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Temperature affects acute mayfly responses to elevated salinity: implications for toxicity of road de-icing salts

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Salinity in freshwater ecosystems has increased significantly at numerous locations throughout the world, and this increase often reflects the use or production of salts from road de-icing, mining/oil and gas drilling activities, or agricultural production. When related to de-icing salts, highest salinity often occurs in winter when water temperature is often low relative to mean annual temperature at a site. Our study examined acute (96 h) responses to elevated salinity (NaCl) concentrations at five to seven temperature treatments (5–25°C) for four mayfly species (Baetidae: *Neocloeon triangulifer*, *Procloeon fragile*; Heptageniidae: *Maccaffertium modestum*; Leptophlebiidae: *Leptophlebia cupida*) that are widely distributed across eastern North America. Based on acute LC50s at 20°C, *P. fragile* was most sensitive (LC50 = 767 mg l^{-1}, 1447 µS cm^{-1}), followed by *N. triangulifer* (2755 mg l^{-1}, 5104 µS cm^{-1}), *M. modestum* (2760 mg l^{-1}, 5118 µS cm^{-1}) and *L. cupida* (4588 mg l^{-1}, 8485 µS cm^{-1}). Acute LC50s decreased as temperature increased for all four species ($n = 5–7$, $R^2 = 0.65–0.88$, $p = 0.052–0.002$). Thus, acute salt toxicity is strongly temperature dependent for the mayfly species we tested, which suggests that brief periods of elevated salinity during cold seasons or in colder locations may be ecologically less toxic than predicted by standard 20 or 25°C laboratory bioassays.

This article is part of the theme issue 'Salt in freshwaters: causes, ecological consequences and future prospects'.

1. Introduction

Salinity in fresh waters is naturally variable, primarily reflecting differences in concentrations of dissolved inorganic cations calcium, magnesium and sodium, and anions carbonate, sulfate and chloride [1,2]. The differences in ion concentrations among fresh waters primarily reflect the weathering of soil and bedrock underlying a watershed, atmospheric deposition, and the evaporation–precipitation cycle. Sodium is generally less common than calcium and magnesium, and chloride is generally less common than carbonate or sulfate in natural waters. Elevated Na and Cl concentrations have been observed in effluents from wastewater treatment plants that reflect use of water softeners, table salt in the human diet, and disinfection before discharge [3,4], in wastewaters from some industrial, coal mining, and oil and gas production activities [5–7], in runoff and groundwater associated with various agricultural practices [8], and in road runoff following applications of de-icing products such as rock salt and anti-icing brines [9–13]. Recent analyses of multi-year data have found that sodium and chloride concentrations in surface waters have been increasing over the last two to five decades, at multiple locations (e.g. [14–18], and more recently [19–21]). This increase in sodium and chloride is part of a worldwide trend for increasing salinity along with pH and alkalinity [22–27], which was recently labelled the Freshwater Salinization Syndrome [28,29].

With these increases in ambient salinity, there has been renewed interest in the toxicity of salt in our aquatic ecosystems. Building on early toxicity tests [30], researchers have again begun examining salt toxicity by focusing on specific ions such as sodium, magnesium, chloride, carbonate, and sulfate (e.g. delivered as NaCl, $MgCl_2$, Na_2SO_4) for a variety of aquatic algae [31], insects and other macroinvertebrates [32–41], mussels [42–46], zooplankton [47,48], amphibians [49,50], and fish [32,51–53]. Other researchers have approached salt toxicity as a function of total salinity (as salt concentration or electrical conductivity), rather than as an ion-specific issue (e.g. [54–57]). The challenge in both cases is general applicability of findings as it is well known that ion composition is important to overall salt toxicity [30,58–62]. Additional references can be found in review articles [63–69]. Salt toxicity has been found to vary greatly among aquatic species, with recent data showing that some mayflies and juvenile mussels are among the most sensitive species tested [34,52,56,70]. The combination of salt sensitivity and elevated ambient salinity suggests that, at least at times, salt may reach levels that may have a negative affect on aquatic organisms [10,31,71–76].

