BEFORE THE ILLINOIS POLLUTION CONTROL BOARD

IN THE MATTER OF:	)	
	)	
AMENDMENTS TO THE GENERAL USE	)	R18-32
WATER QUALITY STANDARDS	)	(Rulemaking – Water)
FOR CHLORIDE	)	

NOTICE OF FILING

To: Don Brown, Clerk
Illinois Pollution Control Board
James R. Thompson Center
100 West Randolph Street, Suite 11-500
Chicago, IL 60601

PLEASE TAKE NOTICE that I have today electronically filed with the Illinois Pollution Control Board, Openlands' testimony of Laura Barghusen concerning the Petition in R18-32, a copy of which is herewith served upon you.

Dated: December 28, 2018

Openlands

By: ____________________
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Testimony of Laura Barghusen, Openlands
(December 28, 2018)

I. Introduction.

My name is Laura Barghusen and I am an Aquatic Ecologist for Openlands. Founded in 1963, Openlands protects the natural and open spaces of Northeastern Illinois and the surrounding region to ensure cleaner air and water, protect natural habitats and wildlife, and help balance and enrich our lives. For decades, Openlands has supported improvements to the waterways of our region as natural assets, economic drivers and community treasures. Openlands and its supporters recreate on and along waterways that are affected by this proceeding. They paddle, watch wildlife, fish, hike and bike on trails and in parks along the riverbanks, and otherwise enjoy these natural resources.

The experience of Openlands supporters would be diminished by allowing a more lenient standard for chlorides, which would ultimately result in greater pollution in these waters, making it more hostile for aquatic life to live and reach their potential. In waterways where chlorides are at issue, more Openlands supporters would recreate on these systems if the quality continued to improve and become "fishable and swimmable" as required by the Clean Water Act. Openlands is concerned with rising levels of chloride in Illinois waters that are important to and relied upon by both people and wildlife.

Overall, Openlands assists government, non-profits, and community groups to enhance and protect water quality and aquatic habitats through planning, education, restoration projects, land preservation, and monitoring of streams and rivers throughout northeastern Illinois and the surrounding region.

As part of connecting people to nature where they live, Openlands partners with government agencies, municipalities and other organizations to make our region's waterways more open and accessible for recreational use. Openlands is one of the original organizations to partner with Illinois Department of Natural Resources to develop and promote the first water trails throughout northeastern Illinois. It has partnered with communities and agencies to install canoe and kayak launches and signage, and hold workshops and events to engage local communities in paddling and stewarding their rivers, streams and lakes. Openlands developed and offers canoeing and kayaking maps and brochures and maintains an interactive website for the public to learn about opportunities to paddle and enjoy particular trails across the region.

For decades, Openlands has provided technical assistance to agencies and communities to acquire and preserve land to protect streams across the region. Openlands has conducted over $50 million in wetland and stream restoration initiatives, improving aquatic habitat in degraded systems. Openlands provides technical assistance in acquiring riparian corridors to protect waterways, ranging from the pristine Kishwaukee and Nippersink Rivers to the Chicago River.

Openlands has utilized its experience to raise scientific evidence before the Illinois Pollution Control Board in past proceedings, such as the Water Quality Standards rulemaking (R08-09) to strengthen necessary protections for existing and attainable species that depend upon the quality of our region's waterways. As an aquatic ecologist, I testified during the CAWS and Lower Des Plaines River water quality standards proceedings about how water quality affects aquatic life throughout the system, including connected higher quality rivers and tributaries. I provided evidence of the tremendous growth of recreational uses within the context of our paddling programs and the myriad of opportunities for people across the Chicagoland area to connect to and access this valuable resource. Since the Illinois Pollution Control Board adopted its new use designations and standards for the CAWS, the quality and use of the waterways has continued to rise.

My testimony is informed by years of involvement in scientific studies and field work on the health of headwater streams and conditions for mussel populations as indicator species. I have an M.S. degree in Zoology from Miami University, Oxford, Ohio and an M.A. in Environmental and Urban Geography from the University of Illinois at Chicago. I represent Openlands in serving as the lead agency for the Chicago Wilderness Priority Species effort to improve conditions for the ellipse mussel to increase its population numbers throughout the Chicago Wilderness Region. Several agencies are coordinating to research and protect mussels as part of the Chicago Wilderness ellipse management group, including the Illinois Natural History Survey, the Wisconsin Department of Natural Resources, the Indiana Department of Natural Resources, the Forest Preserve District of DuPage County, and the Forest Preserve District of DeKalb County. This group documents the existence of the ellipse in Chicago Wilderness rivers and streams, estimates the size of ellipse populations, and suggests management actions that would benefit ellipses through knowledge gained from research and statistical modeling of ellipse populations.

As Openlands staff, I also led in the creation of the Field Guide to the Freshwater Mussels of Chicago Wilderness, a detailed field guide to the identification of the 40 species of native freshwater mussels that live in the Chicago Wilderness region. The guide raises awareness of land managers and community groups of freshwater mussels as taxa in urgent need of conservation. It encourages natural resource agencies and community groups to conduct mussel surveys, using indices to assess the mussel resource value of streams in Chicago Wilderness.

In addition, Openlands works to protect headwater streams throughout the Chicago Wilderness region, which harbor the kinds of aquatic life at issue in this proceeding. In 2017, Openlands published the report *Headwater Streams of Chicago Wilderness: Status and Recommendations* as a resource for local governments and land managers to better understand the value of headwater streams and the biodiversity they support. Through the guide and our work, Openlands demonstrates land acquisition, restoration and policy strategies to preserve and protect these resources.

The purpose of my testimony in this proceeding is, with this background, to share and discuss scientific findings that indicate the suggested chlorides standards will not protect existing or attainable aquatic life in waterways subject to the rulemaking. More recent studies produced after ones relied upon in the Huff & Huff proposal indicate that sensitive aquatic species in these river systems would be adversely affected by the proposed chloride levels. Studies show that greater acute and chronic exposure to chlorides could especially be detrimental to certain species of intolerant glochidia and juvenile mussels. Moreover, the proposal does not adequately account for softer water and warmer temperatures, especially downstate, which are projected to increase as our climate continues to change.

II. Proposed Chloride Standards Would Not Protect Known Aquatic Life in Illinois Waters

A. <u>Recent Data Supports Lower Water Quality Standards for Chloride to Protect Mussel Species in Illinois Waterways</u>

Freshwater mussels are one of the most imperiled groups of organisms. Nearly 70% of these species are designated either as threatened, endangered or in decline (Williams et al 1993). Recent studies have shown that for some contaminants, freshwater mussel glochidia and juveniles are more sensitive than standard test organisms, leading to concerns that water quality regulations do not adequately protect freshwater mussels (Wang et al. 2018a, 2018b). Gillis (2011) points out that for glochidia, the end point for studies of acute chloride toxicity is not death, but loss of ability to attach to a host species, which is necessary for their survival, and renders them "effectively dead." In their study of acute chloride toxicity of Fat Mucket, *Lampsilis siliquoidea*, juveniles and glochidia, Wang et al. (2018b) state that including their more recent mussel data in the toxicity database would "likely lower the [Water Quality Criteria] and [Water Quality Standards] for [Chlorides]" (Wang et al. 2018b). Wang et al (2018a) made a similar statement in their study of the chronic chloride toxicity of the Fat Mucket, in which they state "inclusion of the data from the present study and recent publications to update the national chronic water quality criterion or Iowa chronic water quality standard would likely lower the criterion or standard."

In terms of chronic long term effects on invertebrate assemblages, Wallace and Biastoch (2016) found that in streams in Toronto, Canada, the macroinvertebrate community demonstrated the most taxa changes (declining frequency and abundance of taxa sensitive to chloride and increasing frequency and abundance of taxa tolerant of chloride) at a threshold of approximately 50 to 90 mg Cl- /L. The authors point out that this is below the Canadian Water Quality Guideline of 120 mg CL-/L for chronic exposure and suggest that chloride may be having nonlethal effects on the benthic macroinvertebrate communities in the Toronto, Ontario region.

The chronic and acute thresholds in these newer studies suggest that the proposed revisions to the state chloride standards are too high to protect known aquatic life species, especially mussels in early life stages. In the equation on page 97 of the Huff proposal, *Lampsilis siliquoidea* is entered as having a species mean acute value to chlorides of 2764.4 mg Cl-/L. While it is unclear what life stage is associated with this figure, it is significantly higher than the EC50s that Wang et al 2018b and Gillis 2011 found for juveniles and larva, especially in softer waters where the EC50s for glochidia were as low for acute as 441 mg Cl-/L for *Lampsilis siliquoidea* (Wang et al 2018b) at 50 hardness, and as low as 131 mg Cl-/L in the case of the Wavy rayed lampmussel (*Lampsilis fasciola*) (Gillis 2011) at 95-115 hardness. Therefore, the number 2764.4 mg Cl-/L used in the equation is not consistent with current data for the most sensitive life stages of *Lampsilis siliquoidea* or the most sensitive life stages of other sensitive mussels of the genus *Lampsilis.*

B. **The Proposed Winter Standard Is Not Proven to Protect Existing Aquatic Life Because Sensitive Species Were Not Tested at 10° C**

