Effects of Road Salts on Ephemeral Wetland Ecosystems

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5. Schoell, M, E Nelson, T Rittenhouse, AM Helton. 2015. Poster: Effects of road salts on ephemeral wetland ecosystems: Water chemistry results from mesocosm experiments. Society of Wetland Scientists Annual Meeting. Providence, RI.
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Proposal Title: *Effects of road salts on ephemeral wetland ecosystems*

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Introduction/Research Objective:

Ephemeral wetlands, including seasonal, vernal, and other temporary ponds, are widespread in the Northeast United States (Tiner 2003). They are not connected to permanent surface water bodies, such as lakes, rivers, or streams. As a result they hold water only temporarily, periodically drying out. Although ephemeral wetlands are typically smaller in size than their permanent counterparts, in the Northeast United States they can make up a large portion of the aquatic landscape (e.g., Cormier et al. 2013), and are protected in many states, including Connecticut (Connecticut Inland Wetlands and Watercourses Act, sections 22a-36 through 22a-45 of the General Statutes of Connecticut).

Ephemeral wetlands provide critical fishless habitat for amphibian and macroinvertebrate reproduction (Semlitsch and Bodie 1998), and play particularly important roles in the processing of carbon and retention of nitrogen on the forest floor (Capps et al. 2014). Ephemeral wetlands are often filled during land development, used as storm water detention basins, and receive drainage from agricultural fields or residential areas (Tiner 2003). Human activities can severely alter population and community dynamics in isolated wetlands, by fragmentation of the surrounding terrestrial landscape and by direct surface water contamination (Gibbons 2003; Willson and Hopkins 2013).

Surrounding land use dramatically changes both the chemical and hydrologic regimes of ephemeral wetlands. Deforestation associated with development decreases leaf litter input to wetlands in developed catchments (France et al. 1996), decreasing basal carbon resources to temporary wetland ecosystems. Development increases overland runoff to aquatic systems since impervious surfaces reduce surface water infiltration to groundwater (Walsh et al. 2005). Increased runoff volume and direct routing of storm water increases the amount of time ephemeral wetlands experience standing surface water. Aquatic systems receiving runoff from developed land also typically have higher concentrations of nutrients, salt, and other pollutants (Walsh et al. 2005). Indeed, water quality is commonly degraded in temporary wetlands near human development (e.g., Atkinson et al. 2011).

In the Northeast United States, where chemical deicer is used on roadways in winter months, salinization of surface waters and groundwater (Kaushal et al. 2005; Cassanelli and Robbins 2013) is a pervasive pollutant in temporary wetlands. Road salt use has increased from 7.5 to over 22 million tons per year across the U.S. from 1975 to 2005, with the most dramatic increases across the glaciated northern U.S. (Findlay and Kelly 2011). The mean maximum concentration of chloride regularly exceeds the USEPA recommended chronic criterion for aquatic life in surface waters of urban areas of the glaciated northern United States, and chloride concentrations in surface waters are strongly correlated metrics indicative of paved areas, including percent development, road density, and impervious cover (Findlay and Kelly 2011).

Road salts have a wide range of direct impact on temporary wetland ecosystems. Elevated salinities in roadside wetlands 1) may influence microbial processing of C and N (Groffman et al. 1995), and resulting trace gas emissions, 2) are correlated with shifts in invertebrate communities to more salttolerant species (Petranka and Doyle 2010), and 3) can disrupt osmoregulation of eggs (Karraker 2011) and reduce larval survival of wood frogs and spotted salamanders (Karraker et al 2008).

For this proposal, our objectives were:

1. Quantify the microbial response to salinity by measuring potential denitrification rates and in situ greenhouse gas concentrations across wetlands with a range of road salt exposure.
2. Determine the abundance and shift in species of mosquitos across wetlands with a range of road salt exposure.
3. Quantify amphibian response to road salts across a range of road salt exposure and with controlled mesocosm experiments.

