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Comparing the Impacts of Common Deicing agents NaCl and MgCl2 on the Mobility Behavior, Embryonic Deposition Behavior, and Embryonic Development of the Freshwater Gastropod Physa acuta

Sophia Weldi

Comparing the Impacts of Common Deicing Agents NaCl and $MgCl_2$ on the Mobility Behavior, Embryonic Deposition Behavior, and Embryonic Development of the Freshwater Gastropod *Physa acuta*

S. Weldi, C. Crowell, L. Giancarlo, T. Frankel, University of Mary Washington / Earth and Environmental Sciences

University of Mary Washington - Department of Earth and Environmental Sciences, 1301 College Ave, Fredericksburg, VA 22401

Introduction

Road salt runoff is increasing the salinity of U.S. waterways well above the EPA's recommended limits. In the U.S., over 24.5 million tons of road deicers are applied every year (American Geosciences Institute, 2017), with NaCl and $MgCl_2$ accounting for over 90% of applied salts (Vítzová, et.al., 2016). The EPA recommends surface waters do not exceed a four-day average of 230mg/L Cl^- when associated with Na^+ more than once every three years. However, it has been found that some waterways contain average daily concentrations that exceed 250mg/L NaCl and winter concentrations of 600mg/L NaCl(Kaushal et al., 2005). Road salt runoff is a major contributor to this increase in salinity as 55% of road salt enters waterways as runoff without infiltrating the soil (Venner, 2004). Few studies have investigated and compared the effects of environmentally relevant concentrations of NaCl and $MgCl_2$ on sublethal endpoints in freshwater aquatic invertebrate species.

Objectives

- Determine the effect of environmentally relevant NaCl and $MgCl_2$ concentrations on adult *Physa acuta* mobility behavior endpoints: speed, time spent frozen, distance traveled, acceleration.
- Determine the effect of NaCl and $MgCl_2$ on adult *Physa acuta* embryonic deposition behavior
- Determine the effect of NaCl and $MgCl_2$ on *Physa acuta* embryo clutch development and viability

Hypotheses

Mobility Behavior Studies

- Individuals exposed to elevated NaCl and $MgCl_2$ concentrations expected to display slower speed, more time spent frozen, shorter distance travelled and slower acceleration.

Embryonic Deposition Behavior

- Adults exposed to elevated NaCl and $MgCl_2$ concentrations expected to deposit fewer embryos and deposit less frequently.

Embryonic Development Exposures

- Increase in embryonic mortality and dose dependent developmental delays expected due to NaCl and $MgCl_2$ exposure

Materials and Methods

Mobility Behavior Studies

- Adult *P. acuta* were randomly assigned a treatment level (0mg/L, 100mg/L, 250mg/L, 500mg/L, 1000mg/L NaCl or $MgCl_2$ (n = 9)) and placed individually in 100mL beakers containing 100mL of assigned solution.
- 100% static replacement conducted every 48hrs, beakers maintained in an incubator at 24°C.
- On days 1, 3 and 7 snails were placed in a glass petri dish with 50ml of synthetic water, acclimated for 5 min., and movement recorded using an overhead USB camera for 3 min.
- Recordings were then analyzed using ToxTrac (v. 2.83) for mobility behavior endpoints (n=9).

Embryonic Deposition Behavior

- Embryos deposited by the mobility behavior study individuals during exposure were collected. The number of clutches and embryos in each clutch were recorded.

Embryonic Development Exposures

- Embryo clutches were collected less than 24hrs after deposition, viability of each embryo was confirmed.
- Clutches randomly assigned to 0mg/L, 100mg/L, 250mg/L, 500mg/L NaCl or $MgCl_2$(n=9).
- Embryos photographed every 24hrs. Viability, developmental stage, and successful hatching was recorded daily.

