Freshwater salinization of seasonal ponds: High salinity and stratification threaten critical, overlooked habitats

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Abstract

Nearly a century of road salt use in the snowbelt region of North America has led to substantial increases in salinity levels in freshwater habitats. Salt pollution in lakes and rivers is well characterized. Lacking are broad insights for seasonal ponds. As critical habitats for many endemic species, these small and often poorly flushed surface waters are especially vulnerable to accumulating high levels of salts and other pollutants. Here, we measured salinity in 165 seasonal ponds, characterizing salt pollution patterns across space, through time, and over depth within ponds. We found that 70% of ponds within 37 m of a road contained salinity levels exceeding Canadian federal guidelines. 54% of ponds within 25 m exceeded less conservative US federal guidelines. Within ponds, the water column was stratified due to the combined density effects of salt and temperature. Bottom waters of polluted ponds were about 57% saltier than near surface waters, though many were much saltier than this. Compared to lakes and rivers, far more seasonal ponds appear to be compromised by deicing salt, and overall, the concentration of salt appears to be substantially higher. Among aquatic habitats, seasonal ponds are experiencing the most severe impacts of freshwater salinization with consequent impacts on sensitive aquatic organisms.

Synopsis

Seasonal ponds near roads in the Northeast experience severe freshwater salinization, with over half exceeding U.S. and Canadian chloride criteria.

Introduction

Freshwater salinization is an emerging global environmental problem [1]. Over the past century, many of our fresh surface and ground waters have become polluted with salt from human activities. Sources of salt pollution are many, including intrusion from sea level rise, effluent from mining and industry, runoff from agriculture and dust suppression, and evaporation and irrigation effects due to climate change [1,2]. However, freshwater salinization is particularly pervasive in snowbelt regions of the world where winter deicing practices play a major role. Salts applied to melt ice and snow on roads, driveways, parking lots, and sidewalks are carried with runoff into adjacent surface waters where they increase salinity and often enter ground waters [3].

The ecological impacts of freshwater salinization threaten countless habitats and aquatic organisms across the globe [1,4]. Numerous studies report climbing salt levels in streams, rivers, and lakes [5-8], the latter of which can become stratified by salt, modifying turnover dynamics [9]. Many studies have also reported the negative effects of salt exposure on freshwater organisms [10,11]. Countless species of amphibians, fish, and macro and micro-invertebrates show sensitivity to salt pollution reviewed by [11,12]. Salinization of freshwater habitats is often high enough to produce sublethal effects on physiology and behavior, along with lethal effects on survival and reproduction, all of which can lead to population declines and ecosystem impacts [12]. While salt *per se* is not a federally regulated contaminant in the US or Canada, its chief anion, chloride is. Yet chloride concentrations in salinized freshwaters often exceed federal water quality criteria [5,8,13] and there is little recourse for mediation. More alarmingly, these criteria are often too high to protect aquatic organisms from harm [14].

Unlike for lakes and rivers, there exist no long-term water quality monitoring programs for seasonal ponds or other small surface waters, leaving a key gap in our understanding of freshwater salinization [4]. This gap is critical because small water bodies are by far the most numerous type of lentic habitat on the planet. Globally, 91% of all lakes are < 0.01 km^2 in surface area, averaging just 0.0025 km^2, the size of two Olympic swimming pools [15]. Of special interest are seasonal ponds such as vernal pools, which are small, shallow water features (ranging in size ~ 10 m^2 to 1 ha and typically < 70 cm deep), many of which have only intermittent hydrological connectivity with other surface waters or ground water [16]. Seasonal ponds serve as critical habitats for many endemic, rare, and often sensitive species [17], and deliver ecosystem services such as nutrient cycling, carbon sequestration, and water regulation [18]. Many seasonal ponds have been modified or destroyed through anthropogenic land development and habitat conversion [19]. Formal regulations protecting these habitats are generally lacking [20]. Still, seasonal ponds remain very numerous, sometimes comprising 10% of a landscape's surface area [21].

Unfortunately, because seasonal ponds have small water volumes, pollution can more easily reach high concentrations and harm many aquatic organisms [22]. Residence time of pollution likely depends on hydrological connectivity, with more poorly flushed pools retaining salinity and other runoff contaminants longer and thus increasing the risk and duration of exposure. Also, because salt is not significantly degraded or sequestered through biological or chemical pathways, it has the potential to accumulate to a greater degree than most other contaminants. Taken together, these hydrological and salt pollution characteristics render seasonal ponds highly vulnerable to the negative impacts of freshwater salinization.

Here, to begin to fill the gap in our knowledge about salt pollution in small surface waters, we measured salinity in 165 seasonal ponds and two stormwater ponds in New England, a region with intense winter deicing. We asked several research questions: How salty are seasonal ponds near roads and what proportion of them exceed federal guidelines for chloride? How does salinity vary with road proximity, and does salinity decline throughout the year? Are seasonal ponds stratified with salt? Next, we analyzed high-temporal resolution data from deployed loggers to characterize salt stratification patterns within ponds and throughout the year. Finally, we modeled the relation between salinity, temperature, and water density to help interpret our results and to provide a framework for predicting the interaction of salt and temperature as relative drivers of salinity gradients in small surface waters.

Methods

Study sites and natural history

Over the past two decades, we measured conductivity in suite of small, freshwater ponds distributed across a road-proximity gradient in northeastern USA (Fig. S1). Background conductivity values in our study region are low (i.e., tens of microsiemens/cm) – typical of forested seasonal ponds – and thus elevated conductivity can be used to interpret salt pollution and estimate chloride concentrations [23]. Land use and land cover (LULC) surrounding our study ponds is characterized by limited residential development amid secondary forests dominated by mixed hardwoods. Surficial geology in the entire region is largely the result of Laurentide glaciation. Soils are generally sandy loams, though some may have larger amounts of silt and clay. Indeed, the existence of persistent surface water bodies may indicate more poorly drained soils high in fines at least in the immediate vicinity of the ponds. Ponds varied in terms of their canopy cover, surface area, hydroperiod, emergent vegetation, and depth. Most ponds were wadable when full, typically less than 90 cm deep. 154 of these sites were forested seasonal ponds considered to be ‘vernal pools’, tending to fill with water in late fall and dry completely in mid-summer. 11 of the ponds could be classified as freshwater wetlands rather than vernal pools because they contained emergent vegetation but also showed seasonal hydrology similar to vernal pools. All of these ponds acted as breeding sites (or were considered suitable habitat) for vernal pool species such as wood frogs (*Rana sylvatica*), spotted salamanders (*Ambystoma maculatum*), and diverse invertebrates [24]. Finally, we included two best management practice (BMP) ponds that were engineered for stormwater detention but shared characteristics of vernal pools in size and depth. Seasonal pond hydrology is described in Supporting Text.

Road proximity effect on pond conductivity

We initially selected the seasonal ponds studied here for amphibian experiments, population surveys, and water quality monitoring studies [10, 25, 26]. This initial selection process was conducted by surveying a large suite of candidate ponds that were identified using a combination of wetland maps, satellite imagery, driving searches, and walking searches. Over the years, we have conducted amphibian experiments and surveys in three different regions: southern Connecticut, northeastern Connecticut, and the central valley of Vermont and New Hampshire. In each region, we repeated the process of identifying candidate ponds and narrowing to a subset of experimental ponds. For our amphibian experiments, we generally selected five or six ‘roadside’ ponds located < 15 m from the edge of a paved road that contained the highest conductivity levels among candidate ponds. These ponds were paired with ‘woodland’ ponds located > 150 m from the road, a distance at which chloride is expected to fall to background concentrations [27]. Conductivity in experimental ponds was often measured multiple times per year. Additionally, we routinely conducted amphibian and conductivity surveys across a broader suite of seasonal ponds located across a road-proximity gradient of 2 - 1255 m. Finally, we occasionally measured roadside ponds that we encountered fortuitously during fieldwork (i.e. with no prior searching). We included all these ponds in our analysis of road proximity effects with the goal of compiling the broadest suite of ponds from which to evaluate the relationship between road proximity and conductivity.

