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Short communication

Road deicing salt irreversibly disrupts osmoregulation of salamander egg clutches

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ABSTRACT

It has been postulated that road deicing salts are sufficiently diluted by spring rains to ameliorate any physiological impacts to amphibians breeding in wetlands near roads. We tested this conjecture by exposing clutches of the spotted salamander (*Ambystoma maculatum*) to three chloride concentrations (1 mg/L, 145 mg/L, 945 mg/L) for nine days, then transferred clutches to control water for nine days, and measured change in mass at three-day intervals. We measured mass change because water uptake by clutches reduces risks to embryos associated with freezing, predation, and disease. Clutches in controls sequestered water asymptotically. Those in the moderate concentrations lost 18% mass initially and regained 14% after transfer to control water. Clutches in high concentration lost 33% mass and then lost an additional 8% after transfer. Our results suggest that spring rains do not ameliorate the effects of deicing salts in wetlands with extremely high chloride concentrations.

1. Introduction

Road deicing salts, widely applied in winter to roads and highways in Europe and North America, can be transported as runoff up to 170 m from roads and have been shown to impact water quality and amphibians in wetlands (Karraker et al., 2008). Road salts reduce embryonic and larval survival of at least one species of frog and one salamander (Turtle, 2000; Sanzo and Hecnar, 2006; Karraker et al., 2008), and increase the frequency of malformations in embryos of another frog in northeastern North America (Karraker, 2007). In addition, increasing salinization of wetlands in Australia associated with intensive irrigation in agricultural areas (Bui, 2000) has been shown to reduce survival of larval amphibians (Christy and Dickman, 2002; Chinathamby et al., 2006). The embryos of one species, the spotted salamander (*Ambystoma maculatum*) showed no evidence of having adapted to elevated salinity in breeding pools (Turtle, 2000), despite local populations having been exposed for up to 60 years.

Ambystoma maculatum are broadly distributed throughout central and eastern Canada and the United States (Petranka, 1998). They breed in ephemeral, semi-permanent, and occasionally in permanent wetlands following melting of ice and snow and during the first rainy period of spring. As snow and ice melt and rain begins falling, road deicing salts that have accumulated in soils and snow piles next to roads are carried into the surrounding landscape, causing elevated conductivity and chloride concentrations in nearby wetlands (Karraker et al., 2008). Thus egg clutches of *A. maculatum* and other amphibians breeding in wetlands near roads become exposed to this road-associated contaminant. As the developmental period is generally five to six weeks (Karraker et al., 2008), depending upon water temperature, *A. maculatum* embryos in wetlands near roads may be exposed to elevated chloride concentrations for much of their development.

Clutches of *A. maculatum* are globular, and individual egg capsules are embedded within a larger jelly matrix. In a given breeding season, usually lasting two weeks to one month (Karraker et al., 2008), females attach 2–4 compact egg clutches to sticks, vegetation, or leaves in ponds. After oviposition, clutches become increasingly turgid as they take up water and may increase in size by as much as four times (N. Karraker, pers. obs.). This dramatic uptake of water increases the distance between individual eggs, which improves respiration within the clutch, and may decrease the risk of predation and disease to individual eggs (Gomez-Mestre et al., 2006). In a previous study (Karraker and Ruthig, 2009), we documented an increased frequency of malformations in *A. maculatum* hatchlings when embryos were exposed to elevated salinity concentrations associated with the use of road deicing salts. That study and observations of highly turgid egg masses in pools far from roads and flaccid clutches in pools near roads (Fig. 1), compelled us to examine whether loss of water from clutches in