There are two challenges in understanding the potential salinity toxicity under field conditions in colder climates where de-icing salts can increase salinity dramatically during snow/ice storms. First, most salt toxicity studies have been conducted at constant 17–25°C, which are the recommended test temperatures for standard acute and chronic toxicity tests for many species, [77,78]. However, in colder climates where de-icing salts are frequently used, water temperature can vary naturally across seasons, with winter lows of 0–10°C versus summer highs of 20–30°C (e.g. figure 1). In addition, there can be significant differences among years (e.g. an interannual range of 10°C or more; figure 1). It has been found that temperature can affect toxicity of many chemicals [79–86]. For most toxins and species, the relationship between temperature and toxicity is positive—increases in temperature result in increased toxicity (i.e. a lower LC50). Mayer & Ellersieck [80] summarized the relationship as a 10°C increase in temperature results in a two- to fourfold decrease in the LC50. Second, streams and rivers that exhibit a long-term increase in Na and Cl concentrations also often exhibit a strong seasonal cycle that includes frequent, short-term snow and ice events when salinity can be many times greater than at base flow (figure 2) [87,88]. This is a sharp contrast to streams with little urbanization (e.g. figure 1 and [88]). Unfortunately, the magnitude and duration of these events are often not well quantified in the historic data because these data are primarily periodic grab samples while snow and ice events are better described with a continuously recording sensor. While the recent studies of salt toxicity have addressed the range of conditions needed to set regulatory limits [47,89], they have not included seasonal temperature variation as part of their analyses.

This paper describes a series of experiments that examine lethal responses of mayfly (Ephemeroptera) larvae in acute (96 h) exposures to elevated salinity (i.e. NaCl added to moderately hard source water) at five to seven different temperatures. The results show how understanding the experimental relationship between temperature and salt toxicity can provide important insight into the toxicity of ambient salt concentrations, especially those originating from winter de-icing programmes.

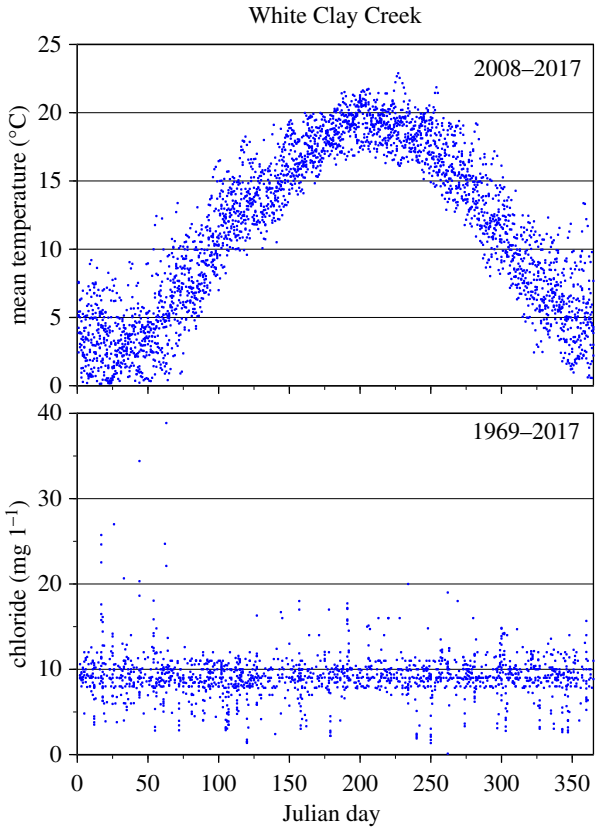

Figure 1. Long-term seasonal variation (date plotted as Julian day) in water temperature (mean daily from various continuous recorders, 2008–2017) and chloride concentration (from grab samples, 1969–2017) for White Clay Creek at the Stroud Water Research Center, 39°51′38.41″ N, 75°47′01.96″ W. Values greater than 20 mg Cl l^{-1} are presumably evidence of local de-icing efforts during winter. (Online version in colour.)

2. Methods

(a) Source water

Water for all tests was collected from White Clay Creek at the Stroud Water Research Center (39°51′38.41″ N, 75°47′01.96″ W), Chester Co. Pennsylvania, a limestone-influenced, headwater stream that drains a 7 km^2, rural (less than 0.5% developed) watershed and is moderately hard (mean 97 mg CO_3^{2-} l^{-1}) with relatively low salinity (143.8 mg l^{-1}, table 1). Seasonal patterns in temperature and chloride (as an indicator of de-icing salts affecting background salinity) from long-term data for White Clay Creek are shown in figure 1. The temperature treatments (see below) are representative of the range of conditions these test mayfly populations have experienced for generations in White Clay Creek. In contrast, the relatively low salinity in the historic data suggests that these wild mayfly populations from White Clay Creek have not been exposed to sodium or chloride concentrations similar to those in our experimental treatments in the last 50 years. Background concentrations on four dates when water was collected for laboratory bioassays averaged 6.6 mg l^{-1} for sodium and 12.3 mg l^{-1} for chloride (table 1).

