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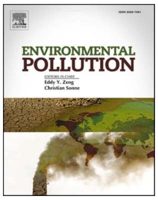

Road salt is more toxic to wood frog embryos from polluted ponds[☆]

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ABSTRACT

Organisms that rely on aquatic habitats in roaded landscapes face a growing array of consequences from pollution, especially due to freshwater salinization. Critically, these consequences can vary from population to population depending on exposure histories and evolutionary responses. Prior studies using transplant and common garden experiments have found that aquatic-stage wood frogs (*Rana sylvatica*) from roadside populations are less fit in the wild and more sensitive to road salt than their counterparts from woodland populations away from roads. While this pattern is consistent with local maladaptation, unresolved insights into the timing and duration of these effects leave open the possibility that negative outcomes are countered during development. Here, we asked whether the survival disadvantage of roadside wood frogs is stage-specific, and whether this disadvantage reverses before metamorphosis. We used a common garden road salt exposure experiment and a field-based reciprocal transplant experiment to examine differences in survival across life-history stage and with respect to population type. In each experimental context, roadside embryos showed a survival disadvantage relative to woodland embryos, and this disadvantage was not reversed prior to metamorphosis. We also found that salt exposure delayed metamorphosis more strongly for roadside than woodland populations. Together, these results suggest that local maladaptation in aquatic-stage wood frogs is driven by embryonic sensitivity to salt and that roadside populations are further compromised by delayed developmental rates. Future studies should consider which embryonic traits fail to adapt to salt toxicity, and how those traits might correlate with terrestrial trait variation.

1. Introduction

Roads and their uses deliver a suite of runoff contaminants to adjacent and downstream aquatic habitats. Common pollutants include heavy metals, polycyclic aromatic hydrocarbons, and road salt used for de-icing (Hwang et al., 2016; Hwang et al., 2019; Kaushal et al., 2005). Road salt is an especially notable contaminant because unlike many other road runoff pollutants, it is applied intentionally and in high abundance each year. For instance in the United States, over 18 million metric tons of salt are used annually for winter road de-icing (Jackson and Jobbágy, 2005). Because salt is conserved in aquatic systems and not subject to appreciable degradation, sodium and chloride ion concentrations have increased in freshwaters throughout many temperate regions, causing widespread salinization of freshwater habitats and drinking water sources (Corsi et al., 2010; Dugan et al., 2017; Godwin et al., 2003; Kaushal et al., 2005). For instance in the North American Lakes Region, chloride concentrations have been increasing in most lakes with >1% impervious land cover, and by 2050 many lakes in that region are predicted to exceed the U.S. EPA's chloride criterion of 230 mg/L Cl-for protecting aquatic life (Dugan et al., 2017).

The intense use and subsequent accumulation of road salt in our surface and ground waters has many impacts on freshwater organisms (Hintz and Relyea, 2019). These effects include osmotic stress (Gomez-Mestre et al., 2004), changes in growth and developmental rates (Hintz and Relyea, 2017), increased disease susceptibility and severity (Buss and Hua, 2018; Hall et al., 2020; Mangahas et al., 2019), and decreased survival (Arnott et al., 2020), all of which can reduce population fitness and modify ecosystem function (Van Meter et al., 2011a, 2011b).

Among freshwater organisms, amphibian populations have received considerable attention in the study of roads and runoff pollution (Egea-Serrano et al., 2012; Glista et al., 2008; Snodgrass et al., 2008). Many amphibians rely on temporary aquatic habitats (e.g., vernal pools), where salt exposure can be severe, especially when these habitats

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are adjacent to roads. For instance, conductivity values (a common salt proxy) in roadside pools used by breeding amphibians in the northeastern U.S. can reach about 4000 μS/cm compared to about 30 μS/cm in woodland pools. These values equate to about 1330 mg/L and 10 mg/L of Cl-respectively (Brady, 2013, 2017), corresponding to about a 130-fold increase in salt concentration and far exceeding values known to cause toxicity in amphibians. Larval spotted salamanders (*Ambystoma maculatum*) for example survive at lower rates when experimentally exposed to 500 μS/cm (Karraker et al., 2008) or when developing in ponds in the wild with conductivity values elevated to as little as 360 μS/cm (Turtle, 2000). These values correspond to roughly 167 and 120 mg/L Cl-respectively, considerably lower than the EPA criterion mentioned above.