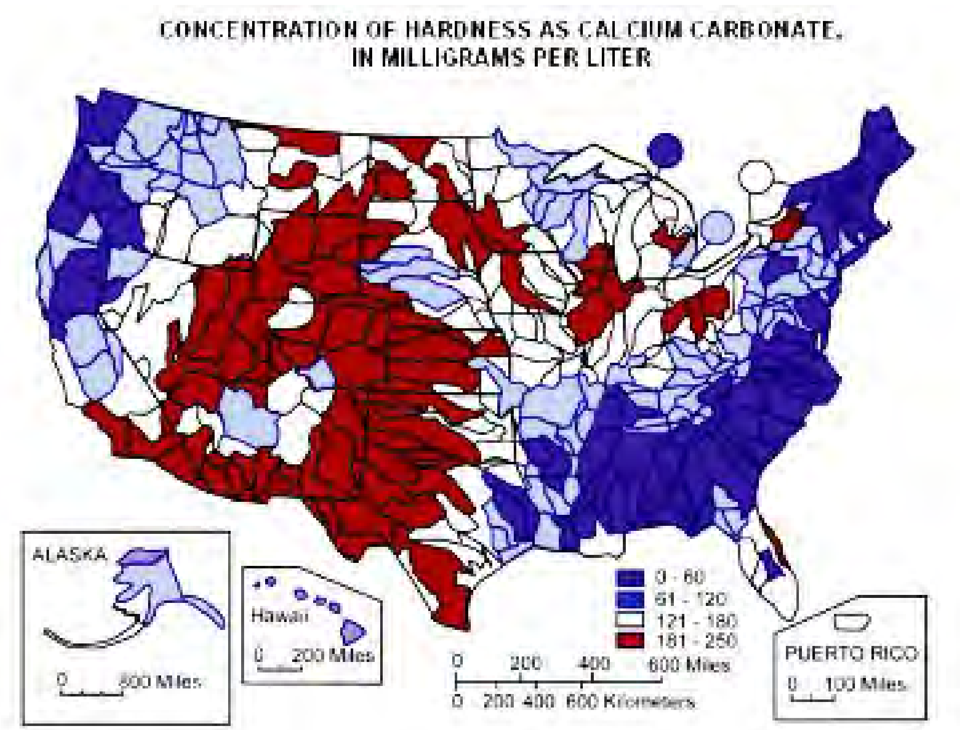
There is no evidence that the proposed acute winter standard of 1,010 mg Cl-L would adequately protect chloride sensitive glochidia of the Fat Mucket (*Lampsilis siliquoidea*), Plain Pocketbook (*Lampsilis cardium*), Wavy-rayed lampmussel (*Lampilis fasciola*), and Northern riffleshell (*Epiblasma torulosa rangiana*) because their chloride toxicity has not been tested at 10° C. Larval freshwater mussels will be in the waterways during the winter months. Freshwater mussel glochidia are known to occur on fishes in the winter, and several species of freshwater mussels tested in a study released glochidia twice a year, resulting in a summer and a winter brood, with some species releasing glochidia in waters that were 5° C, (Watters and O'Dee 2000). Glochica will also be present in the brood chambers of adult mussels during the winter months.

C. **Higher Water Temperatures Earlier in the Year and Softer Water in Places Like Southern Illinois Render Existing Sensitive Aquatic Life More Susceptible to Harm under The Proposed Chloride Standards**

Typical water temperatures in April in Illinois streams vary with location, and can have ranges and averages well above 10° C. A study of temperatures of streams and lakes in Illinois (Harmeson and Schnepper 1965) measured both average temperature and temperature ranges for each month of the year. Looking at the month of April in this study, stream water temperatures ranged up to a high of 70° F (21° C) in April (at a stream station in Springfield, IL). The mean temperature for that same station in Springfield for April was 58° F (14.4° C). Of the 38 stations monitored in the is study, 19 (50%) *ranged* up to temperatures of 58° F (14.4° C) or higher in April and 5 (13%) *averaged* 58 (14.4° C) or higher in April, these included central and southern Illinois locations such as Springfield, Sparta, Murphysboro, Fairfield and Carmi, with the highest average being 59° F (15° C) at Fairfield.

Analyses of long term stream datasets from Wisconsin (1992 to 2009) suggest a warming trend in stream temperatures over that period (Mitro et al. 2011). Since 10° C in April in all Illinois streams is unrealistic, especially in light of established warming trends, the proposed winter chloride standards is not proven to be protective.

Recent studies indicate that softer water compounds impacts of chloride toxicity on aquatic life, especially in warmer temperatures. The hardness of water (measured as CaCO3) has been shown to have significant effects on chloride toxicity to four of five species sensitive to chlorides and tested at a range of water hardness (Soucek 2011), with harder waters ameliorating the toxic effects of chlorides. The species tested include the water flea *Ceriodaphnia dubia*, two species of fingernail clam, *Sphaerium simile and Musculium transversum*, the snail *Gyraulis parvus* and the worm *Tubifex tubifex*. The snail was the only species for which water hardness did not significantly affect toxicity of chlorides. Recent research (Wang et al 2018a, and 2018b) has shown that there is a three and a half fold difference in chloride toxicity to one of the most sensitive macroinvertebrates (the Fat Mucket mussel, *Lampsilis siliquoidea*) at different water hardnesses. The State of Illinois is known to have streams and rivers of varying hardness, with softer water occurring in the southern part of the state and in some western areas of the state. The extreme south eastern part of the state has waters that range into the 0-60 hardness category (0-60 represents the softest waters) according to USGS.



More recent studies indicate Fat Mucket mussels would not tolerate proposed chloride levels, especially in softer waters. While the Fat Mucket mussel, *Lampsilis siliquoidea*, is included in the Huff proposal, it is associated with a higher chloride toxicity tolerance than has been reported in recent studies (Wang et al

2018a, 2018b and Gillis 2011). It is questionable whether the Fat Mucket, which is widely distributed in Illinois, would be protected by the proposed summer acute standard in softer waters. At 23° C, a study by Wang et al (2018a) found that Fat Mucket larva had an acute toxicity (EC50) of 441 mg Cl-/L at 50 hardness, and an acute EC50 of 544 mg Cl-/L at 100 hardness, and an acute EC50 of 728 mg Cl-/L in "Moderately Hard Reconstituted Water." All of these are lower than the proposed acute summer proposed value of 860 mg Cl-/L.

In addition to temperature and hardness, the concentration of other ions, including sulfate concentration, in the water can also affect the toxicity of chlorides to aquatic life. This suggests that revised standards should take more than just temperature into account.

D. Fat Mucket Larva Sensitivity Indicates Potential Harm to Other Mussel Larva Species from Relaxing the Chloride Levels in the Proposed Standards

The sensitivity of Fat Mucket larva may very well indicate the response of other kinds of mussel larva, including untested species, to the higher levels of chlorides in the Huff proposal. Gillis (2011) found that larval mussels of the Plain Pocket Book (*Lampsilis cardium)*, which is present in Illinois, had an acute EC50 817 Cl-/L in reconstituted water of 100 hardness. Gillis (2011) also found that Wavy-rayed lampmussel larva (*Lampsilis fasciola*), which occur in Illinois throughout the Vermillion and Wabash River Basins, had an acute EC50 of 113 Cl-/L in tests conducted in 2008, and of 285 Cl-/L in tests conducted in 2009. The wavy-rayed lampmussel is an endangered species in Illinois. In addition, Gillis (2011) found that, *Epioblasma torulosa rangiana*, the Northern riffleshell mussel, which is listed as endangered federally (and as endangered in the state of Illinois), had an acute LC50 of 244 mg Cl-/L. These tests were all performed in reconstituted water of 100 hardness at 21° C. Every one of the acute chloride toxicities is below the proposed summer acute and chronic chloride standards proposed as well as the proposed winter chronic standards. In addition, some of them are below the proposed summer chronic standard.

E. Relaxed Chloride Standards Threaten the Recovery of Translocated Northern Riffleshell Mussels into the Vermillion River Basin

Three thousand six hundred and ninety nine (3,699) federally endangered Northern riffleshell mussels (along with 4,166 federally endangered clubshells) were translocated from Pennsylvania from 2010 to 2016 into the Vermillion River basin in Vermillion and Champaign Counties in Illinois as part of the recovery plan for this species (Tiemann et al. 2017). This is the only place that Northern riffleshells are known to occur in Illinois currently, with the reintroduced population being the only population as far as we know (INHS, December 5, 2018). The success of this effort will be dependent upon Illinois offering suitable habitat (including water quality) for this species.

F. Winter Ice Can Result in Aquatic Life Exposure to Greater Chloride Concentrations

Todd and Kaltenecker (2012) showed in a long term study in Southern Ontario that Chloride concentrations were often higher in the ice-free than in the ice-over season, demonstrating that winter road salt applications contribute to year round elevated Chloride levels. Chloride can seep into the groundwater and be released into creeks during summer months. Juvenile mussels buried in the sediment would be at risk from chlorides in upwelling ground water. Dugan et al (2017) point out that when ice forms in small lakes and wetlands, Chloride is excluded from the ice, and concentrates in the remaining unfrozen water during the winter. This tendency should be taken into account when setting chloride standards for winter, especially for small lakes. They state:

> The compounding effects of road salt runoff and ice formation should be considered in the management of water quality and ecosystem health in shallow urban water bodies or waterbodies receiving road salt runoff from nearby roadways.

Gillis (2011) notes that potential for chloride toxicity is greater in lakes and other habitats of relatively still water. Chloride contributes to desimetric stratification of receiving waters, resulting in higher chloride concentrations just above the sediment water interface in static or slow moving water bodies. Such stratification could exacerbate the risk of acute chloride toxicity for freshwater mussels living in embayments and lakes subject to salt run off. This harmful effect does not appear to be accounted for in the proposed chloride standards.