Results

Mobility Behavior Studies

Figure 1: Salt treatment averages for each mobility behavior endpoint for Day 3 and Day 7.(A) Average Mobile Speed (mm/s) Day 3.(B) Average Mobile Speed (mm/s) Day 7.(C) Average Speed (mm/s) Day 3.(D) Average Speed (mm/s) Day 7.(E) Time Spent Frozen (sec) Day 3.(F) Time Spent Frozen (sec) Day 7.(G) Distance Travelled (mm) Day 3.(H) Distance Travelled (mm) Day 7. (I) Acceleration (mm/s^2) Day 3.(J) Acceleration (mm/s^2) Day 7.

Embryonic Deposition Behavior

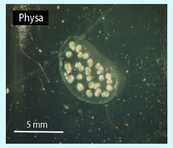


Figure 2: Image of *Physa acuta* embryo clutch with scale.

Figure 3: Image of *Physa acuta* adult.

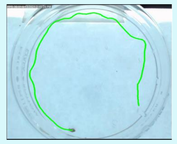
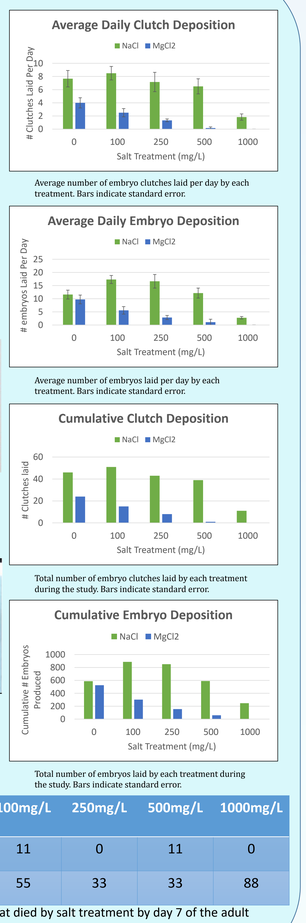


Figure 4: Image of ToxTrac (v. 2.83) output. The green line represents the path traveled by the subject



Average number of embryo clutches laid per day by each treatment. Bars indicate standard error.

Average number of embryos laid per day by each treatment. Bars indicate standard error.

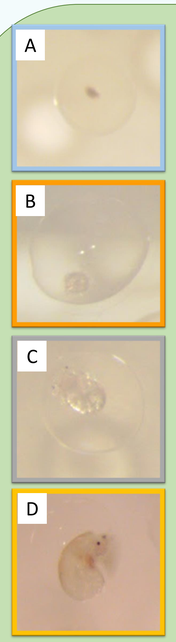
Total number of embryo clutches laid by each treatment during the study. Bars indicate standard error.

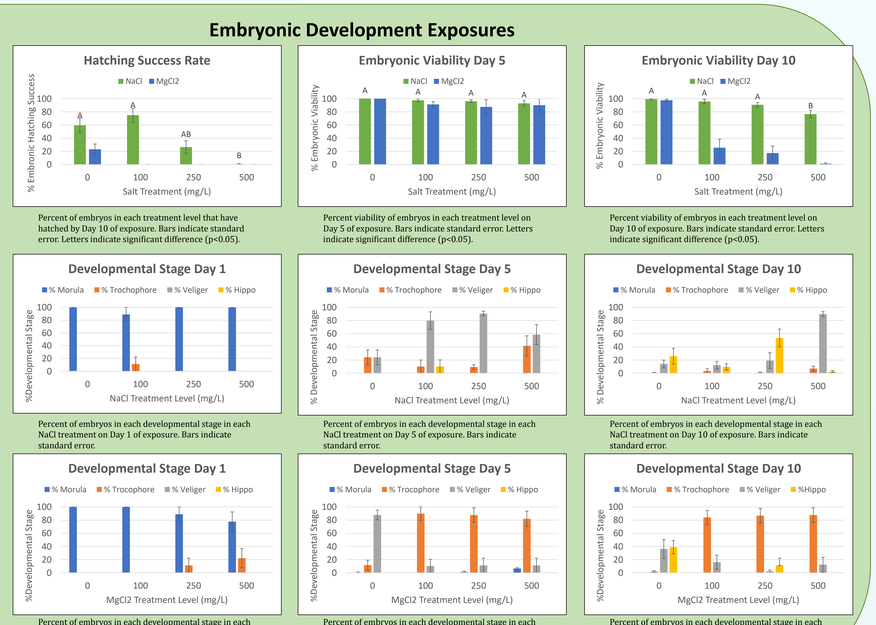
Total number of embryos laid by each treatment during the study. Bars indicate standard error.

