ARTICLE IN PRESS

Environmental Pollution xxx (2016) 1–7

Contents lists available at ScienceDirect

Environmental Pollution

journal homepage: www.elsevier.com/locate/envpol

Rapid evolution of tolerance to road salt in zooplankton☆

Kayla D. Coldsnow*, Brian M. Mattes, William D. Hintz, Rick A. Relyea

[a] Department of Biological Sciences, Rensselaer Polytechnic Institute, 110 8th St., Troy, NY 12180, USA

ARTICLE INFO

Article history:
Received 29 July 2016
Received in revised form
2 December 2016
Accepted 3 December 2016
Available online xxx

Keywords:
Adaptation
Cladoceran
Contaminants
Ecotoxicology
Run-off

ABSTRACT

Organisms around the globe are experiencing novel environments created by human activities. One such disturbance of growing concern is the salinization of freshwater habitats from the application of road deicing salts, which creates salinity levels not experienced within the recent evolutionary history of most freshwater organisms. Moreover, salinization can induce trophic cascades and alter the structure of freshwater communities, but knowledge is still scarce about the ability of freshwater organisms to adapt to elevated salinity. We examined if a common zooplankton of freshwater lakes (*Daphnia pulex*) could evolve a tolerance to the most commonly used road deicing salt (sodium chloride, NaCl). Using a mesocosm experiment, we exposed freshwater communities containing *Daphnia* to five levels of NaCl (15, 100, 200, 500, and 1000 mg Cl$^-$ L^{-1}). After 2.5 months, we collected *Daphnia* from each mesocosm and raised them in the lab for three generations under low salt conditions (15 mg Cl$^-$ L^{-1}). We then conducted a time-to-death experiment with varying concentrations of NaCl (30, 1300, 1500, 1700, 1900 mg Cl$^-$ L^{-1}) to test for evolved tolerance. All *Daphnia* populations exhibited high survival when subsequently exposed to the lowest salt concentration (30 mg Cl$^-$ L^{-1}). At the intermediate concentration (1300 mg Cl$^-$ L^{-1}), however, populations previously exposed to elevated concentrations (i.e.100 −1000 mg Cl$^-$ L^{-1}) had higher survival than populations previously exposed to natural background levels (15 mg Cl$^-$ L^{-1}). All populations survived poorly when subsequently exposed to the highest concentrations (1500, 1700, and 1900 mg Cl$^-$ L^{-1}). Our results show that the evolution of tolerance to moderate levels of salt can occur within 2.5 months, or 5–10 generations, in *Daphnia*. Given the importance of *Daphnia* in freshwater food webs, such evolved tolerance might allow *Daphnia* to buffer food webs from the impacts of freshwater salinization.

1. Introduction

Anthropogenic disturbances generate novel environments and human-induced evolutionary change is occurring worldwide (Palumbi, 2001; Sih et al., 2010). A lack of adaptation to novel environments may threaten biodiversity and ecosystem services (Dudgeon et al., 2006; McGill et al., 2015). Chemical contamination of aquatic ecosystems is one of the most common types of anthropogenic disturbances. Many organisms targeted by chemicals like pesticides and antibiotics have developed a resistance to such chemicals, sometimes within a decade of introduction (Hoy, 1998; Palumbi, 2001). Less attention, however, has been given to how chemical contaminants affect non-target organisms. Recent research has shown that some non-target species in communities are able to adapt to chemicals like pesticides (e.g., Hua et al., 2013, 2015; Bendis and Relyea, 2014), which stabilizes communities and prevents trophic cascades (Bendis and Relyea, 2016).

Salinization of freshwater ecosystems is an emergent environmental and ecological issue worldwide (Cañedo-Argüelles et al., 2016). Salinization is caused by agriculture, mining, and the application of road deicing salts (Thunqvist, 2004; Corsi et al., 2010; Cañedo-Argüelles et al., 2013, 2016). In regions that experience cold winters, the application of road salt has caused the salinity of many freshwater ecosystems to rise sharply over the past few decades (Cañedo-Argüelles et al., 2013). In the United States, road deicing salt consumption has increased from approximately 0.20 million metric tons per year in 1944 to 24.5 million metric tons per year in 2014 (Harris and Tucker, 1947; Bolen, 2016). Expansion of

---

☆ This paper has been recommended for acceptance by Dr. Sarah Michele Harmon.

* Corresponding author.

E-mail addresses: coldsk@rpi.edu (K.D. Coldsnow), matteb2@rpi.edu (B.M. Mattes), hintzwd@gmail.com (W.D. Hintz), relyer@rpi.edu (R.A. Relyea).

http://dx.doi.org/10.1016/j.envpol.2016.12.024
0269-7491/© 2016 Elsevier Ltd. All rights reserved.

Please cite this article in press as: Coldsnow, K.D., et al., Rapid evolution of tolerance to road salt in zooplankton, Environmental Pollution (2016), http://dx.doi.org/10.1016/j.envpol.2016.12.024

impervious surface area from urbanization (Bester et al., 2006) and increased precipitation in cold regions from climate change will only increase the application rates of road salts (Novotny and Stefan, 2010).

Currently because of road salt application, the salinity levels of many lakes, streams, and wetlands exceed the chronic and acute thresholds established for chloride (Cl^-). In the USA, the chronic threshold is 230 mg Cl^- L^{-1} and the acute threshold is 860 mg Cl^- L^{-1} (Benoit and Stephan, 1988). In major metropolitan areas in the northern USA, 51% of stream sampled during the winter months had chloride concentrations above the chronic level, while 23% experienced concentrations above the acute level (Corsi et al., 2010). Many lakes and wetlands in northern USA and Canada also experience chloride concentrations above chronic levels (e.g., Evans and Frick, 2001; Judd et al., 2005). Longer water retention times in lakes and wetlands compared to streams also results in longer exposure times for freshwater organisms (Evans and Frick, 2001), which are typically adapted to low salt concentrations (0–20 mg Cl^- L^{-1}; Kelting and Laxson, 2010). Yet, it is unknown whether freshwater organisms can adapt to salinity levels far above those encountered in their recent evolutionary history.