Methods/Procedures/Progress:

Surveys of wetlands: We selected 16 wetlands within the towns surrounding the University of Connecticut Storrs Campus. Wetlands were sampled seven times from 10 April 2015 to 3 August 2015. On the last day of sampling all but one ephemeral wetland was completely dry. During each visit, we measured specific conductance and water temperature. Surface water samples were immediately filtered and analyzed for nitrate, ammonium, soluble reactive phosphorus, total carbon, total nitrogen, and chloride according to standard methods (APHA 1998) at the Center for Environmental Sciences and Engineering (CESE). We also filtered samples to measure chlorophyll A as a surrogate for algal biomass using standard methods (APHA 1998) with a Turner Fluorometer at CESE. We collected additional surface water samples in evacuated vials and used headspace equilibration techniques to extract dissolved gas samples (Hudson 2004; Helton et al. 2014). Gas samples were analyzed on a Perkin Elmer Clarus 580 gas chromatograph customized to measure CO_2, CH_4, and N_2O located at CESE. Soil cores were collected in six of the wetlands on 23 June 2015. Soil cores were divided into 0-5 cm and 5-10 cm depth increments, sieved (2mm, #10 mesh), and homogenized. We analyzed soil cores for potential rates of denitrification using denitrifying enzyme activity (DEA; Groffman et al. 1999), as well as soil moisture and organic matter content using the loss on ignition method.

Mosquitos were counted on four dates from 22 April 2015 to 30 June 2015 using the partial submersion technique (O'Malley 1989) to sample invertebrate larvae in the water column, where a dipper is used to sample equal volumes near the surface of the water column. All samples were concentrated through a small mesh screen using a concentrator cup and preserved with 90% ethanol. Mosquitos were identified by the Connecticut Agricultural Experiment Station for samples collected on 22 April 2015. Mosquitos collected on the three additional sampling dates are preserved and stored in A.M. Helton's lab at CESE. Wood frog and spotted salamander egg masses were also counted using the double observer method (Scherer 2008) and repeat sampling for five sampling dates from 10 April 2015 to 30 June 2015.

Mesocosm experiments: In a fully randomized and replicated experiment, we randomly assigned one of 12 treatments to each 1000-liter mesocosm (N = 48 mesocosms) such that tanks had all possible combinations of species (LISY, PSCR, and BOTH), temperature (ambient or elevated), and salt (ambient or elevated). Tanks were also organized into four spatial blocks to account for potential environmental gradients at our study site. Lithobates (Rana) sylvaticus (LISY; LeConte, 1825; Frost et al., 2006) is a terrestrial frog with a wide geographic range and a complex life cycle (Berven, 1990). Pseudacris crucifer (PSCR; Weid-Neuweid, 1838) is a semi-arboreal frog with a wide geographic range covering all of the United States east of the Mississippi and as far north as Hudson Bay (Lovett, 2013).

Each mesocosm represented a natural wetland. Thus, we filled mesocosms with ground water, which we allowed to age for two days. To each mesocosm, we added 1 kg of leaf litter collected from a mixed hardwood forest located within the University of Connecticut's Fenton Tract (Wharton et al., 2009; Parent and Volin, 2014). We covered mesocosms with 50% shade cloth lids to represent canopy cover and to prevent other amphibian species and dragonflies from ovipositing in the water. We then inoculated each mesocosm with a concentrated mixture of phyto- and zooplankton collected from multiple natural ponds on 17 April. Zooplankton do not serve as a food source for tadpoles, but are important components of the complex communities found in natural ponds (Schell et al., 2001).