For each pond, distance to the nearest road was measured using Google Earth. We also used a digital elevation model in ArcGIS to score each pond as being either upslope or downslope from the road. Conductivity in all ponds was typically measured during the spring (coinciding with amphibian breeding season) between the months of March and June. Conductivity was measured at a depth of 10 cm at the deepest point in the pond. In some cases, we also measured conductivity in the bottom waters (at the sediment-water interface; SWI) and across multiple 10 cm depths to characterize intra-pond variation. This full data set of ponds and conductivity values is available in Supplemental Item 1. A map of all ponds is shown in Figure S1. Here and throughout, conductivity was standardized to 25 deg C (i.e. ‘specific conductivity’). In each pond, conductivity was measured with calibrated, handheld meters (YSI ProDSS, Oaton PCTEstr 35, or Oakton PCTSTester 50) up to 30 times (mean=4.1, SD=5.4) across multiple years. In addition to handheld meters, we deployed conductivity loggers in three ponds for a period ranging from five days to one year to characterize high temporal resolution and vertical conductivity dynamics (see details in *Stratification patterns* below). Here and throughout, statistical analyses for each subsection are described in Supporting Text.

General salinity patterns in seasonal ponds

We used a bootstrapping approach to estimate mean salinity of seasonal ponds in the northeastern US situated in relatively undeveloped landscapes. Ponds were stratified into 25 m distance classes up to 500 m away from the road. This distance was chosen because relatively few ponds were sampled beyond 500 m. Random sampling with replacement was performed 1000 times. Means were calculated with each bootstrap to estimate an unbiased overall mean salinity and 95% CIs. This process was repeated separately to estimate salinity in both surface and bottom waters.

Seasonal conductivity patterns

To quantify temporal trends across spring months when vernal pools are most actively used as habitat, we analyzed conductivity from a subset of 12 experimental ponds in northeastern Connecticut. These ponds were monitored for conductivity during four consecutive years of amphibian eco-evolutionary experiments on road proximity effects [25, 28]. Six ponds were located < 10 m from the road (i.e., ‘roadside’ ponds) and six ponds were located > 150 m from the road (i.e., ‘woodland’ ponds). Conductivity in these ponds was measured multiple times each spring in each of four consecutive years from 2008-2011, except in one woodland pond (‘rhb’) added to the experimental suite and measured from 2009-2011.

Stratification patterns

To broadly characterize salinity stratification patterns, we asked whether conductivity differed between surface waters (at 10 cm depth) and bottom waters (at the SWI). We first analyzed whether conductivity differed between surface and bottom waters for all ponds where bottom conductivity was ≥150 µS/cm (N=99 ponds) from among the full suite of ponds. We repeated this analysis for all ponds with bottom conductivity < 150 µS/cm (N = 30). (We note that at times in the field, we did not measure bottom water conductivity in ponds where surface water salinity was close to background levels because we found that bottom and surface values were nearly identical in most cases where salinity was not elevated above background levels.) In each case, we used ‘lmer’ to compose a linear mixed-effects model to analyze conductivity as a function of depth (i.e., surface versus bottom). We included pond ID as a random effect to account for repeated measures. P-values were calculated using the Satterthwaite method for degrees of freedom implemented in the ‘lmerTest’ package [29] and 95% Wald confidence intervals were calculated for each parameter.

Next, to characterize salt stratification patterns at high temporal and spatial resolution, we monitored conductivity across a vertical gradient within each of three ponds, with deployment periods ranging from one week to one year. Full methods for these long-term deployments and analyses are provided in Supporting Text.

Results

Road proximity effects on pond conductivity

Maximum conductivity values at the top of the water column exceeded equivalent Cl^- thresholds for US and Canadian criteria in ponds located up to 19 m and 37 m from the road, respectively. Using these distances as reference points, 42% of ponds within 19 m of the nearest road had surface waters that exceeded the US criterion while 64% of ponds within 37 m exceeded the Canadian criterion (Fig. 1A). In bottom waters, maximum conductivity exceeded US and Canadian thresholds up to 25 m and 37 m from the road, representing 54% and 70% of ponds found within these distances, respectively (Fig. 1B).

For conductivity at the top of the pond, the exponential decay model (Fig. 2A) estimated a lower limit asymptotic value of 34 μS/cm (95% CI: -112 – 183 μS/cm; $P = 0.635$, i.e. not different from 0), an intercept upper limit (i.e., where distance to road is zero) of 890 μS/cm (95% CI: 690 – 1090 μS/cm; $P < 0.001$), and a decay parameter of 33 μS/cm (95% CI: 5.8 – 60 μS/cm; $P = 0.018$). For conductivity at the bottom of the pond, the exponential decay model (Fig. 2B) estimated a lower limit asymptotic value of 34 (95% CI: -111 – 183; $P = 0.635$), an upper limit initial value of 1320 μS/cm (95% CI: 880 – 1770 μS/cm; $P < 0.001$), and a decay parameter of 22 μS/cm(95% CI: -1.26 – 45 μS/cm; $P = 0.067$).

For the linear mixed-effects analyses (using $\log_{10}$ transformations of conductivity and distance-to-nearest-road), the model with random effects for pond and year was preferred over the model with a single random effect for pond, for both surface-water conductivity (delta AIC = 15.8, $\chi^2_1 = 17.8$, $P < 0.001$) and bottom-water conductivity (delta AIC = 39.3, $\chi^2_1 = 41.3$, $P < 0.001$). Surface and bottom-water conductivity varied as a function of both distance to the road and microtopography (Fig. S2A-B). In surface waters, $\log_{10}$ conductivity declined at a rate of -0.60 (95% CI: -0.68 – -0.52; $F_{1,143.1} = 233.7$, $P < 0.001$) with each $\log_{10}$ unit of distance from the road. At the bottoms of ponds, $\log_{10}$ conductivity declined at a rate of -0.64 (95% CI: -0.75 – -0.53; $F_{1,92.7} = 138.9$ $P < 0.001$) with each $\log_{10}$ unit of distance from the road. This means that for every tenfold increase in distance from the road, conductivity decreased by about 76% and 77% percent for surface and bottom waters, respectively. In terms of microtopography, ponds downslope of the road were saltier that ponds on the upslope side. Surface waters downslope were 52% saltier than those upslope ($F_{1,142.4} = 7.49$, $P = .007$), while downslope bottom waters were 76% saltier than those upslope ($F_{1,142.4} = 7.49$, $P = .007$).

General salinity patterns in seasonal ponds

Across all ponds, there was a huge range of saltiness. Mean conductivity ranged from 12.0 to 3,300 µS/cm (an estimated 3.3 – 920 mg/L Cl^-) in surface waters and 12.0 to 4,200 µS/cm (an estimated 3.3 – 1180 mg/L Cl^-) in bottom waters. Across bootstrapped samples of ponds located within 500 m of a road, mean surface water conductivity was 103 µS/cm (95% CI: 55. – 240), an estimated 29 mg/L Cl^- (95% CI: 15.3 – 66), compared to a bottom water average of 150 µS/cm (95% CI: 49. – 390), an estimated 42 mg/L Cl^- (95% CI: 13.7 – 109). These values are higher than typical background Cl^- levels of around 7 mg/L found in ponds far from roads.

