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ACUTE TOXICITY OF SODIUM CHLORIDE AND POTASSIUM CHLORIDE TO A UNIONID MUSSEL (*LAMPSILIS SILIQUOIDEA*) IN WATER EXPOSURES

Ning Wang^{†,*}, Christopher D. Ivey[†], Rebecca A. Dorman[†], Christopher G. Ingersoll[†], Jeffery Steevens[†], Edward J. Hammer[‡], Candice R. Bauer[‡], David R. Mount[§]

[†]U.S. Geological Survey, Columbia Environmental Research Center, Columbia, Missouri

[‡]U. S. Environmental Protection Agency, Region 5, Water Quality Branch, Chicago, Illinois

[§]U. S. Environmental Protection Agency, Environmental Effects Research Laboratory, Duluth, Minnesota

Abstract

Freshwater mussels (order Unionoida) are one of the most imperiled groups of animals in the world. However, many ambient water quality criteria and other environmental guideline values do not include data for freshwater mussels, in part because mussel toxicity test methods are comparatively new and data may not have been available when criteria and guidelines were derived. The objectives of the present study were to evaluate the acute toxicity of sodium chloride (NaCl) and potassium chloride (KCl) to larvae (glochidia) and/or juveniles of a unionid mussel (fatmucket, *Lampsilis siliquoidea*), and to determine the potential influences of water hardness (50, 100, 200, and 300 mg/L as CaCO₃) and other major ions (Ca, K, SO₄, or HCO₃) on the acute toxicity of NaCl to the mussels. From the KCl test, the 50% effect concentration (EC50) for fatmucket glochidia was 30 mg K/L, similar to or slightly lower than EC50s for juvenile fatmucket (37–46 mg K/L) tested previously in our laboratory. From the NaCl tests, the EC50s for glochidia increased from 441 to 1,597 mg Cl/L and the EC50s for juvenile mussels increased from 911 to 3,092 mg Cl/L with increasing water hardness from 50 to 300 mg/L. Increasing K from 0.4 to 1.9 mg/L, SO₄ from 13 to 40 mg/L, or HCO₃ from 44 to 200 mg/L in the 50 mg/L hardness water did not substantially change the NaCl EC50s for juvenile mussels, whereas increasing Ca from 9.9 to 42 mg/L increased the EC50s by a factor of 2. The overall results indicate that glochidia were equally or more sensitive to NaCl and KCl compared to juvenile mussels, and the increased water hardness ameliorated the acute toxicity of NaCl to glochidia and juveniles. These responses rank fatmucket among the most acutely sensitive freshwater organisms to NaCl and KCl.

*To whom correspondence may be addressed (nwang@usgs.gov). **Corresponding author:** Ning Wang, Columbia Environmental Research Center, U.S. Geological Survey, 4200 New Haven Road, Columbia, MO 65201, USA, Phone: 573/441-2946, fax: 573/876-1896, nwang@usgs.gov.

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Keywords

Glochidia; Juvenile mussels; Major ion toxicity; Species sensitivity; Water quality criteria

INTRODUCTION

Sodium (Na), chloride (Cl) and potassium (K) occur naturally in aquatic environments. Natural concentrations can be elevated by human activities, such as mineral mining, road deicing, urban and agricultural runoff, oil and gas extraction, water treatment, and industrial wastewater discharge. The US Environmental Protection Agency (USEPA) published national ambient water quality criteria (WQC) for Cl (based on NaCl toxicity data) in 1988, with a single-value acute criterion of 860 mg/L and a chronic criterion of 230 mg/L (USEPA 1988). Later studies have indicated that hardness (more specifically calcium) influences the toxicity of NaCl to several aquatic organisms (Mount et al. 1997, 2016; Elphick et al. 2011; Soucek et al. 2011; Gillis 2011). In 2009, the State of Iowa (USA) published hardness- and sulfate-dependent water quality standards (WQS) for Cl (based on NaCl toxicity data; Iowa Department of Natural Resources 2009). National WQC or State water quality standards (WQS) for K have not been developed in the US.

Freshwater mussels (order Unionoida) are one of the most imperiled groups of animals in the world, and environmental contamination has been linked as a contributing factor to the decline of mussel populations (Lydeard et al. 2004; Strayer et al. 2004; Haag 2012; Lopes-Lima et al. 2017). Studies have demonstrated that mussels are among the most sensitive freshwater species in the USA to a variety of contaminants, including ammonia, metals, and major cations and major anions (Bringolf et al. 2007; March et al. 2007; Wang et al. 2007ab; 2010, 2016, 2017; Cope et al. 2008; Miao et al. 2010; Gillis 2011). However, freshwater mussels are generally under-represented in toxicity databases used to derive the national WQC and State WQS for the protection of aquatic life (Augspurger et al. 2007; March et al. 2007; Wang et al. 2010, 2017), in part because of the relatively recent standardization of toxicity test methods for mussels. The inclusion of NaCl toxicity data from recent freshwater mussel studies in the derivation of the Canadian water quality guideline for Cl resulted in a lower guideline value (Canadian Council of Ministers of the Environment 2011). Similarly, a recent study indicates that including NaCl toxicity data from recent mussel studies in a revision to the 1988 WQC for Cl would likely lower the acute criterion (Wang et al. 2017). The previous study also indicates that 3 of 4 mussel species were among the 4 most sensitive species to KCl for all freshwater organisms tested (Wang et al. 2017).

Limited information is available about the influence of water hardness on the toxicity of NaCl to freshwater mussels. Gillis (2011) found that the toxicity of NaCl to larvae (glochidia) of a unionid mussel (fatmucket, *Lampsilis siliquoidea*) decreased by a factor of 2 with increased hardness from 47 to 99 mg/L as CaCO₃, but toxicity did not decrease further as hardness was increased to 322 mg/L. With the growing evidence that NaCl toxicity is hardness dependent, further studies of the relationship between hardness and NaCl toxicity across species could be useful in creating future WQC that are hardness-dependent. Furthermore, while hardness is used as a surrogate for the major ions affecting the toxicity

of NaCl, it is also important to assess the relative roles of individual major ions in producing the hardness effect on NaCl toxicity (Mount et al. 2016).