Little research has investigated the impacts of road salt on freshwater communities. Some field studies suggest an inverse relationship between salinity and biodiversity in freshwater systems (reviewed in Evans and Frick, 2001). In particular, zooplankton abundance and diversity decrease as salinity increases (e.g. Schallenberg et al., 2003). Experiments manipulating salt concentrations commonly observe increases in rotifers and declines in cladocerans and copepods (Van Meter et al., 2011; Van Meter and Swan, 2014; Dalinsky et al., 2014; Dananay et al., 2015; and Kefford et al., 2016). Because cladocerans are major consumers of phytoplankton, their decline commonly leads to increases in phytoplankton, which may result in a loss of ecosystem services (e.g., Walsh et al., 2016). Cladocerans in the genus *Daphnia* are one of the more sensitive groups of zooplankton (Bona et al., 2014), yet they are vital to aquatic ecosystems as a preferred food source for many planktivorous fish and a primary consumer of phytoplankton (Ebert, 2005; Seda and Petrusek, 2011). Because of their important role in freshwater communities and food webs, it is essential to evaluate if zooplankton, and *Daphnia* in particular, can adapt to high salinities resulting from road salt contamination.

We investigated if *Daphnia pulex*, a dominant cladoceran species in many aquatic ecosystems, could evolve a tolerance to road salt contamination. We performed a two-phase experiment. In Phase 1, we raised *Daphnia* in outdoor mesocosms, embedded within a lake food web, under varying levels of the road salt (Hintz et al., *in press*). After 2.5 months of exposure (i.e. 5–10 generations), we transferred the *Daphnia* to the laboratory. After being raised under low salt conditions for three generations (1.5 months), Phase 2 of the experiment tested whether populations previously exposed to elevated salt concentrations during Phase 1 exhibited increased salt tolerance during Phase 2. If zooplankton were able to evolve a tolerance to road salt, we predicted that populations previously exposed to a wide range of salt concentrations for several generations would all exhibit high survival when subsequently exposed to a low concentration of salt (Fig. 1A). In contrast, we predicted that populations previously exposed to elevated salt concentrations would exhibit higher survival (relative to the control) when subsequently exposed to moderate concentrations of salt (Fig. 1B). Finally, we predicted that populations previously exposed to any salt concentrations would exhibit low survival when subsequently exposed to high concentrations of salt (Fig. 1C).

2. Methods

2.1. Phase 1—initial salt exposure

Phase 1 of the experiment was part of a larger study, which took place outside at the Rensselaer Aquatic Lab (Troy, New York, USA) during the summer of 2015 (Hintz et al., *in press*). The experimental design consisted of five salt treatments: 15 (control), 100, 250, 500, and 1000 mg Cl^- L^{-1}. We chose these road salt concentrations because they represented concentrations near the EPA thresholds for aquatic ecosystems (Benoit and Stephan, 1988), as well as concentrations that have been detected in lakes (Judd et al., 2005; Novotny et al., 2008; Novotny and Stefan, 2010; Sibert et al., 2015), streams (Kaushal et al., 2005; Corsi et al., 2010), and wetlands (Evans and Frick, 2001). We used Solar Salt (Morton® Salt, Chicago, IL, USA), which is 99.8% pure NaCl. We chose NaCl because it is the most widely used road deicer in North America (Kelting and Laxson, 2010). The treatments were replicated four times for a total of 20 experimental units. The experimental units were 1200-L polyethylene cattle tanks (mesocosms) covered with a 60% shade cloth to prevent organisms from departing or colonizing the tanks.

We filled each mesocosm with a sand substrate, leaf litter, and water from Lake George (New York, USA), which had a chloride concentration of 15 mg Cl^- L^{-1}. Each mesocosm contained a food web consisting of pouch snails (*Physa acuta*), amphipods (*Hyalella azteca*), isopods (*Asellus aquaticus*), banded mystery snails (*Viviparus georgianus*), and fingernail clams (*Sphaerium simile*). We also added two concentrated 450-mL aliquots of a zooplankton and phytoplankton mix from Lake George to serve as a source of plankton. Plankton were collected from Lake George using a vertical, integrated plankton tow (64-μm mesh) at various places (throughout Northwest Bay, Lake George) starting just below the thermocline and up to the lake's surface. Once the organisms had adjusted to tank conditions for 2 d, we applied the salt treatments on 10 July. Additional details of the food web experiment can be found in Hintz et al. (*in press*).

2.2. Animal collection and husbandry

After 2.5 months of the food webs being exposed to the different salt concentrations, we collected samples of zooplankton from the 20 mesocosms using aquarium dip nets with a 100-μm mesh. Within 30 min of collection, we transported the zooplankton to a lab where they were raised under the low-salt conditions of Lake George (15 mg Cl^- L^{-1}). From each zooplankton sample collected from a given mesocosm, we selected 20 large *Daphnia pulex* (body length: 1.5–2.0 mm), all of which were reproductively active females. We placed 10 of them into each of two 400-mL experimental jars. Each jar contained 300 mL of Lake George water, which was filtered using glass microfiber filters (1.2-μm pore size; Whatman, Inc.) to remove any zooplankton. Populations exposed to the five salt concentrations from Phase 1 were raised separately by mesocosm until later pooled within a concentration for Phase 2. Populations were pooled because the timing of reproduction between jars did not line up in a way that made it possible to have enough *Daphnia* for Phase 2 in all replicates. The *Daphnia* were placed in an animal room with a light cycle of 12 L:12 D and at constant temperature (20.5 ± 0.2 °C). Each jar of *Daphnia* was sufficiently fed 150 μL of concentrated algae (*Raphidocelis subcapitata*) every 2 d. A single batch of *R. subcapitata* algae was used for the duration of *Daphnia* husbandry. This algae was grown in high phosphorus COMBO medium prior to the start of the experiment (Kilham et al., 1998). We added new water when needed to keep the water level constant and removed any debris to keep the water clean. Each population was reared in the lab for three generations to reduce any