Seasonal conductivity patterns

The model containing the random slope for pond and random intercepts for pond and year (AIC = 3455.0, $X^2_1 = 9.17$, $P = 0.002$) was preferred over the alternative models with 1) year nested within slope (AIC = 3470) and 2) a single random intercept for pond (AIC = 3470). Conductivity varied as a function of the interaction between pond type (i.e., roadside vs. woodland) and date ($X^2_1 = 9.17$, $P = 0.002$). Conductivity in roadside ponds was high (estimate = 1730 µS/cm, 95% CI = 1160 – 2300 µS/cm) and declined at a rate of -6.33 µS/cm (95% CI = -9.3 – -3.4 µS/cm) per day (Fig. S3). In woodland ponds, conductivity was low (estimate = 22.4 µS/cm, 95% CI = -560 – 610 µS/cm) and remained level over time (coefficient = 0.08, 95% CI= -2.96 – 3.12 µS/cm). All fixed and random effect estimates and CIs are provided in Supplemental Item 2. Variation in conductivity among ponds accounted for 89 % of the total variance. Marginal R-squared (i.e. based on fixed effects alone) was 0.61 compared to a conditional R-squared (i.e., based on fixed and random effects) of 0.89.

Stratification patterns

Conductivity varied between surface and bottom waters in ponds with salinity ≥ 150 µS/cm ($F_{1, 414.0} = 75.2$, $P < 0.001$; Fig. 3A) but not in ponds where conductivity was below this level ($F_{1, 112.9} = 1.64$, $P = 0.204$). Conductivity was estimated to be 960 µS/cm (95% CI: 820 – 1110 µS/cm) at the bottom of the water column compared to 610 µS/cm (95% CI = 530 – 690 µS/cm) at the surface, making bottom waters about 57% saltier than surface waters. Variation in conductivity among ponds accounted for 58% of the total variance observed in the data. Marginal R-squared (i.e. based on fixed effects alone) was 0.06 compared to the conditional R-squared (i.e., based on fixed and random effects) of 0.61. Fixed and random effect estimates and CIs are provided in Supplemental Item 3.

Our most detailed measurements of salinity are for the vernal pool called Crystal H (CH) located in northeastern Connecticut 3.9 m from the nearest roadway (Fig. 3B). Here our profile data covered a period of just over a year and revealed a pattern of sharp stratification beginning with the salting season in December, growing through March, and extending to August, long after salting had ceased (typically in late March). The pond held water from the time of deployment until August, when all but the deepest part of the basin was dry (Fig. 3B). Finally in late fall, the pond refilled with water (typical of seasonal pools in our area) and the cycle appeared to begin anew. In this system, the water column was generally vertically isothermal, and the stable stratification over a distance of only a few tens of cm was apparently caused almost entirely by density differences driven by salinity variations (Fig. 3C). To tease out the crucial role of salinity, we removed the effect of temperature, which changed only temporally but was constant vertically. Without this adjustment, the density scale is suppressed, concealing the dominant role of salinity. This plot covers only the period of spring stratification which is the amphibian breeding season and also when salt dominates water density.

Detailed time-depth records were also collected for two other ponds over weeklong periods in May. Pond SC was stratified from 40 cm to the bottom throughout the period of measurement (Fig. 4A). Whitney was nearly homogenous vertically at the beginning of the measurement period (Fig. 4B), a time just after 4.5 cm of rain fell. After two days, stratification developed from the surface to the maximum depth of 110 cm, continuing until the end of the measurement period. There was a maximum in salinity near 80 cm, which could exist at this intermediate depth because temperature stratification caused maximum density to extend right to the bottom.

Despite significant differences among the many sites we studied, there was a clear tendency for stratification to exist and for salt levels to increase with depth, whether considered collectively (Fig. 3A) or in more detail within individual ponds (Fig. 4C).

Water density model

For fresh waters, density depends almost equally on temperature and salinity over the ranges we encountered for these two parameters. Figure 4D shows water density in temperature-salinity space for our systems. As shown in the plot, density generally increases with salt level and declines with temperature. Considering only the spring breeding season, water temperature rarely exceeds 10 degrees. As the maximum in water density occurs at 4 degrees, there is relatively little influence of temperature around this value, and the total range is less than 1 part per thousand (Fig. 4D). Conductivity varied widely among the many ponds we evaluated. For example, the highest salt levels we tested were close to 4,000 μS/cm. Because of the relative insensitivity of density to temperature during the breeding season, and the high levels of salinity we sometimes observed, it is important to consider salt as a factor influencing stratification in seasonal ponds.

Discussion

Seasonal ponds in the northeastern US have become heavily salinized from road deicing salt. This effect is driven in part by road proximity. Salinity appears to reach peak levels in late winter or early spring and declines modestly into summer for the subset of ponds shown in Figure 3. Within ponds, bottom waters are considerably saltier than surface waters, with stratification persisting throughout much of the hydroperiod. Taken together, these results show that most seasonal ponds near roads in the northeastern US are heavily polluted and stratified by salt, that salt variation within and among ponds is high, and that seasonal pond habitats are particularly vulnerable to freshwater salinization effects.

In our sample, a staggering 70% of seasonal ponds located within 37 m of a road exceeded the Canadian water quality criterion for Cl^- and 54% within 25 m exceeded the US criterion. Disconcertingly, federal criteria for chloride fail to adequately protect many species from harm [14]. Moreover, our estimate of the proportion of seasonal ponds impacted by road salt is likely conservative across the snowbelt region because most of the ponds studied here were located in relatively undeveloped and rural settings, where roads were the primary source of deicing salt. Urbanized landscapes have many more impervious surfaces that are deiced. Parking lots alone cover an estimated 5.5% of developed land area in the U.S [30]. Driveways and sidewalks are also important sources of deicing salt. But unlike public roadways, the amount of deicing salt applied to these surfaces is determined by commercial contractors and private residents, and rates of application often exceed guidelines provided by municipalities. For instance, our preliminary observations show that salt applied to sidewalks by our university is 10-100x higher than recommended by state guidelines (G. Benoit, unpublished data). Thus, small ponds in urbanized landscapes likely contain even higher salinity levels than those studied here, and a higher proportion likely have exceedances.

In the US, while just 1% of the landscape is covered by road surfaces [31], their impacts are projected to occur across 20% of the landscape [32]. An estimated 20% of land area is located within 127 m of the nearest road [33]. Many roads throughout the US and Canada are treated with deicing salt in winter [34]. Taken together, it seems likely that a high fraction of seasonal ponds in the snowbelt regions of the US and Canada are salinized, with the most severe impacts occurring in ponds within tens of meters of a road.

Compared to other aquatic habitats, seasonal ponds appear to be the most severely salinized surface waters due to salt pollution, which is perhaps not surprising because of their small size and poorly flushed hydrology. For example, lakes in the North American lakes region have average Cl^- concentrations ranging from 0.18 – 240 mg/L Cl^- [8]. Estimates for rivers in rural watersheds like the ones studied here range up to about 50 – 100 mg/L Cl^- [5, 7] (though in urban watersheds maximum values have been shown to reach up to about 265 mg/L [35], 600 mg/L [36], and 3000 mg/L [37]). Here, we found that average Cl^- concentration in small ponds ranged from 3.3 mg/L to 920 mg/L, nearly four times the highest average found in the North American lakes region and nine to 18 times the amount found in rural streams and rivers. Presumably seasonal ponds are also the most heavily impacted by all runoff contaminants, not just salt, underscoring the vulnerability of these critical habitats to pollution.