The objectives of the present study were to evaluate (1) the acute toxicity of NaCl and fatmucket glochidia and juveniles; (2) the potential influence of water hardness and other co-occurring major ions on the acute toxicity of NaCl to the early life stages of fatmucket; and (3) to evaluate the comparative toxicity of KCl to fatmucket glochidia. A companion study was conducted to evaluate the chronic toxicity of NaCl and KCl to juvenile fatmucket (Wang et al. in review).

For toxicity testing, major ions like Na and Cl can only be added in salt form, such that the effect of one ion cannot be tested in isolation of the other. NaCl has been the primary salt used to develop existing regulatory guidance from USEPA and some US states, but this guidance has focused specifically on Cl concentration, rather than the combination of ions. While some earlier work suggested that Cl might be primarily responsible for the toxicity of NaCl (e.g., Mount et al. 1997), more recent work (Mount et al. 2016; Erickson et al. 2018) has suggested that Cl is not the sole cause of toxicity (at least to some organisms) and that toxicity of NaCl is more an aggregate effect of both Na and Cl ions. In the case of KCl, there is evidence to suggest that K is more, if not solely, responsible for the toxicity of KCl to the cladoceran (*Ceriodaphnia dubia*; Mount et al. 2016) and likely additional organisms (D.R. Mount, unpublished data). To facilitate comparison to other published data and regulatory guidelines, the results from the present study are expressed as Cl (for NaCl) and K (for KCl) concentrations, but this should not be taken as an assertion that these are the only appropriate exposure metrics, especially for NaCl.

MATERIALS AND METHODS

Acute toxicity tests with glochidia and juvenile mussels were conducted in accordance with the ASTM standard methods (ASTM 2017). Test conditions are summarized in Supplemental Data Table S1.

Test organisms

The fatmucket, like most freshwater mussels, has a complex reproductive cycle involving a parasitic stage on fish. The fatmucket is a long-term brooder, which spawns in late summer and brood glochidia over winter for release of mature glochidia the following spring. Sperm released by a male enters a female through the incurrent siphon, and fertilized eggs develop to larvae called glochidia that mature in specialized chambers (marsupia) of the mussel gills. Glochidia are released into the water and must attach to the gills or fins of a suitable host fish. Largemouth bass (*Micropterus salmoides*) is one species of host fish for fatmucket. After one to several weeks of the parasitic stage, glochidia transform to juvenile mussels, detach from the fish, and drop to the sediment to begin the free-living juvenile stage.

Gravid female fatmucket brooding mature glochidia were collected in February 2013 from the Silver Fork of Perche Creek (Boone County, MO, USA). The adult mussels were transported to the Columbia Environmental Research Center (CERC), Columbia, MO, USA, and held in a 600-L flow-through fiberglass tank with CERC well water (hardness 300 mg/L

as CaCO₃, alkalinity 250 mg/L as CaCO₃, pH 8.0) at a flow rate of 2 L/min. Water was aerated and maintained at 10 to 12°C to prevent the mussels from releasing glochidia. Plastic containers (35×24×23 cm) with a 10 cm layer of creek gravel (0.2 to 1.5 cm diameter) were submerged in the tank. Up to 5 adult mussels were placed in each container. The adult mussels were fed ad libitum with a commercial non-viable microalgal *Nannochloropsis* concentrate and Shellfish Diet (a unique mix of 4 microalgae, *Tisochrysis lutea*, *Pavlova* sp., *Tetraselmis* sp., *Thalassiosira weissflogii*; Reed Mariculture, Campbell, CA, USA). Other conditions for holding and feeding female mussels were as described in a previous publication (Wang et al. 2007a).

To collect glochidia for toxicity testing, roughly equal numbers of glochidia were gently flushed from the marsupial gills of each of 6 female mussels into a 300-mL crystallizing dish using a 1-mm needle and 35-mL syringe filled with the mussel culture water. The viability of glochidia isolated from each female mussel was examined under a dissecting microscope following ASTM International standard methods (ASTM 2017). Three subsamples of roughly 100 glochidia were impartially selected and transferred to each of 3 wells of a 24-well polystyrene tissue-culture plate filled with 2 mL of the culture water. One drop of a saturated NaCl solution (~360 g/L) was added into the well, and the response of glochidia (valve closure) within 1 minute was recorded. Open and closed glochidia were calculated as described in the standard methods (ASTM 2017):

$$\text{Viability (\%)} = 100 \times (\text{number of closed glochidia after adding NaCl solution} - \text{number of closed glochidia before adding NaCl solution}) \div (\text{total number of open and closed glochidia after adding NaCl solution}).$$

The viability of glochidia from the 6 female mussels ranged from 95 to 99%, and met the test acceptability requirement of >80%, preferably >90% (ASTM 2017). The remaining glochidia isolated from the 6 mussels were pooled in 1-L beakers and mixed for the glochidia toxicity test. Glochidia were acclimated to test water and temperature by 30% water replacement with test water (details on the preparation of the test water follow) over 2 to 4 h before the start of a toxicity test.

For the propagation of juvenile mussels used in toxicity tests, the gravid female mussels were transported to Missouri State University, Springfield, MO, and held in moderately-hard reconstituted water (80-100 mg hardness as CaCO₃/L, pH ~7.8; USEPA 2002). Roughly equal numbers of glochidia were removed from each of 3 to 6 adult mussels by gently flushing the mussel marsupial gills. The viability of glochidia isolated from each adult mussel was determined as described previously and exceeded 90% in all samples. The glochidia isolated from the adult mussels were pooled and placed on hatchery-reared largemouth bass for metamorphosis. The bass were infested with glochidia for 15 min in water containing about 4,000 glochidia/L. The host fish were maintained at 22°C in a recirculating system designed to collect transformed juvenile mussels. The transformed juvenile mussels left the host fish approximately 2 weeks following fish infestation. Juveniles were collected during the peak drop-off days (typically 2 to 4 days).

Newly transformed mussels (<5 d old) were shipped overnight to the CERC. The mussels were held in a recirculating mussel culture system (Barnhart 2006) with test water and

temperature for at least 48 h before the start of a toxicity test. Juvenile mussels were fed an algal mixture twice daily in the early morning and later afternoon, which maintained an algal concentration of 2 to 5 nl cell volume/mL for at least 10 h per day. The algal mixture was prepared daily by adding 4 mL of the *Nannochloropsis* concentrate and 6 mL of Shellfish Diet into 1.8 L of water. Ambient laboratory light of about 500 lux with 16:8 h light:dark photoperiod was used during the acclimation and toxicity testing.

