambystoma maculatum*) because it causes delayed hatching as well as increased deformities in hatchlings<sup>5</sup>
- Egg clutches of spotted salamanders are surrounded by multiple protective gelatinous layers, often eaten away by predators such as wood frog tadpoles (*Lithobates sylvaticus*).
- Predators has been shown to have an impact on the hatching timing of amphibian hatchlings<sup>4</sup>



**Figure 1** Adult Spotted Salamander (*Ambystoma maculatum*)



**Figure 2** Adult wood frog (*Lithobates sylvaticus*)

## Hypotheses

1) Salt pollution decreases hatching success and delays development, therefore prolonging hatching time, and decreases size of salamander embryos.

- Hatchlings that hatch early are smaller, less developed, and have been found to experience more deformities than hatchlings that did not hatch early<sup>5</sup>

2) Presence of wood frog tadpoles, in addition to the absence of jelly layers, would exacerbate the effect of salt pollution.

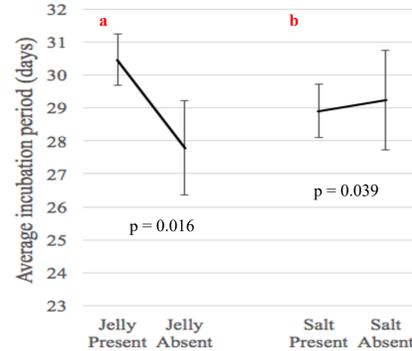
- Salamander embryos have been found to have decreased survivorship when in the presence of wood frog tadpoles<sup>5</sup>

## References

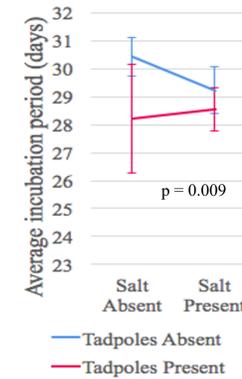
- <sup>1</sup>Environment Canada. (2001). Canadian Environmental Protection Act, 1999: Priority Substance List Assessment Report – Road Salts. Environment Canada, Hull, Quebec.
- <sup>2</sup>Harrison, R. (1969) Harrison Stages and Description of Normal Development of the Spotted Salamander, *Ambystoma punctatum* (Linn). In: Wilens, S., Ed., Organization and Development of the Embryo, Yale University Press, New Haven, 44-66.
- <sup>3</sup>Touchon, J. C., Urbina, J., & Warkentin, K. M. (2010). Habitat-specific constraints on induced hatching in a treefrog with reproductive mode plasticity. *Behavioral Ecology*, 22(1), 169-175.
- <sup>4</sup>Touchon, J. C., McCoy, M. W., Vonesh, J. R., & Warkentin, K. M. (2013). Effects of plastic hatching timing carry over through metamorphosis in red-eyed treefrogs. *Ecology*, 94(4), 850-860.
- <sup>5</sup>Ocampo, M. & Takahashi, M. (in prep). The effects of road salt (NaCl), predation, and competition on the growth and development of spotted salamanders (*Ambystoma maculatum*) and wood frogs (*Lithobates sylvaticus*).

## Results

### Incubation Period

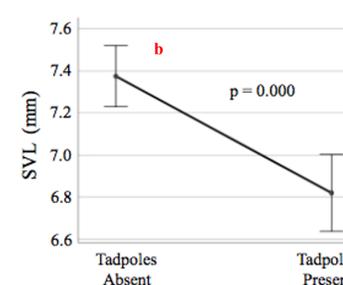
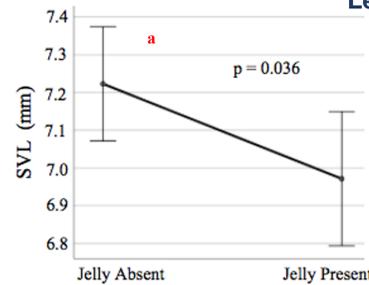


**Figure 3** Independent effects of jelly layer (a) and salt (b) on incubation period.



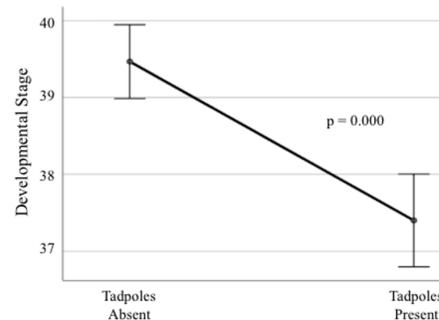
**Figure 4** Interactive effect of salt and tadpoles on incubation period.