Road proximity is a primary driver of increased salinity in seasonal ponds, with salinity declining exponentially with distance from roads. However, significant variation in conductivity was observed among roadside ponds, even those <10 m apart or on opposite sides of the same road. Some variation can be attributed to microtopography [38], with downslope ponds being saltier (Fig. S2A-B), and modifications such as drainage channels exacerbating pollution. Not all roadside ponds had high conductivity; factors like pond size, hydrology, and orientation (e.g., parallel vs. perpendicular to roads) may influence salinity. Smaller, isolated ponds and those with long axes parallel to roads were generally saltier, while larger ponds or those with freshwater inputs from upland areas had lower salinity. Modeling these variables could improve predictions of pond salinity.

In three ponds, we measured vertical profiles of salt and temperature over periods of time ranging from a week to more than a year. Of these, patterns of salinity in the two natural vernal pools matched each other during the period when both were measured. The vertical profile that was measured for a week in May in SC (Fig. 4A) is very similar to the pattern in CH for the same interval although the total amount of salt differs (Fig. 3B). For both, there was a monotonic increase in conductivity with depth, reaching a maximum at the SWI. In SC, the very deepest measurement depth (81 cm) exhibited an increase in conductivity over the period of measurement (Fig. 4A), but we believe this reflects diffusion of salt in the sediments and in the diffusive boundary layer to the logger's sensor rather than a change in salt within the water column. Conductivity at all other depths remained unchanged (within the measurement uncertainty) over the week of measurements. For both ponds we interpret the time-depth salinity patterns as reflecting inputs from either surface runoff or ground-water inflow during the winter salting period with the influent salty water flowing downward in the pond to a level matching its density as caused by salinity and temperature. This generally means that the saltiest water is found at the deepest level in the ponds. The resultant stratification seems remarkably persistent over very short distances in the face of external forces with the potential to cause mixing such as wind or surface water inflow. This persistence means that organisms that live just a few cm from the surface can be exposed to high levels of salt long after the deicing season.

Pond CH was measured the longest and deserves further discussion. From the start of the salting season in December, a vertical salt profile was established that continued through the rest of the hydroperiod (Fig. 3B). At shallower depths (0 – 60 cm), salt at first increased then gradually declined, whereas at an intermediate depth (70 cm) salt was high and remained constant. Nearest the bottom (90 cm), salt steadily increased from winter through the summer (Fig. 3B). Considering that it is unlikely that surface runoff contained high amounts of salt long after the deicing season, it seems more probable that the increase at depth reflects ground water sources. This hypothesis could be tested in the future by direct measurements of seepage. Combined with temperature, which had a lesser effect, salt caused a weak but persistent density stratification that lasted for months, notably during the breeding season (Fig. 3B). Surprisingly, this stratification was the result of density differences much less than one part per thousand. We are aware of no other freshwater body where such a small but significant effect has been documented.

The BMP pond (Whitney; Fig. 4B) exhibited a different and more complicated pattern than CH (Fig. 3B) and SC (Fig. 4A), and is discussed in Supporting Text.

Small but critical differences in density caused by a combination of salt and temperature explain why the ponds remain stratified, often over periods of months. Denser water on the pond bottoms does not mix with less dense water above it. Furthermore, the bottom water is likely to be saltier, for two separate reasons. First, salt itself increases water density and is likely to flow downward. Second, water carrying deicing salts is likely to be added in the winter when water temperatures are lower and the fluid denser, again leading to saltier bottom waters. Stratification reduces mixing and can cause high levels of salinity to persist well past the deicing season, increasing the time when aquatic organisms might be exposed to harmful levels of salt. It is important to remember that both saltiness and temperature can play an important role in influencing water density and causing stratification. This is especially true as the two parameters often correlate, with colder waters being saltier because deicing substances are applied in the winter months. Also, as fresh water reaches its maximum density at about 4° C, the change in water density is less sensitive to thermal variations at lower temperatures (Fig. 4D); lines of constant density are nearly horizontal in this low temperature zone shown in the figure. Outside this area, say in the range from 10 to 25 degrees, a one-degree change in temperature has roughly the same effect on density as a change of 200 μS/cm in conductivity. In extreme cases, each parameter can cause a variation in density of as much as 10 ppth (1%) over the range that they vary. Thus, both parameters can play an important role in causing stratification, depending on the values of each.

The degree of salinization in seasonal ponds should raise significant concern for conservation. Not only are seasonal ponds the most salinized freshwater habitats, the species that live there are often more sensitive to salt than congeners and other aquatic species found in rivers and lakes [11]. Stratification of salt in seasonal ponds should heighten this conservation concern because many seasonal pond organisms spend much of their time in the benthic zone (e.g., foraging on detritus and evading predation) where salt is most concentrated. To make matters worse, benthic dwelling—a common behavior in early life stages of amphibians—places them at higher risk, as these stages are generally far less tolerant of salt than adults [39].

Roads are a leading source of freshwater salinization. A variety of salts are used, especially NaCl, $MgCl_2$, and $CaCl_2$, with NaCl applied in the greatest amount, both as rock salt and brine [40]. In contrast to lakes, rivers, estuaries, and oceans, there are no formal efforts to monitor water quality impacts of human activities on seasonal ponds and other small surface waters. Yet these surface waters are the most numerous lentic freshwater habitats on the planet, provide key ecosystem services, and serve as critical habitat for endemic species across the globe. The severe degree of salt pollution in these habitats should prompt close consideration from regulatory bodies and raise additional concern about the intensity of other runoff contaminants polluting seasonal ponds.

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Author contributions

SPB and GB designed the study, collected and analyzed the data, and wrote the manuscript.

Competing interests

The authors declare no competing interests.

Figure 1. Study site and cumulative distribution of ponds exceeding federal chloride guidelines. The cumulative proportion of ponds exceeding federal water quality criteria for Cl^- is shown for (A) surface waters and (B) bottom waters. For the scatterplots, each point represents a pond with an exceedance up to the maximum distance detected (indicated by vertical dashed lines). Red and blue points correspond to US and Canadian criteria respectively, fitted lines are predicted from asymptotic regressions. Ponds are ordered by distance to the road to show the rate at which proportion of exceedances accumulate across ponds. The relative frequency of those exceedances is shown as bars in 10 m bins, with blue bars corresponding to the Canadian criterion and red bars corresponding to the US criterion.