We created the elevated salt treatment by adding road salt obtained from the Department of Transportation storage facility in Mansfield, CT. We dissolved road salt into 15 L of water and then stirred salt water into the mesocosms until we reached a concentration of 1600 mg/L on 21 April. We created the elevated by 3 ºC temperature treatment using JBJ True Temp Heating Systems (Model T3-1000) which consists of a 1000 watt titanium heating rod and digital controller. We floated the heated rod 10 cm below the surface of the water, such that a natural temperature gradient occurred with the warmest water at the surface and coolest water at the bottom. The digital controller provided a set point temperature, but could not be programed to follow daily temperature fluctuations, and the thermometer linked to the controller was placed at the bottom of the tank. We adjusted temperature set points in the morning, by programing the set-point temperature to be 3 ºC warmer than the current morning temperature at the bottom of control tanks. Set-point temperatures were not changed daily, but rather every three to five days. Adjustments, both increasing and decreasing the set-point, were needed following weather fronts causing large shifts in daily high or low temperatures and when rain fell which added water of a different temperature to the mesocosms.

We stocked mesocosm tanks on 27 April with 30 tadpoles of LISY, PSCR, or BOTH species; mesocosms assigned to BOTH received 30 tadpoles of each species for a total of 60 tadpoles. This density is well within the range of larval anuran densities in natural wetlands (Morin, 1983). We removed animals when at least one front leg erupted (stage 42; Gosner 1960), and these individuals were identified as surviving the larval lifestage. We placed metamorphosed individuals into plastic sandwich containers with a small amount of water from the mesocosm to protect against desiccation during tail absorption and positioned the containers to allow for movement of metamorphs in and out of water. We labeled containers with the corresponding mesocosm tank number and held the animals in the adjacent animal care facility through tail resorption. We checked the containers daily to assess progress through metamorphosis. We recorded the date of metamorphosis and mass at the completion of resorption. We calculated all "days to metamorphosis" using the date of complete tail resorption, rather than capture date, because some metamorphs were captured at different stages in the tail resorption process.

Water temperature, specific conductivity, and dissolved oxygen (DO) were measured biweekly in all mesocosms using a handheld sonde (Yellow Springs Instruments, YSI 556 MPS). Water samples were collected biweekly and filtered immediately through 0.7um GF/F Whatman filters in syringe filter holders. Filtered samples were collected in acid washed and field rinsed bottles, transported to the lab on ice, and frozen until analysis. Filtered samples were analyzed according to standard methods (APHA, 1998) for soluble reactive phosphorus (SRP; ascorbic acid method), ammonium (NH4+; phenate method), and nitrate (NO3-; cadmium reduction method) at the Center for Environmental Sciences and Engineering Nutrients Laboratory. Dissolved inorganic nitrogen (DIN) was calculated by summing NH4+ and NO3- concentrations. Filters were preserved by freezing, and chlorophyll a concentrations were measured by acetone extraction on a Turner Designs fluorometer (Trilogy 7000-000).

Results/Significance:

Surveys of wetlands: The wetlands surveyed ranged in their average specific conductivities from 0.034 to 1.102 mS cm $^{-1}$ (Table 1). Wetlands also varied in their water chemistry and greenhouse gas concentrations, with several wetlands having particularly high nitrous oxide concentrations (Table 1). We are currently analyzing this dataset, and summary statistics are available in Table 1.

Table 1. Average and Standard Deviation of water chemistry, Chlorophyll A, and dissolved greenhouse gases collected from ephemeral wetlands during 2015.