To provide context for laboratory results, field data were collected every 5 min (30 Mar 2017–1 May 2018) with a Decagon CTD-10 (electrical conductivity or specific conductance corrected to 25°C, temperature, depth) sensor in Rocky Run, First State National Historic Park, New Castle County, Delaware, USA (39°49′00.45″ N and 75°33′02.84″ W), which drains a highly urbanized (60% developed), 2 km^2 watershed about 20 km from the Stroud Water Research Center. Salinity for Rocky Run was estimated from the conductivity : salinity relationship used in our experiments with White Clay Creek water, where salinity =

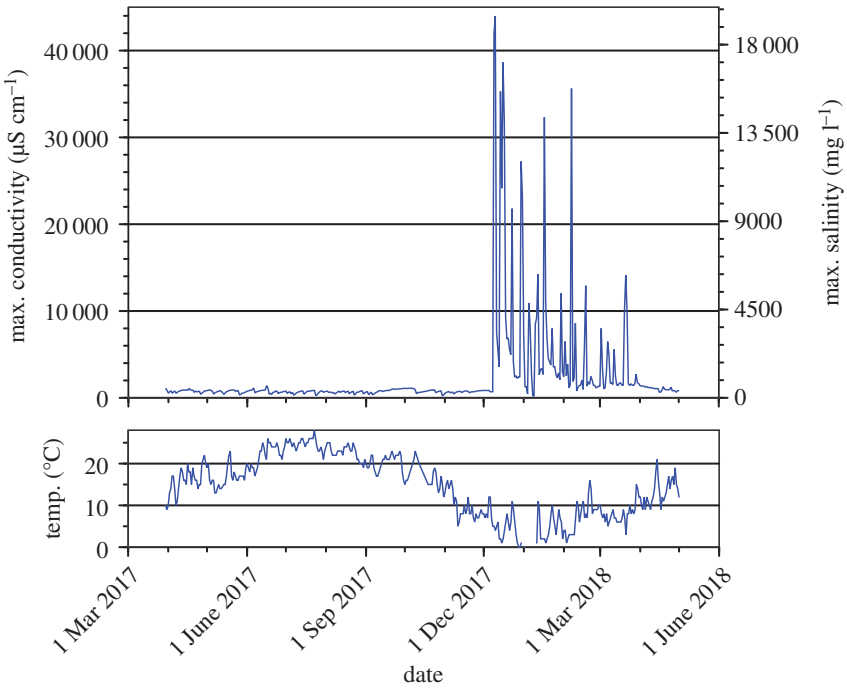

Figure 2. Seasonal variation (from 30 March 2017 to 1 May 2018) in maximum daily salinity as conductivity (μS cm^{-1}) and mg l^{-1}, and maximum daily temperature (°C) for Rocky Run, First State National Historic Park, New Castle County, Delaware. (Online version in colour.)

Table 1. Chemical characteristics of moderately hard water from White Clay Creek, PA used in acute toxicity tests in 2016. TDS, total dissolved salts.

date:	4 Apr 2016	16 Apr 2016	12 May 2016	5 June 2016	
time:	06.30	07.00	08.30	11.45	mean
pH	7.7	8.2	7.7	7.8	7.9
conductivity (μS cm^{-1})	238	232	239	241	238
alkalinity (mg l^{-1})	71.3	66.0	67.8	69.2	68.6
hardness (mg CO_3^{2-} l^{-1})	96	93	97	100	97
Ca^{2+} (mg l^{-1})	23.9	23.9	24.7	26.6	24.8
Mg^{2+} (mg l^{-1})	8.8	8.0	8.6	8.2	8.4
K^+ (mg l^{-1})	2.0	1.6	1.7	1.9	1.8
Na^+ (mg l^{-1})	7.1	6.1	6.5	6.5	6.6
Cl^- (mg l^{-1})	13.0	12.1	12.0	12.2	12.3
SO_4^{2-} (mg l^{-1})	17.0	17.6	17.5	17.2	17.3
TDS (mg l^{-1})	139	160	152	152	152.5

(electrical conductivity − 23.099)/1.844, where salinity is in mg l^{-1}, and electrical conductivity is in μS cm^{-1} at 25°C.