Acute toxicity tests with glochidia

The 24-h KCl or NaCl toxicity test with fatmucket glochidia was conducted under static conditions in 300-mL glass beakers, each containing about 100 mL of test solution. At the beginning of each exposure, about 500 glochidia were impartially transferred from the pooled sample of glochidia into each of 3 replicate beakers. Test beakers were held in temperature-controlled water baths at $20 \pm 1^\circ\text{C}$. Because glochidia added into test chambers generally were not 100% viable, initial viability of glochidia was estimated by determining the viability of glochidia in the control replicates at the beginning of a test and used to adjust the control viability at the end of the 24-h test (i.e., percent of the initial mean control viability; Wang et al. 2007a). For the viability determination, a subsample of roughly 100 glochidia were impartially taken from a replicate chamber using a 2-mm wide-bore pipette and placed into one well of the polystyrene tissue-culture plate. One drop of the saturated NaCl solution was added into the well, and the response of glochidia (valve closure) within 1 minute was recorded and the viability rate was calculated as described previously. For the NaCl test, a saturated KCl solution was also used to determine glochidia viability in one treatment (100 mg/L hardness water) to evaluate any potential influence of the use of NaCl or KCl solution on viability determination.

The 24-h KCl toxicity test was conducted in May 2013 in a diluted well water, which was prepared by diluting CERC well water of hardness 300 mg/L as CaCO_3 with deionized water to a hardness of 100 mg/L as CaCO_3 . American Chemical Society-grade KCl (99.7% purity; Fisher Scientific, Fair Lawn, New Jersey) was used for test solution preparation. Five K concentrations (50% serial dilution; nominal 6.25, 12.5, 25, 50, and 100 mg K/L) plus a control were tested.

The 24-h NaCl toxicity test was conducted in March 2013 in moderately-hard reconstituted water (hardness ranging from 80 to 100 mg/L as CaCO_3 ; USEPA 2002). The use of the reconstituted water was to compare the response of glochidia from the present study to those tested in the same water in a previous study (Gillis 2011), in which glochidia of several mussel species were found to be highly sensitive to NaCl (Gillis 2011). Concurrently, an additional four NaCl tests were conducted with glochidia at 4 different levels of hardness in CERC well water and 3 diluted well waters to evaluate influence of hardness on NaCl toxicity to glochidia. The moderately-hard reconstituted water was prepared by adding reagent-grade salts ($\text{CaSO}_4 \cdot 2\text{H}_2\text{O}$, MgSO_4 , KCl, and NaHCO_3 ; EM Science, Gibbstown, NJ, USA) into deionized water (USEPA 2002). Well waters of different hardnesses were prepared by diluting the CERC well water with deionized water to a hardness of 50, 100, and 200 mg/L as CaCO_3 (i.e., 50, 100, and 200 hard water). The waters were prepared and maintained in a 35-L polypropylene container at 20°C . American Chemical Society-grade

NaCl (99.0% purity, Sigma-Aldrich, St. Louis, MO) was used to prepare NaCl concentrations. Seven NaCl concentrations (50% serial dilution; nominal concentrations 160, 320, 630, 1250, 2500, 5000, and 10000 mg NaCl/L) plus a control were tested in the acute tests. Each NaCl solution was prepared by spiking NaCl in 1 L of water and was held in the dark at 4°C for 48 h before initiation of an exposure (Gillis 2011).

Acute toxicity tests with juvenile mussels

Eight acute 96-h NaCl tests were conducted concurrently in September 2013 with newly transformed fatmucket (~10 d old). Four of the 8 NaCl tests were conducted in the 50, 100, 200, and 300 hard waters to evaluate influence of hardness on NaCl toxicity to juvenile mussels. The 4 test waters were prepared as described previously for the glochidia tests. The other 4 NaCl tests were conducted in the 50 hard water with an addition of one major ion salt to determine whether a specific major ion influenced NaCl toxicity in the 4 hardness waters. Specifically, the test waters were prepared by adding KCl, CaCl₂, Na₂SO₄, or NaHCO₃ into the 50 hard water to match the level of K, Ca, SO₄, or HCO₃ in the 200 hard water. In each case, the counter ion for the manipulated ion was either Na or Cl, an addition of which was negligible compared to the much higher concentrations from the added NaCl in the exposure solutions. Thus, these test waters should have effectively isolated the influence of individual ions.

Five test concentrations of NaCl with a 50% serial dilution plus a control were used for each test. A solution of the highest exposure concentration was prepared in a 5-L glass jar. Fifty percent manual dilutions were performed with half of the high solution to create the lower exposure concentrations. The control water and solutions were held in the dark at 4°C and warmed to test temperature in a water bath for use at the beginning of a test and for water renewal at 48 h. At the beginning of each static-renewal test, five juvenile mussels were impartially selected and transferred into each of four 50-mL replicate glass beakers containing 30 mL of water. Test beakers were held in a plastic container (30 × 18 × 10 cm) with a cover to reduce evaporation. The containers were held in a water bath at 23±1°C. Water temperature was monitored daily. Test organisms were not fed during 96-h exposures. About 75% of the water in each replicate beaker was removed and renewed after 48 h. At the end of exposures, mussels in each beaker were examined under a dissecting microscope. Effect was defined as either mortality (empty shell or gaped shell containing decomposed tissue) or immobility (no foot or shell movement within a 5-minute observation period).

Water quality and chemical analyses

Water quality (dissolved oxygen, pH, conductivity, hardness, and alkalinity) were determined using standard methods (Eaton et al. 2005) on composite water samples collected from the replicates in the control, medium, and/or high exposure concentrations at the beginning and the end of acute toxicity tests. Composite water samples for analyses of major cations (Ca, K, Mg, Na) and major anions (Cl and SO₄) were collected from the control water at the start of tests. Water samples for major cation analyses were preserved within a few hours by adding a sufficient volume of concentrated house-distilled nitric acid (16 M) to each sample to result in a final acid concentration of 1 to 2 % (v/v). Quantitative analyses of major cations were performed using inductively coupled plasma mass

spectrometry (ICP-MS; ELAN DRC-e, PerkinElmer, Shelton, CT, USA). The ICP-MS methods were similar to USEPA method 6020B (USEPA 2014). Water samples for major anion analyses were stored at 4°C for up to 28 days before analysis and were analyzed by ion chromatography (ICS-1100, Dionex Corporation, Sunnyvale, CA) using a method similar to USEPA method 9056A (USEPA 2007).

