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Physiological consequences of exposure to salinized roadside ponds on wood frog larvae and adults

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ABSTRACT

Aquatic organisms living adjacent to roads face many challenges posed by exposure to toxic runoff. The use of de-icing road salt across northern latitudes has salinized nearby freshwater habitats. Freshwater amphibians exposed to road salt at early life stages typically experience reduced survival; however, few studies address later-life effects. One study investigating whether populations can adapt to roadside ponds found survival in wood frog embryos raised in roadside environments was lowest in individuals with parents from roadside ponds. We investigated whether this negative response is also exhibited in late larval stages in reciprocally transplanted individuals from roadside populations and those located away from roads ('woodland' populations). We found reduced growth rates and more variable developmental rates in larvae raised in roadside ponds relative to woodland ponds regardless of origin, but no difference in survival between rearing environments. Laboratory exposure to road salt at relevant concentrations (0.6 ppt salinity) reduced larval activity and foraging behaviors, which may explain slower growth observed in the transplant experiment. Physiological assays of adult males migrating to breed revealed increased water retention in roadside populations. Further, these bloated males exhibited elevated resting plasma corticosterone levels and reduced capacity to secrete corticosterone when stimulated. Potential fitness consequences, such as reduced longevity and fecundity post metamorphosis through adulthood, of the roadside habitat could affect wood frog demography. Taken together, we provide evidence that the conditions experienced in the roadside environment pose challenges across life stages that have implications for persistence if populations are challenged with further stressors.

1. Introduction

The global road network has expanded rapidly over the last half-century and is projected to increase 60% by 2050 (Dulac, 2013). Roads induce a suite of ecological consequences including roadkill, habitat fragmentation, noise disruption, and contamination of adjacent habitats (Forman and Alexander, 1998; Tennessen et al., 2014; Trombulak and Frissell, 2000). In northern latitudes of the United States the salinization of freshwater habitats due to runoff of de-icing salts is of particular concern (Jackson and Jobbagy, 2005). The intensity of this salinization can be severe, with some road-adjacent wetlands seeing salinity levels 25% that of seawater (Kaushal et al., 2005). Moreover, increases in salinity equivalent to 1% seawater can affect physiological processes of freshwater organisms and the structure of biological communities (Findlay and Kelly, 2011).

Because of their apparent narrow physiological tolerance to osmotic change, amphibians have received considerable attention in the study of the effects of de-icing salt runoff on wildlife. Many experimental studies show reduced embryonic and hatchling survival, growth, or developmental rates following exposure to elevated salinity levels (see review in Hopkins and Brodie, 2015). Based on these findings, demographic models predict that roadside populations have a higher probability of experiencing declines (Karraker et al., 2008); but as these and other authors (Dananay et al., 2015) point out, higher embryonic mortality could also reduce density and therefore, elevated salinity could positively affect fitness traits like size at metamorphosis. Fewer studies, however, quantify physiological effects across life stages in natural populations affected by roadside runoff (e.g., Karraker et al., 2008).

Although many amphibian species have adapted to coastal brackish environments, the energetic cost of osmoregulation is hypothesized to be significant, especially for embryonic life stages with limited osmoregulatory tolerances (Hopkins and Brodie, 2015). As larvae develop, they accrue physiological adaptations to elevated salinity (i.e., internal gills and integumentary ion pumps), however, increased tolerance comes at the cost of size and time to metamorphosis (Gomez-Mestre et al., 2004; Kearney et al., 2014). Additionally, road salt effects of the larval environment can carry over to juvenile stages (Dananay et al.,

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2015), and juveniles and adults are exposed to de-icing salt runoff in breeding ponds or the surrounding forests (Findlay and Kelly, 2011). Therefore, continual exposure over life stages could pose osmoregulatory challenges that decrease survival, reproductive success, and physiological health of adults, and in turn, affect offspring performance mediated by parental environmental effects (Galloway, 2001).

In addition to the proximate impacts, it is important to consider the evolutionary responses of roadside populations. Strong selective pressures derived from recent environmental change have been observed to cause rapid evolutionary responses (Hendry et al., 2008; Sih et al., 2011). In the case of road runoff, selection imposed by osmotic changes can lead to differentiation of local populations in their capacity to tolerate increased salinity, as seen in the spotted salamander (Brady, 2012) and amphibians that inhabit brackish wetlands in coastal areas (e.g., Gomez-Mestre and Tejedo, 2003). Alternatively, populations could respond maladaptively, where fitness is reduced in their natal environment, a phenomenon that may be emerging as a result of rapid environmental change (Rolshausen et al., 2015). In order to predict population responses to recent environmental change, better estimates of the evolutionary and physiological capacities of natural populations are needed.