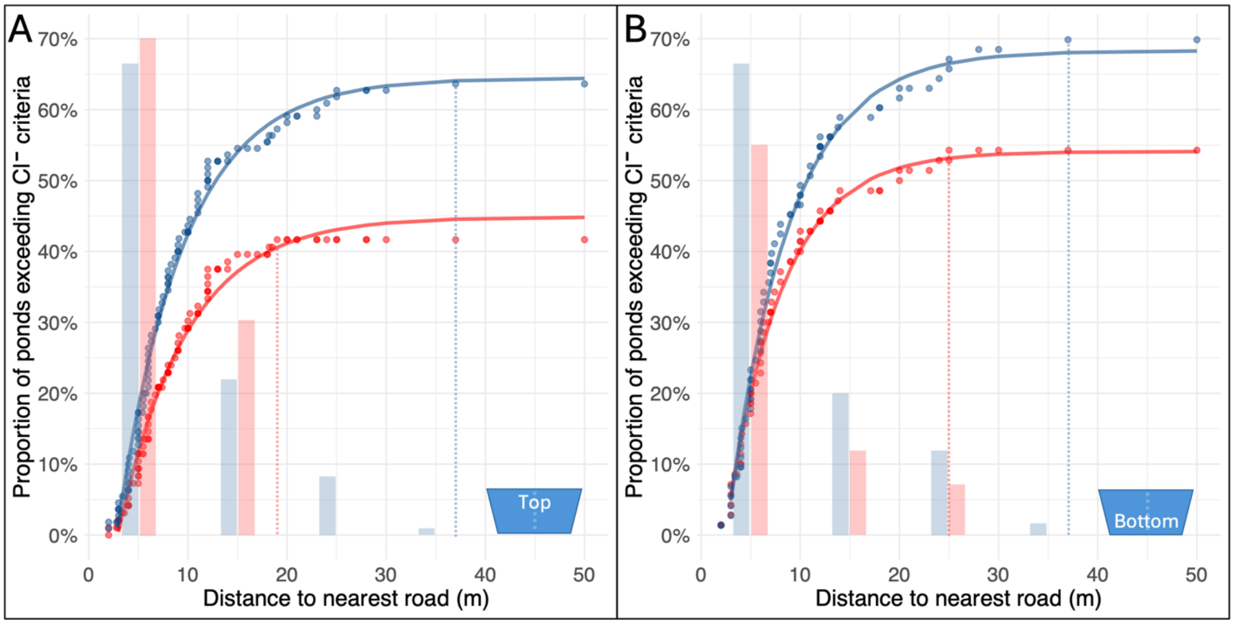


Figure 2. Pond conductivity as a function of distance from the road. In each panel, all conductivity observations are shown as gray circles, mean conductivity for each pond (N=167) is shown as blue diamonds, and model predictions are shown as fitted lines from the exponential decay model. Values are shown for (A) surface and (B) bottom bottom waters. Main panels are zoomed in to show variation. Insets show the full dataset and model results. Results of the log-log analysis are visualized in Supporting Information (Fig. S2).

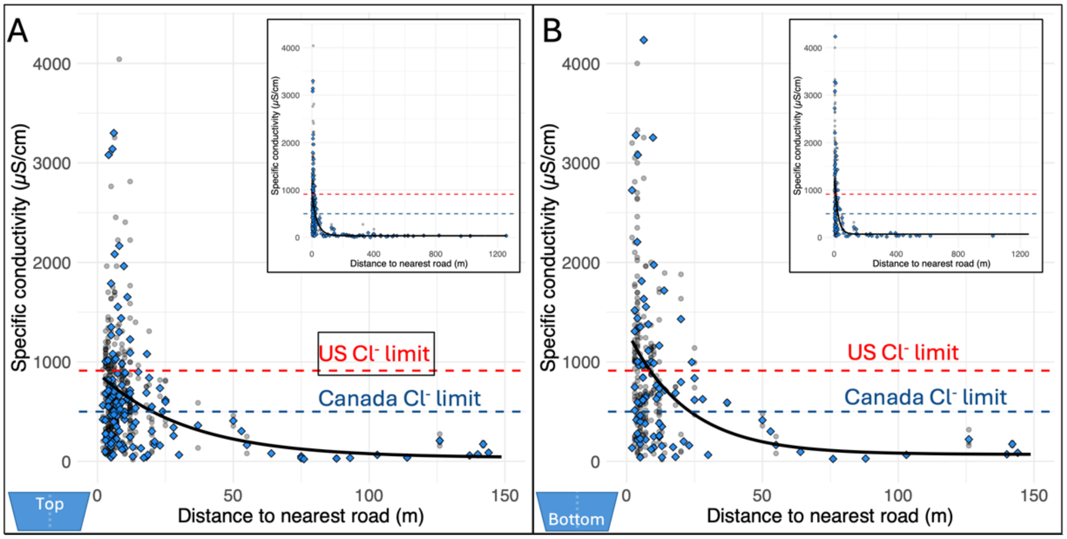


Figure 3. Salinity stratification patterns in small ponds. (A) Overall patterns of conductivity in surface versus bottom waters shows that stratification is common. Pond-level means are shown in blue and model estimates (with 95% CIs) are shown as gray diamonds. Ponds with values greater than 2,000 µS/cm were excluded (N=6 bottom, N=5 surface) from the plot to better visualize effect size. (B) Yearlong conductivity profile in a natural roadside pond (CH) shows the seasonality of stratification and the minor effects of precipitation events. (C) Salinity driven density values were calculated for Pond CH. Over the period shown, temperature caused a large variation in total density, which changed over time, but was constant vertically on any date. To better reveal the dominant role of salinity in determining vertical density differences and stratification during this period, we have calculated and removed the temperature effect.