Please cite this article in press as: Coldsnow, K.D., et al., Rapid evolution of tolerance to road salt in zooplankton, Environmental Pollution (2016), http://dx.doi.org/10.1016/j.envpol.2016.12.024

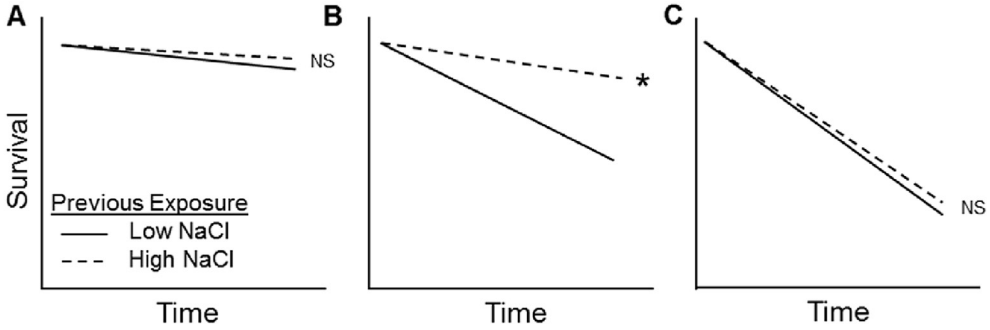

Fig. 1. Predictions of evolved tolerance to road salt (NaCl). The solid line represents a population of zooplankton that has been previously exposed to low salt levels. The dashed line represents a population of zooplankton that has been previously exposed to high salt levels. An asterisk indicates a hypothesized difference between the two lines, while NS means there is no significant difference between the two lines. (A) If these populations were exposed to low levels of salt, their survival would be similar. At low levels, there is no threat to either population. (B) If these populations were exposed to moderate levels of salt, their survival would differ. The high exposed population would survive better than the low exposed population because of the development of tolerance. (C) If these populations were exposed to high levels of salt, their survival would be similar. At high levels, the threat would be the same, regardless of tolerance level.

environmental and maternal effects. The original mothers, as well as each consecutive mother, was removed from each jar once young *Daphnia* were released to ensure that we were working with only one generation at a time.

2.3. *Phase 2—testing for evolved tolerance*

We began Phase 2 of the experiment on 5 November. We crossed the five original treatments from Phase 1 with five new treatments [30 (control), 1300, 1500, 1700, 1900 mg Cl^- L^{-1}]. The goal for these concentrations was to have intermediate, varying levels of survival across the Phase-1 populations over a 48-hr timeframe. We based these concentrations on a pilot study of *Daphnia* tolerance, as well as previous reports of acute ($\leq$48 h) concentrations of salt that cause an effect (EC_{50}) or death (LC_{50}) to *Daphnia* species. There is immense variation among these studies sometimes based on other factors (i.e. temperature, food availability) or the population's background chloride concentration. For example, *D. magna* has many reported values for the LC_{50} of NaCl from 2024 mg Cl^- L^{-1} (Benoit and Stephan, 1988) to 4704 mg Cl^- L^{-1} (Cowgill and Milazzo, 1990), while *D. pulex* has reported NaCl LC_{50}s ranging from 1470 mg Cl^- L^{-1} (Benoit and Stephan, 1988) to 2042 mg Cl^- L^{-1} (Gardner and Royer, 2010).

Using a randomized design, we replicated each of the 25 treatment combinations three times, for a total of 75 experimental units. The experimental units were 250-mL glass beakers, each filled with 200 mL of one of the five new salt concentrations. We prepared the water using filtered Lake George water, which when collected had a concentration of 30 mg Cl^- L^{-1}. This 15 mg Cl^- L^{-1} increase was not a concern since it was less than our lowest elevated Phase-1 concentration (i.e. 100 mg Cl^- L^{-1}) and our next highest Phase-2 concentration (i.e. 1300 mg Cl^- L^{-1}). The increase was likely caused by runoff from a nearby stream, causing localized fluctuations in Cl^- concentration. We measured the salinity of each salt concentration with the Traceable® Salinity Meter (VWR International). The actual concentrations of the water were, on average, within 1.0% of the nominal, so throughout our results we refer to the nominal concentrations. Using third-generation *Daphnia,* we collected eight large individuals from the populations exposed to the salt concentrations in Phase 1 and placed them in a beaker containing one of the five new concentrations. We then monitored *Daphnia* mortality every 2 h for 48 h. Mortality was defined as no movement before or after being tapped gently with a pipette two times. *Daphnia* were not fed during the 48-hr trials.