III. Conclusion

In sum, while the Huff petition raises excellent points about chloride toxicities, and how they are affected by temperature in several sensitive species, it does not adequately account for recent research on the sensitivity of the glochidia of several resident mussel species in Illinois waters. It also does not adequately address the variability of hardness and water temperatures in different parts of the state, both of which appear to be critical in terms of how toxic chlorides are to sensitive species. More recent studies produced for the U.S. EPA and U.S.G.S. (Wang et al 2018a and 2018b) assert if new data for sensitive aquatic species, such as Fat Mucket mussel (*Lampsilis siliquoidea*), were added to databases utilized to calculate water quality criteria and standards for waterways, then the permissible chloride levels would likely need to be lower to protect these species. Gillis (2011) also found that glochidia of several mussel species had acute chloride toxicities that indicated they may be among the most sensitive species to chlorides, including the Plain Pocket Book (*Lampsilis cardium)*, thet Wavy-rayed lampmussel larva (*Lampsilis fasciola*), and the Northern riffleshell, (*Epioblasma torulosa rangiana)*. These species are all in Illinois waters and should be taken into account when setting chloride standards. Also, phenomena such as the tendency for winter salt to seep into groundwater and continue to pollute surface waters during the warmer months and the tendency for chloride ions to concentrate in unfrozen water as ice forms should be considered when setting chloride standards for winter.

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LITERATURE CITED

Dugan, HA, G Helmueller, JJ Magnuson. 2017. Ice formation and the risk of chloride toxicity in shallow wetlands and lakes. Limnology and Oceanography Letters 2: 150-158.

Gillis, PL. 2011. Assessing the toxicity of sodium chloride to the glochidia of freshwater mussels: implications for salinization of surface waters. Environmental pollution 159: 1702-108.

Harmeson, RH, and VM Schnepper. 1965. Temperatures of surface waters in Illinois. Report of Investigation 49, Illinois State Water Survey, Urbana, Illinois.

Mitro, M., J. Lyons, S. Sharma. 2010. Coldwater fish and fisheries working group report. Wisconsin Initiative on Climate Change Impacts, Wisconsin Department of Natural Resources, Madison, Wisconsin.

Soucek, DJ, TK Linton, CD Tarr, A Dickinson, N Wickramanayake, CG Delos and LA Cruz. 2011. Influence of water hardness and sulfate on the acute toxicity of chloride to sensitive freshwater invertebrates. Environ Toxicol Chem April; 30(4): 930-8. Doi: 10.1002/etc.454. Epub 2011 Feb 11.

Todd, AK, and MG Kaltenecker. 2012. Warm season chloride concentrations in stream habitats of freshwater mussel species at risk. Environmental Pollution 171: 199-206.

USGS Water Quality Information. Concentration of hardness as calcium carbonate in milligrams per liter. Map. Edited by USEPA, 2005. Modified from Briggs and others, 1977. Originally from Briggs, JC, and JF Ficke. 1977. Quality of Rivers of the United States, 1975 Water Year -- Based on the National Stream Quality Accounting Network (NASQAN): U.S. Geological Survey Open-File Report 78-200, 436 p. accessed at https://water.usgs.gov/owq/hardness-alkalinity.html#map, October 25, 2018.

Wallace, AM and RG Biastoch. 2016. Detecting changes in the benthic invertebrate community in response to increasing chloride in streams in Toronto, Canada. Freshwater Science 35(1): 353-363.

Wang, N, CD Ivey, RA Dorman, CG Ingersoll, J Steevens, EJ Hammer, CR Bauer, and DR Mount. 2018b. Acute toxicity of sodium chloride and potassium chloride to a unionid mussel (Lampsilis siliquoidea) in water exposures. Environmental Toxicology and Chemistry. First published: 19 June 2018 https://doi.org/10.1002/etc.4206

Wang, N, JL Kunz, RA Dorman, CG Ingersoll, JA Steevens, EJ Hammer, and CR Bauer. 2018a. Evaluating chronic toxicity of sodium chloride or potassium chloride to a unionid mussel

(Lamsilis siliquoidea) in water exposures using standard and refined toxicity testing methods. Environmental Toxicology and Chemistry. 2018 Aug 21. doi: 10.1002/etc.4258. [Epub ahead of print].

Williams, JD, ML Warren, KS Cummings, JL Harris, RJ Neves. 1993. Conservation status of freshwater mussels of the United States and Canada. Fisheries 18, 6-22.

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EXPERIENCE

Aquatic Ecologist, Openlands **(Chicago, IL, 9/2004 – Present)**

Leads volunteers in sampling streams for aquatic biodiversity to monitor stream quality, advocate for stream health, and assess the impact of restoration practices on biodiversity. This includes sampling and analysis of macroinvertebrates, mussels and fish to gauge the health and recovery of aquatic systems.

Funded and led the development of a Field Guide to the Freshwater Mussels of Chicago Wilderness to assist professionals (e.g. land managers and consultants) and volunteers in evaluating the health of mussel populations.

Co-authored the Headwater Streams of the Chicago Wilderness Region: Status and Recommendations to raise awareness of the value of headwaters and encourage the assessment, preservation and restoration of such waterways.

Implements the Northeastern Illinois Regional Water Trail Plan by improving canoe and kayak access on waterways in the Chicago region through planning, advocacy, events, outreach, and assisting volunteer Water TrailKeepers in organizing clean ups and monitoring water trail conditions.

Created, updates and maintains paddleillinoiswatertrails.org, an Openlands interactive website that is a guide to paddling water trails throughout the Chicago Region.

Collaborates with Friends of the Chicago River and other environmental groups to strengthen water quality standards for Chicago Area Waterways to better protect people that recreate on and in our rivers. Provided aquatic life use testimony during Water Quality Standards hearings before the Illinois Pollution Control Board (R08-09), which explored the importance of connectivity and interplay between habitat and water quality.

Lead the Chicago Wilderness Ellipse Management Recovery Team for this Chicago Wilderness priority mussel species. Coordinate with other natural resource agencies to create and implement a plan to increase ellipse population numbers and improve habitat for the ellipses, including estimating ellipse population sizes and considering modeling results to make project decisions to benefit ellipses.

Environmental Planner & GIS Specialist, **(Chicago, IL, 12/2000-9/2004)**
Northeastern Illinois Planning Comn., Natural Resources Dept.

Constructed GIS models to predict the highest habitat values and potential impacts from infrastructure for use in natural resource planning.

Mapped and assessed Kane County wetlands for habitat value and stormwater storage value for an Advanced Identification of Aquatic Resources (ADID) in collaboration with U.S. EPA, Kane County Department of Stormwater Management, and U.S. FWS.

Led an inventory of stream restoration projects undertaken in Chicago Wilderness to assess what techniques functioned best under different circumstances in collaboration with U.S.G.S., U.S. FWS, and Openlands.

Provided technical assistance to local governments and watershed groups in watershed planning.

Environmental and Conservation Programs Intern & GIS Technician, The Field Museum of Natural History **(Chicago, IL, 08/1999-11/2000)**

Used land use maps and remote sensing to analyze land cover of the Chicago region.

Authored reports for researchers and land managers to understand the different methodologies of the project to calculate acreages to contribute an understanding of losses or gains of various land cover types over time.

Fisheries Intern, Salmon-Challis National Forest **(Salmon, ID, 05/1999-08/1999)**

Monitored water chemistry in incubators located on private and National Forest Service land containing steelhead eggs and assisted in placing eggs in incubators.

Engaged landowners regarding their choice to host incubators.

Teaching Assistant, University of Illinois at Chicago **(Chicago, IL 08/1998-12/1998 and 08/1999-12/1999)**

Science Curriculum Specialist, American School **(Lansing, IL 09/1996-08/1998)**

EDUCATION

Master of Arts, Environmental and Urban Geography, 2001
University of Illinois at Chicago, Chicago, IL

Master of Science, Zoology, 1994
Miami University, Oxford, OH

Bachelor of Arts with General Honors, History, 1987
University of Chicago, Chicago, IL

SELECTED PUBLICATIONS AND REPORTS

Anderson, D and L Barghusen. 2017. *Headwater Streams of the Chicago Wilderness Region: Status and Recommendations.* Chicago, Illinois: Openlands.

Barghusen, L and RJ Klocek. 2015. *Biological Richness of Headwater Streams*: report for the Indiana Lake Michigan Coastal Program Grant CZ213, granted to Openlands. Chicago, Illinois: Openlands.

Klocek, RJ and L Barghusen. 2014. Aquatic Faunal Surveys at the Hadley Valley Forest Preserve, Spring Creek, Will County, Illinois, 2014 Results. Chicago, Illinois: Openlands.

Klocek, RJ, J Bland, and L Barghusen. 2008. *A Field Guide to the Freshwater Mussels of Chicago Wilderness.* Chicago, Illinois: Openlands.

Barghusen, Laura. *Endangered Animals and Habitats: The Bear*. San Diego: Lucent Books, 1999.

Barghusen, L., and N. Toomy, 1998. *Study Guide for Ecology*. Chicago, Illinois: American School.

Barghusen, L.E., D.L. Claussen, M.S. Anderson and A.J. Bailer. 1997. The effects of temperature on the web-building behavior of the common house spider, *Achaearanea tepidariorum*. *Functional Ecology* 11:4-10.

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Assessing the toxicity of sodium chloride to the glochidia of freshwater mussels: Implications for salinization of surface waters

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Freshwater mussel larvae were acutely sensitive to sodium chloride, such that chloride levels in some Canadian rivers may pose a threat to the survival of this early life stage.

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ABSTRACT

Chloride concentrations in surface waters have increased significantly, a rise attributed to road salt use. In Canada, this may be a concern for endangered freshwater mussels, many with ranges limited to southern Ontario, Canada's most road-dense region. The acute toxicity of NaCl was determined for glochidia, the mussel's larval stage. The 24 h EC50s of four (including two Canadian endangered) species ranged from 113–1430 mg Cl L^{-1} (reconstituted water, 100 mg $CaCO_3$ L^{-1}). To determine how mussels would respond to a chloride pulse, natural river water (hardness 278–322 mg $CaCO_3$ L^{-1}) was augmented with salt. *Lampsilis fasciola* glochidia were significantly less sensitive to salt in natural water (EC50s 1265–1559 mg Cl L^{-1}) than in reconstituted water (EC50 285 mg L^{-1}). Chloride data from mussel habitats revealed chloride reaches levels acutely toxic to glochidia (1300 mg L^{-1}). The increased salinization of freshwater could negatively impact freshwater mussels, including numerous species at risk.