Site Name	Specific Conductivity (mS / cm)		Water Temperature (°C)		Ammonium (mg N / L)		Ortho-phosphate (mg P / L)		Nitrate (mg N / L)		Dissolved organic carbon(mg / L)		Chlorophyll A (ug/L)		Dissolved methane (ug / L)		Dissolved carbon dioxide(ug / L)		Dissolved nitrous oxide (ug / L)	
	Avg.	Std. Dev	Avg.	Std. Dev	Avg.	Std. Dev	Avg.	Std. Dev	Avg.	Std. Dev	Avg.	Std. Dev	Avg.	Std. Dev	Avg.	Std. Dev	Avg.	Std. Dev	Avg.	Std. Dev
Andover	0.498	0.433	18.9	1.7	0.225	0.406	0.017	0.015	0.011	0.011	7.47	2.30	13.34	15.02	274.9	403.2	12057	4694	76	47
BackRd	0.047	0.013	16.1	5.3	0.009	0.017	0.006	0.006	0.000	0.017	8.10	5.10	1.71	1.41	211.2	362.2	26215	21639	66	26
BrownRd	0.382	0.129	12.4	4.2	0.013	0.009	0.005	0.005	0.658	0.890	5.29	4.41	10.51	5.92	35.9	38.4	35697	38089	508	108
Chaffee	0.044	0.012	14.1	5.6	0.075	0.086	0.027	0.027	0.000	0.004	9.49	8.12	2.79	1.63	75.0	81.6	17929	12079	72	29
Erdoni	0.037	0.009	16.8	2.5	0.037	0.044	0.004	0.004	0.034	0.073	5.76	2.19	2.52	2.88	128.1	109.5	18201	23943	47	28
GehringRd	1.102	0.321	17.3	2.9	0.020	0.019	0.013	0.005	0.000	0.022	12.95	3.80	9.08	5.87	393.7	366.0	23514	16561	66	27
GrantHill	0.618	0.351	15.5	3.2	0.109	0.098	0.013	0.011	0.194	0.279	5.67	3.01	2.21	1.25	113.3	109.2	20934	15360	368	223
Heidi1	0.432	0.095	14.3	5.9	0.040	0.048	0.015	0.011	0.016	0.064	9.01	2.89	2.90	1.97	242.5	176.4	23341	14611	64	31
Heidi2	0.491	0.066	15.4	6.1	0.061	0.047	0.011	0.008	0.088	0.167	4.57	1.16	16.05	28.43	52.4	31.2	16578	11657	59	24
MS1	0.222	0.269	11.7	5.1	0.082	0.083	0.014	0.015	0.000	0.026	20.15	12.99	3.03	1.86	273.1	126.2	32211	28097	64	30
MS2	0.034	0.005	12.4	4.6	0.068	0.142	0.020	0.023	0.000	0.013	10.45	7.56	2.73	3.46	319.7	611.3	38624	35211	67	27
NH	0.116	0.025	14.4	2.5	0.008	0.008	0.004	0.003	0.000	0.013	7.77	3.87	4.10	4.54	323.3	287.4	30081	25178	60	16
UF1	0.165	0.024	10.0	5.4	0.010	0.007	0.007	0.007	0.045	0.048	2.91	0.38	0.55	0.34	1.7	0.5	8973	1580	75	8
UF2	0.034	0.012	13.6	9.4	0.039	0.034	0.040	0.057	0.000	0.028	23.89	15.41	2.79	1.55	39.4	6.9	14830	14267	57	22
UF3	0.087	0.023	11.2	6.4	0.005	0.007	0.004	0.006	0.013	0.024	2.87	1.99	1.00	0.19	1.7	2.2	15624	10793	157	127
UF4	0.076	0.026	13.5	3.3	0.026	0.033	0.008	0.005	0.000	0.010	2.93	1.42	2.00	1.26	172.1	197.2	18261	15805	67	25

Soil cores were collected from six of the 16 wetlands that covered the full range of measured average salinities. We found that potential denitrification was significantly positively related to organic matter content ($r^2 = 0.50$, $p<0.05$) and soil moisture content ($r^2 = 0.79$, $p<0.05$). We also found that potential denitrification rates were significantly negatively correlated with specific conductance (Figure 1), *suggesting that elevated salinity suppresses potential rates of denitrification in ephemeral wetlands.*

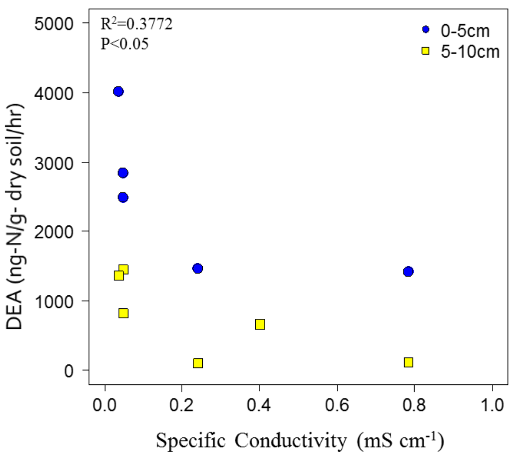

Figure 1. Potential denitrification rates versus specific conductivity for wetland soil cores.