Amid the many insights into road salt toxicity, a growing interest in ‘evolutionary toxicology’ points to the need to incorporate evolutionary perspectives to fully understand the effects of salt and other environmental pollutants on wild populations (Bickham, 2011; Brady et al., 2017a). Indeed such approaches have revealed remarkable variation—not only between groups of species but among populations of the same species—in their capacity to tolerate salt (Brady et al., 2017b). For one pair of amphibians, evolutionary perspectives have shown complex and seemingly contrasting responses to road runoff pollution. In the context of a 10-population reciprocal transplant experiment, embryos and hatchlings from roadside spotted salamander populations have been found to survive at higher rates compared to those imported from foreign woodland populations, suggesting that roadside populations have become locally adapted to roadside environments (Brady, 2012). In contrast, wood frog (*Rana sylvatica*) embryos and hatchlings in these same habitats show no such pattern. Instead, roadside wood frogs show evidence of maladaptation to roadside dwelling, surviving less, developing more slowly, and accruing more malformations than their woodland counterparts in the contexts of transplant experiments and salt exposure experiments (Brady, 2013, 2017).

Interestingly, despite these negative effects, we have observed that roadside wood frog populations in the wild show no differences in population size compared to woodland populations. Thus, the negative effects observed at embryonic and hatchling stages might entail one component of a tradeoff, with improved performance at later life-history stages. Such an outcome could explain the apparent local maladaptation of embryos and hatchlings, for instance if later-stage larvae and/or metamorphs are in fact more tolerant to salt. Relatedly, the exact timing of the survival disadvantage remains unknown, and thus we have little understanding of mechanisms or trait variation influencing differences in survival. One possibility is that the survival disadvantage is stage-dependent, for instance having a strong effect on early-stage embryos during critical developmental periods. Here, we evaluated these different possibilities with data from a common garden chronic road salt exposure experiment and a field-based reciprocal transplant experiment. We hypothesized that any survival disadvantage experienced by roadside populations would be stage-dependent, affecting embryonic and/or hatchling stages most strongly, but that these effects would be reversed during larval stages prior to metamorphosis, pointing to a potential tradeoff.

2. Methods

2.1. Natural history

The wood frog is one of the most widespread amphibians in North America, with a range extending from the eastern, southern Appalachian Mountains north and westward to the Alaska-Yukon region (Green et al., 2014; Martof and Humphries, 1959). Within local populations, individuals migrate seasonally between aquatic and terrestrial environments, but generally remain closely associated with their pond of origin where they return each spring to breed (Berven and Grudzien, 1990). Breeding is explosive, typically lasting 1–2 weeks. Males amplex females and fertilize eggs externally during oviposition. Each female lays a single clutch as an egg mass containing approximately 800–1100 eggs. Embryos develop over 2–3 weeks before hatching. This early developmental period tends to coincide with the highest concentrations of road salt in natal ponds due to winter accumulation and subsequent snowmelt and runoff. After hatching, individuals continue to develop as aquatic larvae throughout spring and early summer until they metamorphose into terrestrial juveniles, which disperse into upland habitat. Adults can live for 5–6 years (Berven, 2009; Brady et al., 2019), and apart from annual breeding, tend to spend most of their lives in terrestrial habitat. Juvenile wood frogs show strong natal site fidelity and, as adults, strong breeding site fidelity (Berven and Grudzien, 1990). Thus, each breeding pool is generally considered to represent a single population. Local populations have been estimated to exhibit neutral genetic differentiation at distances of about 1000 m to 10,000 m (Berven and Grudzien, 1990; Newman and Squire, 2001), and at shorter distances of 100s of meters when selection gradients are taken into account (Richardson and Urban, 2013). Differences in local population structure may also be intensified by anthropogenic features, such as roads, which limit local movement (Crosby et al., 2009).

2.2. Salt exposure experiment

In April 2019 we collected four to six wood frog egg masses within two days of oviposition from each of 11 populations (N = 5 roadside, 6 woodland) in and near Norwich, Vermont. One woodland population was inadvertently sampled twice, yielding 11 total egg masses from this one pond. This brief period of embryonic exposure to natal pond water does not induce maladaptive survival in offspring (Brady, 2017) and thus would not affect inference into survival differences. Woodland populations were located >500 m from the nearest paved road, and conductivity in these ponds averaged 30 μS/cm (range 25–35 μS/cm). Roadside populations were located <30 m from the nearest paved road, and conductivity in these ponds averaged 522 μS/cm (range 341–622 μS/cm). Distances between individual ponds ranged from 100 to 6380 m. When possible, egg masses were collected from different locations within the selected pond to reduce the already low chance of genetic similarity from multiple paternity. Egg masses were transported from the field to the lab in coolers with ice packs and held overnight at 4 deg C. From each egg mass, 12 embryos were haphazardly selected and carefully separated out for stocking. Gosner developmental stage (Gosner, 1960) was scored for each egg using a stereo dissecting microscope. We allocated these 12 embryos to four road salt treatments, with three replicates per treatment. Four treatments were made with NaCl road salt (which did not contain additives such as anti-caking agents) obtained from the Connecticut Department of Transportation. Road salt was mixed with tap water that was dechlorinated and aged for a minimum of 24 h to create the following treatments: 275 μS/cm (control without added NaCl), 1000 μS/cm, 4000 μS/cm, and 7000 μS/cm. The values of 1000 and 4000 μS/cm were chosen to represent the approximate average and maximum conductivity found in roadside ponds in our region (Brady, 2013; Brady et al., 2019), while 7000 μS/cm was chosen to provide inference into more extreme conditions.