Here, we assessed the physiological responses to road salt runoff across life history stages of the wood frog (*Lithobates sylvaticus*) in natural populations adjacent to roads that receive road salt application and those distant from roads (hereafter 'woodland ponds'). Brady (2013) showed that wood frog hatchlings grow, develop, and survive at lower rates in roadside ponds with elevated salinity compared to woodland ponds and hatchlings from parents collected from roadside ponds experienced lower survival compared to those with parents from woodland ponds when reared in roadside ponds. This survival disadvantage suggests that they are locally maladapted to their environment or there are negative parental environmental effects. To extend these findings, using the same populations we first conducted a reciprocal transplant experiment in which individuals with parents collected from roadside or woodland environments were reared in either environment type. We hypothesized that traits expressed later in development are influenced by local adaptation or parental environmental effects resulting from exposure to the roadside habitat. Second, we conducted a laboratory experiment to test the hypothesis that road salt exposure alters activity and foraging behavior, as shown in the context of other environmental stressors (Crespi and Denver, 2004; Fraker et al., 2009). Further, it has been shown that these behaviors can contribute to growth or developmental rates (Anholt and Werner, 1998; Skelly and Werner, 1990), providing a potential mechanism of responses seen in the field. Finally, we collected adults migrating to breed in roadside and woodland ponds to test the hypothesis that roadside environments are linked to elevated physiological stress in breeding adults.

2. Methods

2.1. Study organism and region

Wood frogs are widely distributed throughout eastern North America and much of Canada (Martof and Humphries, 1959). We conducted our study in Northeast Connecticut in or near Yale Myers Experimental Forest, a managed mixed-hardwood forest in which roads and rural development are the primary anthropogenic features (Fig. 1). Road salt is applied on these paved roads and conductivities of adjacent ponds are known to reach 4000 μs/cm (Brady, 2013). Wood frogs migrate to breeding ponds in early spring, and in some populations site fidelity is nearly 100% among adults and 80% in juveniles (Berven and Grudzien, 1990); and there are phenotypically differentiated populations along environmental gradients that span tens to hundreds of meters at this site (e.g., canopy cover; Skelly, 2004).

2.2. Reciprocal transplant experiment

2.2.1. Site selection

We chose 4 ponds with high conductivity (>400 μs/cm at the time of breeding) within 20 m of a paved road (i.e., roadside), and 4 ponds that were located >200 m from a paved road (i.e., woodland; see Fig. 1). We used ponds with a breeding population in excess of 10 wood frog pairs so we would not dramatically affect populations. We matched roadside and woodland ponds based on similar canopy cover, pool size, and emergent vegetation, resulting in 4 reciprocally transplanted pairs of populations (see Table 1 for site characteristics). Of the abiotic variables we monitored each month throughout the four months of the experiment, only conductivity (a measure of salinity) differed between environment types (repeated-measures analysis of variance [ANOVA]; df = 1, F = 368.47, $P < 0.001$). Specifically, salinity levels were >20 times higher on average (maximum of 2100 μs/cm or ~1.07 ppt) in roadside ponds compared to woodland ponds. This maximum concentration is nearing the concentration predicted to cause extirpation of this species (3000 μs/cm; Karraker et al., 2008), which was evident by little to no activity of wood frogs in the extremely high salinity ponds in our study region (EMH pers. obs.).

2.2.2. Adult collection and breeding

We captured adults migrating into ponds with drift fencing at each of the 8 ponds; males and females from each pond were haphazardly paired and housed in a clear, 5 L container filled with ~1 L of water beside the pond from which they were collected (Apr. 8–15, 2011). Oviposition occurred within 2–11 d of pairing, and eggs from 4 pairs/pond were divided and assigned to treatment groups. Eggs were exposed to pond water for up to 48 h, although this has been shown to have no effect on embryonic survival, growth or developmental rates (Brady, 2016), this early exposure may affect larval vital traits. Protocols were approved by the Institutional Animal Care and Use Committee (IACUC) of Yale University (2011-11024) and collections were approved by the Connecticut Department of Energy and Environmental Protection (CDEEP Scientific Collections Permit #1115003).

2.2.3. Experimental design

We conducted reciprocal transplants between each of the 4 pairs of roadside and woodland pools (see Appendix A: Supplementary Fig. 1 for diagram). We split clusters of ~100 embryos from each egg mass into floating 14 L plastic enclosures in both the natal pond and the reciprocal pond of opposite type. We used a subset of individuals that were measured for embryonic traits in Brady (2013). At feeding stage (Gosner stage 25 [Gosner, 1960]) we transferred larvae into larger enclosures (n = 5 larvae/replicate enclosure/clutch) in the same pond, which were 0.5 m diameter × 1 m tall cylinder made of no-see-um mesh (20 × 20 gauge), submerged with >20 cm above water level, and 4 L of dry leaves to provide substrate for algae growth. We had a total of 16 enclosures in each pond (2 replicates/clutch × 4 clutches × 2 origins), except two cases in which hatchling survival was low: one woodland pond had 3 natal and 4 reciprocal enclosures, and one roadside pond had 4 natal and 8 reciprocal enclosures.

At the start and end (after 20–21 d; June 17–23, 2011) of this experiment, we photographed each individual with a ruled scale and recorded Gosner stage and snout-vent length (SVL) using Image J V1.44p (Rasband 1997–2012). We measured percent survival, and calculated growth and developmental rates as the exponential functions of change in SVL or stage over of days elapsed (e.g., [ln(final size) − ln(initial size)] / period).

2.2.4. Statistical analysis

We analyzed data using R, V. 3.0.2 (R Development Core Team, 2012). We used a logistic regression combined with MCMC