Adult % Mortality	0mg/L	100mg/L	250mg/L	500mg/L	1000mg/L
NaCl	0	11	0	11	0
$MgCl_2$	22	55	33	33	88

Table 1: Percent of adults that died by salt treatment by day 7 of the adult behavior studies.

Embryonic Development Exposures





Percent of embryos in each treatment level that have hatched by Day 10 of exposure. Bars indicate standard error. Letters indicate significant difference (p<0.05).

Percent viability of embryos in each treatment level on Day 5 of exposure. Bars indicate standard error. Letters indicate significant difference (p<0.05).

Percent viability of embryos in each treatment level on Day 10 of exposure. Bars indicate standard error. Letters indicate significant difference (p<0.05).

Percent of embryos in each developmental stage in each NaCl treatment on Day 1 of exposure. Bars indicate standard error.

Percent of embryos in each developmental stage in each NaCl treatment on Day 5 of exposure. Bars indicate standard error.

Percent of embryos in each developmental stage in each NaCl treatment on Day 10 of exposure. Bars indicate standard error.

Percent of embryos in each developmental stage in each $MgCl_2$ treatment on Day 1 of exposure. Bars indicate standard error.

Percent of embryos in each developmental stage in each $MgCl_2$ treatment on Day 5 of exposure. Bars indicate standard error.

Percent of embryos in each developmental stage in each $MgCl_2$ treatment on Day 10 of exposure. Bars indicate standard error.

Figure 3: Photograph examples of *Physa acuta* developmental stages. (A) Morula (B) Trochophore (C) Veliger (D) Hippo

Results (cont.)

Mobility Behavior Study

- No difference for any endpoint for any treatment level on day 1.
- Mobility of *P. acuta* affected in 500mg/L and 1000mg/L NaCl treatment after 3 days. Mobility behavior impacted after 7 days in 1000mg/L NaCl treatment.
- $MgCl_2$ appears to impact mobility behavior in 250, 500, and 1000mg/L treatments after 3 days. Mobility behavior appears to be impacted in all treatment levels after 7 days.
- $MgCl_2$ exposure appears to cause increased adult mortality compared to NaCl.

Embryonic Deposition Behavior

- Average and cumulative clutch and embryo deposition display a dose dependent downward trend in both NaCl and $MgCl_2$ exposures. $MgCl_2$ appears to cause impacts in the 100mg/L $MgCl_2$ treatment as opposed to the apparent effects of NaCl beginning in the 500mg/L treatment.

Embryonic Development Exposures

- Viability decreased for 500mg/L NaCl and appeared to decrease in all $MgCl_2$ treatment levels.
- A dose-dependent delay in development was observed for NaCl . Embryos arrested in the veliger stage in the 500mg/L treatment starting on day 8.
- Embryos in $MgCl_2$ treatment levels appear arrested in the trochophore stage starting on day 4.

Conclusions

Mobility Behavior Study

- Impacts of NaCl and $MgCl_2$ on mobility behavior may negatively impact the fitness of individuals by increasing the risk of predation.
- $MgCl_2$ appears to be more toxic to adult *Physa acuta* than NaCl.

Embryonic Deposition Behavior

- Shift in the number of embryos and clutches laid by exposed individuals may result in increased risk of population decline.

Embryonic Development Exposures

- $MgCl_2$ appears to impact development more heavily than NaCl and appears to decrease embryonic viability at a larger scale than NaCl.
- Embryonic development appears to be the most sensitive endpoint assessed. This suggests that road salt exposure may result in large-scale impacts on populations that are not immediately apparent by decreasing embryonic survival.
- Due to their increased sensitivity embryos may be a good indicator of pollution in the environment.

Future Studies

- Assess common deicing additive sodium ferrocyanide on *P. acuta* adult mobility behavior, embryonic deposition behavior, and embryonic development.

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