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Method development for accessing the impacts of road salts on P. acuta behavior and egg viability

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Method development for accessing the impacts of road salts on *P. acuta behavior and egg viability*

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Introduction

Road salt runoff is increasing the salinity of the United States waterways well above the EPA's recommended limits. In the U.S. 90% of road salt applied to the road is NaCl (Vítzová, et.al., 2016). The EPA recommends that natural bodies of freshwater do not exceed a limit of 230mg/L NaCl for a span of four days once in three years. However, a study conducted in Baltimore discovered that its waterways had an average daily concentration of above 250mg/L NaCl (Kaushal et al., 2005). Road salt runoff is a major contributor to this increase in salinity as 55% of road salt ends up entering waterways without infiltrating the soil (Venner, 2004). In 2014 alone over 24.5 million tons of road salt was applied to roads in the U.S. with over 12.2 million tons of road salt carried into natural waterways as runoff (American Geosciences Institute , 2017). Few studies have investigated the effects of this rapid increase in salinity on freshwater aquatic invertebrate species.

Objectives

Embryonic Development

- Determine the effect of NaCl on *Physa acuta* egg cluster mortality

Behavior Studies

- Determine baseline adult *Physa acuta* behavior, speed, acceleration, and distance traveled.
- Determine the effect of various environmentally relevant NaCl concentrations on adult *Physa acuta* behavior, speed, acceleration, and distance traveled.

Hypotheses

Egg Cluster Exposures

- Frequency of egg mortality was expected to increase with an increase in NaCl concentration.

Behavior Studies

- Baseline behavior was expected to be similar in all individuals.
- Individuals exposed to higher concentrations of NaCl were expected to experience slower movement speeds, less acceleration, and travel shorted distances than the control group.

Materials and Methods

Egg Cluster Exposures

- 4 egg clusters were collected each day for 4 days and observed under light microscopy to count and record the number of eggs present and to determine if any of the eggs were inviable
- one egg cluster from each day was placed in each of the four concentrations of 0mg/L, 100mg/L, 500mg/L, and 1000mg/L NaCl
- The eggs were observed under a microscope daily to count the #number hatched and determine if the eggs were inviable

Behavior Studies

- One adult snail was placed in a large glass petri dish with 50ml of synthetic water. The snail was acclimated for 5 min. and its movements were recorded using an overhead USB camera for 3 min.
- The recording was analyzed using ToxTrac (v. 2.83) to monitor the snail's movements. Six locomotor endpoints were analyzed (Table 2) using seven snails
- Individual adult snails were randomly placed in 5 different treatments (Control (0mg/L), 100mg/L, 250mg/L, 500mg/L, and 1000mg/L NaCl) (n = 6).
- Each snail was individually placed in a 100mL beaker, 100% of the water was changed every 48 hrs. and the snails were kept in an incubator at around 24°C.
- After 3 days and 7 days the snails were removed from their beaker and placed in a glass dish with a lid and 50ml of synthetic water and their movements recorded using the above methods.

Results

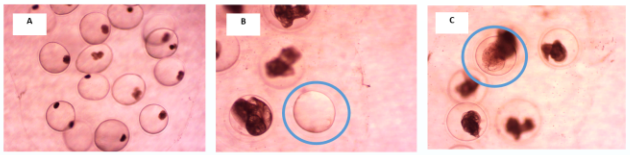


Figure 1: Eggs on Day 1 (A) Example of an inviable egg circled in blue (B), Another example of an inviable egg circled in blue (C).

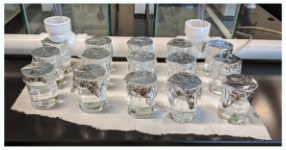


Figure 3: Example of NaCl exposure set up.

Table 1: Egg clusters considered viable or containing 100% inviable eggs after 15 days exposure.

Replicate	0mg/LNaCl	100mg/L NaCl	500mg/L NaCl	1000mg/L NaCl
1	Inviable	Viable	Viable	Inviable
2	Viable	Viable	Viable	Inviable
3	Viable	Viable	Viable	Inviable
4	Inviable	Viable	Viable	Inviable

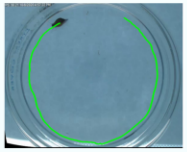


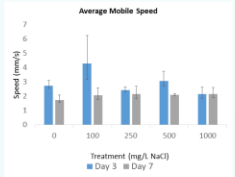
Figure 2: Image of ToxTrac (v. 2.83) output. The green line represents the path traveled by the snail.

Table 2: Snail Movement variables that were recorded and their definitions.

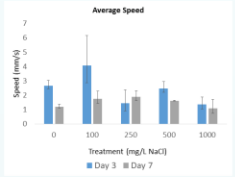
Average Mobile Speed (mm/s)	This is the average speed the snail moved at not including the time that it spent stopped.
Average Speed (mm)	This is the average speed the snail moved at over the entire 3 min. period.
Total Distance Traveled (mm)	This is the distance the snail traveled of the entire 3 min. period.
Acceleration (mm/s^2)	This is the average acceleration that the snail had over the entire 3 min. period.
Number of Frozen Events	This is the number of times that the snails movement stopped.
Time Spent Frozen	This is the total amount of time during the 3 min. that the snail spent stopped.