We stocked a total of 818 embryos into 17 mL 6-well plates (one embryo per 17 mL well), with each plate containing embryos from a single egg mass. All wells on the same plate contained the same salt treatment, and each combination of population and salt concentration was replicated three times (N = 144 plates). Plates were arranged randomly on benches in the lab under ambient temperatures (which range from 15.8 to 25.7 C and average 21.7 C ± 1.36 sd) and with a 14:10 h overhead lighting cycle. One week after stocking, mortality was assessed for each embryo every two to four days by screening for tissue deterioration and arrested progression between developmental stages. After hatching when larvae reached feeding stages (Gosner stage 23–25), each surviving individual was transferred from its well into a 120 mL plastic specimen container filled with 100 mL of matching treatment water. Complete water changes were conducted every three to four days and containers were rotated among benches after each feeding. Immediately after water changes, larvae were fed a 3:1 ration of ground rabbit chow to fish flakes corresponding to approximately 10% average body mass per day. In between feedings and water changes, larval housing containers were stored with lids loosely placed over the container to provide airflow while limiting evaporation. Mortality was assessed every three to four days for each individual until death or the beginning of metamorphosis (i.e., Gosner stage 42, marked by the emergence of a forelimb). Mortality was confirmed by lack of response to physical stimulus and visual indicators of death, such as rigor mortis and tissue deterioration. Dead individuals were preserved in 70% ethanol for later assessment of Gosner developmental stage at mortality.

2.3. *Statistical analyses for salt exposure experiment*

We first analyzed whether overall survival (from embryonic stages until metamorphosis) varied across the interaction of population type and salt concentration. All individuals were included in this analysis (N = 818). Next, to assess whether salt induced stage-dependent mortality, and whether this effect interacted with population type, we analyzed survival during each of three different life-history categories: 'embryos' (Gosner stages 1–20), 'hatchlings' (Gosner stages 21–25), and 'larvae' (Gosner stages 25–42). A subset of individuals (N = 236) were too decomposed at the time of mortality to accurately score developmental stage and were therefore excluded from these analyses of stage-dependent mortality. Separate models were used for the analysis of survival in each of these life-history categories. We used the 'lme4' package (Bates et al., 2015) in R (V. 4.1.0) (R Development Core Team, 2021) to compose generalized mixed models with a logit link to analyze binomial survival (i.e., number of individuals alive at the beginning of each phase, number of individuals surviving that phase) across the interaction of the two fixed effects (population type and salt concentration). Salt concentration was $\log_{10}$ transformed in all models due to the wide range of conductivity values (275–7000 μS/cm) that otherwise caused models to fail to converge. Models were first fit with two different random effect structures: 1) ~ population, or 2) ~ population + ~well plate. In each case, the model preferred by AIC (using a delta AIC threshold of 2) was evaluated for interaction effects. In cases where interaction terms were not significant, selected models were refit with additive main effects. Inference of fixed effects was conducted using likelihood ratio tests between models with and without the effect of interest.

We also used the 'lme4' package to compose a linear mixed model to analyze time to metamorphosis. Two random effect structures were evaluated before inferring the potential interacting effects of population type and salt: 1) ~ population, or ~ population + ~ well plate. AIC selection (using a delta AIC threshold of 2) indicated that the less parameterized model was preferred. The model was refit with additive main effects, which were inferred based on degrees of freedom estimated using the Satterthwaite method (Kuznetsova et al., 2017).