2.4. *Statistical analysis*

To examine whether a previous exposure to 15, 100, 250, 500, and 1000 mg Cl^- L^{-1} affected the ability of later generations to survive at a given salt concentration, we performed a Cox's proportional hazard regression (Cox, 1972). We obtained regression coefficients (b) and hazard ratios (e^b) by using the time-to-death of individual *Daphnia* for each Phase-1 population at a given test concentration (30, 1300, 1500, 1700, 1900 mg Cl^- L^{-1}) as the response variable and the different Phase-1 populations as the predictor with replicate as a stratified covariate. In preliminary analysis, we checked for interactions between Phase-1 populations and replicate number and after finding widespread insignificant interactions, we dropped the interaction but retained the replicate term in the analysis. Within each salt exposure in the lab, a negative coefficient would indicate that a population exposed to higher salt in Phase 1 survived better than the population exposed to the lowest salt concentration in Phase 1, while a positive coefficient would indicate reduced survival. Because the control population (15 mg Cl^- L^{-1}) was the reference population for the comparison of elevated exposures, the regression coefficient is zero. The hazard ratios, or the rate of death, were used to calculate the statistical difference. If the 95% confidence intervals did not overlap with the hazard ratio of the control population (i.e. 1), this would signal a statistically significant difference from the control population. We also obtained percent censored data for each Cox's proportional hazard regression for the Phase-2 concentrations, telling us the percentage of individuals that contributed to the survivor function but not the death (Crawley, 2013). Survival graphs represent the number of individuals alive at each time, visually showing the rate of death for each Phase-1 population at a given Phase-2 concentration. All analyses were completed using the package RcmdrPlugin.survival in RStudio version 0.99.491 (Fox and Carvalho, 2012; R Development Core Team, 2015).

3. Results

Using the Cox's proportional hazard model, we found that survival in the Phase-2 control treatment (30 mg Cl^- L^{-1}) was high (94%) with most populations experiencing no death (Table 1, Fig. 2A). None of the populations previously exposed to elevated salt concentrations (100–1000 mg Cl^- L^{-1}) differed in survival from the Phase-1 control population (15 mg Cl^- L^{-1}; $p \geq 0.21$).

When we examined the Phase-2 treatment of 1300 mg Cl^- L^{-1}, we detected survival differences among the populations previously

Please cite this article in press as: Coldsnow, K.D., et al., Rapid evolution of tolerance to road salt in zooplankton, Environmental Pollution (2016), http://dx.doi.org/10.1016/j.envpol.2016.12.024

raised under different salt concentrations in Phase 1 (Table 1, Fig. 2B). Populations previously exposed to moderate salt concentrations (100 and 250 mg $Cl^- L^{-1}$) exhibited significantly higher survival ($p = 0.01$) and populations previously exposed to 1000 mg $Cl^- L^{-1}$ exhibited marginally higher survival ($p = 0.09$) when compared to the control. The 500 mg $Cl^- L^{-1}$ population was not significantly different from the control ($p = 0.11$).

When we exposed the Phase-1 populations to much higher salt concentrations in Phase 2 (1500, 1700, and 1900 mg $Cl^- L^{-1}$), we found that all populations experienced high mortality (Table 1, Fig. 2C–E). In all populations, survival decreased as the concentration of salt increased (Table 1). Averaged across all Phase-1 concentrations, survival was 69% when exposed to 1500 mg $Cl^- L^{-1}$, 58% when exposed to 1700 mg $Cl^- L^{-1}$, and 26% when exposed to 1900 mg $Cl^- L^{-1}$. The Cox's proportional hazard model showed no significant differences among any Phase-1 populations at 1500 ($p \geq 0.16$), 1700 ($p \geq 0.10$) or 1900 mg $Cl^- L^{-1}$ ($p \geq 0.26$).

4. Discussion

We show that in the span of 2.5 months (i.e. 5–10 generations), *Daphnia* were able to evolve an increased tolerance to road salt. *Daphnia* populations initially reared under high salt conditions were subsequently able to survive relatively high salinities (1300 $Cl^- L^{-1}$) compared to *Daphnia* reared under their natural concentration of 15 mg $Cl^- L^{-1}$. Even initial exposures to moderate levels of salt (100 and 250 mg $Cl^- L^{-1}$) resulted in the evolution of increased tolerance. However, survival was reduced above 1500 mg $Cl^- L^{-1}$, regardless of exposure history, suggesting a limit to evolved tolerance in *Daphnia*.

We are the first to show evolved tolerance to road salt in *Daphnia* within recent evolutionary history. Other studies show that species in the family Daphniidae living at high salinities have a higher tolerance to salt than populations that live in low salinity environments (Weider and Hebert, 1987; Teschner, 1995; Latta et al., 2012; Loureiro et al., 2012; Liao et al., 2015), which demonstrates adaptability and differences between populations and

Table 1
Regression coefficients (b) and hazard ratios of *Daphnia* that were originally exposed to 15, 100, 250, 500, or 1000 mg $Cl^- L^{-1}$ in Phase 1 and then re-exposed several months later to 30, 1300, 1500, 1700, or 1900 mg $Cl^- L^{-1}$ in Phase 2. Percent censored indicates the average percent survival of *Daphnia* after the 48-hr exposure in all the Phase-1 populations for that given Phase-2 concentration. Bolded *p*-values indicate a significant ($p \leq 0.05$) or marginally significant ($p < 0.10$) difference between the rate of death in that population and the lowest concentration (15 mg $Cl^- L^{-1}$).

Phase-2 exposure	Percent censored	Phase-1 exposure	Regression coefficient (b)	Hazard ratio [95% CI]	*p*-value
30	94	15	0	1	–
		100	−20.88	8.53e-10 [0.00, Infinite]	1.00
		250	−0.69	0.50 [0.09, 2.73]	0.42
		500	−20.88	8.53e-10 [0.00, Infinite]	1.00
		1000	−1.41	0.24 [0.03, 2.19]	0.21
1300	74	15	0	1	–
		100	−1.70	0.18 [0.05, 0.64]	**0.01**
		250	−2.13	0.12 [0.03, 0.53]	**0.01**
		500	−0.76	0.47 [0.19, 1.18]	0.11
		1000	−0.85	0.33 [0.16, 1.13]	**0.09**
1500	69	15	0	1	–
		100	−0.48	0.62 [0.22, 1.74]	0.36
		250	−0.21	0.81 [0.31, 2.10]	0.67
		500	−0.07	0.93 [0.37, 2.36]	0.88
		1000	−0.79	0.45 [0.15, 1.35]	0.16
1700	58	15	0	1	–
		100	−0.43	0.65 [0.24, 1.75]	0.39
		250	0.69	1.99 [0.87, 4.58]	0.10
		500	0.40	1.49 [0.64, 3.47]	0.36
		1000	−0.78	0.46 [0.15, 1.36]	0.16
1900	26	15	0	1	–
		100	0.04	1.04 [0.55, 1.96]	0.90
		250	−0.36	0.70 [0.36, 1.37]	0.26
		500	−0.08	0.93 [0.49, 1.76]	0.82
		1000	−0.18	0.84 [0.43, 1.64]	0.61