1. Introduction

The increase in the chloride concentration of North American surface waters over the past 30 years has been correlated with the increased application of de-icing salts on paved surfaces (Kaushal et al., 2005; Jackson and Jobbágy, 2005). Kaushal et al. (2005) demonstrated that chloride levels in rivers and streams were correlated with the percentage of impermeable surfaces in the watershed. This increased salinization of freshwater has implications for both human and ecosystem health. Chloride concentrations in some drinking water reservoirs now exceed the level for potable water (Kaushal et al., 2005) and numerous urban streams frequently exceed the levels considered harmful to aquatic life (Evans and Frick, 2001; Trowbridge et al., 2010). In addition to the seasonal influx of salt in snowmelt and runoff, groundwater (Howard and Haynes, 1993; Kelly et al., 2008; Roy and Bickerton, 2010) and soils (Kincaid and Findlay, 2009) can also act as reservoirs releasing chloride throughout the year. Therefore, it is quite probable that the full impact of freshwater salinization has yet to be realized, not only because millions of tons of road salt are applied each year (Environment Canada and Health Canada, 2001), but also because delayed and longer-term inputs of chloride from contaminated soils and groundwater are expected (Kelly et al., 2008; Kincaid and Findlay, 2009). Kaushal et al., (2005) suggested that baseline salinity in the Northeastern United States is approaching levels where significant changes in ecological communities and ecosystem function are expected. Recent studies suggest that such shifts may in fact already be occurring for some contaminant sensitive groups. For example, Collins and Russell (2009) concluded that exposure to road salt affects amphibian community structure and species richness by excluding salt-sensitive species from high chloride environments.

Freshwater mussels, one of the most imperiled groups of organisms (Ricciardi and Rasmussen, 1999; Lydeard et al., 2004), are also known to be particularly sensitive to some waterborne contaminants. In fact environmental pollution is considered to be one of the factors responsible for their decline (Strayer et al., 2004; Lydeard et al., 2004). Nearly 70% of North American freshwater mussels are designated as either threatened, endangered, or in decline (Williams et al., 1993; Neves et al., 1997). Recent studies have reported that for some contaminants, freshwater mussel larvae and juveniles are much more sensitive than standard test organisms, leading to concerns that water quality regulations may not protect freshwater mussels (Augspurger et al., 2003; Wang et al., 2007, 2009; March et al., 2007). In Canada, the geographical distribution of freshwater mussels is thought to be limited by temperature, either because the mussels themselves or their fish hosts reach their lower limit of thermal tolerance (Metcalfe-Smith et al., 1998). Many species reach the northern limit of their range in

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the lower Great Lakes Basin, with 40 of Canada's 53 freshwater mussels species found in this area (Metcalfe-Smith et al., 1998). Of particular concern is that the range of eight Canadian mussel species classified as federally endangered are limited to the heavily populated and road dense southern Ontario. However, it is unknown whether the contamination of mussel habitats by chloride will affect the mussels, particularly their sensitive early life stages. The parasitic larvae of freshwater mussels, called glochidia, are released from the brooding chambers (marsupia) in the female's gills into the water column in order to make contact with fish hosts. In Canada, most glochidia are released between May and October, depending upon species specific temperature cues for release. Fortunately, the typical release period does not coincide with the seasonal influx of chloride associated with snowmelt runoff, but the steady increase in baseline chloride levels along with periodic summer chloride pulses from stormwater runoff and groundwater upwelling (Howard and Haynes, 1993; Kincaid and Findlay, 2009) may pose a risk to this imperiled, but ecologically significant group of animals.

This study examined acute sodium chloride (NaCl) toxicity in glochidia and compared median effective concentrations (EC50s) to chloride concentrations in the mussel's natural habitat. Specifically, sensitivity was determined for five species of mussels, three of which are designated as federally endangered in Canada. Toxicity tests with glochidia and NaCl were conducted in both standard reconstituted waters and natural waters. Reconstituted water exposures were used to determine the sensitivity of glochidia to chloride in relation to other aquatic organisms and the effect of water hardness on chloride toxicity. Natural water exposures employed water collected from four southern Ontario rivers that support diverse mussel populations (9–34 species). The aim of the natural water exposures was to determine how glochidia would respond to an episodic pulse of chloride in their habitat.

2. Materials and methods

2.1. Mussel collection and laboratory care

Gravid female mussels were collected from streams and rivers in southern Ontario. The period of gravidity varied with species such that *Lampsilis siliquoidea* (Barnes 1823) (fatmucket) and *Lampsilis cardium* (Rafinesque 1820) (plain pocketbook) were collected in May, *Lampsilis fasciola* (Rafinesque 1820) (wavy-rayed lampmussel) in mid-July, and *Epioblasma torulosa rangiana* (Lea 1838) (northern riffleshell) and *Ptychobranchus fasciolaris* (Rafinesque 1820) (kidneyshell) in early September. The endangered *L. fasciola*, *P. fasciolaris* and *E. torulosa rangiana* were collected under Canadian Species at Risk Permits (SECT 08 SCI 007, SECT 73 SARA C&A 09-012). Because the availability of gravid females varied each year, toxicity tests were conducted over two field seasons (2008–2009). In addition, acute chloride sensitivity of *L. siliquoidea* glochidia was assessed using gravid females collected from two different watersheds (one in each of 2008 and 2009) and toxicity tests with *L. fasciola* were also conducted in both years but using different gravid females collected from the same field site. Although mussels for this study were collected in Ontario, all species examined are also found in the U.S. (Parmalee and Bogan, 1998).

Gravid mussels were held at the University of Guelph's Aqualab facility and maintained in a flow-through system with well water held at 10 ± 2 °C (to prevent the glochidia release). Mussels were fed approximately 1.2×10^{10} algae cells per mussel per day with a commercial shellfish diet (Instant Algae Shellfish Diet 1800®, Richmond Hill, ON). Glochidia for testing were collected by flushing the marsupia (i.e., brooding chambers) with a water-filled syringe. The viability of each mussel's glochidia was assessed (described below) prior to use. Prior to initiating an exposure, glochidia collected from gravid mussels held at 10 °C, were gradually (over 2–3 h) acclimated to the exposure temperature (21 °C) through dilutions with room temperature reconstituted water. Glochidia were pooled from a minimum of three gravid females for each experiment. For the endangered species, glochidia were only collected from one marsupium gill, and each mussel was returned to the location from which they were collected to facilitate the release of remaining glochidia in their natural habitat.

2.2. Toxicity testing

Acute toxicity tests with glochidia were modeled after the American Society for Testing and Materials' method for conducting toxicity tests with the early life stages of freshwater mussels (ASTM, 2006). Briefly, the viability of glochidia were evaluated after exposure to waterborne contaminants. In order to parasitize fish, glochidia must be viable, which means they must be able to close their valves and clamp down on a fish's gill in order to encyst. Glochidia viability (i.e., ability to close valves) was assessed prior to exposure and after 24 h of exposure in a sub-sample (100–200) of the glochidia (500–1000) through the addition of a saturated salt solution (NaCl 240 g L^{-1}). Viability was calculated using the following equation: Percent Viability = 100 × (Number of closed glochidia after NaCl addition − Number of closed glochidia before NaCl addition)/(Number of closed glochidia after NaCl addition + Number of open glochidia after NaCl addition). Results are expressed as (chloride) effective median concentrations (EC50) rather than median lethal concentrations (LC50), but as they are obligatory parasites, for practical purposes non-viable glochidia should be considered 'dead' because they would be unable to attach to a host fish and complete their life cycle.

The ASTM (2006) method indicates that glochidia control survival remain above 90%. Therefore, for toxicity tests conducted in reconstituted water, pre-exposure ($t = 0$) and post-exposure ($t = 24$ h) control survival (i.e. viability) were determined. In addition, for toxicity tests conducted in natural waters, 24 h control survival in each river water (without salt augmentation) was determined.

An aqueous stock made from certified ACS grade (Fisher Scientific) sodium chloride (NaCl) was used to create exposure solutions. Waters (reconstituted or field-collected) were spiked with NaCl (nominal, 0–10 g NaCl L^{-1}) and held in the dark at 4 °C for 48 h before initiation of an exposure. Exposures were conducted in 250 mL glass beakers, under a 16:8 light:dark cycle at 21 ± 2 °C. Water quality including dissolved oxygen (DO), pH, alkalinity, dissolved organic carbon (DOC), water hardness as well as the concentration of major ions (Na, K, Ca) and trace metals were assessed at exposure initiation. DO, pH and Cl were also measured upon completion of an exposure. Water analysis was conducted by the Canadian National Laboratory for Environmental Testing (Environment Canada, Burlington, ON). Chloride was measured by Ion Chromatography (detection limit (DL) 0.02 mg L^{-1}). Mean chloride recovery was 100.4% (STD 0.36) using the National Water Research Institute's (NWRI) certified reference material ION-915. Metals, including copper, were measured by ICP-SFMS (copper DL 0.02 μg L^{-1}). Mean copper recovery was 100% (STD 0.16) using the National Research Council of Canada's certified reference material SLRS-4. DOC (DL 0.1 mg L^{-1}) was measured by a UV Persulfate Total Organic Carbon Analyzer. Mean DOC recovery was 95.5% (STD 0.2) using NWRI's certified reference material WINN-02. Major ions (e.g. potassium, DL 0.01 mg L^{-1}) were analyzed by Atomic Absorption Spectrometry. Mean potassium recovery was 99.4% (STD 0.4) using VHG Labs (New Hampshire) certified reference material QWSMIN. Glassware was acid washed with 10% nitric acid (Reagent Grade, Fisher Scientific) prior to use and solutions were made with Millipore™ water.