Water samples for Cl measurements in NaCl toxicity tests or for K measurements in KCl toxicity tests were collected from each test concentration at the beginning of the acute exposures. Chloride in the test solutions was analyzed by chloride ion selective electrode using a Hach HQ440d benchtop dual input, multi-parameter meter (Hach, Loveland, CO, USA). Salinity and conductivity were also measured at the beginning and the end of acute toxicity tests to monitor the exposure concentrations. Potassium in the test solutions was analyzed using inductively coupled plasma mass spectrometry as described previously.

Analyses of tested chemicals were performed by CERC chemistry laboratory, following internal standard operating procedures and quality assurance/quality control protocols developed based on the USEPA documents (USEPA 2007, 2014). Established laboratory quality assurance/quality control procedures and sample types (i.e., second source calibration verification, laboratory spikes, duplicates, reference/laboratory control materials) were used to verify instrument performance, accuracy, and precision throughout the analyses. These established procedures were in place to ensure method performance and instrument suitability. Results underwent data quality review by the chemistry laboratory before use in the present study.

Data analysis

Measured exposure concentrations were used for the calculations of median effect concentrations (EC50). An EC50 was determined based on glochidia viability or juvenile mussel mortality plus immobility (ASTM 2017) using the Toxicity Relationship Analysis Program (version 1.30a; Erickson 2015). The exposure concentrations were log-transformed and the response of each replicate was used for the calculation. A Gaussian (normal) distribution model was used for data analyses. When the data did not meet the requirements of the Gaussian distribution model (at least 2 partial responses), either a Spearman-Kärber or trimmed Spearman-Kärber method was used to determine the EC50s following the flowchart recommended by the USEPA (2002) using TOXSTAT® software (version 3.5, Western EcoSystems Technology).

RESULTS AND DISCUSSION

KCl toxicity to glochidia

Mean measured water quality characteristics in the acute 24-h KCl toxicity test (Table 1) were similar to nominal values of the CERC 100 hard water (Wang et al. 2016). Mean concentration of dissolved oxygen was 8.2 mg/L. As expected, the conductivity increased with increasing exposure concentrations of KCl (Supplemental Data, Table S2). The measured concentrations of K in the 6 treatments of acute exposures were similar to nominal concentrations (the differences typically within 20%; Table S2). Mean viability of the

glochidia in the controls was 93% at the beginning of the acute test and was 94% at the end of the test (Table S2). The adjusted control viability at the end of the test was 100% and met the test acceptability criterion (TAC) of 90% control viability in acute 24-h exposures with glochidia (ASTM 2017).

Low viability (<50%) was observed at the two high concentrations at the end of the test (Table S2), and acute EC50 was 30 mg K/L (Table 1). The acute EC50 for fatmucket glochidia was similar to or slightly lower than an acute EC50 for newly transformed (a few days old) juvenile fatmucket in a previous 96-h KCl exposure (46 mg K/L; Wang et al. 2017) and an EC50 for 2-month-old juvenile fatmucket in the first 4 d of a chronic 28-d KCl exposure in the companion study (37 mg K/L; Wang et al. in review). All these present and previous tests were conducted in the CERC diluted well water. The results indicate that the glochidia were equally or more sensitive to KCl than juvenile mussels.

Although the potential influence of hardness on KCl toxicity was not tested in the current study, 2 unpublished studies evaluated the influence of hardness on acute 96-h KCl toxicity to juvenile fatmucket in diluted CERC well waters (N. Wang) or in reconstituted waters prepared by adding reagent-grade salts into deionized water (Richard Lockwood, Ramboll Environ, Brentwood, TN, USA, personal communication), representing a hardness range of a stream potentially contaminated by manufacturing plant effluent with elevated K. The results of these 2 studies indicated that the acute KCl toxicity decreased by a factor of 2 with increasing hardness from 35 to 300 mg/L (N. Wang, unpublished data) or with increasing hardness from 100 to 400 mg/L (Richard Lockwood, unpublished data). Because the concentrations of all major ions increased with increasing hardness in these experiments, it is unclear which aspect of water composition may be responsible for the amelioration of KCl toxicity. For the cladoceran *C. dubia*, Mount et al. (2016) showed that increased water hardness ameliorated the acute toxicity of several major ion salts. For Na and Mg salts, most or all of this effect was attributable specifically to Ca, but the effect of hardness on KCl toxicity was primarily a result of covarying Na concentration; KCl toxicity decreased by a factor of 6 when Na concentrations were increased from 1.6 to 300 mg/L, all at the same hardness (Mount et al. 2016). Thus, more studies are needed to further evaluate the influence of background water chemistry on K toxicity to the early life-stages of mussels.

NaCl toxicity to glochidia

Measured water quality characteristics in acute 24-h NaCl toxicity tests with glochidia are summarized in Table 2 and Supplemental Data, Table S3. Mean measured hardness of 87 mg/L in the moderately hard reconstituted water was within the nominal range of 80 to 100 mg/L (USEPA 2002), and mean measured hardness in the 50 to 300 hard waters were similar to nominal hardness (Table 2). The measured concentrations of Cl in all tests were also similar to nominal concentrations (the differences typically within 10%; Supplemental Data, Table S3).

Mean control viability of glochidia in the 5 test waters ranged from 96 to 98% at the beginning of the tests and from 95 to 99% at the end of the tests (Supplemental Data, Table S3). The adjusted control viability at the end of the test for all 5 test waters was 96% and met the ASTM TAC of 90% control viability (ASTM 2017). The viability of glochidia

determined by the saturated NaCl and KCl solutions in each of 8 treatments with the 100 hard water test was similar (Supplemental Data, Table S3); the EC50 of 603 mg Cl/L based on viability determined with the saturated KCl solution was close to (within 10% difference) the EC50 of 544 mg Cl/L determined with the saturated NaCl solution (with overlapping of the 95% confidence limits; Table 2), indicating no substantial difference of using the NaCl or KCl solution to determine the viability of glochidia in the NaCl toxicity test.