(b) Study species

Mayflies were chosen for this study because Ephemeroptera are ecologically significant in most streams and rivers, and they are considered pollution sensitive and have historically played important roles in water quality monitoring programmes [90–92]. We quantified acute responses to short-term (96 h) chloride exposures for four mayfly species that are common in White Clay Creek (where test species were collected) and widely distributed in eastern North America. *Neocloeon triangulifer* (McDunnough 1931) was until recently called *Centroptilum triangulifer* [93] and before that *Cloeon triangulifer* [94]. It is a parthenogenetic (clonal) mayfly species [95,96] that is most abundant during summer, when it has a relatively rapid larval development (egg hatch to adult in 25–30 days at 20°C). We worked with Stroud Water Research Center (SWRC) Clone WCC-2®, which occurs in low larval numbers during the winter, with minimal growth below 9.6°C. This specific clone has also been recently used in a number of experiments examining the toxic effects of cadmium, mercury, selenium and zinc [97–102], and chloride and sulfate salts [34,36,38–40]. *Procloeon fragile* (McDunnough 1923) was for many years called *Centroptilum fragile* [94]. It is a sexual mayfly species that exhibits a life history similar to that of *N. triangulifer* except that it has a winter egg diapause. *Maccaffertium modestum* (Banks, 1910) was long known as *Stenonema modestum*, but was recently reclassified [103]. It is a sexual

Table 2. Acute (96 h) salinity toxicity (LC50; geometric mean with 95% CI) expressed as mg l^{-1} and μS cm^{-1} for four mayfly species exposed to elevated NaCl in five to seven constant temperature (°C) treatments.

	LC50 per temperature treatment (geometric mean with 95% CI, mg l^{-1} and μS cm^{-1})						
species	5°C	7.5°C	10°C	12.5°C	15°C	20°C	25°C
salinity (mg l^{-1})							
N. triangulifer	9655 (8751 – 10 653)	10 462 (9655 – 11 337)	6719 (5541 – 8148)	5101 (4843 – 5373)	2573 (389 – 17 017)	2755 (2018 – 3762)	364 (171 – 773)
P. fragile			6874 (4595 – 10 284)	4115 (3111 – 5443)	3239 (1662 – 6312)	767 (187 – 3146)	766 (748 – 784)
L. cupida	10 086 (9037 – 11 257)	10 152 (9299 – 11 083)	10 439 (8551 – 12 743)	11 908 (11 650 – 12 172)	8368 (6955 – 10 067)	4588 (3108 – 6774)	3216 (1821 – 5678)
M. modestum		7236 (6005 – 8719)	5429 (2442 – 12 067)	7792 (5483 – 11 075)	7808 (3742 – 16 293)	2760 (1436 – 5303)	1656 (680 – 4035)
electrical cond. (μS cm^{-1})							
N. triangulifer	17 829 (16 161 – 19 668)	19 317 (17 829 – 20 929)	12 414 (10 241 – 15 049)	9430 (8953 – 9932)	4788 (739 – 31 042)	5104 (3745 – 6958)	698 (335 – 1453)
P. fragile			12 700 (8495 – 18 986)	7612 (5759 – 10 060)	5997 (3086 – 11 656)	1447 (367 – 5707)	1435 (1402 – 1469)
L. cupida	18 623 (16 688 – 20 782)	18 744 (17 172 – 20 461)	19 273 (15 792 – 23 522)	21 983 (21 506 – 22 470)	15 454 (12 849 – 18 587)	8485 (5754 – 12 514)	5955 (3381 – 10 487)
M. modestum		13 366 (11 096 – 16 102)	10 036 (4525 – 22 260)	14 393 (10 133 – 20 444)	14 425 (6923 – 30 053)	5118 (2676 – 9788)	3081 (1274 – 7451)

species that exhibits a bivoltine or multivoltine life history at White Clay Creek, with larval development of about 80 days at 20°C. *Leptophlebia cupida* (Say 1923) is a sexual mayfly species that exhibits a univoltine life history that begins with eggs hatching in mid-June and adult emergence the following April.

(i) Experimental treatments

We quantified acute responses of four mayfly species in short-term (96 h) exposures to elevated NaCl (A.C.S. reagent; J.T. Baker 33624-05). NaCl was chosen for these experiments because it represents 90–98% of the rock salt (halite) used for de-icing roads [104]. We conducted 100 temperature-specific acute tests (each test had one replicate of 20 individuals for each salinity treatment), with 20 newly hatched 1st instar larvae for *N. triangulifer*, *P. fragile*, *L. cupida* or *M. modestum* placed in a 30 ml beaker containing 15 ml of treatment solution. Newly hatched larvae were chosen because younger/smaller individuals are often more sensitive than older/larger individuals of the same species [55,105–107].