2.4. *Field-based reciprocal transplant and population survey*

We used data from a field-based reciprocal transplant experiment conducted across 10 populations (N = 5 roadside, 5 woodland) in Northeastern, Connecticut to assess survival across the interaction of population type X environment type, and to contrast these outcomes between embryonic and larval stages. Methods and results of the embryonic phase of this experiment were previously reported (see Brady, 2013). Briefly, roadside pools were located <10 m from a paved road while woodland pools were located >200 m from the nearest paved road. Distance between pairs ranged from 880 to 6060 m. From each population, embryos were collected <36 h after oviposition and raised in predator-free floating enclosures in their natal pond and their assigned transplant pond (forming a 'pond pair' selected to minimize confounding variation in habitat). We sampled 10 egg masses from each population, stocking approximately 100 embryos into each pond (N = 10 ponds X 10 enclosures = 100 enclosures). Each enclosure contained ca. 50 embryos from each of two egg masses (from the natal pond or the paired transplant pond), forming a 'clutch pair' (see Supplemental Fig. 1 for a schematic of stocking design).

For the larval phase of the transplant experiment (first reported here), feeding-stage larvae were transferred into larger enclosures (N = 100 enclosures) while maintaining their original clutch pair grouping. This change in enclosures reduced potential density effects and allowed larvae to access the full water column. Enclosures were constructed using vinyl-coated garden fencing to form a cylindrical frame (1.2 m high X 0.6 m diameter) completely enclosed with Noseum netting (ca. 97 holes per cm^2) to exclude predators but permit water flow and colonization by zooplankton. Each enclosure was stocked with ca. 5 L of submerged leaf litter collected from the local breeding pool and allowed to air-dry for at least one week to remove invertebrate predators. We stocked a target of 20 larvae per enclosure. Due to low embryonic survival in one roadside pond, six enclosures were stocked with either 2, 11, or 18 individuals. Across the experiment, the remaining 94 enclosures were each stocked with 20 individuals. Enclosures were staked in place, with one natal enclosure and one transplant enclosure sharing a stake to form an experimental block. Larvae were raised until the most developed individuals began approaching metamorphosis at approximately Gosner stage 35, at which time survival was assessed for all enclosures. This developmental stage was chosen to allow as long a developmental period as possible while minimizing the risk of pond drying and catastrophic loss of experimental units. In 2011, one week after breeding was complete, we waded through each of the 10 experimental ponds to visually count all naturally laid wood frog egg masses. Because each female lays one egg mass, we use this value as an estimate of population size in terms of the number of breeding females.

2.5. *Statistical analyses for field-based reciprocal transplant and population survey*

We composed a suite of candidate generalized linear mixed models to evaluate the potential effects of population type, environment type, and their interaction on larval survival. Candidate models included up to three different random effects using all combinations of pond pair, clutch pair, and block where each model contained the minimal random effect of pond pair. Preferred random effect structure was selected using AIC, with a delta AIC value of 2. Random effects were first evaluated for models containing the interaction between population type and environment, and because interactions terms were not supported, this selection process was repeated using only additive main effects in the model. Survival was modeled as a binomial response (number of larvae stocked, number of larvae survived). Models were fit with a logit link transformation in the R (V 4.1.0) package (R Development Core Team, 2021) 'lme4 (Bates et al., 2015). Inference was conducted using likelihood ratio tests between models. We used a simple linear model to evaluate whether egg mass counts varied between population types. Values for egg mass counts were divided by pond area prior to analysis to account for differences in the size of ponds.

3. Results

3.1. *Salt exposure experiment*

Overall survival to metamorphosis varied with respect to population type (roadside population type coefficient = −0.661, $X^2{}_{1,}$ = 4.29, P = 0.038) and $\log_{10}$ salt concentration (coefficient = −1.22, $X^2{}_{1,}$ = 69.1, P < 0.001), and these terms did not interact ($X^2{}_{1,}$ = 1.41, P = 0.234). Logit scores for survival declined by about 0.116 with every 10% increase in salt concentration, and across all concentrations logit scores for roadside populations were about 0.66 units lower than woodland populations (Fig. 1). For instance, at 1000 μS, roadside populations had a 13% probability of surviving to metamorphosis compared to 22% for woodland populations.