### Length

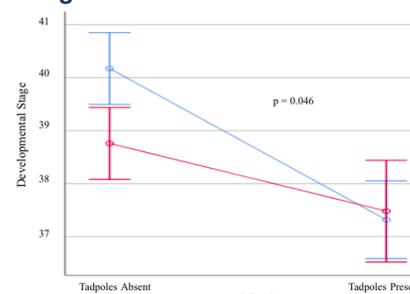


**Figure 5** Independent effect of jelly (a) and tadpoles (b) on SVL

### Developmental Stage

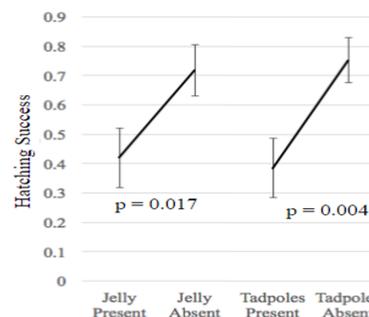


**Figure 6** Independent effect of tadpoles on developmental stage.



**Figure 7** interactive effect of tadpoles and jelly layers on developmental stage.

### Hatching Success



**Figure 8** Independent effects of jelly layer and tadpoles on hatching success.

## Methods

### Sampling and Data Collection

	1	2	3	4	5	6	7	8
<b>Salt</b>	Y	Y	Y	N	N	N	N	Y
<b>Tad</b>	Y	Y	N	N	N	Y	Y	N
<b>Jelly</b>	Y	N	N	N	Y	Y	N	Y

**Figure 9** Factorial experimental design. The experiment was initiated on April 21, each treatment had four replicates. Salt concentration was 1000 mg/L within the environmentally relevant range<sup>1</sup>

- Collected salamander and wood frog egg masses from vernal ponds in Bald Eagle State Forest (April 2018)
- Incubator had an average of 12°C temperature and 88% humidity.
- Recorded the hatching success and incubation period daily from the date of the first hatchling (April 25) to the last hatchling (May 1). Hatchlings preserved for later analysis.

### Analysis

- Hatchlings anesthetized immediately after hatching and staged according to *Ambystoma punctatum* staging series<sup>2</sup>
- Ran a series of 3-way ANOVAs for the effects of salt, tadpoles, and jelly layer on developmental stage, length, incubation period, and hatching success

## Conclusions

1) Salt pollution would decrease hatching success and delay development, therefore prolonging hatching time and size of embryos.

**No support:** Salt pollution had no significant effect on hatching success, development, or body size. It also shortened hatching time.

2) Presence of wood frog tadpoles, in addition to the absence of jelly layers, would exacerbate the effect of salt pollution.

### Mixed support:

- Jelly layers alone significantly prolonged incubation period.
- Tadpoles did not affect incubation period, but developmental stage decreased in the presence of tadpoles.
- Tadpoles and jelly layers independently decreased hatching success and interactively affected developmental stage of the salamander hatchlings.

**Conclusion:** Salt pollution shortened incubation period. However, the shortened incubation period did not result in negative effects on developmental stage and body size at hatching. The overall lack of significant effects of salt pollution on salamander hatching success and development contradicts the findings of the previous studies. This may be because our experiment was done in a laboratory setting whereas the previous studies were conducted using mesocosms in which salt concentration was likely greater at the bottom.

**Further Research:** The long-term effects of the results from this experiment are still unknown— the inclusion of metamorphosed salamanders should be considered.

## Acknowledgements

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