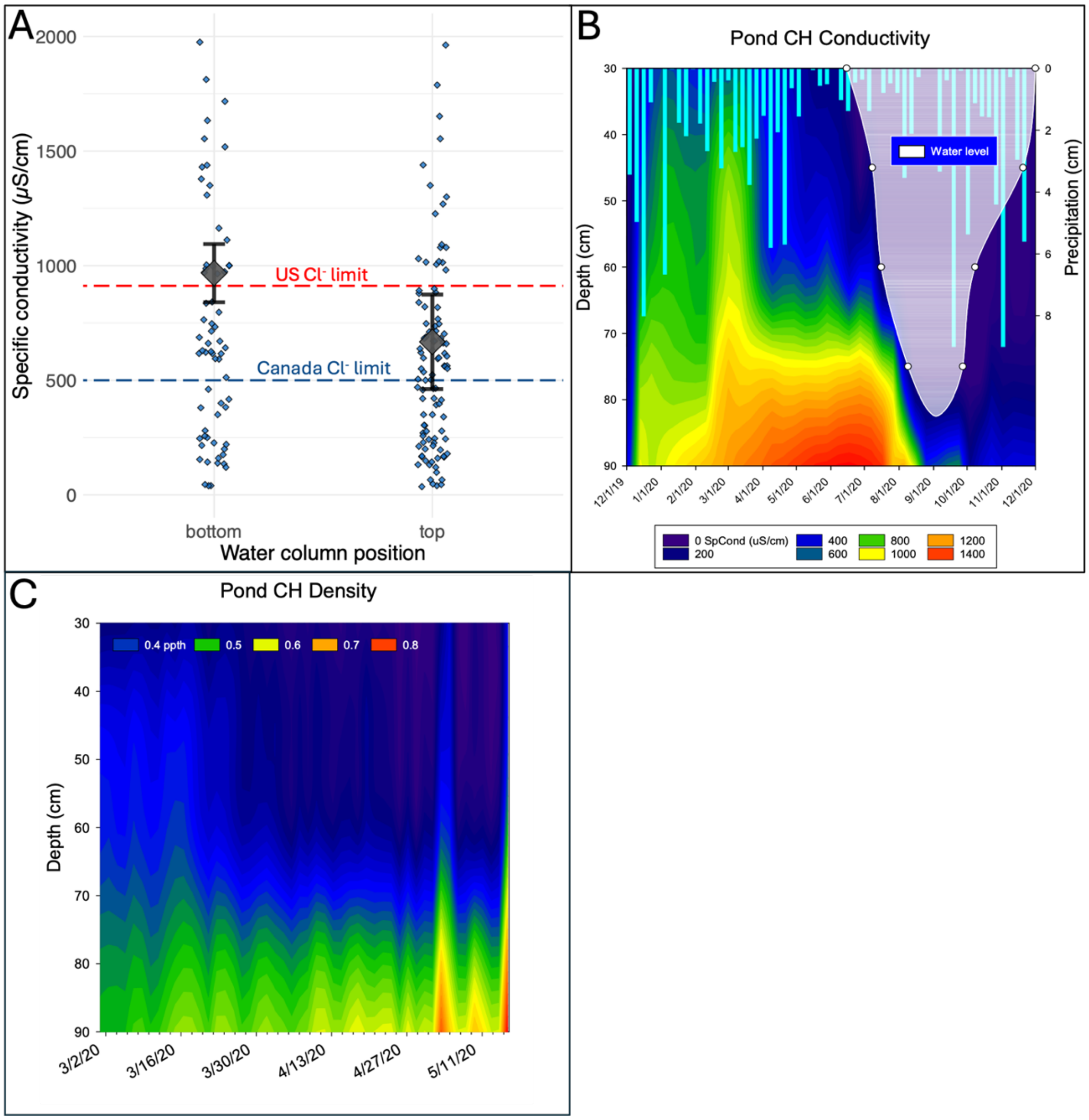
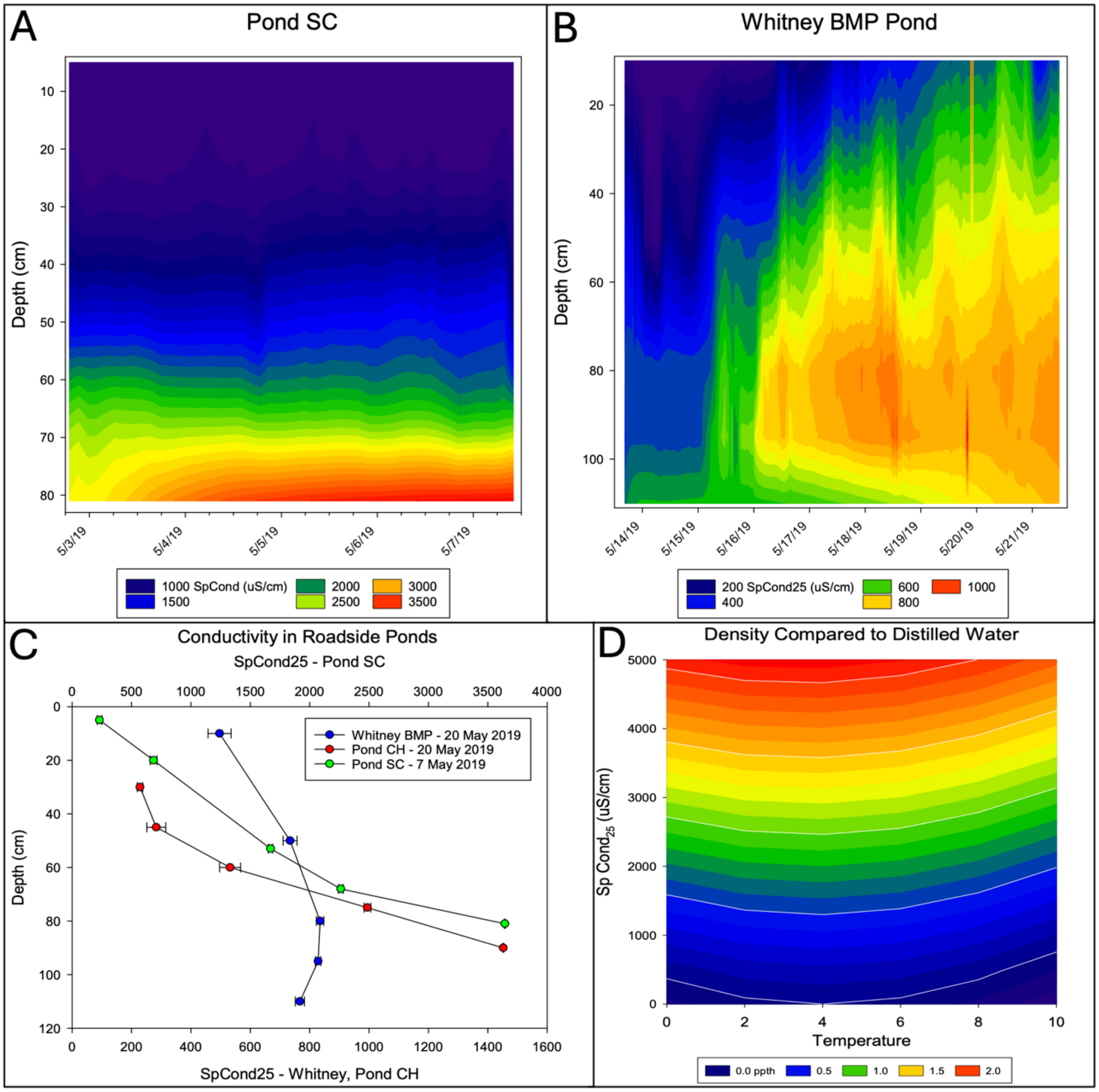


Figure 4. Conductivity profiles and density model. Detailed profiles are shown for A) SC and B) Whitney BMP. C) Average salinity profiles from similar dates in spring are shown for all three ponds that were monitored with deployed loggers. D) Modeled density effects of temperature and salinity provide a framework for interpreting the role of temperature versus salinity in driving stratification in seasonal ponds.



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Supporting Text

Seasonal pond hydrology

Often, seasonal ponds form at low points in mound and depression topography. Their hydrology can change seasonally and is governed by surface and ground water flows [37]. Specifically, their quantitative water balance can be described by the standard mass balance equation [38]:

$$S = P + SWI + GWI - ET - SWO - GWO$$

Where:

P is direct precipitation to the pond's surface,
SWI is surface water inflow,
GWI is ground water inflow,
ET is evapotranspiration,
SWO is surface water outflow, and
GWO is ground water outflow.
S is the resulting change in water storage.

In our study region, only the term ET changes significantly with time – peaking in the summer – and often causing S to approach or reach zero seasonally.

Statistical analyses for road proximity effects on conductivity

All analyses here and throughout were conducted in R v. 4.2.3 [44]. Visual inspection of the relation between conductivity and distance from road showed a nonlinear pattern. High conductivity values decreased rapidly with increasing road distance before stabilizing to background levels. We therefore used an exponential regression model to analyze the relation between conductivity and road proximity. Initial attempts to fit a non-linear mixed effects model (in the package *nlme* [45] to the raw conductivity data (with pond as a random effect) failed to solve. Instead, we used the function 'drm' from the package *drc* [46] to model the average conductivity value in each pond as an exponential function of distance to the nearest road. We used the 'EXD.3' function to allow a non-zero asymptote because background conductivity levels in ponds in our study region tend to be in the low tens of µS/cm rather than zero. Next, because most variation in conductivity levels was found within the first 50 m of a road, we also performed a log-log linear analysis. We used a linear mixed-effects model to analyze $\log_{10}$ transformed conductivity as a function of $\log_{10}$ distance to road. To test the effect of microtopography on conductivity, we included a categorical covariate based on the digital elevation analysis indicating whether the nearest road drains to the pond. Pond was specified as a random effect to account for repeated measurements made at many ponds. For the three ponds where deployed loggers were used ('CH', 'SC', and 'WhitneyBMP'), mean conductivity values were used in these analyses.