Survival patterns in relation to salt concentration and population type also differed between life history stages (Fig. 2). Embryo survival (Fig. 2A) varied across the interaction of population type and $\log_{10}$ salt concentration (coefficient for interaction term for roadside population type X $\log_{10}$ salt $= -1.25$, $X^2{}_{1,} = 8.71$, $P = 0.003$). Logit scores for survival of roadside populations relative to woodland populations declined by about 0.119 with every 10% increase in salt concentration. For instance, at 1000 μS, roadside populations had a 74% probability of surviving through the embryonic period compared to 89% for woodland populations. At 4000 μS/cm, roadside populations had a 57% probability of surviving through the embryonic period compared to 88% for woodland populations. Survival among hatchlings (Fig. 2B) was negatively influenced by $\log_{10}$ salt concentration (coefficient $= -1.28$, $X^2{}_{1,} = 46.5$, $P < 0.001$), but did not vary with respect to population type ($X^2{}_{1,} = 0.46$, $P = 0.496$), nor across the interaction of these two factors ($X^2{}_{1,} = 0.466$, $P = 0.495$). Thus, for hatchlings, salt decreased survival by about 0.12 logits for each 10% increase in salt, and this effect was equivalent between population types. A qualitatively similar pattern was found for larvae (Fig. 2C), as larval survival did not vary across the interaction between population type and salt concentration ($X^2{}_{1,} = 0.128$, $P = 0.721$), but declined with increasing salt concentration (coefficient: -1.78, $X^2{}_{1,} = 28.0$, $P < 0.001$), and this effect did not differ between population types ($_{1,} = 0.010$, $P = 0.920$). Thus, for larvae, salt decreased survival by about 0.170 logits for each 10% increase in salt, and this effect was equivalent between population types.

Time to metamorphosis was influenced by the additive effects of population type (roadside population type coefficient $= 2.58$, $F_{1,\ 6.8} = 9.24$, $P = 0.020$) and $\log_{10}$ salt concentration (coefficient $= 3.19$, $F_{1,\ 235.7} = 21.8$, $P < 0.001$), and these terms did not interact ($F_{1,\ 234.0} = 0.036$, $P = 0.849$). Across all populations, a 10% increase in salt concentration caused frogs to reach metamorphosis about 0.3 days later (Fig. 3), and across all treatments, roadside populations took 2.58 days longer to metamorphose compared to woodland populations. For instance, in control conditions, woodland populations metamorphosed at an estimated 64.1 days compared to 66.7 days for roadside populations, whereas at 4000 μS/cm, woodland populations metamorphosed after 67.9 days compared to 70.4 days for roadside populations.

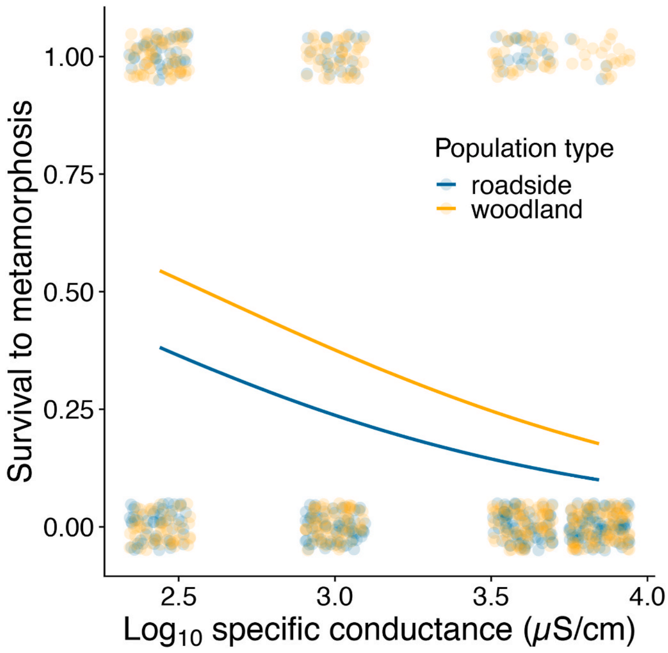

Fig. 1. Survival to metamorphosis. Each point indicates whether or not an individual survived to metamorphosis. Points – shown for each individual in each treatment and for each population type – were jittered to reduce overlap. Fitted lines represent predicted values reverse-transformed from logit scores to probability.

3.2. *Reciprocal transplant experiment and population survey*

As previously reported, the initial phase of the transplant experiment showed that around the time of hatching when early-stage larvae reach feeding stages, roadside wood frogs survived at lower rates than their woodland counterparts, not only when transplanted into woodland environments, but also when reared in their natal environments (Brady, 2013). For the larval phase of the experiment, larval survival under field-based reciprocal transplant conditions did not vary across the interaction of population type X environment type ($X_1 = 0.319$, $P = 0.572$), nor across either of the main effects (population type: $X_1 = 0.034$, $P = 0.854$; environment type: $X_1 = 1.98$, $P = 0.159$; Fig. 4). Population size measured in terms of the number of egg masses per pond did not vary between roadside and woodland populations ($F_{1,10} = 0.026$, $P = 0.876$).