The EC50s ranged from 441 to 1,597 mg Cl/L in the 5 test waters (Table 2) and increased with increasing hardness from 50 to 300 mg/L in the 4 diluted well waters (Figure 1). The EC50 of 728 mg Cl/L for fatmucket glochidia tested in the MHRW of the present study (Table 2) was between the EC50s of 168 and 1,430 mg Cl/L from 2 previous acute NaCl tests with fatmucket glochidia in a MHRW (Gillis 2011). However, the glochidia viability in the controls was 77.4% and 93.2%, respectively, at the end of each test in the previous study (Gillis 2011), and the author pointed out that the poor quality of glochidia (77.4% control survival) might have resulted in the lower EC50 of 168 mg Cl/L. Gillis (2011) also found that NaCl toxicity to fatmucket glochidia decreased with increasing hardness; this is consistent with the current study, though our study with diluted well water showed a much stronger correlation between water hardness and NaCl toxicity (Figure 1) than the Gillis study (Gillis 2011) which used reconstituted waters based on USEPA formulas (USEPA 2002). The EC50s increased linearly with increasing hardness from 61 to 299 mg/L in the present study (Figure 1), whereas, in the Gillis study, the EC50s increased with increasing water hardness from 47 to 172 mg/L, but did not decrease with further hardness increases from 172 to 322 mg/L (Supplemental Data, Figure S1). In addition, the EC50s obtained in different test waters in the present study were equal to or less than the EC50s from previous 24-h NaCl toxicity tests with fatmucket glochidia (Gillis 2011; Bringolf et al. 2007; Cope et al. 2008; Hazelton et al. 2012; Roy et al. 2015) that met the test acceptability criteria (e.g., 90% control survival; Supplemental Data, Figure S1). An exception was a low EC50 of 334 mg Cl/L at hardness of 181 mg/L reported by Bringolf et al. (2007). However, two additional NaCl toxicity tests were conducted later at the same laboratory under similar test conditions (Cope et al. 2008) and resulted in >4-fold higher EC50s (Supplemental Data, Figure S1).

When compared on a molar basis, the toxicity of KCl to glochidia ($EC_{50} = 0.76 \text{ mM/L}$) was more than 20-fold higher than the toxicity of NaCl to glochidia ($EC_{50} = 20.5 \text{ mM/L}$) tested in the same water (100 hard), which suggests that the toxicity of KCl to fatmucket was attributable primarily to K rather than Cl. This is consistent with the results in previous studies on acute toxicity of major ion salts to other aquatic organisms (Mount et al. 1997, 2016).

NaCl toxicity to juvenile mussels

Water quality characteristics in acute NaCl toxicity tests with the newly transformed juvenile mussels are summarized in Table 3 and Supplemental Data, Table S4. Mean measured hardness values in the different test waters were similar to the nominals, with the exception of the hardness in the 50 hard water with the addition of CaCl_2 which increased, as expected, to 130 mg/L (Table 3). In the 50 hard water prepared to match the K, Ca, SO_4 , or

HCO₃ concentrations of the 200 hard water, measured ion concentrations were close to the target values (differences <7%; Table 3). The measured concentrations of Cl in all treatments were similar to nominal concentrations (the differences typically within 10%; Supplemental Data, Table S4).

Mean control survival in all 8 tests was 100% (Supplemental Data, Table S4) and met the TAC of 90% control survival (ASTM 2017). The EC50s ranged from 911 to 3,092 mg Cl/L in the 4 hardness waters of 50 to 300 mg/L (Tests 1 to 4 in Table 3). As observed in tests with glochidia, the EC50s for the juvenile mussels increased significantly with increasing water hardness, and the slope of the regression line for juvenile mussels was similar to the slope for glochidia (Figure 1). In the 50 hard water with the elevated K, SO₄, or HCO₃ (Tests 5, 7, and 8 in Table 3), the EC50s ranged from 937 to 1,164 mg Cl/L, and all had confidence limits overlapping those for the unamended 50 hard water test (911 mg Cl/L; Test 1 in Table 3). In contrast, the EC50 from the 50 hard water with the elevated Ca (2,106 mg Cl/L; Test 6 in Table 3) increased by a factor of 2 in comparison to the EC50 from the 50 hard water (Test 1; with no overlapping of the 95% confidence limits), and was about equal to the EC50 from the 200 hard water (2,075 mg Cl/L; Test 3). The results indicate that the influence of hardness on the toxicity of NaCl to juvenile mussels was primarily an effect of Ca (rather than other ions that co-vary with hardness), which was consistent with results from previous studies with *C. dubia* (Mount et al. 2016; Erickson et al. 2018). However, the slope of the hardness (or Ca) relationship for juvenile fatmucket was somewhat steeper than for *C. dubia*, the latter showing only about 50% increase in acute EC50 over a similar range in Ca (Erickson et al. 2018). The results from the present mussel study also suggest that normalizing NaCl toxicity across waters may be more accurate if done on the basis of Ca rather than hardness (Mount et al. 2016; Erickson et al. 2018).

The results from the present study indicate that there was no influence of increasing SO₄ from 13 to 40 mg/L on the NaCl toxicity to the mussels in the 50 mg/L hardness water (Table 3) and confirms the finding from a previous NaCl toxicity study with *C. dubia* (Soucek et al. 2011) in which acute EC50s for NaCl did not substantially change over a low SO₄ concentration range of 25 to 200 mg/L in a 300 mg/L hardness water. However, a recent study on the toxicity of NaCl and Na₂SO₄ mixtures to juvenile fatmucket in the CERC 100 hard water showed that the acute toxicity of NaCl significantly increased with increasing concentrations of SO₄ from 350 to 1,800 mg/L (C.D. Ivey, unpublished data). The additive toxicity of the NaCl and Na₂SO₄ mixtures have been also observed in acute testing with *C. dubia* (Erickson et al. 2017) and other freshwater organisms (D.R. Mount, unpublished data; D.J. Soucek, Illinois Natural History Survey, Champaign, IL, USA, unpublished data). Therefore, environmental guidelines derived individually for Cl or SO₄ might be under-protective if there are substantial co-occurring concentrations of either ion.