Each toxicity test had six treatments: a control (0 mg NaCl l^{-1} added to White Clay water) and five elevated salinity treatments that represented a 50% dilution series (i.e. 412, 824, 1649, 3297, 6594 mg NaCl l^{-1} added to White Clay water for *N. triangulifer* and *P. fragile*, and 824, 1649, 3297, 6594, 13 188 mg NaCl l^{-1} added to White Clay water for *L. cupida* and *M. modestum*). These were static (no renewal) experiments, conducted at five to seven constant ($\pm$0.1°C) temperature treatments (i.e. 10, 12.5, 15, 20, 25°C for all species, with the addition of a 7.5°C treatment for *M. modestum* and 5 and 7.5°C treatments for *N. triangulifer* and *L. cupida*). A diatom slurry (i.e. *ca* 20 µl of biofilm scrapings suspended in White Clay water) was provided as food in each test vessel for *N. triangulifer* and *P. fragile*. Food was not provided in *L. cupida* and *M. modestum* tests. Four replicate tests were run for each temperature treatment. Photoperiod (light : dark) was 16 : 8 h during the tests. Temperature in the rearing system was recorded every 5 min, and calibrated with a certified thermometer. Salinity across treatments was monitored with a calibrated conductivity meter.

Mayfly response was reported as survivorship after 96 h, and summarized as the lethal salinity associated with 50% mortality (or LC50) estimated using the nonparametric trimmed-Spearman–Karber method [76,108] of test population at a specific temperature. The relationship between temperature and LC50 for each species was assessed with a simple linear regression of geometric means. Linear regressions were used because it was a simple assessment of the relationship between five to seven temperature treatments and salinity toxicity, and because regression slope was consistent across the temperature range, which facilitates interpretation and incorporation into regulatory standards.

3. Results and discussion

(a) Interspecific differences in mayfly sensitivity to elevated salinity

Control survival was greater than 90% in most of the acute toxicity tests reported for *P. fragile*, *N. triangulifer* and *L. cupida*, and those tests with slightly higher control mortality were still included in these analyses as their dose-responses were similar to other tests. Survival was less than 90% for many tests with *M. modestum* (suggesting this species should be fed during 96 h tests), but the response to temperature was similar to the other mayfly species and is included in this report. However, because of low control survival, the LC50s for *M. modestum* should be used with caution until further verification.

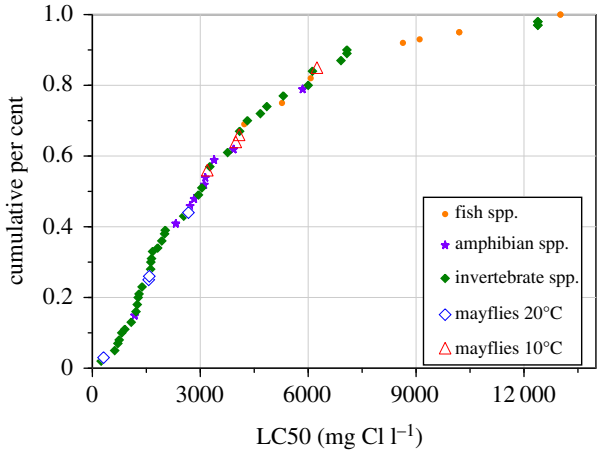

Figure 3. Relative sensitivities for the mayflies *N. triangulifer*, *P. fragile*, *M. modestum*, and *L. cupida* based on LC50s (expressed as mg Cl l^{-1}, electronic supplementary material, table S1) for 10 and 20°C (table 2) plotted with fish, amphibian and invertebrate data included in fig. 3 from [52].

Mean LC50s estimated by the nonparametric trimmed-Spearman–Karber method are expressed as salinity (mg l^{-1}) and electrical conductance (µS cm^{-1}) in table 2. We prefer to compare toxicities among mayflies at 20°C because it appears in some mayfly species we have examined that 25°C is physiologically stressful, independent of the chemical stressor being evaluated. Based on acute LC50s at 20°C, *P. fragile* was most sensitive (LC50 = 767 mg l^{-1}, 1447 µS cm^{-1}), followed by *N. triangulifer* (2755 mg l^{-1}, 5104 µS cm^{-1}) and *M. modestum* (2760 mg l^{-1}, 5118 µS cm^{-1}), and finally *L. cupida* (4588 mg l^{-1}, 8485 µS cm^{-1}) (table 2). NaCl toxicity for *N. triangulifer* has been examined in earlier studies [34,36,39], but all at 25°C. The acute LC50 for *N. triangulifer* at 25°C in our study was markedly lower than LC50 we observed at 20°C as well as the LC50s estimated by Soucek & Dickinson [34], Struewing *et al*. [36], and Soucek *et al*. [39]. Our LC50s for 25°C for all four mayfly species were not out of line with LC50s from colder temperature treatments, and the temperature versus LC50 regressions fitted the data relatively well (see below), so we do not currently have an explanation for differences observed among the studies of *N. triangulifer*. When salinity was expressed as electrical conductivity (µS cm^{-1} or mS cm^{-1}), the LC50s we observed for the baetids *P. fragile* and *N. triangulifer* (1447–5104 µS cm^{-1}) were similar to those observed for the baetid *Centroptilum* sp. (1.8–5.6 mS cm^{-1} in [59], and 10 mS cm^{-1} in [57]), and less than was observed for the baetid *Cloeon* sp. (21 mS cm^{-1} in [57]).