Please cite this article in press as: Coldsnow, K.D., et al., Rapid evolution of tolerance to road salt in zooplankton, Environmental Pollution (2016), http://dx.doi.org/10.1016/j.envpol.2016.12.024

genotypes. However, these studies examine salinity tolerance in close proximity to saltwater (e.g., brackish water, total salts >2000 mg L^{-1}). No study has shown that a salinity increase from anthropogenic sources is capable of inducing an evolved tolerance to high salinities in such a short period of time. Loureiro et al. (2015) showed that *Daphnia* previously exposed to 1000 mg Cl^- L^{-1} for one, three, or nine generations had little influence on their survival time at 6000 mg Cl^- L^{-1}. This is contrary to what we found, but the test concentration in Loureiro et al. (2015) was much higher than the exposure concentration, potentially accounting for the differences in our results and those of Loureiro et al. (2015). It is known that zooplankton are able to evolve tolerance to other freshwater contaminants. For example, *Daphnia pulex* and *Simocephalus vetulus* evolved a tolerance to pesticides, but the level of tolerance was dependent on distance to agriculture and exposure history over a relatively long period of time (Bendis and Relyea, 2014). In the present study, we show that evolved tolerance to an aquatic contaminant can occur over a short period of time even after an abrupt change in concentration.

While we pooled the original Phase-1 populations before going into the Phase-2 experiment, we still saw evolved tolerance to NaCl. Because each Phase-1 population was exposed to a control environment that only varied in salinity levels, we would expect there to be little differences between Phase-1 replicates within a concentration and in turn little issue with pooling the populations in order to gain the numbers needed for the experiment. Further, we see what appears to be a grouping of the moderate pre-exposed populations (100 and 250 mg Cl^- L^{-1}) and the high pre-exposed populations (500 and 1000 mg Cl^- L^{-1}) specifically shown by high significance and marginal or non-significance respectively (Table 1, Fig. 2B). Because of this graded response in evolved tolerance, rather than random response, we feel the pooling of the populations still allows us to see the evolved tolerance in the populations. Future studies should involve looking at separate populations that were independently exposed, as well as investigating other traits that may follow a similar graded pattern as seen here.

The mechanism behind evolved salt tolerance in zooplankton remains unclear. Changes in the expression of genes related to osmoregulation and ATP activity are thought to play a role (Kefford et al., 2016). In fact, Latta et al. (2012) found that genes responsible for the sodium/potassium ATPase activity in *D. pulex* were upregulated in individuals adapted to higher salt concentrations. This enzyme is important in osmoregulation in most eukaryotic organisms by transporting salts into and out of the cells, allowing them to survive in different salinity environments (Ituarte et al., 2008). It is possible that differences in these genes, either within the population or within an individual, are responsible for the evolved tolerance discovered in the present study. Because the tolerance was present after three generations (1.5 months) in the lab, the tolerance could be a result of a change in allele frequency through selection of more tolerant individuals within the population exposed during Phase 1 of the experiment. The alternative could be a long lasting epigenetic change passed through generations. Epigenetic changes, such as methylation, have been shown to occur when *Daphnia* are exposed to other toxins such as heavy metals (Vandegehuchte et al., 2009; Harris et al., 2012). Some of these changes in gene expression, such as the gene responsible for ATPase activity, may affect more than just salinity tolerance (e.g., life history traits).

Besides mortality and increased tolerance, salinity increases can affect other traits by causing increased developmental abnormalities, reduced feeding rates (Evans and Frick, 2001), decreased reproduction (Sarma et al., 2006; Gonçalves et al., 2007; Stoks et al., 2014), and decreased respiration (Arnér and Koivisto, 1992). *Daphnia* populations that are more tolerant to salt or survive in higher salt environments have smaller bodies and experience reduced reproduction when compared to *Daphnia* populations