2.3. Chloride sensitivity in reconstituted waters

A series of toxicity tests were conducted with NaCl and reconstituted waters (ASTM, 2003). For each species studied at least one acute toxicity test was conducted in moderately-hard reconstituted water (95–115 mg $CaCO_3$ L^{-1}). In addition, a series of exposures were conducted in reconstituted waters of varying hardness (range 47–322 mg $CaCO_3$ L^{-1}) using *L. siliquoidea* glochidia.

2.4. Chloride sensitivity in natural waters

A series of toxicity tests were conducted with water collected from four significant mussel habitats in southern Ontario (Table 1). River water (10 L) was collected just below the surface where the water was visibly flowing. Water samples were held in the dark at 4 °C until used in an exposure (maximum one week). Acute exposures in NaCl-spiked natural waters were conducted with *L. fasciola* glochidia as described above.

In addition to the *L. fasciola* natural water exposures, another natural water test was conducted with *P. fasciolaris* glochidia. Unlike the other species examined which release free glochidia, *P. fasciolaris* produces conglutinates. These small packets of glochidia (100–200) resemble fish prey and serve to enhance infection of host fish. Two intact conglutinates were used in each replicate test concentration. One conglutinate was opened (by gently tearing casing with fine forceps) after 24 h and the other after 48 h of exposure to assess the viability of the encased glochidia (24 h data shown). Because the number of conglutinates was limited, an exposure with chloride-spiked natural water was selected as the most ecologically relevant test to conduct with this endangered species.

2.5. Statistical analysis

Chloride EC50s and EC20s were determined by Probit Analysis (Statistical Package for the Social Sciences (SPSS)) version 11.0 using measured chloride concentrations and presented with 95% confidence intervals (CI) (e.g. EC50 (95% CI). EC50s and EC20s were considered to be significantly different when their 95% CI did not overlap (Environment Canada, 2005). Linear regression analysis was conducted (SigmaStat version 3.2) to examine the relationship between water hardness and chloride toxicity (EC50s). Note: Although EC50s and EC20s are reported with respect to the chloride component of NaCl, no attempt was made to determine the toxic

Table 1
Summary of selected water chemistry[a] parameters for four Canadian rivers used in acute sodium chloride exposures with *Lampsilis fasciola* glochidia, along with pre-exposure ($t = 0$) viability, and post-exposure ($t = 24$ h) reconstituted water and un-spiked river water control survival.

Water source[b]	Chloride (mg L^{-1})	Potassium (mg L^{-1})	Copper (μg L^{-1})	DOC (mg L^{-1})	pH	Water hardness (mg $CaCO_3$ L^{-1})	% Viability, $t = 0$	% Viability reconstituted water, $t = 24$	% Viability river water, $t = 24$ h
Sydenham River	34.1	4.6	6.0	4.5	8.25	292	91.5	91.7	92.8
Grand River	66.8	3.3	4.9	6.2	8.62	278	91.5	91.7	89.1
Maitland River	57.4	5.8	5.6	6.7	8.32	322	91.0	87.1	85.1
Thames River	75.2	4.7	4.9	4.4	8.29	306	91.0	87.1	78.1

[a] Measured chloride, potassium, copper, dissolved organic carbon (DOC), pH, and water hardness values represent background concentrations in un-spiked river water.
[b] Rivers located in Ontario, Canada.

mode of action of NaCl in glochidia, therefore this study cannot distinguish whether glochidia are responding to the chloride ion or the sodium ion.

2.6. Chloride concentrations and mussel distribution data in southern Ontario

To assess the potential threat that chloride poses to freshwater mussels, the chloride levels in key mussel habitats in southern Ontario were examined. In Ontario, watersheds are managed locally by Conservation Authorities. The Canadian Department of Fisheries and Oceans has produced distribution lists of endangered mussels and fish species for each Conservation Authority (CA). Therefore, mussel distribution data and chloride concentrations are presented according to CA. Four CAs were selected for in-depth analysis of field-measured chloride levels and laboratory toxicity tests with waters from these habitats. The CAs selected along with their main mussel habitat (i.e. river) were the Grand River CA (Grand River), St. Clair Region CA (Sydenham River), Maitland Valley CA (Maitland River), and Upper Thames River and Lower Thames Valley CAs (Thames River). For the purposes of this summary, data from the Upper and Lower Thames CAs were combined. Chloride concentrations measured from 1998 to 2008 at 105 sites across the CAs were determined by the (Ontario) Provincial Water Quality Monitoring Network (PWQMN) and provided by the Ontario Ministry of the Environment (PWQMN, 2009).

Individual chloride concentrations at each site were averaged over time. These 'site averages' were then averaged to determine an overall mean for each CA, referred herein to as a "CA Mean". Site averages, rather than individual readings were used to calculate each CA Mean to prevent skewing of the mean by differences in sampling frequency or extreme readings. The 'CA Range' demonstrates the maximum and minimum individual chloride concentrations across the CA over the 10 years examined. The number of endangered mussel species reported for each CA was obtained from Canadian Department of Fisheries and Oceans (DFO) maps (DFO, 2010).

3. Results

3.1. Chloride sensitivity in reconstituted waters

Glochidia control survival (24 h) for the four mussel species employed in NaCl exposures with moderately-hard reconstituted water is presented in Table 2. With one exception (*L. siliquoidea*, 2008), all tests met the ASTM (2006) requirement of less than 10% drop in control survival. The 24 h chloride EC50s ranged from 113 mg Cl L^{-1} for *L. fasciola* (2008) to 1430 mg Cl L^{-1} for *L. siliquoidea* (2009) (Fig. 1). In addition to interspecific variation, *L. siliquoidea* glochidia collected from different water bodies exhibited significantly different EC50s. Those collected from Cox Creek (2008) produced an EC50 of 168 (135–198) mg Cl L^{-1}, while those collected from the Maitland River (2009) produced an EC50 of 1430 (1350–2953) mg Cl L^{-1}. In contrast, both tests (2008, 2009) of *L. fasciola* glochidia from a single field site produced relatively similar EC50s (113 (63–163), 285 (163–451) mg Cl L^{-1}, respectively).

A series of exposures with *L. siliquoidea* glochidia demonstrated that chloride sensitivity is influenced by water hardness (Table 3). A linear relationship between the 24 h chloride EC50s and water hardness ($r^2 = 0.97$) was observed for water hardness between 47 and 172 mg $CaCO_3$ L^{-1}, but no further protection was afforded when hardness increased to 322 mg $CaCO_3$ L^{-1}.

3.2. Chloride sensitivity in natural waters

Control survival of *L. fasciola* glochidia in the field-collected waters was more variable (78–93%) than in reconstituted water (87–92%) (Table 1). The 24 h chloride EC50 values for *L. fasciola* glochidia were similar across the natural waters tested (1265–1559 mg Cl L^{-1}) (Table 4), but all were significantly higher than the EC50 (285 (163–451) mg Cl L^{-1}) produced in reconstituted water with glochidia from the same gravid females. The 24 h natural water control survival for *P. fasciolaris's* conglutinate encased glochidia was 95% and the EC50 was 3416 (3059–3835) mg Cl L^{-1}.

Table 2
Pre-exposure ($t = 0$) viability and post-exposure ($t = 24$ h) control survival for freshwater mussel glochidia as well as observed 24 h chloride EC50s (95% confidence intervals) from sodium chloride exposures conducted in reconstituted water.

Mussel species	% Viability ($t = 0$)	% Viability ($t = 24$)	EC50 (95% CI) (mg Cl L^{-1})
Lampsilis siliquoidea[a] (2008)	91.3	77.4	168 (135–189)
Lampsilis siliquoidea (2009)	93.4	93.2	1430 (1350–2953)
Lampsilis cardium	91.1	88.3	817 (770–869)
Lampsilis fasciola[b] (2008)	91.9	92.2	113 (63–163)
Lampsilis fasciola (2009)	93.8	91.4	285 (163–451)
Epioblasma torulosa rangiana	95.2	91.3	244 (230–260)

[a] Gravid *L. siliquoidea* were collected from different water bodies in 2008 and 2009.
[b] Gravid *L. fasciola* were collected from the same site in 2008 and 2009.

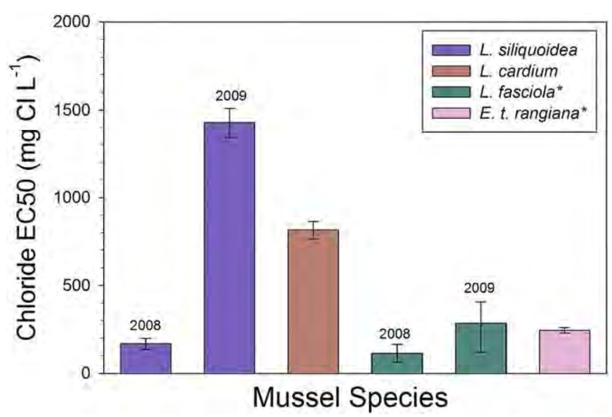

Fig. 1. Chloride EC50s (24 h) for glochidia (larvae) of four species of freshwater mussels. Exposures were conducted in reconstituted moderately-hard water (95–115 mg $CaCO_3$ mg L^{-1}). Error bars represent 95% confidence intervals around the EC50. Asterisks indicate Canadian endangered species. Toxicity tests with *Lampsilis siliquoidea* and *Lampsilis fasciola* were conducted in both 2008 and 2009. *L. siliquoidea* were collected from different water bodies. *L. fasciola* were collected from the same field site both years.