Mayflies are generally considered pollution sensitive, and are important contributors to metrics used to assess pollution impacts [90–92]. When we compared the LC50s for our mayflies at 20°C (expressed as mg Cl l^{-1}, electronic supplementary material, table S1) relative to the acute LC50s included in fig. 3 of [52], *P. fragile* was among the most sensitive species, *M. modestum* and *N. triangulifer* was moderately sensitive (*ca* 25th percentile) and *L. cupida* was average (45th percentile) (figure 3). Relative sensitivity for mayflies in our study would be even higher if we used LC50s from the common test temperature of 25°C (table 2)—*P. fragile*, *N. triangulifer* and *M. modestum* would be among the most sensitive, and *L. cupida* would be moderately sensitive.

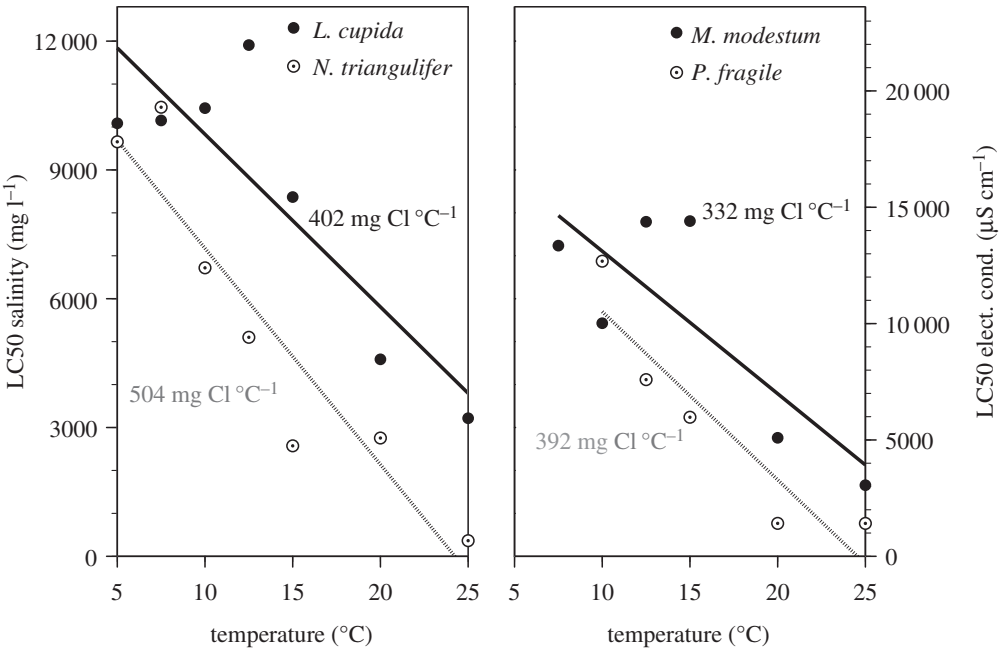

Figure 4. Simple linear regressions describing the relationship between temperature and acute salinity LC50s for the mayflies *L. cupida*, *N. triangulifer*, *M. modestum* and *P. fragile*.

Table 3. Simple linear regression results for figure 4 describing the relationship between temperature and acute salinity toxicity expressed as LC50 (geometric means, mg l^{-1}).

species	*N*	Pr > F	R^2	intercept	slope
N. triangulifer	7	0.002	0.881	12 211.2	−503.7
P. fragile	5	0.026	0.850	9616.0	−391.8
L. cupida	7	0.011	0.756	13 851.5	−402.2
M. modestum	6	0.052	0.653	10 425.3	−331.9

Conversely, the mayflies in our study would not be considered sensitive if we used LC50s from the 10°C test temperature (figure 3) We saw similar relative sensitivity when our study mayflies were compared to the mayflies and other macroinvertebrates presented in Wang *et al*. [70], and in the broader global survey of salinity sensitivity for mayflies and other macroinvertebrates in Kefford *et al*. [56].