Mesocosm experiments: Detailed results of the mesocosm experiments are available in D. Macklem (2016). Briefly, LISY and PSCR survival was significantly influenced by salinity (Figure 2). The number of LISY tadpoles surviving to metamorphosis was reduced by both elevated road salt ($p < 0.001$) and elevated temperature ($p < 0.039$). Overall, road salt additions decreased average LISY survival from 90.4% to 62.3% and average PSCR survival from 56% to 14%.

We also found elevated chlorophyll a in both LISY and PSCR salt treatments. Chlorophyll a was higher in LISY elevated salt treatments ($F_{1,26} = 18.07$, $p < 0.001$). This may be due to elevated soluble reactive phosphorus concentrations in both LISY and PSCR salt treatments, due to sand with elevated phosphorus commonly added to road salts.

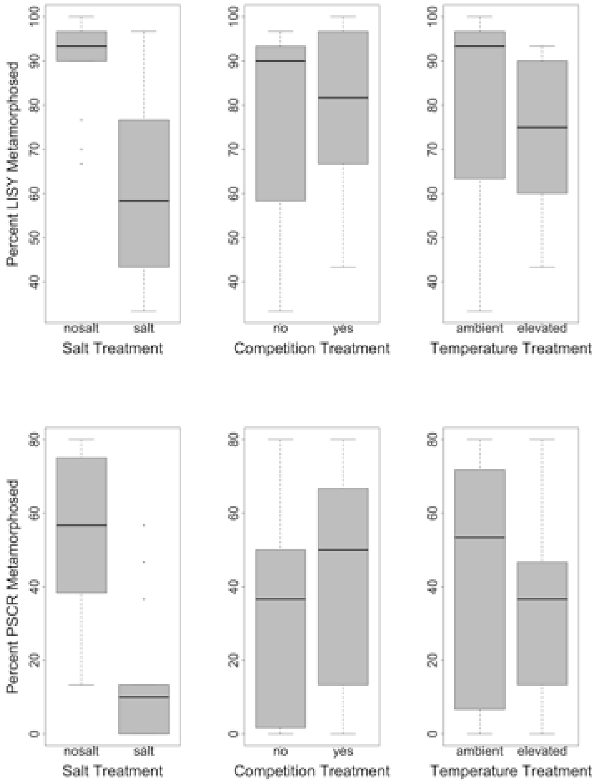

Figure 2. The percent of LISY and PSCR tadpoles surviving to metamorphosis under the various treatments.

References:

APHA (1998) Standard methods for the examination of water and wastewater, Washington DC, American Public Health Association Publication, APHA, AWWA, WEF

Atkinson CL, Golladay SW, and First MR (2011) Water quality and planktonic microbial assemblages of isolated wetlands in an agricultural landscape. Wetlands 35:885-894.

Capps KA, Rancatti R, Tomczyk N, TB Parr, Calhoun AJ, Hunter M (2014) Biogeochemical hotspots in forested landscapes: The role of vernal pools in denitrification and organic matter processing. Ecosystems. doi 10.1007/s10021-014-9807-z

Cassanelli JP and Robbins GA (2013) Effects of road salt on Connecticut's groundwater: A statewide centennial perspective. *Journal of Environmental Quality* 42(3): 737-748.

Cormier TA, Congalton RG, and Babbitt KJ (2013) Spatio-statistical predictions of vernal pool locations in Massachusetts: Incorporating the spatial component in ecological modeling. Photogrammetric Engineering & Remote Sensing 79 (1): 25–35.

Findlay SEG, Kelly VR (2011) Emerging indirect and long-term road salt effects on ecosystems. Annals of the New York Academy of Sciences, The Year in Ecology and Conservation Biology 1123: 58-68.