Table 3
Concentrations of chloride, potassium, and water hardness for reconstituted waters employed in acute sodium chloride exposures with *Lampsilis siliquoidea* glochidia as well the pre-exposure ($t = 0$) viability, post-exposure ($t = 24$ h) control survival, and observed 24 h chloride EC50.

Reconstituted water	Chloride (mg L^{-1})	Potassium (mg L^{-1})	Water hardness (mg $CaCO_3$ L^{-1})	% Viability ($t = 0$)	% Viability ($t = 24$)	EC50 (95% CI) (mg Cl L^{-1})
Soft	1.8	1.0	47	89.5	87.7	763 (523–1214)
Moderately-hard	2.8	2.4	99	93.4	93.2	1430 (1350–1518)
Hard	5.5	4.7	172	89.5	86.4	1962 (1447–2953)
Very hard	8.9	9.4	322	93.4	90.7	1870 (1595–2225)

3.3. *Chloride concentrations and mussel distribution in southern Ontario*

A summary of chloride concentrations in four rivers in southern Ontario, along with the number of mussels species found in each habitat is presented in Table 4. Water hardness for the selected rivers ranged from 278 to 322 mg $CaCO_3$ L^{-1}. Although the range in mean chloride concentration was narrow (38–58 mg Cl L^{-1}), the range of individual measured chloride concentrations over the 10 years examined was much broader, covering 2–1300 mg L^{-1}.

4. Discussion

4.1. *Chloride sensitivity in reconstituted waters*

Acute toxicity testing in reconstituted water revealed that glochidia were sensitive to chloride, although significant interspecific and in one case intraspecific variation was observed. The EC50 values for free glochidia of the four mussel species tested ranged from 113 to 1430 mg Cl L^{-1} (Fig. 1). This 13 fold difference in chloride sensitivity between mussel species was not unlike the variation observed by Wang et al. (2007) (12 fold for 9 species) and Gillis et al. (2008) (5 fold for 8 species) in the acute sensitivity of glochidia to copper. Although chloride toxicity data for glochidia is limited, NaCl has been used as a reference toxicant for glochidia toxicity tests. Bringolf et al. (2007) reported EC50s from 0.55 to 3.3 g NaCl L^{-1} (334–2008 mg Cl L^{-1}) for five species of mussel glochidia, Valenti et al. (2007) reported EC50s from 2.68 to 3.08 g NaCl L^{-1} (1625–1868 mg Cl L^{-1}) for three species, and finally Cope et al. (2008) reported EC50s of 2.0 and 2.7 g NaCl L^{-1} (1213–1638 mg Cl L^{-1}) for *L. siliquoidea* glochidia. In this study there also appears to be intraspecific variation in chloride sensitivity. Although *L. fasciola* collected from the same site (Grand River, ON) on two different occasions produced somewhat similar EC50s (113 and 285 mg Cl L^{-1}), *L. siliquoidea* glochidia from two separate water bodies produced EC50s that varied by eight fold (Maitland River, 1430 mg L^{-1}; Cox Creek 168 mg L^{-1}). Perhaps the discrepancy is simply due to the fact that one batch of glochidia was healthier (Maitland River, control survival 93.2%) than the other (Cox Creek, control survival 77.4%) or perhaps prior exposure or even acquired tolerance may alter the response of glochidia to contaminants. But regardless, these data indicate that mussels from different water bodies may respond differently to chloride. While this observation was only based on the chloride sensitivity of one mussel species from two watersheds, possible differences in contaminant sensitivity across watersheds should be considered when selecting gravid females for toxicity testing with glochidia.

Even taking the variability between species into account, glochidia are still notably more sensitive to chloride than most previously tested aquatic organisms. While a full review of chloride toxicity in freshwater organisms is beyond the scope of this paper, Table 5 illustrates that compared to other groups, freshwater mussel larvae, were more sensitive to chloride. Particularly interesting is that some species of mussel glochidia (Fig. 1) experience chloride toxicity at a fraction of the concentration required to kill *Daphnia* (Mount et al., 1997; Harmon et al., 2003), a standard test organism often used to assess the toxicity of chemicals and effluents. Implications of this sensitivity for natural populations of freshwater mussels are discussed below.

4.2. *Effect of water hardness*

Water hardness had a significant effect on the sensitivity of glochidia to chloride. A two fold increase in the EC50 was observed when hardness increased from 47 to 99 mg $CaCO_3$ L^{-1}, but further increases in hardness were less effective at protecting glochidia (Table 3). The ameliorating effect of water hardness on chloride toxicity has been previously documented, in fact the state of Iowa has recently (2009) updated water quality criteria for chloride to adjust for water hardness (Iowa Department of Natural Resources,

Table 4
Summary of chloride concentrations in four significant mussel habitats in southern Ontario, the total number of mussel species and species at risk found in each habitat, as well as the observed 24 h chloride EC50s and EC20s for *Lampsilis faciola* glochidia in toxicity tests conducted with salt-spiked samples of those waters.

Conservation authority	CA mean chloride (mg L^{-1})	CA range chloride (mg L^{-1})	Observed EC50 (mg L^{-1})	Observed EC20 (mg L^{-1})	Total mussel species	Mussels species at risk[a,b]
Grand River	53 (1), $n = 45$	2–507	1313 (1239–1394)	432 (365–496)	25[c]	9
St. Clair Region	42 (14), $n = 9$	8–149	1559 (1338–1824)	403 (155–617)	34[d]	12
Maitland Valley	38 (29), $n = 13$	7–212	1391 (1308–1481)	261 (174–342)	9[e]	2
Upper Thames River & LowerThames Valley	58 (38), $n = 38$	6–1300	1265 (1167–1372)	153 (34–258)	26[f]	11

Watersheds in Ontario are organized by Conservation Authority (CA). Chloride data provided by the Ontario Ministry of the Environment (2009). Mean chloride values and ranges are for data collected from 1998 to 2008. Values reported as 'Mean' are the average of all site averages (repeated sampling at one site over time) for each CA. The number of individual site averages used to determine a 'CA Mean' (with standard deviation) is reported as *n*.
[a] Endangered species in Canada are designated by the Committee on the Status of Endangered Wildlife in Canada (COSEWIC, 2007).
[b] Endangered species data, Department of Fisheries and Oceans, 2010.
[c] Metcalfe-Smith et al., 2000.
[d] Jacques Whitford Environment Limited, 2004.
[e] D.J. McGoldrick, J.L. Metcalfe-Smith, Environment Canada, Burlington, ON, Canada, unpublished data.
[f] Morris and Edwards, 2007.

Table 5
Acute toxicity of chloride (LC50s or EC50s) to various aquatic organisms illustrating the range of previously reported[a] sensitivities for each group.

Taxonomic Group	Species	Exposure duration (h)	LC50 (mg Cl L^{-1})	Reference
Molluscs	Glochidia[b] (4 species)	24	113–1430	Current study
	Glochidia[b] (5 species)	24	334–2008	Bringolf et al. (2007)
	Glochidia[b] (3 species)	24	1625–1868	Valenti et al. (2007)
	Glochidia[b] (1 species)	24	1213–1638	Cope et al. (2008)
	Physa sp. (snail)	96	3257	Clemens and Jones (1954)
Cladocerans	*Daphnia ambigua*	48	1213	Harmon et al. (2003)
	Daphnia magna	48	2893	Mount et al. (1997)
Amphibians	*Ambystoma maculatum* (larvae)	96	1178	Collins and Russell (2009)
	Bufo americanus (larvae)	96	3926	Collins and Russell (2009)
Fish	*Pimephales promelas*	96	3876	Mount et al. (1997)
	Fundulus kansae	96	9706	Clemens and Jones (1954)

[a] Data were limited to peer-reviewed publications.
[b] Free glochidia (i.e. not encased in conglutinates).

2009). The protection provided by hard water is beneficial for the freshwater mussels of southern Ontario as many key mussel habitats have very hard water (Table 1).

4.3. Chloride sensitivity in natural waters

L. fasciola glochidia were significantly less sensitive to salt in natural water than in reconstituted water. Some of the discrepancy can be explained by difference in water hardness because all of the natural waters tested were much harder (278–322 mg $CaCO_3$ L^{-1}) than the moderately-hard reconstituted water (100 $CaCO_3$ L^{-1}) used in the *L. fasciola* exposures. However, the four fold difference in EC50s is much larger than would be expected based solely on the difference in hardness because the *L. siliquoidea* exposures with a similar increase (100–322 mg $CaCO_3$ L^{-1}) produced less than a 30% difference in the chloride EC50. These data suggest that in addition to the protection provided by elevated water hardness that other water chemistry factors contributed to the reduced toxicity of chloride in natural waters.

The EC50 (3416 mg Cl L^{-1}) of conglutinate encased *P. fasciolaris* glochidia exposed to the salt-augmented water of the Grand River is nearly three times that of *L. fasciola* glochidia in the same water. This could simply be another example of intraspecific variation in glochidia contaminant sensitivity, or it could indicate that the life history strategy of encasing glochidia in conglutinates not only facilitates host transfer, but may also provide protection for the encased glochidia from chloride and potentially other ionic waterborne contaminants. For the current study it is not possible to determine the reason for the higher EC50 in conglutinate encased glochidia, although a previous study demonstrated that *P. fasciolaris* conglutinate encased glochidia were four fold less sensitive to copper than glochidia released from their conglutinate (Gillis et al., 2008).