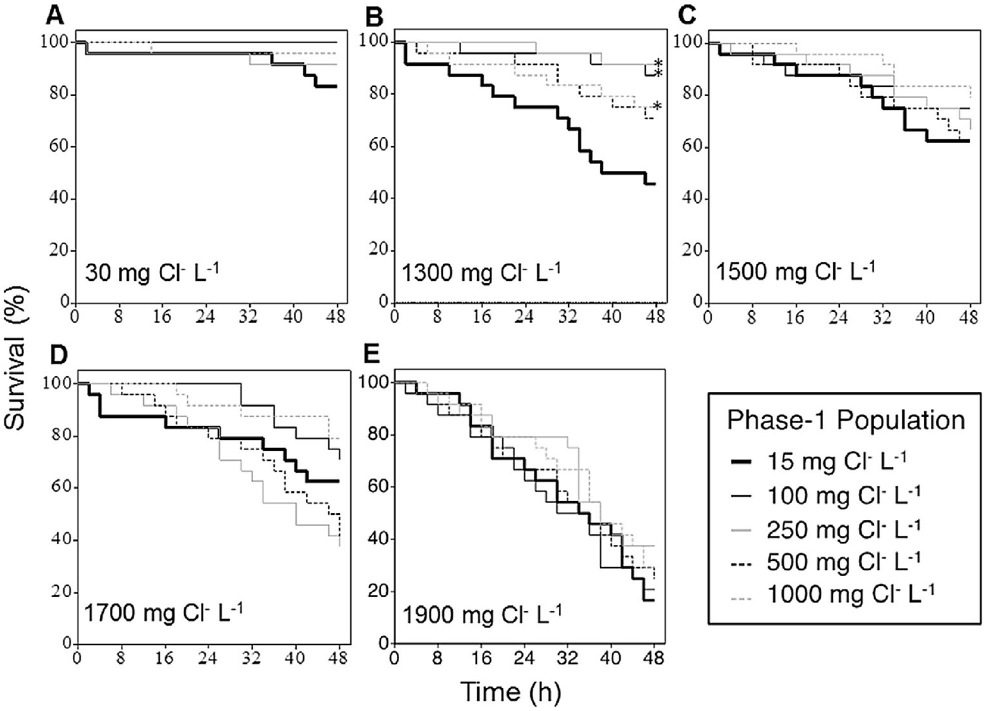

Fig. 2. The survival of five *Daphnia* populations that were previously exposed to different levels of salt (15, 100, 250, 500, and 1000 mg Cl^- L^{-1}) in Phase 1 and then in Phase 2, exposed to five new salt concentrations: (A) 30, (B) 1300 (C) 1500, (D) 1700, and (E) 1900 mg Cl^- L^{-1}. The asterisks indicate that there is a significant difference between the hazard ratio, or rate of death, of that population and the control population.

Please cite this article in press as: Coldsnow, K.D., et al., Rapid evolution of tolerance to road salt in zooplankton, Environmental Pollution (2016), http://dx.doi.org/10.1016/j.envpol.2016.12.024

from low salt waters, despite having time to evolve mechanisms to cope with the trade offs of higher salt tolerance (Ghazy et al., 2009; Latta et al., 2012). More tolerant zooplankton also show changes in gene expression in genes other than ATPase activity, such as important predator sensing genes (Latta et al., 2012), hinting at indirect effects from salt exposure. If the costs are less than the benefits, the exposed population and community may be able to benefit from the survival of a tolerant species.

Understanding the impacts of contaminants on zooplankton is essential because of their critical role in aquatic food webs. Zooplankton are important for transferring energy from primary producers to higher trophic levels (Carpenter et al., 1985) and maintaining ecosystem services like water clarity (Walsh et al., 2016). As such, an evolved tolerance to road salt could mitigate a salt-induced trophic cascade whereby the reduction of zooplankton abundance would lead to elevated phytoplankton levels (e.g., Hintz et al., *in press*). This may be particularly important for urban lakes where salinity levels are high from road salt inputs (e.g., Novotny et al., 2008) because of the ecosystem services they provide. The ability to mitigate a trophic cascade has already been shown in zooplankton with an evolved tolerance to pesticides (Bendis and Relyea, 2016), but this has yet to be tested with salt-tolerant zooplankton.

The existence of evolved tolerance to road salts opens up a number of fascinating possibilities for future research. For example, the prevalence of evolved tolerance in organisms living in natural freshwater systems is currently unknown but may be common. Further, it may be that evolution of increased tolerance to a common road salt also confers increased tolerance to other roads salts (e.g., $CaCl_2$, $MgCl_2$). It is also important to determine how long evolved tolerance persists in freshwater organisms. Road salt enters freshwater systems in pulses during the spring snowmelt but elevated salinity levels can remain year round in some ecosystems (Langen et al., 2006; Corsi et al., 2010). The current study discovered that a press disturbance of high salinity triggered evolved tolerance, but future research should examine whether repeated salt pulses also trigger evolved tolerance. Finally, future work should also consider the potential life history costs of evolved salt tolerance as well as potential consequences such as changes in behavior, species interactions, community structure, or ecosystem services.

Funding sources

This study was funded by the Jefferson Project at Lake George, which is supported by Rensselaer Polytechnic Institute, International Business Machines (IBM), and The Fund for Lake George.

Disclosure statement

The authors state that there are no competing financial or personal interests.

Acknowledgements

We thank Alex Brooks, Jeevan Narendran, and Kelsey Sudol for help with setting up and executing both phases of the experiment and Devin Jones for assistance with the analysis.

References

Arnér, M., Koivisto, S., 1992. Effects of salinity on metabolism and life history characteristics of *Daphnia magna*. Hydrobiologia 259, 69–77.

Bendis, R.J., Relyea, R.A., 2014. Living on the edge: populations of two zooplankton species living closer to agricultural fields are more resistant to a common insecticide. Environ. Toxicol. Chem. 33, 2835–2841.

Bendis, R.J., Relyea, R.A., 2016. Wetland defense: naturally occurring pesticide resistance in zooplankton populations protects the stability of aquatic communities. Oecologia 181, 487–498.

Benoit, D.A., Stephan, C.E., 1988. Ambient water quality criteria for chloride. U.S. Environmental Protection Agency Office of Research and Development Environmental Research Laboratory, Duluth, MN. Report no. PB88–175 047.

Bester, M.L., Frind, E.O., Molson, J.W., Rudolph, D.L., 2006. Numerical investigation of road salt impact on an urban wellfield. Ground Water 44, 165–175.

Bolen, W.P., 2016. 2014 minerals yearbook: salt. U.S. Geol. Surv. 63, 1–22.

Bona, M.D., Di Leva, V., De Liguoro, M., 2014. The sensitivity of *Daphnia magna* and *Daphnia curvirostris* to 10 veterinary antibacterials and to some of their binary mixtures. Chemosphere 115, 67–74.

Carpenter, S.R., Kitchell, J.F., Hodgson, J.R., 1985. Cascading trophic interactions and lake productivity: fish predation and herbivory can regulate lake ecosystems. BioScience 35, 634–639.