France R, Culbert H, and Peters R (1996) Decreased carbon and nutrient input to boreal lakes from particulate organic matter following riparian clear-cutting. Environmental Management. 20 (4). 579-583.

Frost, D.R., T. Grant, J. Faivovich, R.H. Bain, A. Haas, C.F.B. Haddad, R.O. De Sá, A. Channing, M. Wilkinson, S.C. Donnellan, C.J. Raxworthy, J.A. Campbell, B.L. Blotto, P. Moler, R.C. Drewes, R.A. Nussbaum, J.D. Lynch, D.M. Green, and W.C. Wheeler. 2006. The amphibian tree of life. Bulletin of the American Museum of Natural History 297:1-291.

Gibbons JW (2003) Terrestrial habitat: A vital component for herpetofauna of isolated wetlands. Wetlands 23(3): 630-635.

Gosner, K.L. 1960. A simplified table for staging anuran embryos and larvae with notes on identification. Herpetologica 16:183-190.

Groffman PM, Gold AJ, Howard G (1995) Hydrologic tracer effects on soil microbial activities. Soil Science Society of America Journal 59(2): 478-481.

Hudson F (2004) Standard Operating Procedure: sample preparation and calculations for dissolved gas analysis in water samples using a GC headspace equilibration technique. U. E. P. Agency. Washington, DC.

Karraker NE and Gibbs JP (2011) Road deicing salt irreversibly disrupts osmoregulation of salamander egg clutches. Environmental Pollution 159:833-835.

Karraker NE, Gibbs JP and Vonesh JR (2008) Impacts of road deicing salt on the demography of vernal pool-breeding amphibians. Ecological Applications 18:724-734.

Kaushal SS, Groffman PM, Likens GE, et al. (2005) Increased salinization of fresh water in the northeastern United States. PNAS 102(38) 12517-13520.

LeConte, J. 1825. Remarks on the American species of the genera Hyla and Rana. Annals of the Lyceum of Natural History of New York 1:278-282.

Morin, P.J. 1983. Predation, competition, and the composition of larval anuran guilds. Ecological Monographs 53:119-138.

O'Malley C (1989) Guidelines for larval surveillance. Proceedings of the Seventy-Sixth Annual Meeting of the New Jersey Mosquito Control Association, Inc. pp 44-55.

Parent, J., and J.C. Volin. 2014. Assessing the potential for leaf-off LiDAR data to model canopy closure in temperature deciduous forests. ISPRS Journal of Photogrammetry and Remote Sensing 95:134-145.

Petranka JW and Doyle EJ (2010) Effects of road salts on the composition of seasonal pond communities: can the use of road salts enhance mosquito recruitment? Aquatic Ecology 44:155-166.

Petranka JW and RA Francis (2013) Effects of Road Salts on Seasonal Wetlands: Poor Prey Performance May Compromise Growth of Predatory Salamanders. Wetlands 33:707-715.

Scherer RD (2008) Detection of wood frog egg masses and implications for monitoring amphibian populations. Copeia 2008:669-672.

Semlitsch RD and Bodie JR (1998) Are small, isolated wetlands expendable? Conservation Biology 12(5): 1129-1133.

Tiner RW (2003) Geographically isolated wetlands of the United States. Wetlands 23(3): 494-516. http://dx.doi.org/10.1672/0277-5212(2003)023[0494:GIWOTU]2.0.CO;2.

Walsh CJ, Roy AH, Feminella JW, et al. (2005) The urban stream syndrome: current knowledge and the search for a cure. Journal of the North American Benthological Society 24(3): 706-723.

Wharton, E.H., R.H. Widmann, C.L. Alerich, C.J. Barnett, A.J. Lister, T.W. Lister, D. Smith, and F. Borman. 2004. The forest of Connecticut. USDA Forest Service. Resource Bulletin NE-160.

Willson JD and WA Hopkins (2013) Beyond the wetland: evaluating the effects of anthropogenic stressors on source-sink dynamics in pond-breeding amphibians. Conservation Biology 27:595-604.