The advantage of using reconstituted waters in toxicity tests is that they provide consistency and permit comparison between studies and between species; the disadvantage is that EC50s produced in reconstituted water may not necessarily predict how an organism will respond to that contaminant in its natural environment. On the other hand, one disadvantage of natural water exposures is that other contaminants may be present which can contribute to toxicity. Perhaps the variable (78–93%) control survival in the natural waters examined was due to other contaminants. One such contaminant of concern is potassium which is much more toxic than chloride. Imlay (1973) observed that only 2 of 10 rivers in the United States with potassium concentrations greater than 4 mg L^{-1} supported freshwater mussels, whereas 28 of 39 rivers with levels less than 4 mg L^{-1} were found to support mussels. All four natural waters tested were at or near this apparent threshold (Table 1). Moreover, preliminary data (Gillis unpublished) indicate that glochidia are sensitivity to potassium (*L. fasciola* 24 h moderately-hard reconstituted water LC50, 10 mg K L^{-1}). The potential effect of elevated potassium on freshwater mussel recovery requires further study especially because potassium chloride is currently being used as an alternative to sodium chloride for winter road maintenance (Evans and Frick, 2001). There have also been concerns that in some water bodies copper may be negatively impacting freshwater mussels (March et al., 2007; Ward et al., 2007). Background copper levels in the natural waters tested ranged from 5 to 6 μg L^{-1} (Table 1), but considering the level of DOC in these waters (>4 mg C L^{-1}) it is unlikely that copper contributed to the observed toxicity (Gillis et al., 2010; Wang et al., 2009). Unfortunately no comment can be made on the potential contribution of organic contaminants (such as pesticides) to the variation in control survival because these water samples were not analyzed for organics.

4.4. Implications for native populations of mussels

The rivers and streams of the lower Great Lakes Basin contain the richest assemblage of freshwater mussels in Canada (Metcalfe-Smith et al., 1998). After surveying historic (pre-1960) and more recent (up to 1996) mussel distribution data for southern Ontario, Metcalfe-Smith et al. (1998) concluded that significant species losses (15–30%) had already occurred, thereby verifying that the freshwater mussel decline documented in the U.S. (Neves et al., 1997) is also occurring in Canada. Although, many factors from exotic species to habitat loss (Williams et al., 1993; Bogan, 1993; Gillis and Mackie, 1994) are thought to have contributed to the decline of freshwater mussels, the role of waterborne contaminants remains uncertain. The chloride levels in the mussel habitats examined along with the heightened sensitivity of glochidia to NaCl suggest that chloride may in fact be impacting freshwater mussels in the lower Great Lake Basin. Even though the 'Mean' chloride concentrations (10–50 mg Cl L^{-1}) indicate that during the majority of the year, chloride levels are below the EC50, there are many documented instances where chloride concentrations would be toxic to glochidia. Even considering the higher EC50s produced in field-collected water (1265–1559 mg Cl L^{-1}), some rivers such as the Thames River, a habitat that supports eleven federally endangered species of mussels can exceed (1300 mg L^{-1}) the level found to be toxic to 50% of the glochidia.

Based on the results of this study, it is clear that even short-term spikes in chloride during the period of glochidia release would have a negative impact on the successful reproduction of freshwater mussels. Fortunately for most species, timing appears to be in the glochidia's favor, because the largest chloride spikes typically coincide with snowmelt (PWQMN, 2009), months before glochidia are released into the water column. However, many mussel species are referred to as long term brooders. This means that glochidia are held in the marsupial gills throughout the winter and released the following spring (Barnhart et al., 2008). Although there is evidence that brooding glochidia are less sensitive to waterborne copper than those released to the water column (Jacobson et al., 1997), it is unknown whether brooding glochidia are affected by the chloride laden waters their mothers are exposed to in early spring. While glochidia are only present in the water column during the spring and summer months, juvenile mussels, which have also been shown to be sensitive to some waterborne contaminants (Wang et al., 2007) would be exposed to chloride throughout the year. Another potential and likely year-round source of chloride is contaminated groundwater (Kincaid and Findlay, 2009). Juvenile mussels, because they remain burrowed in the sediment for their first few years of life, would be most at risk from elevated chloride from groundwater upwelling. Although the present study examined the potential risk of chloride toxicity in lotic habitats of freshwater mussels, urban basin analysis suggests the potential for chloride toxicity may be even greater in lentic habitats. Chloride contributes to densimetric stratification of receiving waters (Marsalek, 2003; Eyles and Meriano, 2010) which results in higher chloride concentrations just above the sediment-water interface in static or slow moving water bodies. Such stratification could exacerbate the risk of acute chloride toxicity for freshwater mussels living in embayments and lakes subjected to road salt runoff.

5. Conclusion

This study has demonstrated that compared to most other aquatic organisms that glochidia are very sensitive to chloride. It has also been demonstrated that increased water hardness and natural river water offer 'protection' from acute chloride toxicity. But even considering these ameliorating factors, the level of chloride, likely from road salt runoff, in key mussel habitats in the lower Great Lakes Basin, may pose a threat to the successful reproduction and thus recovery of endangered freshwater mussels in this area.

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References

ASTM E729-96, 2003. Standard Guide for Conducting Acute Toxicity Tests on Test Materials with Fishes, Macroinvertebrates and Amphibians. ASTM International, West Conshohocken, PA.

ASTM E2455-05, 2006. Standard Guide for Conducting Laboratory Toxicity Tests with Freshwater Mussels. ASTM International, West Conshohocken, PA.

Augspurger, T., Keller, A.E., Black, M.C., Cope, W.G., Dwyer, F.J., 2003. Water quality guidance for the protection of freshwater mussels (Unionidae) from ammonia exposure. Environmental Toxicology and Chemistry 22, 2569–2575.

Barnhart, M.C., Haag, W.R., Roston, W.N., 2008. Adaptations to host infection and larval parasitism in Unionoida. Journal of the North American Benthological Society 27, 370–394.

Bogan, A.E., 1993. Freshwater bivalve extinctions (Mollusca: Unionidae): a search for causes. American Zoologist 33, 599–609.

Bringolf, R.B., Cope, W.G., Barnhart, M.C., Mosher, S., Lazaro, P.R., Shea, D., 2007. Acute and chronic toxicity of pesticide formulations (atrazine, chlorpyrifos and permethrin) to glochidia and juveniles of *Lampsilis siliquoidea*. Environmental Toxicology and Chemistry 26, 2086–2093.

Clemens, H.P., Jones, W.H., 1954. Toxicity of brine water from oil wells. Transactions of the American Fisheries Society 84, 97–109.

Collins, S.J., Russell, R.W., 2009. Toxicity of road salt to Nova Scotia amphibians. Environmental Pollution 157, 320–324.

Cope, W.G., Bringolf, R.B., Buchwalter, D.B., Newton, T.J., Ingersoll, C.G., Wang, N., Augspurger, T., Dwyer, F.J., Barnhart, F.C., Neves, R.J., Hammer, E., 2008. Differential exposure, duration, and sensitivity of unionoidean bivalve life stages to environmental contaminants. Journal of the North American Benthological Society 27, 451–462.

COSEWIC, 2007. Canadian Species at Risk. Available at. Committee on the Status of Endangered Wildlife in Canada. www.cosewic.gc.ca.

Department of Fisheries and Oceans, 2010. Distribution maps for mussel and fish species at risk. Available at. www.conservation-ontario.on.ca/projects/DFO.html.

Environment Canada and Health Canada, 2001. Priority Substances List Assessment Report for Road Salts, ISBN 0-662-31018-7 Cat. No. En40-215/63E.

Environment Canada, 2005. Guidance Document on Statistical Methods for Environmental Toxicity Tests. Method Development and Applications Section. Environmental Technology Centre, Environment Canada. Report EPS 1/RM/46.

Evans, M., Frick, C., 2001. The effects of road salts on aquatic ecosystems. National Water Research Institute Contribution Series No. 02-308.

Eyles, N., Meriano, M., 2010. Road-impacted sediment and water in a Lake Ontario watershed and lagoon, City of Pickering, Ontario, Canada: an example of urban basin analysis. Sedimentary Geology 224, 15–28.

Gillis, P.L., Mackie, G.L., 1994. Impact of the zebra mussel, *Dreissena polymorpha*, on populations of Unionidae (Bivalvia) in Lake St. Clair. Canadian Journal of Zoology 72, 1260–1271.

Gillis, P.L., Mitchell, R.J., Schwalb, A.N., McNichols, K.A., Mackie, G.L., Wood, C.M., Ackerman, J.D., 2008. Sensitivity of the glochidia (larvae) of freshwater mussels to copper: assessing the effect of water hardness and dissolved organic carbon on the sensitivity of endangered species. Aquatic Toxicology 88, 137–145.

Gillis, P.L., McGeer, J.C., Wilkie, W.P., Mackie, G.L., Ackerman, J.D., 2010. The effect of natural dissolved organic carbon on the acute toxicity of copper to larval freshwater mussels (glochidia). Environmental Toxicology and Chemistry 29, 2519–2528.

Harmon, S.M., Specht, W.L., Chandler, G.T., 2003. A comparison of the Daphnids *Ceriodaphnia dubia* and *Daphnia ambigua* for their utilization in routine toxicity testing in the Southeastern United States. Archives of Environmental Contamination and Toxicology 45, 79–85.

Howard, K.W.F., Haynes, J., 1993. Groundwater contamination due to road de-icing chemicals – salt balance implications. Geoscience Canada 20, 1–8.

Imlay, M.J., 1973. Effects of potassium on survival and distribution of freshwater mussels. Malacologia 2, 97–113.

Iowa Department of Natural Resources, 2009. Water quality standards review: chloride, sulfate and total dissolved solids. Available at. http://www.iowadnr.gov/water/standards/chloride.html.

Jackson, R.B., Jobbágy, E.G., 2005. From icy roads to salty streams. Proceedings of the National Academy of Sciences of the United States of America 102, 14487–14488.