Cañedo-Argüelles, M., Hawkins, C.P., Kefford, B.J., Schaefer, R.B., Dyack, B.J., Brucet, S., Buchwalter, D., Dunlop, J., Froer, O., Lazorchak, J., Coring, E., Fernandez, H.R., Goodfellow, W., Gonzalez Achem, A.L., Hatfield-Dodds, S., Karimov, B.K., Mensah, P., Olson, J.R., Piscart, C., Prat, N., Ponsa, S., Schulz, C.J., Timpano, A.J., 2016. Saving freshwater from salts. Science 351, 914–916.

Cañedo-Argüelles, M., Kefford, B.J., Piscart, C., Prat, N., Schäfer, R.B., Schulz, C., 2013. Salinisation of rivers: an urgent ecological issue. Environ. Pollut. 173, 157–167.

Corsi, S.R., Graczyk, D.J., Geis, S.W., Booth, N.L., Richards, K.D., 2010. A fresh Look at road salt: aquatic toxicity and water-quality impacts on local, regional, and national scales. Environ. Sci. Technol. 44, 7376–7382.

Cowgill, U.M., Milazzo, D.P., 1990. The sensitivity of two cladocerans to water quality variables: salinity and hardness. Arch. für Hydrobiol. 120, 185–196.

Cox, D.R., 1972. Regression models and life-tables. J. R. Stat. Soc. 34, 187–220.

Crawley, M.J., 2013. The R Book. John Wiley & Sons, Inc, Hoboken, NJ, USA.

Dalinsky, S.A., Lolya, L.M., Maguder, J.L., Pierce, J.L.B., Kelting, D.L., Laxson, C.L., Patrick, D.A., 2014. Comparing the effects of aquatic stressors on model temperate freshwater aquatic communities. Water, Air, Soil Pollut. 225, 1–15.

Dananay, K.L., Krynak, K.L., Krynak, T.J., Benard, M.F., 2015. Legacy of road salt: apparent positive larval effects counteracted by negative post-metamorphic effects in wood frogs. Environ. Toxicol. Chem. 34, 2417–2424.

Dudgeon, D., Arthington, A.H., Gessner, M.O., Kawabata, Z., Knowler, D.J., Lévêque, C., Naiman, R.J., Prieur-Richard, A., Soto, D., Stiassny, M.L.J., Sullivan, C.A., 2006. Freshwater biodiversity: importance, threats, status and conservation challenges. Biol. Rev. Camb. Philosophical Soc. 81, 163–182.

Ebert, D., 2005. Ecology, epidemiology, and evolution of parasitism in *Daphnia*. National Library of Medicine (US), National Center for Biotechnology Information, Bethesda (MD).

Evans, M., Frick, C., 2001. The Effects of Road Salts on Aquatic Ecosystems. Environment Canada, Water Science and Technology Directorate. Report no. 02–308.

Fox, J., Carvalho, M.S., 2012. The RcmdrPlugin.survival package: extending the R Commander interface to survival analysis. J. Stat. Softw. 49, 1–32.

Gardner, K.M., Royer, T.V., 2010. Effect of road salt application on seasonal chloride concentrations and toxicity in South-Central Indiana streams. J. Environ. Qual. 39, 1036–1042.

Ghazy, M.M.E., Habashy, M.M., Kossa, F.I., Mohammady, E.Y., 2009. Effects of salinity on survival, growth and reproduction of the water flea, *Daphnia magna*. Nat. Sci. 7, 28–42.

Gonçalves, A.M.M., Castro, B.B., Pardal, M.A., Gonçalves, F., 2007. Salinity effects on survival and life history of two freshwater cladocerans (*Daphnia magna* and *Daphnia longispina*). Ann. de Limnologie - Int. J. Limnol. 43, 13–20.

Harris, K.D.M., Bartlett, N.J., Lloyd, V.K., 2012. *Daphnia* as an emerging epigenetic model organism. Genet. Res. Int. 2012, 1–8.

Harris, F.E., Tucker, E.M., 1947. Minerals Yearbook 1945: Salt. United States Government Printing Office, Washington, pp. 1513–1527.

Hintz, W.D., Mattes, B.M., Schuler, M.S., Jones, D.K., Stoler, A.B., Lind, L., Relyea R.A, In press. Salinization triggers a trophic cascade in experimental freshwater communities with varying food-chain length, Ecol. Appl.

Hoy, M., 1998. Myths, models and mitigation of resistance to pesticides. Philosophical Trans. R. Soc. B 353, 1787–1795.

Hua, J., Morehouse, N.I., Relyea, R.A., 2013. Pesticide tolerance in amphibians: induced tolerance in susceptible populations, constitutive tolerance in tolerant populations. Evol. Appl. 6, 1028–1040.

Hua, J., Jones, D.K., Mattes, B.M., Cothran, R.D., Relyea, R.A., Hoverman, J.T., 2015. The contribution of phenotypic plasticity to the evolution of insecticide tolerance in amphibian populations. Evol. Appl. 8, 586–596.

Ituarte, R.B., López Mañanes, A.A., Spivak, E.D., Anger, K., 2008. Activity of Na^+,K^+-ATPase in a 'freshwater shrimp', *Palaemonetes argentinus* (Caridea, Palaemonidae): ontogenetic and salinity-induced changes. Aquat. Biol. 3, 283–290.

Judd, K.E., Adams, H.E., Bosch, N.S., Kostrzewski, J.M., Scott, C.E., Schultz, B.M., Wang, D.H., Kling, G.W., 2005. A case history: effects of mixing regime on nutrient dynamics and community structure in Third Sister Lake, Michigan during late winter and early spring 2003. Lake Reserv. Manag. 21, 316–329.

Kaushal, S.S., Groffman, P.M., Likens, G.E., Belt, K.T., Stack, W.P., Kelly, V.R., Band, L.E., Fisher, G.T., 2005. Increased salinization of fresh water in the northeastern United States. Proc. Natl. Acad. Sci. U. S. A. 102, 13517–13520.