Conductivity *per se* is not regulated in freshwater by the EPA or other agencies but chloride is, and the two are strongly correlated. To provide regulatory context for interpreting our results, we estimated equivalent conductivity values of the national recommended water quality criteria for chloride in the US (230 mg Cl^- / L) and Canada (120 mg Cl^- / L), using a previously established linear relationship calculated from direct measurements of NaCl and conductivity [47]. Ambient water quality criteria, established by federal agencies, represent the highest concentrations expected to pose no harm to aquatic life. However, we note that emerging evidence indicates that these criteria are inadequate to protect aquatic life in lakes [14]. Likewise, our previous experiments and those of others show negative effects of conductivity on amphibians below these thresholds [40, 41, 48]. We examined the maximum distance at which ponds experienced values above US and Canadian Cl^- criteria and used these distances to summarize the proportion of ponds with exceedances. We also fit asymptotic regression models to visualize the cumulative proportion of ponds—as a function of distance to road–containing Cl^- values (inferred from conductivity) greater than US and Canadian federal water criteria. These regressions were limited to ponds within 50 m of a road, providing enough range for levels to reach asymptotes.

Statistical analyses for seasonal conductivity patterns

We used the function 'lmer' in the *lme4* library to compose a linear-mixed effects model with the 'bobyqa' optimizer to analyze conductivity as a function of the interaction between date (specified as Julian day) and pond type. We included random intercepts for year and pond. Initially we allowed each pond to have a random slope that varied within each year, i.e., conductivity ~ Julian date X pond type+ (1 | year) + (Julian date | year : pond), expecting that interannual variation might influence the rate of conductivity change. However, the model fit was singular suggesting an overparameterized random effects structure. We therefore refit the model with a random effect for year and random slopes over time for each pond, which were not allowed to vary across years, i.e., + (1 | year) + (Julian date | pond). Next, we composed a model with random intercepts for year and pond, i.e., + (1 | year) + (Julian date | pond), and finally a minimally parameterized model with a random intercept for pond, i.e., + (1 | year). We used AIC to evaluate model performance, selecting for inference the model with the lowest AIC value that was at least 2 AICs away from the next lowest AIC model (i.e. delta AIC > 2). A log-likelihood test was used to infer the interaction between pond type and date by comparing the selected model with a reduced model with identical random effects but containing additive rather than interactive main effects.

Long-term deployments to study stratification

We constructed a profiling device from a PVC pipe (12.7 cm X 120 cm) fitted with five Onset brand Hobo model Conductivity Loggers at various distances. Loggers were positioned at depths to span the water column of each pond, with more loggers placed closer to the bottom based on previous observations that bottom waters of roadside ponds can be much saltier than surface waters. We deployed the profiler in three different ponds. In each deployment, the PVC pipe supporting the loggers was slid over a wooden garden stake inserted into the deepest portion of the pond allowing the bottom of the pipe and its lowest data logger to rest on the sediment. We acknowledge that the lowest sensor may have been located in the sediment or in the diffusive boundary layer adjacent to the SWI.

We made a yearlong deployment in a roadside pond ('Crystal H'; CH) located 3.9 m from the road in northeastern CT. CH is approximately 0.1 ha in area and up to 90 cm deep. Apart from the adjacent road, nearby LULC is almost exclusively second growth forest, with only one residential property located within a 150 m radius of the pond. There is no apparent surface inlet (apart from a runoff drainage path leading from the road) or outlet. Loggers were positioned at heights of 0, 15, 30, 45, and 60 cm above the bottom of the pond, and measurements were made every 6 hrs from 23 Nov. 2019 – 27 Dec. 2020. We also made two, weeklong deployments, the first in a roadside pond ('Spincycle'; SC) located 4.0 m from the road in southern CT from 02 – 07 May 2019 and situated in a landscape with mixed secondary forest (including a nature reserve) and residential development. SC is 0.5 ha and up to 90 cm deep. Loggers were positioned at heights of 0, 13, 28, 61, and 76 cm above the bottom of the pond, and measurements were made every minute. The second weeklong deployment was in a BMP pond ('WhitneyBMP'), located 28 m from the road in southern CT, from 13 – 21 May 2019. WhitneyBMP has a watershed of 8.1 ha mainly residential LULC. It was intentionally dug to below the seasonal high-water table to maintain year-round wet conditions. High flows can partially bypass the pond, which has a discrete outlet that leads to a drinking water reservoir (Lake Whitney, Hamden, CT). Loggers were positioned at heights of 0, 15, 30, 60, and 100 cm above the bottom of the pond, and measurements were made every 30 minutes.

We analyzed the long-term deployment in CH using a linear model to test the interaction between time and depth on conductivity. Following a significant interaction effect, and because conductivity is not expected to follow a specific linear or non-linear pattern over a year, we interpreted conductivity trends visually using contour plots overlain with daily precipitation records as hyetographs. Precipitation data were downloaded from the National Centers for Environmental Information (https://www.ncei.noaa.gov/) using the nearest airport (Bradley International Airport in Windsor Locks, CT). For SC and whitneyBMP, we used separate mixed effect models to analyze the effect of depth on conductivity in each pond. Time was specified as a random effect rather than a fixed effect because of the relatively short deployment period in these two ponds. Separately, we modeled water density across a relevant range of salinity and temperature values to guide our interpretation of stratification (see ‘Water density model’ text below).