The EC50 of 1,733 mg Cl/L for the newly transformed juvenile fatmucket tested in the 100 hard water in the present study (Table 3) was similar to the EC50s (1,897 to 2,246 mg Cl/L; n=5) for newly transformed fatmucket tested in the 100 hard water in a previous study (Wang et al. 2017), and was also close to the EC50 of 1,500 mg Cl/L for newly transformed fatmucket in moderately hard reconstituted water (hardness 90 mg/L) in another previous study (Roy et al. 2015). The EC50s for juvenile fatmucket from the present study across a

broad hardness range of 50 to 300 mg/L were consistently 2-fold greater than the EC50s for glochidia (Figure 1), indicating that the glochidia were more sensitive to NaCl than the juvenile mussels. Higher sensitivity of glochidia than juvenile mussels of the same species was also found in acute exposure to chlorine in a previous study with several unionid mussels, including fatmucket (Wang et al. 2007b). However, other studies comparing sensitivity of glochidia and juvenile mussels of the same species showed that glochidia were equally sensitive to copper and ammonia (Wang et al. 2007b), but less sensitive to cadmium, lead, and zinc than juveniles of the same species (Wang et al. 2010). Thus, there was no consistent pattern in the sensitivity of glochidia relative to the sensitivity of juvenile mussels across different toxicants (Wang et al. 2010).

Implications for water quality criteria and standards in the US

A previous study demonstrated that freshwater mussels are among the most sensitive species in a compiled acute KCl toxicity database for all tested freshwater organisms (Wang et al. 2017). With the additional fatmucket glochidia data from the present study and juvenile data from the companion study (Wang et al. in review), fatmucket would be the second most sensitive species among tested freshwater organisms to K, and the 4 most sensitive genera would all be mussels. USEPA WQC or State WQS for K have not been developed. However, to protect freshwater mussels, some States have considered developing a site-specific standard for K or requiring mussel testing as part of the permit process for effluents that contain elevated K (Suzanne Dunn, US Fish and Wildlife Services, Tulsa, OK; Tom Augspurger, US Fish and Wildlife Services, Raleigh, NC; personal communication).

In the present NaCl study, the EC50s for Cl at different hardnesses of 50 to 300 mg/L were up to 4-fold below the final acute value (FAV) used for deriving the 1988 USEPA acute WQC for Cl (i.e., 1/2 FAV; USEPA 1988), and were equal to or 2-fold below the hardness- and sulfate-dependent FAVs in the Iowa WQS for Cl (Iowa Department of Natural Resources 2009; Figure 1). From the juvenile tests, the EC50 for Cl at the low hardness of 50 mg/L was also below FAVs in the WQC and WQS for Cl (Figure 1). The results provide additional data to support the conclusion that inclusion of the mussel data in the toxicity database would likely lower the WQC and WQS for Cl (Wang et al. 2017).

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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REFERENCES

- ASTM International. 2017 Standard guide for conducting laboratory toxicity tests with freshwater mussels (E2455-06 (2013)) Annual Book of ASTM Standards, Volume 11.06 ASTM International, West Conshohocken, PA.
- Augsburger T, Dwyer FJ, Ingersoll CG, Kane CM. 2007 Advances and opportunities in assessing contaminant sensitivity of freshwater mussel (Unionidae) early life stages. *Environ Toxicol Chem* 26:2025–2028. [PubMed: 17867884]
- Barnhart MC. 2006 Bucket of mucklets: A compact recirculating system for rearing juvenile freshwater mussels. *Aquaculture* 254:227–233.
- Bringolf RB, Cope WG, Barnhart MC, Mosher S, Lazaro PR, Shea D. 2007 Acute and chronic toxicity of pesticide formulations (atrazine, chlorpyrifos and permethrin) to glochidia and juveniles of *Lampsilis siliquoidea*. *Environ Toxicol Chem* 26, 2086–2093. [PubMed: 17867872]
- Canadian Council of Ministers of the Environment. 2011 Canadian water quality guidelines for the protection of aquatic life: Chloride. Canadian Council of Ministers of the Environment, Winnipeg.
- Cope WG, Bringolf RB, Buchwalter DB, Newton TJ, Ingersoll CG, Wang N, Augspurger T, Dwyer FJ, Barnhart FC, Neves RJ, Hammer E. 2008 Differential exposure, duration, and sensitivity of unionoidean bivalve life stages to environmental contaminants. *J N Am Benthol Soc* 27, 451e462.
- Eaton AD, Clesceri LS, Rice EW, Greenberg AE. 2005 Standard Methods for the Examination of Water and Wastewater, 21st ed.: Washington, D.C., American Public Health Association, Water Environment Federation, American Water Works Association, 1,368 p.
- Elphick JR, Bergh KD, Bailey HC. 2011 Chronic toxicity of chloride to freshwater species: effects of hardness and implications for water quality guidelines. *Environ Toxicol Chem* 30:239–246. [PubMed: 20872898]
- Erickson RJ. 2015 Toxicity Relationship Analysis Program (TRAP), Ver 1.30a. EPA/600/C-11/002 U.S. Environmental Protection Agency, Washington, DC.
- Erickson RJ, Mount DR, Highland TL, Hockett JR, Hoff DJ, Jenson CT, Norberg-King TJ, Peterson KN. 2017 The acute toxicity of major ion salts to *Ceriodaphnia dubia*. II. Empirical relationships in binary salt mixtures. *Environ Toxicol Chem* 36:1525–1537. [PubMed: 27800634]
- Erickson RJ, Mount DR, Highland TL, Hockett JR, Hoff DJ, Jenson CT, Norberg-King TJ, Peterson KN. 2018 The acute toxicity of major ion salts to *Ceriodaphnia dubia*. III. Mathematical models for mixture toxicity. *Environ Toxicol Chem* 37:247–259. [PubMed: 28833416]
- Gillis PL. Assessing the toxicity of sodium chloride to the glochidia of freshwater mussels: implications for salinization of surface waters. 2011 *Environ Pollut* 159: 1702–1708. [PubMed: 21429642]
- Haag WR. 2012 North American freshwater mussels: natural history, ecology, and conservation. New York (NY): Cambridge University Press.
- Hazelton PD, Cope WG, Pandolfo TJ, Mosher S, Strynar MJ, Barnhart CM, Bringolf RB. 2012 Partial life-cycle and acute toxicity of perfluoroalkyl acids to freshwater mussels. *Environ Toxicol Chem* 31:1611–20. [PubMed: 22553110]
- Iowa Department of Natural Resources. 2009 Revising Criteria for Chloride, Sulfate and Total Dissolved Solids. [cited 2018 January 12]. Available from www.iowadnr.gov/portals/idnr/uploads/water/standards/ws_fact.pdf.
- Lopes-Lima M, Sousa R, Geist J, Aldridge DC, Araujo R, Bergengren J, Bernalaya Y, Bodis E, Burlakova L, Van Damme D, Douda K, Froufe E, Georgiev D, Gumpinger C, Karatayev A, Kebapci U, Killeen I, Lajtner J, Larsen BM, Lauceri R, Legakis A, Lois S, Lundberg S, Moorkens E, Motte G, Nagel KO, Ondina P, Outeiro A, Paunovic M, Prie V, von Proschwitz T, Riccardi N, Rudzite M, Rudzitis M, Scheder C, Seddon M, Sereflisan H, Simic V, Sokolov S, Stoeckl K, Taskinen J, Teixeira A, Thielen F, Trichkova T, Varandas S, Vicentini H, Zajac K, Zajac T, Zogaris S 2017 Conservation status of freshwater mussels in Europe: state of the art and future challenges. *Biol Rev* 92:572–607. [PubMed: 26727244]
- Lydeard C, Cowie RH, Ponder WF, Bogan AE, Bouchet P, Clark SA, Cummings KS, Frest TJ, Gargominy O, Herbert DG, Hershler R, Perez KE, Roth B, Seddon M, Strong EE, Thompson FG. 2004 The global decline of nonmarine mollusks. *BioScience* 54:321–330.