Kefford, B.J., Buchwalter, D., Cañedo-Argùelles, M., Davis, J., Duncan, R.P., Hoffmann, A., Thompson, R., 2016. Salinized rivers: degraded systems or new habitats for salt-tolerant faunas? Biol. Lett. 12, 1–7.

Kelting, D.L., Laxson, C.L., 2010. Review of effects and costs of road de-icing with

Please cite this article in press as: Coldsnow, K.D., et al., Rapid evolution of tolerance to road salt in zooplankton, Environmental Pollution (2016), http://dx.doi.org/10.1016/j.envpol.2016.12.024

recommendations for winter road management in the Adirondack Park. Adirondack Watershed Institute, Paul Smith's College, Paul Smiths, NY, USA. Report no. AWI2010–01.

Kilham, S.S., Kreeger, D.A., Lynn, S.G., Goulden, C.E., Herrera, L., 1998. COMBO: a defined freshwater culture medium for algae and zooplankton. Hydrobiologia 377, 147–159.

Langen, T.A., Twiss, M., Young, T., Janoyan, K., Stager, J.C., Osso, J., Prutzman, H., Green, B., 2006. Environmental Impacts of Winter Road Management at the Cascade Lakes and Chapel Pond. Clarkson Center for the Environment.

Latta, L.C., Weider, L.J., Colbourne, J.K., Pfrender, M.E., 2012. The evolution of salinity tolerance in *Daphnia*: a functional genomics approach. Ecol. Lett. 15, 794–802.

Liao, Y., Faulks, L.K., Östman, Ö., 2015. Stress tolerance and population stability of rock pool *Daphnia* in relation to local conditions and population isolation. Hydrobiologia 742, 267–278.

Loureiro, C., Castro, B.B., Claro, M.T., Alves, A., Pedrosa, M.A., Gonçalves, F., 2012. Genetic variability in the tolerance of natural populations of *Simocephalus vetulus* (Müller, 1776) to lethal levels of sodium chloride. Ann. de Limnologie - Int. J. Limnol. 48, 95–103.

Loureiro, C., Cuco, A.P., Claro, M.T., Santos, J.I., Pedrosa, M.A., Gonçalves, F., Castro, B.B., 2015. Progressive acclimation alters interaction between salinity and temperature in experimental *Daphnia* populations. Chemosphere 139, 126–132.

McGill, B.J., Dornelas, M., Gotelli, N.J., Magurran, A.E., 2015. Fifteen forms of biodiversity trend in the Anthropocene. Trends Ecol. Evol. 30, 104–113.

Novotny, E.V., Murphy, D., Stefan, H.G., 2008. Increase of urban lake salinity by road deicing salt. Sci. Total Environ. 406, 131–144.

Novotny, E.V., Stefan, H.G., 2010. Projections of chloride concentrations in urban lakes receiving road de-icing salt. Water, Air, Soil Pollut. 211, 261–271.

Palumbi, S.R., 2001. Humans as the world's greatest evolutionary force. Science 293, 1786–1790.

R Development Core Team, 2015. R: a Language and Environment for Statistical Computing. R Foundation for Statistical Computing, Vienna, Austria.

Sarma, S.S.S., Nandini, S., Morales-Ventura, J., Delgado-Martínez, I., González-Valverde, L., 2006. Effects of NaCl salinity on the population dynamics of freshwater zooplankton (rotifers and cladocerans). Aquat. Ecol. 40, 349–360.

Schallenberg, M., Hall, C.J., Burns, C.W., 2003. Consequences of climate-induced salinity increases on zooplankton abundance and diversity in coastal lakes. Mar. Ecol. Prog. Ser. 251, 181–189.

Seda, J., Petrusek, A., 2011. *Daphnia* as a model organism in limnology and aquatic biology: introductory remarks. J. Limnol. 70, 337–344.

Sibert, R.J., Koretsky, C.M., Wyman, D.A., 2015. Cultural meromixis: effects of road salt on the chemical stratification of an urban kettle lake. Chem. Geol. 395, 126–137.

Sih, A., Ferrari, M.C.O., Harris, D.J., 2010. Evolution and behavioural responses to human induced rapid environmental change. Evol. Appl. 4, 367–387.

Stoks, R., Geerts, A.N., De Meester, L., 2014. Evolutionary and plastic responses of freshwater invertebrates to climate change: realized patterns and future potential. Evol. Appl. 7, 42–55.

Teschner, M., 1995. Effects of salinity on the life history and fitness of *Daphnia magna*: variability within and between populations. Hydrobiologia 307, 33–41.

Thunqvist, E., 2004. Regional increase of mean chloride concentration in water due to the application of deicing salt. Sci. Total Environ. 325, 29–37.

Vandegehuchte, M.B., Lemière, F., Janssen, C.R., 2009. Quantitative DNA-methylation in *Daphnia magna* and effects of multigeneration Zn exposure. Comp. Biochem. Physiology, Part C 150, 343–348.

Van Meter, R.J., Swan, C.M., 2014. Road salts as environmental constraints in urban pond food webs. Plos One 9, 1–12.

Van Meter, R.J., Swan, C.M., Leips, J., Snodgrass, J.W., 2011. Road salt stress induces novel food web structure and interactions. Wetlands 31, 843–851.

Walsh, J.R., Carpenter, S.R., Vander Zanden, M.J., 2016. Invasive species triggers a massive loss of ecosystem services through a trophic cascade. Proc. Natl. Acad. Sci. U. S. A. 113, 4081–4085.

Weider, L.J., Hebert, P.D.N., 1987. Ecological and physiological differentiation among low-Arctic clones of Daphnia pulex. Ecology 68, 188–198.

Please cite this article in press as: Coldsnow, K.D., et al., Rapid evolution of tolerance to road salt in zooplankton, Environmental Pollution (2016), http://dx.doi.org/10.1016/j.envpol.2016.12.024