The four mayfly species included in our study were not selected based on presumed or known pollution sensitivity. In fact, it is possible there are mayfly species that are as or more sensitive to elevated salinity than the species we examined. Our data, in combination with other published observations such as Wang *et al*. [70] and Kefford *et al*. [56], support the general belief that mayflies as a group are relatively sensitive to elevated salinity, although the physiological mechanisms surrounding mayfly sensitivity to salt remain to be determined [109]. Cormier *et al*. [110] defined a maximum acute benchmark of 680µS cm^{-1} for salinity derived from field observations of occurrence for 142 stream macroinvertebrate genera and annual chemistry data. While this hypothetical benchmark might not be directly comparable with our laboratory studies (Cormier *et al*. [110] eliminated several sites with high chloride), 680 µS cm^{-1} (=369 mg l^{-1} in our study) would appear to be over-protective for all species based on the LC50s at 5–10°C, and protective for *N. triangulifer*, *M. modestum* and *L. cupida*, and possibly *P. fragile*, based on the LC50s at 20°C. The benchmark might not be protective for *P. fragile* and *N. triangulifer* based on the LC50s at 25°C.

It is important to note that salinity toxicity is known to vary among salts and dilution waters tested [30,39,41,47,58–61], so our toxicities for elevated salinity that is predominately NaCl must be used with caution when referring to other de-icing and anti-icing salts such as $MgCl_2$, $CaCl_2$, KCl or calcium magnesium acetate ($CaMg_2(CH_3COO)_6$), to the 'chemical cocktail' that characterizes the Freshwater Salinization Syndrome [29], or to ambient waters with natural salinities that are markedly lower or higher than in White Clay Creek (e.g. a soft-water stream or a limestone stream).

(b) Changes in salinity toxicity in response to temperature

The relationship between salinity toxicity and temperature is important because, in regions where de-icing salts are frequently used, water temperature can change significantly with seasons (figure 1). Moreover, salinity from de-icing efforts peaks following snow and ice events when stream temperature is often nearest its lowest level, and well below the 20 or 25°C temperature used in standard bioassays (figure 2). We observed a significant or nearly significant ($n = 5–7$, $R^2 = 0.65–0.88$, $p = 0.052–0.002$) decrease in toxicity (i.e. acute LC50s increased) as temperature decreased for all four species (figure 4 and table 3). Based on the

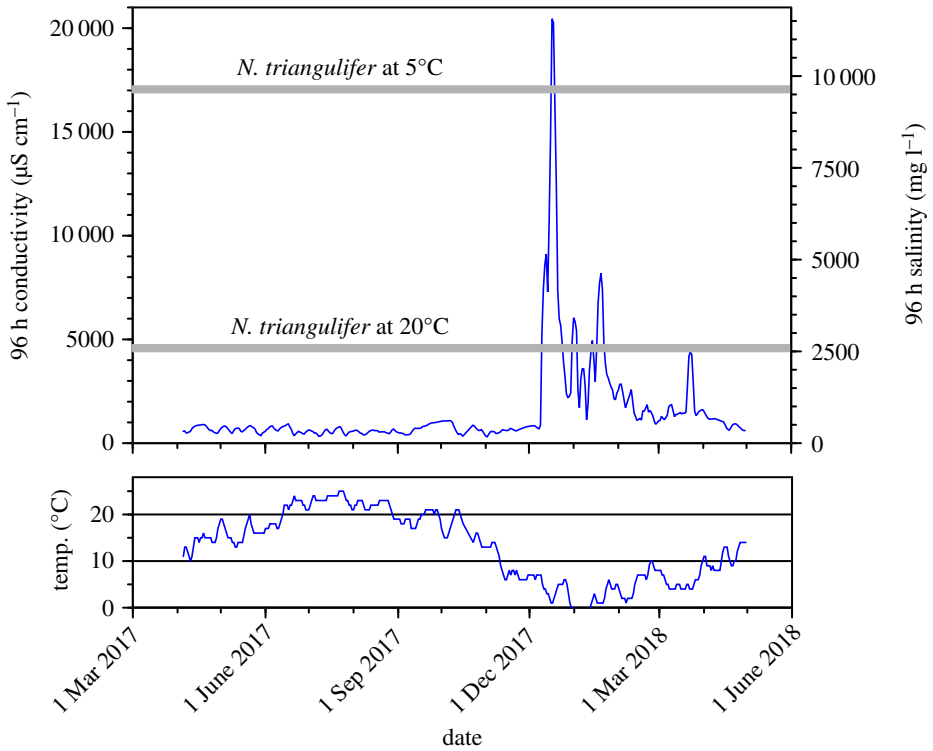

Figure 5. Plot showing mean 96 h LC50 for *N. triangulifer* at 5 and 20°C with seasonal variation (from 30 March 2017 to 1 May 2018) in 96 h running salinity as conductivity (µS cm^{-1}) and mg l^{-1}, and 96 h mean running daily temperature (°C) for Rocky Run, First State National Historic Park, New Castle County, Delaware, USA. (Online version in colour.)