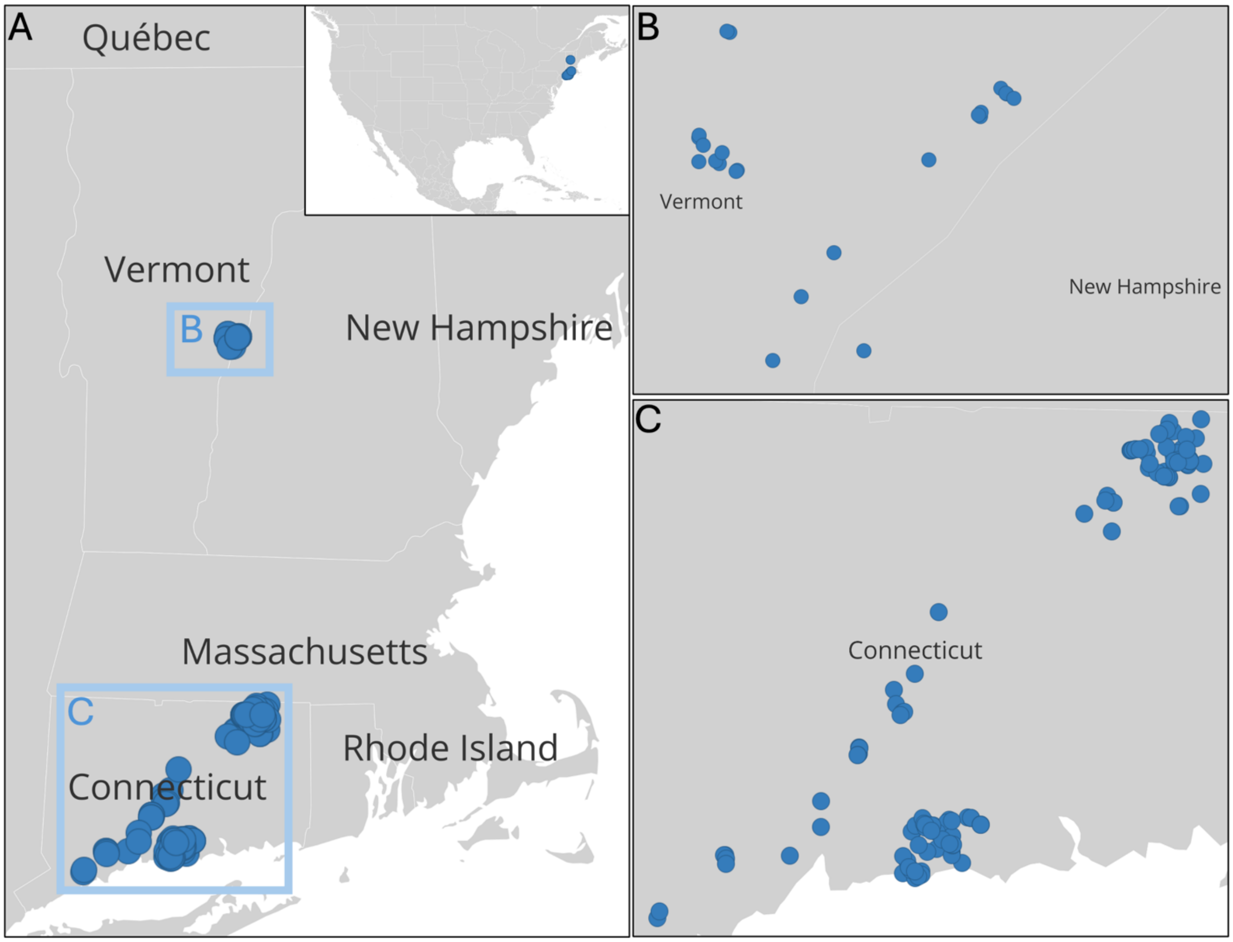
Water density model

Lentic water bodies, like lakes and ponds, can become stratified because of density differences caused by temperature and salinity. These variations are often slight, amounting to only a few parts per thousand (ppth) or even a few tenths. But they can cause stratification and lack of mixing for extended periods of time. To better understand this effect in our ponds, we used a standard correlation equation [51] to calculate density values from temperature and specific conductivity. We were especially interested in understanding how much of the effect can be attributed to each of the drivers, which vary from pond to pond and over time. It is notable that these ponds are often stratified over very short distances (a few 10s of cm) for long periods of time (months).

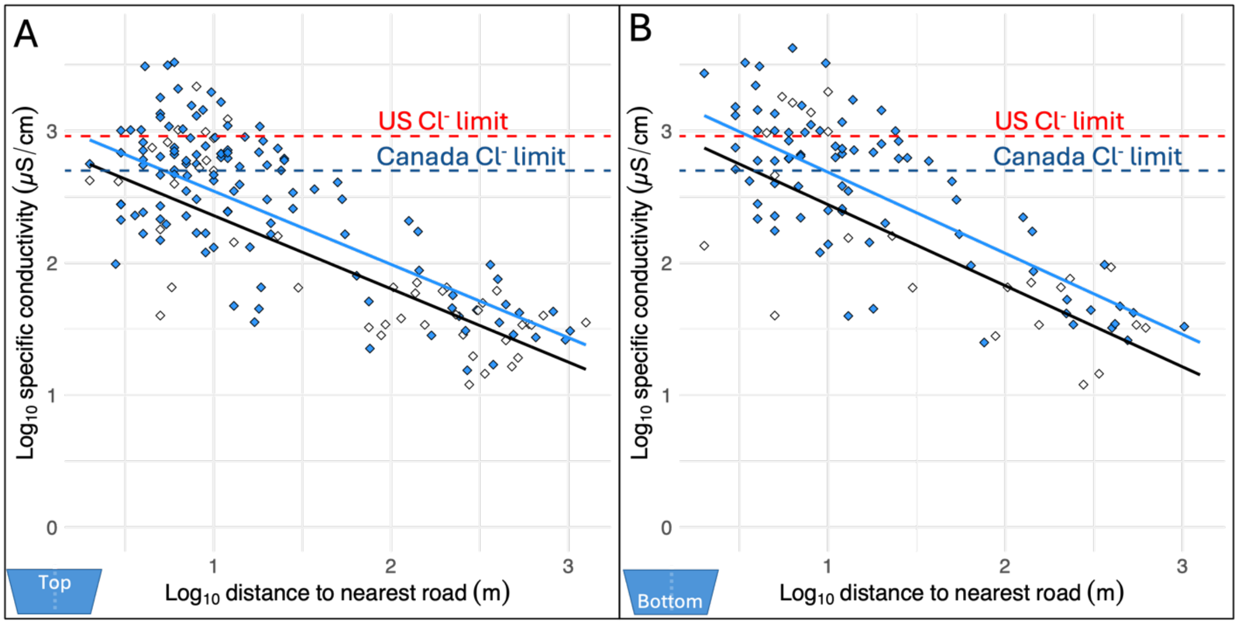
BMP pond discussion

Following significant rain that fell just before the measurement period, the pond was only weakly stratified, but within two days, a much stronger stratification was re-established. That change must have been the result of salty water entering the pond either from the watershed or nearby ground water. We believe that the limited available data support the latter mechanism. Between rain events, flows from the watershed decrease to near zero in volume and are likely to be relatively fresh, yet the greater part of the new salt in Whitney arrived two or more days after the rain. We believe that this pattern reflects the pond re-equilibrating with the shallow ground water in which it is embedded. Indeed, modeling has shown that contributions from shallow ground-water are necessary to explain observed hydrological patterns in many vernal pools [39]. Measurements of inflowing water and of salt profiles in nearby shallow ground water could resolve this question. Including temperature in this analysis also explains the perhaps unexpected vertical maximum in conductivity near a depth of 80 cm in Whitney BMP. In fact, density increases all the way to the bottom because of the influence of temperature in addition to salt in controlling water density.

Supporting Figure S1. Study sites. Conductivity data was measured in seasonal ponds throughout (A) New England. Panels B and C show zoomed in views to better display sites.



Supporting Figure S2. Pond conductivity as a function of distance from the road. In each panel, all conductivity observations are shown as gray circles, mean conductivity for each pond (N=167) is shown as blue diamonds, and model predictions are shown as fitted lines. Values are shown for (A) surface waters at a depth of 10 cm and (B) bottom waters measured at the surface water interface. Fitted lines are from linear-mixed effects models of $\log_{10}$ transformed conductivity and $\log_{10}$ transformed distance to the nearest road. Intercepts for upslope versus downslope are indicated as blue and black lines, respectively. Dashed lines indicate water quality criteria for US (red) and Canada (blue).



Supporting Figure S3. Seasonal conductivity trends. Data are from a subset of 12 ponds used for amphibian experiments where conductivity was monitored throughout the spring. Specific conductivity declined in roadside ponds (all located <10 m from the road) from early spring to summer in each of four years. Specific conductivity also varied between years. Julian day was modeled as a fixed effect (interacting with pond type) and its predicted values are shown as a heavy line in each plot (black=roadside, gray=woodland). Pond was modeled as a random effect, with a slope that was allowed to interact with Julian date and year. These random slopes are shown for each pond. A color palette distinguishes roadside ponds. To increase color contrast between roadside ponds, woodland ponds are shaded gray because slopes were flat and intercepts showed little variation. Dashed lines indicate water quality criteria for US (red) and Canada (blue).