4. Discussion

In the face of chronic salt exposure throughout their aquatic life history, roadside wood frogs survived less than woodland wood frogs. This relative survival disadvantage occurred during embryonic development and failed to reverse prior to metamorphosis. Hatchling and larval stage survival effects were similar between populations, while salt exposure strongly affected embryonic survival in roadside wood frogs, indicating that the embryonic stage is the most sensitive life stage for survival under salt contaminated conditions. Roadside individuals also took longer to metamorphose than their woodland counterparts. A similar survival effect was found in wild populations within a reciprocal transplant experiment, whereby roadside embryos survived at lower rates than woodland embryos, and this survival disadvantage was not reversed during larval development. Together, these results show that a legacy of exposure to roadside ponds experienced by prior generations predisposes wood frogs to increased road salt toxicity, leading to lower survival during embryonic development and delayed metamorphosis. The similarity between lab and field results supports the likelihood that road salt is an important contaminant responsible for the survival disadvantage in wild roadside populations of the wood frog. Strikingly, these results indicate that the populations actually encountering road salt pollution in the wild are least tolerant of its effects.

Our findings that roadside populations reach metamorphosis with a survival disadvantage and developmental delay help resolve prior uncertainty about the timing and persistence of the effects of salt contamination. Previous work has shown that wood frogs from roadside populations were less fit in roadside aquatic conditions compared to nearby populations that lack a history of exposure to roadside environments or contaminants (Brady, 2013, 2017), prompting the intriguing possibility that roadside populations are locally maladapted to roadside dwelling. However, studies of these effects had been limited to embryonic/hatchling periods and/or had occurred in the context of short-term ‘acute’ salt exposures. More complete insights into the full aquatic lifecycle of these organisms have been lacking. Thus, an alternative explanation to maladaptation was that roadside populations might be locally adapted to roadside conditions if the survival disadvantage of embryos/hatchlings was reversed prior to metamorphosis. Here, we show that this was not the case. Instead, roadside wood frogs developed a substantial survival disadvantage during embryonic development, and this disadvantage was never compensated prior to metamorphosis.