regression slopes, the rate of change was similar for *P. fragile*, *M. modestum* and *L. cupida*. Their LC50s decreased between 332 and 402 mg l^{-1} for each 1°C increase in temperature. The response for *N. triangulifer* was somewhat stronger and its LC50s decreased 504 mg l^{-1} for each 1°C increase in temperature. The LC50s for *L. cupida* and *M. modestum* increased 1.7–1.9-fold for each 10°C decrease in temperature while the LC50s for *N. triangulifer* and *P. fragile* increased 3.5–3.6-fold for each 10°C decrease. This difference between *L. cupida* and *M. modestum* versus *P. fragile* reflects the estimated LC50s relative to the rate of change per °C. The species with lowest LC50 (*P. fragile*) increased proportionally more per °C than species with higher LC50s (*L. cupida* and *M. modestum*). The higher proportional change for *N. triangulifer* reflects a moderately low LC50 with a higher rate of change per °C. Our results almost match the summarization by Mayer & Ellersieck [80] that a 10°C increase in temperature results in a two- to fourfold decrease in the LC50. There are a few studies where reduced salt toxicity has been observed at lower versus higher temperature [73,111,112], but the relationship between acute salt toxicity and temperature has not been quantified in a manner that can be applied to water quality criteria (table 3).

To illustrate how the interaction between temperature and salinity toxicity provides important perspective to understanding aquatic ecosystems receiving de-icing salts, we took the raw data used to generate figure 2 and calculated 96 h (i.e. the duration of the acute toxicity tests) running mean values for conductivity, salinity (from conductivity) and temperature (figure 5). We then added the LC50 for *N. triangulifer* at 5°C and 20°C to figure 5. Based on the LC50 at 20°C, there were 21 dates that were preceded by 96 h with an average salinity that exceeded the LC50 at 20°C. In contrast, based on the LC50 at 5°C (which is more representative of thermal conditions at the time of elevated salinity), there were only two dates that were preceded by 96 h with an average salinity that exceeded the LC50 at 5°C. Thus, accounting for lower salt toxicity for an acute exposure at low temperature can change one's perspective on the apparent toxicity of ambient conditions during winter. However, it is important to note that, even after accounting for lower toxicity at 5–10°C, salinity in Rocky Run still appears to have been acutely toxic (i.e. ≥50% mortality in a 96 h period) for all four mayflies we examined. This suggests that elevated salinity (e.g. averaging 9500–11 500 mg l^{-1} for 96 h) during winter when snow and ice management programmes are being implemented may contribute to the overall impairment of the macroinvertebrate assemblage in Rocky Run, and probably other small urban streams that receive salt-laden runoff from roads, car parks and pavements. However, this is not to suggest that elevated (but not peak) salt concentrations during winter are not contributing to overall impairment. These non-peak exposures are more frequent (i.e. exposure time can be longer), and based on results for polar marine invertebrates, exposure time must be considered in the evaluation and interpretation of potential impact of toxicants at cold temperature [113,114].

(c) Regulatory and management implications of the relationship between salinity toxicity and temperature

As salinization of freshwater ecosystems resulting from de-icing and anti-icing salts continues, the regulatory and management challenge for winter road maintenance programmes will be to balance the need to protect public safety and reduce the economic costs of winter storms with the need to protect environmental health and infrastructure integrity related to excess salt, and to address potential drinking water/public health related to increased dietary intake of

sodium [4,21,115,116]. Our study found low temperature can reduce the frequency or intensity of salt-related toxic events expected based on winter de-icing activities that increase NaCl concentrations. But it also shows that NaCl concentrations during winter can be so high that NaCl-related toxic events may still occur even after accounting for low temperature. Our results can also be applied to other activities that result in acute exposure to elevated salt. For example, spills or discharges of high salinity wastewaters such as oil and gas brine [6,7] may have more of an impact in summer, when both the stored wastewater and receiving stream water are seasonally warmer, than in winter, when both are cool. The negative relationship between temperature and salt toxicity we observed highlights the potential importance in considering water temperature when interpreting current environmental conditions or events, or setting regulatory standards for salinity or NaCl.

Data accessibility. Data are available as electronic supplementary material.

Authors' contributions. Overall project and experimental design: J.K.J. and D.H.F.; experimental set-up and data collection: D.H.F.; data analyses and interpretation: J.K.J. and D.H.F.; drafted manuscript: J.K.J.; edited manuscript: J.K.J. and D.H.F.

Competing interests. We declare we have no competing interests.

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