- March FA, Dwyer FJ, Augspurger T, Ingersoll CG, Wang N, Mebane CA. 2007 An evaluation of freshwater mussel toxicity data in the derivation of water quality guidance and standards for copper. *Environ Toxicol Chem* 26:2066–2074. [PubMed: 17867876]
- Miao J, Barnhart MC, Brunson EL, Hardesty DK, Ingersoll CG, Wang N. 2010 An evaluation of the influence of substrate on the response of juvenile freshwater mussels (*Fatmucket, Lampsilis siliquioidea*) in acute water exposure to ammonia. *Environ Toxicol Chem* 29:2112–2116. [PubMed: 20821670]
- Mount DR, Erickson RJ, Highland TL, Hockett JR, Hoff DJ, Jenson CT, Norberg-King TJ, Peterson KN, Wisniewski S. 2016 The acute toxicity of major ion salts to *Ceriodaphnia dubia*: I. Influence of background water chemistry. *Environ Toxicol Chem* 35:3039–3057. [PubMed: 27167636]
- Mount DR, Gulley DD, Hockett JR, Garrison TD, Evans JM. 1997 Statistical models to predict the toxicity of major ions to *Ceriodaphnia dubia*, *Daphnia magna*, and *Pimephales promelas* (fathead minnows). *Environ Toxicol Chem* 16:2009–2019.
- Roy JW, McInnis R, Bickerton G, Gillis PL. 2015 Assessing potential toxicity of chloride-affected groundwater discharging to an urban stream using juvenile freshwater mussels (*Lampsilis siliquioidea*). *Sci Total Environ* 532: 309–315. [PubMed: 26081733]
- Soucek DJ, Linton TK, Tarr CD, Dickinson A, Wickramanayake N, Delos CG, Cruz LA. 2011 Influence of water hardness and sulfate on the acute toxicity of chloride to sensitive freshwater invertebrates. *Environ Toxicol Chem* 30:930–938. [PubMed: 21191883]
- Strayer DL, Downing JA, Haag WR, King TL, Layzer JB, Newton TJ, Nichols SJ. 2004 Changing perspectives on pearly mussels, North America's most imperiled animals. *BioScience* 54:429–439.
- U.S. Environmental Protection Agency. 1988 Ambient water quality criteria for chloride. EPA-440-5-88-001 Office of Water, Washington, DC.
- U.S. Environmental Protection Agency. 2002 Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms, 5th ed EPA-821-R-02-012 Office of Water, Washington, DC.
- US Environmental Protection Agency. 2007 Method 9056A: Determination of inorganic anions by ion chromatography. Washington, DC.
- US Environmental Protection Agency. 2014 Method 6020B: Inductively coupled plasma-mass spectrometry. Washington, DC.
- Wang N, Augspurger T, Barnhart MC, Bidwell JR, Cope WG, Dwyer FJ, Geis S, Greer IE, Ingersoll CG, Kane CM, May TW, Neves RJ, Newton TJ, Roberts AD, Whites DW. 2007a Intra- and inter-laboratory variability in acute toxicity tests with glochidia and juveniles of freshwater mussels (Unionidae). *Environ Toxicol Chem* 26:2029–2035. [PubMed: 17867871]
- Wang N, Ingersoll CG, Hardesty DK, Ivey CD, Kunz JL, May TW, Dwyer FJ, Roberts AD, Augspurger T, Kane CM, Neves RJ, Barnhart MC. 2007b Acute toxicity of copper, ammonia, and chlorine to glochidia and juveniles of freshwater mussels (Unionidae). *Environ Toxicol Chem* 26:2036–2047. [PubMed: 17867873]
- Wang N, Ingersoll CG, Ivey CD, Hardesty DK, May TW, Augspurger T, Roberts AD, van Genderen E, Barnhart MC. 2010 Sensitivity of early life stages of freshwater mussels (Unionidae) to acute and chronic toxicity of lead, cadmium, and zinc in water. *Environ Toxicol Chem* 29:2053–2063. [PubMed: 20821663]
- Wang N, Dorman RA, Ingersoll CG, Hardesty DK, Brumbaugh WG, Hammer EJ, Bauer CR, Mount DR. 2016 Acute and chronic toxicity of sodium sulfate to four freshwater organisms in water-only exposures. *Environ Toxicol Chem* 35:115–127. [PubMed: 26139383]
- Wang N, Ivey CD, Ingersoll CG, Brumbaugh WG, Alvarez D, Hammer EJ, Bauer CR, Augspurger T, Raimondo S, Barnhart MC. 2017 Acute sensitivity of a broad range of freshwater mussels to chemicals with different modes of toxic action. *Environ Toxicol Chem* 36:786–796. [PubMed: 27699830]
- Wang N, Kunz JK, Dorman RA, Ingersoll CG, Steevens JA, Hammer EJ, Bauer CR. Chronic toxicity of sodium chloride and potassium chloride to a unionid mussel (*Lampsilis siliquioidea*) in water exposures. *Environ Toxicol Chem*. In Review (Manuscript ID ETCJ-Apr-18-00184).