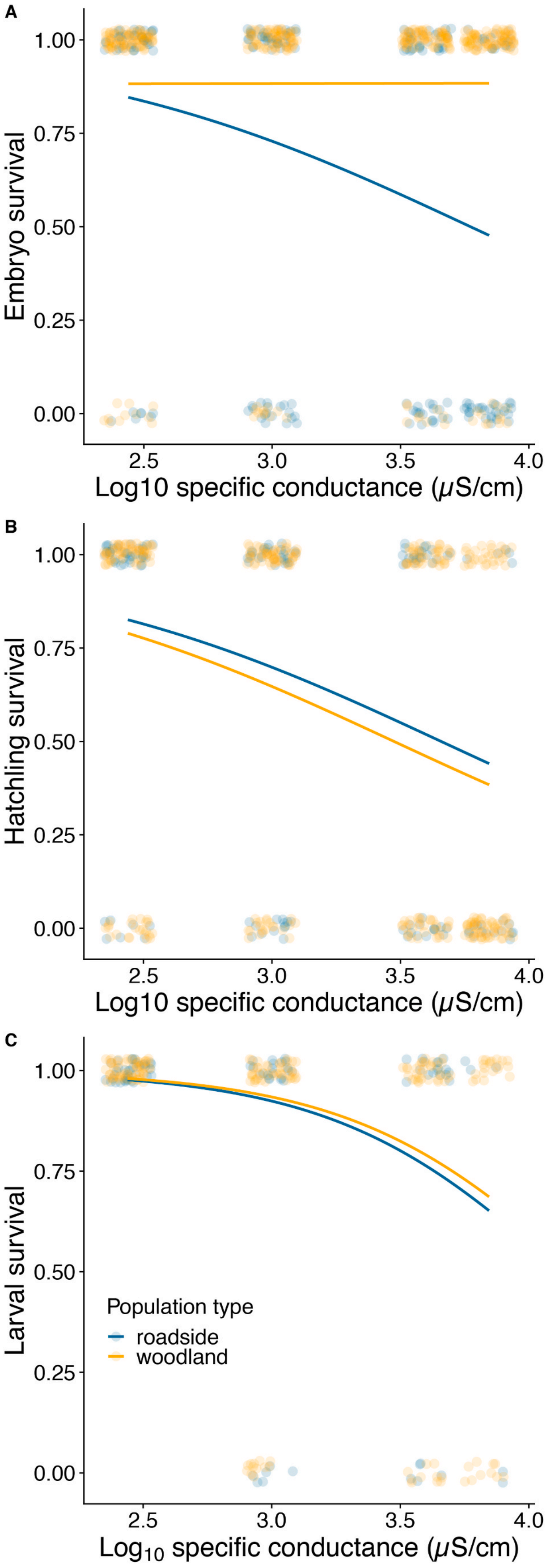

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Fig. 2. Effect of salt on survival for each of three different life history stages from embryo to metamorphosis. Salt concentration values are shown as $\log_{10}$-transformed specific conductivity (μS/cm). For each life history stage, circles represent raw data points showing survival (coded as 1) or mortality (coded as 0) for each individual that was alive at the start of that stage. Lines represent fitted values that were reverse-transformed from logit scores to probability from each model. Fitted lines are shown for each population type for visual comparison. Population type interacted with salt for embryos, however population type did not have a significant effect on survival for hatchlings or larvae. (A) Embryo survival shows survival from the start of the experiment until hatching. (B) Hatchling survival shows outcomes for tadpoles stage 21–25 that had survived through embryo stages. (C) Larval survival shows outcomes for tadpoles from Gosner stage 26–41 that had survived through hatchling stages. X-axis is shown as log-transformed values, which were used for model convergence. Untransformed values of treatments correspond to 275, 1,000, 4,000, and 7000 μS/cm.

Roadside populations reached metamorphosis more slowly than woodland populations during chronic road salt exposure. Such delays in the wild could have substantial effects on fitness, especially because wood frog embryos and larvae develop in temporary habitats prone to seasonal drying. Indeed, the frequency and timing of drying in these habitats exert strong selective pressure on developmental traits (Rowe and Dunson, 1995; Wellborn et al., 1996), and individuals that do not metamorphose before their natal ponds dry are almost certain to perish (Semlitsch, 1987). Moreover, delayed development should increase the amount of time roadside larvae spend in polluted conditions, and as a result, individuals from roadside populations should experience longer periods of contaminant exposure. Additionally, the effects of delayed development could be further exacerbated in the wild if salt concentration increases throughout the developmental period, for instance due to evaporative water loss as ponds dry.

Conceivably, such added exposure could further decrease developmental rate, leading to a positive feedback between exposure and developmental delays and adding to the burden of salt toxicity in later development stages. Longer exposures to roadside pollution and/or delayed development might have carryover effects after metamorphosis that negatively influence performance or survival as juveniles or adults. For instance, Dananay et al. (2015) found that experimental salt exposure in larvae was associated with increased body mass in late-stage larvae, and larger body mass at metamorphosis was associated with higher rates of mortality following metamorphosis. While we did not assess body size at metamorphosis here, Brady et al. (2019) found that adult wood frogs in roadside populations are, on average, larger than woodland adults. The larger average size of salt-exposed roadside populations might be an artifact of larger body size at metamorphosis associated with salt exposure during pre-metamorphic development and/or might be caused by edema, which itself also appears to incur fitness costs in salt-exposed populations (Frymus et al., 2021).

Wood frogs are philopatric, showing strong natal site fidelity as adults and, to a somewhat lesser degree, as juveniles (Berven and Grudzien, 1990). Further, across microgeographic spatial scales, wood frogs show phenotypic divergence (Brady and Goedert, 2017; Skelly, 2004) and genetic divergence (Richardson and Urban, 2013) associated with natural and anthropogenic selection pressures. Thus, it is reasonable to expect that wood frogs from roadside populations in this study have a legacy of exposure that occurred during parental and pre-parental generations, and that this legacy is responsible for increased toxicity to road salt and roadside conditions. Mechanisms of transgenerational transmission of reduced survival are yet unknown, and indeed could be the result of true, genetic maladaptation arising from processes such as DNA damage (Friedberg, 2003) or strong selection leading to inbreeding depression (O'Grady et al., 2006). Similarly, transgenerational plasticity (Donelan et al., 2020) or parentally-mediated contaminant transfer (Hopkins et al., 2006) could predispose offspring to relative reductions in embryonic survival and

