Assessing Salt Tolerance in Coastal vs Inland Breeding Spotted Salamanders (*Ambystoma maculatum*) Through Behavioral Avoidance Tests

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Introduction

- Whether amphibians can adapt or tolerate salinization of breeding pools, either by way of road salt runoff in the spring or through sea water intrusion of coastal wetlands due to rising ocean levels, has major implications for population viability of frog and salamander species native to the northeastern United States.[1]
- In Acadia National Park, Maine, USA, a population of Spotted Salamanders (*Ambystoma maculatum*) breeds in vernal pools that are in close proximity to the high tide zone. Breeding adults at this locale are subject to periodic spikes in salinity that occasionally approach full strength sea water (35 ppt).
- As part of a long-term study on the effect of elevated salinity levels on all life history stages of this local population, we employed behavioral avoidance tests to assess the level of salt tolerance between adult salamanders that breed in inland freshwater pools (salinity levels <1 ppt) and adult salamanders that breed in the more euryhaline coastal pools.

Methods

- We collected 48 adult salamanders by hand from five different inland vernal pools on Mount Desert Island, Maine, USA between April 18-25, 2018.
- From this cohort of salamanders, we randomly selected one adult and placed it in one of two adjoining test chambers that contained water with salinity concentrations of 3.9, 5.8, 7.3, 11.6, or 17.5 ppt (made from Instant Ocean® Sea Salt). The adjoining chamber contained freshwater (bottled spring water) (Figure 1). We then recorded time spent by individual salamanders in either chamber over 60 minutes, testing eight salamanders per concentration. As a control, we also recorded time spent by individual salamanders (N=8) over 60 minutes in adjoining chambers that both contained freshwater.
- In an effort to eliminate salamander movement between chambers due to extraneous visual stimuli, we collected behavioral choice data behind a blind and in a darkened room illuminated overhead with a 25 Watt red light (Figure 2).
- Unfortunately, efforts to test coastal breeding salamanders at all five salinity concentrations were curtailed by the onset of cold and dry weather conditions that greatly suppressed amphibian activity midway through the breeding season. We thus could only collect 16 salamanders from the coastal population in Acadia National Park, which we tested at the two highest salinity concentrations, 11.6 and 17.5 ppt.

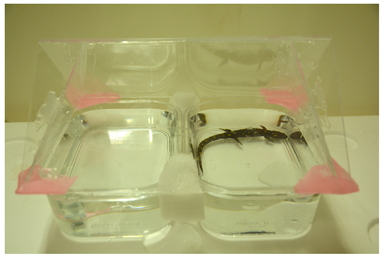


Figure 1. Representative behavioral choice arena. Adjoining chambers each contained 850 mls of liquid. Salamanders could easily traverse the lip between chambers.



Figure 2. Observers recording behavioral choice data behind opaque vertical blind, using reflection from underlying mirror (arrow) to record relative time in either chamber.

Results

- Salinity levels of inlands pools ranged from .05 to 0.54 ppt (data represent single reading per pool recorded at time salamanders were collected). Salinity level of coastal pool from which salamanders were collected for behavioral trials ranged from 0.17 - 31.1 ppt (mean salinity = 11.03 ppt) during peak breeding season (04/13/18 to 04/29/18).
- For inland breeding salamanders, a Kruskal-Wallis test revealed that 17.5 ppt represented the threshold at which salamanders spent significantly less time in that salinity concentration compared to less concentrated salt water and fresh water (Figure 3).
- For coastal breeding salamanders, a Mann-Whitney U-test revealed that individuals spent significantly less time in 17.5 ppt compared to 11.6 ppt (Figure 3).

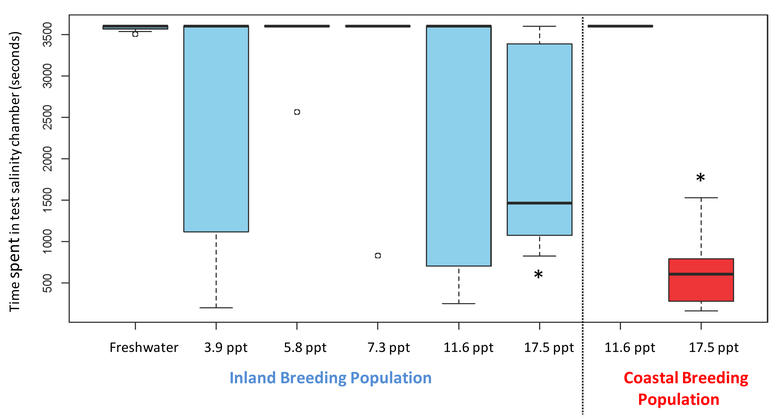


Figure 3. Box plot of data for behavioral avoidance trials. Asterisks denotes amount of time spent in 17.5 ppt is statistically significant ($p < 0.05$) from time spent in all other concentrations for inland breeding population and statistically significant ($p < 0.001$) from time spent in 11.6 ppt for coastal breeding population.

Conclusions

- Coastal breeding salamanders occupied breeding pools that were, on average, approximately two orders of magnitude higher in salt content than that recorded for pools used by inland breeding salamanders.
- Behavioral choice trials suggest that adult salamanders from inland and coastal breeding populations tolerate salinity levels up to 17.5 ppt over the course of an hour. The upper threshold of salt tolerance reported here for both populations is higher than values previously reported for caudate amphibians. [2]
- Although our behavioral avoidance data on coastal breeding salamanders are admittedly much more limited than what we had hoped for at the beginning of our 2018 field season, our findings suggest both coastal and inland breeding Spotted Salamanders tolerate increasing environmental salt concentrations to the same upper threshold.
- This provisional pattern contrasts with previous data which support the notion that salamander populations of some species can locally adapt to saline environments.[3,4]
- Alternatively, additional interpretation of our limited data hint that local adaptation to elevated environmental salt in coastal breeding salamanders could take the form of more rapid avoidance behavior, as evidenced by median time spent in 17.5 ppt by coastal breeding salamanders was 10 minutes compared to 24.5 minutes for inland breeding salamanders.

Literature Cited

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