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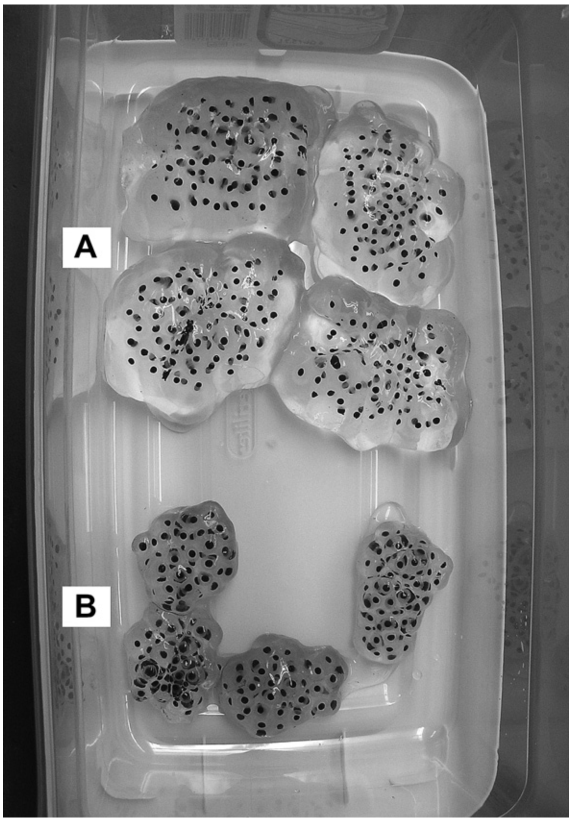

Fig. 1. Photograph of clutches of *A. maculatum* from (A.) pool located >200 m from a highway with chloride concentration of 1 mg/L, and (B.) pool located 11 m from a highway with chloride concentration of 721 mg/L, showing differences in water uptake.

hypersaline environments was permanent or, alternatively, if normal osmoregulation would resume when salinity concentrations were reduced. Significant rainstorm events occur frequently during the breeding season of this species in the spring (April and May), and hence could ameliorate any threat of physiological impacts posed by elevated salinity to amphibians breeding near roads.

2. Methods

In May 2004, we collected 21 egg clutches, which had been deposited the previous night, from three vernal pools not influenced by road deicing salts in the central Adirondack region of New York State in the northeastern United States. Clutches were transported to the laboratory and then mixed together in one container and randomly assigned, such that each treatment had seven replicates, to one of three chloride concentration treatments: 1 mg/l (control), 145 mg/l (moderate), and 945 mg/l (high). Treatments were made by combining road deicing salt (principal component was sodium chloride) obtained from a local highway department with dechlorinated tap water. Control, moderate, and high treatments corresponded to chloride concentrations observed in vernal pools located >200 m from a highway, and the mean and maximum chloride concentrations in pools located within 200 m of a highway, respectively (for details see Karraker et al., 2008).

Clutches were held in a controlled-temperature chamber with the temperature set at 11.0 °C, which was the average water temperature during embryonic periods in three breeding seasons of *A. maculatum* in a previous study (Karraker et al., 2008). We weighed all clutches at the start of the experiment and after three, six, and nine days. At nine days, clutches from all treatments were transferred into control water and were weighed three, six, and nine days after the transfer. We calculated proportional change in mass for each period, before and after transfer to control water. Repeated measures analysis of variance was used to compare changes in mass among clutches by time and salinity treatment. Three-day periods were the repeated measure, and chloride concentration treatment was the grouping factor. Mass data were $\log_{10}$-transformed prior to analyses. Waller-Duncan k-ratio tests were used to determine which means differed, and all tests were considered significant at $\alpha = 0.05$.

3. Results and discussion

Clutches in the control treatment (1 mg/L chloride) increased in mean mass by just over 25% after nine days, compared with a loss of nearly 20% in the moderate treatment (145 mg/L) and nearly 35% in the high treatment (945 mg/L; Fig. 2). After transfer of all clutches to control water, those originally placed in the control treatment lost an average of 2% of their mass, those in the moderate treatment gained nearly 15%, and those in the high treatment lost nearly 10% (Fig. 2). We found an effect ($F_{6,108} = 37.70$, $P < 0.0001$) of time on mass of clutches, with differences during all measurements except at the start of the experiment, and an interaction ($F_{12,108} = 72.75$, $P < 0.0001$) between time and treatment. Mass of clutches differed ($F_{2,18} = 10.85$, $P = 0.0008$) among treatments. Over the entire 18-d period of the experiment, clutches in the control treatment increased in mass by an average of 25%, those in the moderate treatment lost an average mass of 2%, and in the high treatment clutches lost an average mass of nearly 45%.