Table 3: Baseline behavioral data for adult *Physa Acuta*

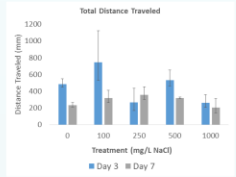
Replicate	Average Mobile Speed (mm/s)	Average Speed (mm/s)	Total Distance Traveled (mm)	Acceleration (mm/s^2)	# of Frozen Events	Time Spent Frozen
1	1.54	0.96	180.1	4.61	6	2.56
2	2.15	1.69	363.21	7.36	5	37
3	2.59	2.34	428.01	4.65	3	34
4	2.57	2.46	447.67	4.65	3	22
5	8.83	8.73	1572.68	11.43	0	0
6	1.78	1.01	190.23	3.43	3	2.19
7	3.37	3.34	604.5	4.11	1	3
Average	3.26	2.93	540.91	5.75	3	14.39
Standard Deviation	2.527940061	2.692023491	478.6736426	2.788251714	2.081665999	16.22379602



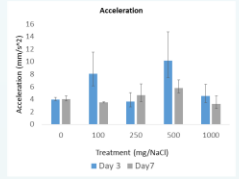
Treatment averages of average mobile speed (mm/s) from day 3 recordings and day 7 recordings. Bars indicate standard error.



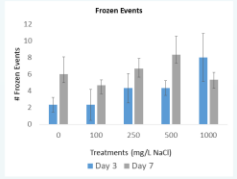
Treatment averages of average speed (mm/s) from day 3 recordings and day 7 recordings. Bars indicate standard error.



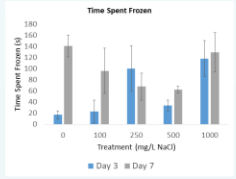
Graph 3: Treatment averages of total distance traveled (mm) from day 3 recordings and day 7 recordings



Graph 4: Treatment averages of average acceleration (mm/s^2) from day 3 recordings and day 7 recordings. Bars indicate standard error.



Graph 5: Treatment averages of average number of frozen events from day 3 recordings and day 7 recordings. Bars indicate standard error.



Graph 6: Treatment averages of average time spent frozen (s) from day 3 recordings and day 7 recordings. Bars indicate standard error.

Results (cont.)

Egg Cluster Exposures

- Inviability was observed in 2 of the controls, it is possible that one factor in causing this was stress, because of this egg clusters will be placed in small plastic petri dishes to avoid stress from movement required for observation.
- Eggs in the 1000mg/L NaCl solution developed slowly and did not hatch

Behavioral Study

- Baseline behavioral measurements of *Physa Acuta* vary by individual.
- Average mobile speed decreased lightly or remained constant between day 3 and day 7 recordings.
- In all treatments except for the 1000mg/L NaCl the number of frozen events increased from the day 3 recordings to the day 7 recordings.

Conclusions

Egg Cluster Exposures

- Results from suggest that 1000mg/L is too high of a concentration to study egg development as it results in mortality. Selecting a sub-lethal concentration (e.g 100mg/L, 250mg/L, etc.) would be more appropriate.

Behavioral Study

- The amount of time that the snails were given to acclimate was enough for them to behave normally. However, multiple behaviors varied from individual to individual. Therefore, it would be more beneficial to collect both pre- and post-exposure mobility data for each individual. Any change could be quantified as a percent change between baseline and day 3 and baseline and day 7. The percent change could then be averaged between all individuals exposed to the same concentration in order receive a more relevant comparison of snail behavior and NaCl concentration.

Future Studies

- Perform egg cluster studies with sublethal concentrations to determine NaCl effect on egg viability and development.
- Perform more behavioral exposures and take baseline recordings of the individuals before exposing them to NaCl.
- Increase sample size of behavioral study.
- Conduct behavioral exposures with a common Road Salt additive Sodium hydrocyanide.

Acknowledgements

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Funding

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References

- American Geosciences Institute. (2017). Roadway deicing in the United States [Fact Sheet]. https://www.americangeosciences.org/sites/default/files/CI_Factsheet_2017_3_Deicing_170712.pdf
- Kaushal, S. S., Groffman, P. M., Likens, G. E., Belt, K. T., Stack, W. P., Kelly, V. R., Band, L. E., & Fisher, G. T. (2005,) September). *Increased salinization of fresh water in the northeastern United States*. Proceedings of the National Academy of Sciences of the United States of America. https://www.pnas.org/content/102/38/13517
- Venner, M. 2004. Environmental stewardship practices, procedures, and policies for highway construction and maintenance. NCHRP Project 25-25 (04) Interim Report. http://onlinepubs.trb.org/onlinepubs/archive/NotesDocs/25-25(4)_FR.pdf, accessed 7/14/20
- Vítzová, M., Vítz, T., & Nitayapat, N. (2016). Effects of de-icing salts on the respiration of the microorganisms of activated sludge. *Water, Air and Soil Pollution, 227*(11), 1-7. doi:http://dx.doi.org.umw.idm.oclc.org/10.1007/s11270-016-3119-1