Figure 1.

Relationships between acute EC50s and water hardness obtained in a 24-h NaCl toxicity test with glochidia of fatmucket (*Lampsilis siliquoidea*) and a 96-h NaCl toxicity test with juvenile fatmucket. Dashed line indicates final acute value (FAV) in the USEPA water quality criteria (USEPA 1988) or in the Iowa State water quality standards (Iowa Department of Natural Resources 2009). Note: The Iowa FAV line was created based on measured hardness and sulfate in the different test waters used in the present acute tests with the glochidia and juvenile mussels.

Table 1.

Mean measured water quality characteristics^a (standard deviation in parentheses) and EC50s (95% confidence limits in parenthesis) in acute 24-h KCl toxicity test with glochidia of fatmucket (*Lampsilis siliquoidea*) conducted in CERC 100 hard well water

Dissolved oxygen (mg/L; n=6)	pH (n=6)	Hardness	Alkalinity	Major cation and anion (mg/L; n=1)						EC50 (mg K/L)
		(mg/L as CaCO ₃ ; n=6)		Ca	K	Mg	Na	Cl	SO ₄	
8.2 (0.2)	8.3 (0.1)	109 (1.2)	91 (0.6)	27	1.2	9.4	11	12	18	30 (28-31)

^aWater quality was measured in the control, medium, and high exposure concentrations at the beginning and end of tests. Major ions measured in the control water at the beginning of the test.

EC50=50% effect concentration; CERC=Columbia Environmental Research Center.

Table 2.

Mean measured water quality characteristics^a (standard deviation in parentheses) of different base waters and EC50s (95% confidence limits in parenthesis) in acute 24-h NaCl toxicity tests with glochidia of fatmucket (*Lampsilis siliquoidea*) conducted in the moderately hard reconstituted water and in CERC 50, 100, 200, and 300 hard well water

Test	Dissolved oxygen (mg/L; n=6)	pH (n=6)	Hardness (mg/L as CaCO ₃ ; n=6)	Major cation and anion (mg/L; n=1)						EC50 (mg C/L)	
				Ca	K	Mg	Na	Cl	SO ₄		
1. MHRW	8.7 (0.3)	8.2 (0.1)	87 (4.9)	72 (3.9)	14	2.5	11	30	3.9	86	728 (675-786)
2. 50 hard	8.6 (0.3)	7.8 (0.3)	61 (7.3)	48 (9.0)	16	0.4	5.0	4.9	7.1	13	441 (379-446)
3. 100 hard	8.6 (0.2)	8.1 (0.2)	103 (7.3)	87 (6.4)	26	0.6	7.7	8.0	11	21	544 (509-580) ^b
4. 200 hard	8.6 (0.3)	8.4 (0.1)	204 (11)	169 (7.5)	52	1.2	15	9.2	14	43	1288 (1205-1377)
5. 300 hard	8.6 (0.2)	8.5 (0.1)	299 (14)	243 (4.7)	78	1.7	23	13	21	67	1597 (1498-1702)

^aWater quality was measured in the control, medium, and high exposure concentrations at the beginning and end of tests. Major ions measured in the control water at the beginning of the test.

^bEC50 was 603 (564-645) when glochidia viability was determined using a saturated KCl solution (rather than NaCl solution).

EC50=50% effect concentration; MHRW=moderately hard reconstituted water; CERC=Columbia Environmental Research Center.

Table 3.

Mean measured water quality characteristics^a (standard deviation in parentheses) and EC50s (95% confidence limits in parentheses) in acute 96-h NaCl toxicity tests with juvenile fatmucket (*Lampsilis siliquoides*) conducted in CERC 50, 100, 200, and 300 hard well water, and in the 50 hard well water with addition of KCl, CaCl₂, Na₂SO₄, or NaHCO₃ to match the level of K, Ca, Na, or HCO₃ in the 200 hard water

Test	Dissolved oxygen (mg/L; n=6)	pH (n=6)	Hardness (mg/L as CaCO ₃ ; n=6)	Major cation and anion (mg/L; n=1)								EC50 (mg Cl/L)
				Ca	K	Mg	Na	Cl	SO ₄	HCO ₃		
1. 50 hard	7.90 (0.26)	7.8 (0.2)	43 (0.6)	9.9	0.4	3.8	4.1	7.3	13	44	911 (812-1022)	
2. 100 hard	7.93 (0.35)	8.2 (0.2)	109 (0.6)	27	1.1	10	11	17	29	115	1733 (1388-2163)	
3. 200 hard	7.89 (0.32)	8.4 (0.1)	193 (1.0)	45	1.9	18	19	24	42	193	2075 [1461-2946] ^b	
4. 300 hard	7.95 (0.34)	8.4 (0.3)	265 (1.7)	63	2.9	26	28	27	49	269	3092 (2674-3576)	
5. 50 hard+K	7.87 (0.34)	7.8 (0.2)	41 (1.0)	9.8	1.9	3.9	4.3	6.0	8.6	46	1164 (945-1434)	
6. 50 hard+Ca	7.95 (0.28)	7.8 (0.2)	130 (1.5)	42	0.4	3.7	4.1	90	8.3	46	2106 [1516-2925] ^b	
7. 50 hard+SO ₄	7.87 (0.41)	7.8 (0.2)	41 (2.8)	9.4	0.4	3.7	19	5.3	40	44	1087 (992-1190)	
8. 50 hard+HCO ₃	7.95 (0.46)	8.4 (0.3)	40 (3.7)	9.2	0.4	3.7	63	4.7	8.5	200	937 (877-1000)	

^aWater quality was measured in the control, medium, and high exposure concentrations at the beginning and end of tests. Major ions measured in the control water at the beginning of the test.

^bAn EC50 could not be calculated due to no partial mortality (Table S4). The geometric mean of the bracketing concentrations with 0% and 100% mortality was calculated to obtain an estimated EC50. The 0% and 100% effect concentrations are provided in brackets as [0%-100% effect concentration].

EC50=50% effect concentration; CERC=Columbia Environmental Research Center.