We found that *A. maculatum* clutches exposed to moderate chloride concentration lost water but then were able to resume water uptake when transferred to water with a low chloride concentration. This indicates that diluting rains in the spring may help to ameliorate the effects of moderate chloride concentrations. However, in years when rainfall inadequately dilutes pools with moderate chloride concentrations, embryos may be at risk due to reduced water uptake. In contrast, clutches exposed to high chloride concentration lost water and then were not able to take up water when transferred to low chloride concentration. This result suggests that high salinity levels associated with deicing salts in breeding habitats may permanently disrupt the ability of *A. maculatum* clutches to take up water. Dilution provided by spring rains, which commonly occur during the breeding season of *A. maculatum*, may not be adequate to reverse the cycle of water loss caused by high chloride levels, increasing the risks of predation, freezing, malformations, and other factors to embryos. We emphasize, however, that it is unlikely that many pools located near roads where deicing salts are applied will have such high concentrations. In a previous study (Karraker et al., 2008), we examined chloride concentrations of 28 pools within 200 m of a highway during April and May, the period of embryonic development of *A. maculatum*, over a three-year period. Of 28 pools,

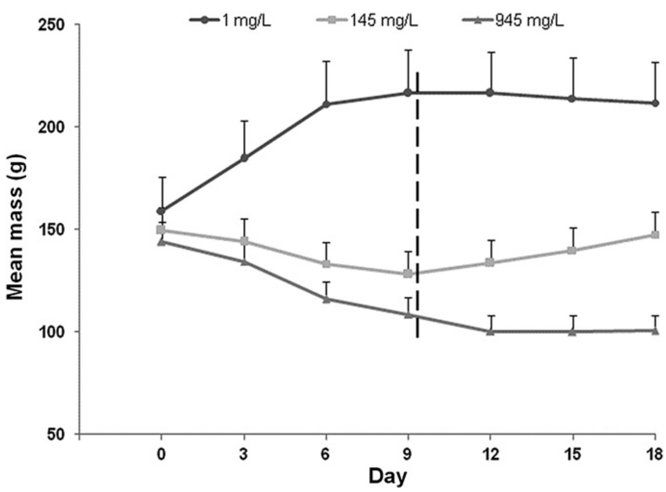

Fig. 2. Mean mass of egg clutches (+SE) of *A. maculatum* by 3-d period in control (1 mg/L chloride), moderate (145 mg/L), and high (945 mg/L) salinity treatments. The dashed line indicates the point at which all clutches were transferred from the salinity treatment (control, moderate, high) to control water.

eight had chloride concentrations above 145 mg/L, the moderate concentration tested in this study, and the three highest concentrations were 573, 656, and 915 mg/L. In the current study, we did not test a concentration intermediate between 145 and 945 mg/L; thus, we cannot predict how intermediate concentrations will affect water uptake. In addition, we do not know the magnitude of dilution that spring rains provide. In the previous study described above, mean chloride concentrations in pools decreased slightly between May (159 mg/L) and June (155 mg/L) over a three-year period (Karraker, 2008). However, this study occurred during a period of relatively low precipitation in the region.

We did not determine the mechanism by which permanent disruption of osmoregulation occurs, but it likely is related to chemical changes in the egg capsule (perivitelline) membrane, as has been documented for eggs in highly acidic conditions (Pierce, 1985). As in acidic conditions, exposure to high chloride concentrations may make the membrane more rigid, reducing permeability, and therefore unable to resume water uptake when solutes become more dilute.

Our experiment encompassed approximately one-third to one-half of the embryonic developmental period of this species. However, it was not of sufficient duration to determine if transfer to control water, despite an apparent inability to take up water, conferred benefits to embryos in terms of survival or incidence of malformations. In previous studies, we found that of embryos held at the high chloride concentration (945 mg/L) for the entire embryonic period, only 3% survived to hatching compared with 84% in the control (1 mg/L) (Karraker et al., 2008) and that the percentage of malformed hatchlings was as high as 17% in the high concentration compared with up to 3% in the control (Karraker and Ruthig, 2009).

Our results suggest that clutches of *A. maculatum* laid in wetlands with high chloride concentrations, associated with road deicing salts from winter application, may not benefit from dilution by spring rains and may experience lower survival and increased frequency of malformations. This study corroborates the general conclusion emerging from other amphibian-road deicing salt studies (e.g. Sanzo and Hecnar, 2006; Karraker et al., 2008; Collins and Russell, 2009) that road salt applications can exert significant negative effects on amphibian populations.

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