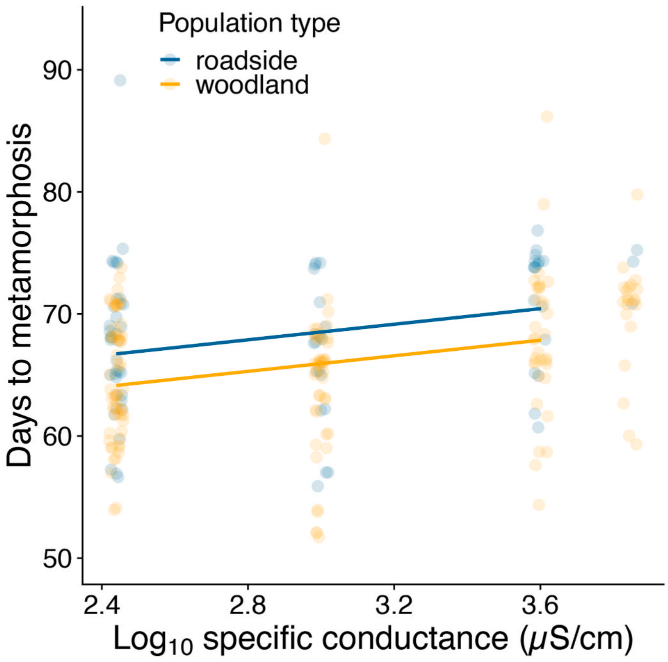

Fig. 3. Time to metamorphosis. For individuals surviving to metamorphosis, both salt concentration and roadside population identity delayed metamorphosis. Increasing salt concentration increased the number of days required to metamorphose, while roadside populations developed more slowly than those from woodland populations. Fitted lines represent predicted values from the model.

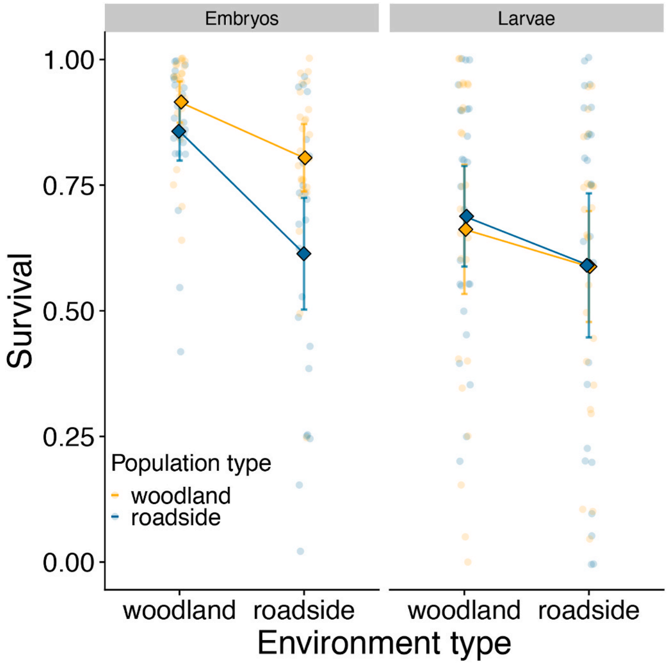

Fig. 4. Survival in reciprocal transplant experiment. Left panel: for roadside populations, embryonic survival was lower than woodland populations in both environments, and the roadside environment decreased survival for both population types (reproduced from Brady, 2013). Right panel: for larvae, survival was equivalent for both population types and in both environments. Circles represent proportion survival for each enclosure while diamonds indicate population type means (±95% CI).

developmental rate. Alternatively, local roadside populations might still be locally adapted to roadside habitats if for instance increased toxicity experienced by embryos trades off with well-adapted traits found in juveniles and/or adults, such as increased fecundity and jumping performance (Brady et al., 2019). Ultimately, future studies aiming to resolve these mechanisms would benefit by examining the correlation between traits expressed during aquatic and terrestrial stages (Goedert and Calsbeek, 2019). Identifying the physiological mechanisms responsible for increased embryonic mortality and developmental delays will also prove useful in understanding the source of differential toxicity between local populations and provide targets for potential genetic inquires.

5. Conclusion

Here, we used a combination of field and laboratory techniques to examine the effects of salt exposure on pre-metamorphic roadside and woodland wood frogs. While salt exposure had detrimental effects across both population types, our results show that roadside individuals performed worse than their woodland counterparts by multiple measures, including reduced survival and delayed development rates. In short, we showed that road salt is more toxic for embryos of wood frogs originating from roadside populations and that this increased relative toxicity is manifest only during embryonic stages. Yet critically, the increased toxicity in roadside embryos is not compensated for by increased relative survival in later hatchling and larval stages. Thus, the effect of salt on embryonic survival is strong enough to reduce the number of individuals metamorphosing from roadside versus woodland populations. Taken together, our results demonstrate for the first time that roadside populations are indeed locally maladapted to salt exposure throughout the entirety of aquatic development, indicating that a lineage of population-level salt exposure compromises tolerance to this widespread pollutant.

Author statement

Mia Forgione: Conceptualization, Methodology, Investigation, Writing- Original draft preparation. Steven Brady: Formal analysis, Writing- Reviewing and Editing, Visualization, Investigation, Resources, Project administration, Supervision.

Data availability

All data will be made available in a public repository upon acceptance.

Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.envpol.2021.118757.

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