Jacobson, P.J., Neves, R.J., Cherry, D.S., Farris, J.L., 1997. Sensitivity of glochidial stages of freshwater mussels (Bivalvia: Unionidae) to copper. Environmental Toxicology and Chemistry 16, 2384–2392.

Jacques Whitford Environment Limited, 2004. Sydenham River Recovery Project: Synthesis and Analysis of Background Data; Project No. ONO50368: Ottawa, ON.

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. Proceedings of the National Academy of Sciences of the United States of America 102, 13515–13520.

Kelly, V.R., Lovett, G.M., Weathers, K.C., Findlay, S.E.G., Strayer, D.L., Burns, D.J., Likens, G.E., 2008. Long-term sodium chloride retention in a rural watershed: legacy effects of road salt on streamwater concentration. Environmental Science and Technology 42, 410–415.

Kincaid, D.W., Findlay, S.E., 2009. Sources of elevated chloride in local streams: groundwater and soil as potential reservoirs. Water, Air, and Soil Pollution 203, 335–342.

Lydeard, C., Cowie, R.H., Onder, W.F., Bogan, A.E., Bouchet, P., Clark, S.A., Cummings, K.S., Fresh, T.J., Gorgominy, O., Herbert, D.G., Hershler, R., Perez, K.E., Roth, B., Seddon, M., Strong, E.E., Thompson, F.G., 2004. The global decline of nonmarine mollusks. BioScience 54, 321–330.

March, F.A., Dwyer, F.J., Augspurger, T., Ingersoll, C.G., Wang, N., Mebane, C.A., 2007. An evaluation of water quality guidance and standards for copper. Environmental Toxicology and Chemistry 26, 2066–2074.

Marsalek, J., 2003. Road salts in urban storm water: an emerging issue in storm water management in cold climates. Water Science and Technology 48, 61–70.

Metcalfe-Smith, J.L., Staton, S.K., Mackie, G.L., Lane, N.M., 1998. Changes in the biodiversity of 9 freshwater mussels in the Canadian waters of the lower Great Lakes drainage basin over the past 140 years. Journal of Great Lakes Research 24, 845–858.

Metcalfe-Smith, J.L., Mackie, G.L., Di Maio, J., Staton, S.K., 2000. Changes over time in the diversity and distribution of freshwater mussels (Unionidae) in the Grand River, Southwestern Ontario. Journal of Great Lakes Research 26, 445–459.

Morris, T.J., Edwards, A., 2007. Freshwater Mussel Communities of the Thames River, Ontario: 2004–2005. Manuscript 2810. Canadian Manuscript Report of Fisheries and Aquatic Sciences, Ottawa, ON.

Mount, D.R., Gulley, D.D., Hockett, J.R., Garrison, T.D., Evans, J.M., 1997. Statistical models to predict the toxicity of major ions to *Ceriodaphia dubia, Daphnia magna*, and *Pimephales promelas* (Fathead minnows). Environmental Toxicology and Chemistry 16, 2009–2019.

Neves, R.J., Bogan, A.E., Williams, J.D., Ahlstedt, S.A., Hartfield, P.W., 1997. Status of aquatic mollusks in the southeastern United States: a downward spiral of diversity. In: Benz, G.W., Collins, D.E. (Eds.), Aquatic Fauna in Peril: The Southeastern Perspective Special Publication 1. Southeast Aquatic Research Institute; Lenz Design and Communications, Decatur, GA, pp. 43–85.

Parmalee, P.W., Bogan, A.E., 1998. The Freshwater Mussels of Tennessee. University of Tennessee Press, Knoxville, TN, pp 328.

PWQMN. Provincial Water Quality Monitoring Network, 2009. Dataset Records: 2009. Available at. Ontario Ministry of the Environment. http://www.ene.gov.on.ca/envision/water/pwqmn/index.htm.

Ricciardi, A., Rasmussen, J.B., 1999. Extinction rates of North American freshwater fauna. Conservation Biology 13, 1220–1222.

Roy, J.W., Bickerton, G., 2010. Proactive screening approach for detecting groundwater contaminants along urban streams at the reach-scale. Environmental Science and Technology 44, 6088–6094.

Strayer, D.L., Downing, J.A., Haag, W.R., King, T.L., Layzer, J.B., Newton, T.J., Nichols, S.J., 2004. Changing perspectives on pearly mussels, North America's most imperiled species. BioScience 54, 429–439.

Trowbridge, P.R., Kahl, J.S., Sassan, D.A., Heath, D.L., Walsh, E.M., 2010. Relating road salt to exceedances of the water quality standard for chloride in New Hampshire streams. Environmental Science and Technology 44, 4903–4909.

Valenti, T.W., Cherry, D.S., Neves, R.J., Locke, B.A., Schmerfeld, J.J., 2007. Case study: sensitivity of mussel glochidia and regulatory test organisms to mercury and a reference toxicant. In: Farris, J.L., Van Hassel, J.H. (Eds.), Freshwater Bivalve Ecotoxicology. CRC Press, Pensacola, FL, pp. 351–367.

Wang, N., Ingersoll, C.G., Hardesty, D.K., Ivey, C.D., Kunz, J.L., May, T.W., Dwyer, F.J., Roberts, A.D., Augspurger, T., Kane, C.M., Neves, R.J., Barnhart, M.C., 2007. Acute toxicity of copper, ammonia, and chlorine to glochidia and juvenile mussels. Environmental Toxicology and Chemistry 26, 2036–2047.

Wang, N., Mebane, C.A., Kunz, J.L., Ingersoll, C.G., May, T.W., Arnold, W.R., Santore, R.C., Augspurger, T., Dwyer, F.J., Barnhart, M.C., 2009. Evaluation of acute copper toxicity to juvenile freshwater mussels (fatmucket, *Lampsilis siliquoidea*) in natural and reconstituted waters. Environmental Toxicology and Chemistry 28, 2367–2377.

Ward, S., Augspurger, T., Dwyer, F.J., Kane, C., Ingersoll, C.G., 2007. Risk assessment of water quality in three North Carolina streams supporting federally-endangered freshwater mussels. Environmental Toxicology and Chemistry 26, 2075–2085.

Williams, J.D., Warren, M.L., Cummings, K.S., Harris, J.L., Neves, R.J., 1993. Conservation status of freshwater mussels of the United States and Canada. Fisheries 18, 6–22.

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Environmental Toxicology

Acute Toxicity of Sodium Chloride and Potassium Chloride to a Unionid Mussel (*Lampsilis siliquoidea*) in Water Exposures

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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 $CaCO_3$) and other major ions (Ca, K, SO_4, or HCO_3) 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 the 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 1597 mg Cl/L and the EC50s for juvenile mussels increased from 911 to 3092 mg Cl/L with increasing water hardness from 50 to 300 mg/L. Increasing K from 0.4 to 1.9 mg/L, SO_4 from 13 to 40 mg/L, or HCO_3 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 with juvenile mussels and that 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. *Environ Toxicol Chem* 2018;9999:1–9. © 2018 SETAC. This article is a US government work and, as such, is in thepublic domain in the United States of America.

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 (US Environmental Protection Agency 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 WQS for K have not been developed in the United States.

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 United States 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. 2007a, 2007b, 2010, 2016, 2017; Cope et al. 2008; Miao et al. 2010; Gillis 2011). However, freshwater mussels are

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generally underrepresented 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). That 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 $CaCO_3$ but that it 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, although 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) 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 (N. Wang et al., unpublished manuscript).

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. The primary salt used to develop existing regulatory guidance from the USEPA and some US states has been NaCl, but this guidance has focused specifically on Cl concentration, rather than the combination of ions. Although 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 International 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 broods 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 1 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 $CaCO_3$, alkalinity 250 mg/L as $CaCO_3$, 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 (0.2–1.5 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 nonviable microalgal *Nannochloropsis* concentrate and Shellfish Diet (a unique mix of 4 microalgae, *Tisochrysis lutea*, *Pavlova* sp., *Tetraselmis* sp., *Thalassiosira weissflogii*; Reed Mariculture). 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, approximately 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 International 2017). Three subsamples of approximately 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 min was recorded. Open and closed glochidia were calculated as described in the standard methods (ASTM International 2017): Viability (%) = 100 × (number of closed glochidia after adding NaCl solution − number of closed glochidia before adding NaCl solution) ÷ (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 International 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, gravid female mussels were transported to Missouri State University (Springfield, MO) and held in moderately hard reconstituted water (80–100 mg hardness as $CaCO_3$/L, pH ~7.8; US Environmental Protection Agency 2002). Approximately 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 in this section 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 approximately 4000 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 wk following fish infestation. Juveniles were collected during the peak drop-off days (typically 2–4 d).

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/d. 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 approximately 500 lux with a 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 approximately 100 mL of test solution. At the beginning of each exposure, approximately 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 ± 1 °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., percentage of the initial mean control viability; Wang et al. 2007a). For the viability determination, a subsample of approximately 100 glochidia was 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 min was recorded and the viability rate calculated as described in the previous section. 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 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) 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 $CaCO_3$; US Environmental Protection Agency 2002). The use of the reconstituted water was to compare the response of glochidia from the present study with the response of those tested in the same water in a previous study, in which glochidia of several mussel species were found to be highly sensitive to NaCl (Gillis 2011). Concurrently, an additional 4 NaCl tests were conducted with glochidia at 4 different levels of hardness in CERC well water and 3 diluted well waters to evaluate the influence of hardness on NaCl toxicity to glochidia. The moderately hard reconstituted water was prepared by adding reagent-grade salts ($CaSO_4 \times 2H_2O$, $MgSO_4$, KCl, and $NaHCO_3$; EM Science) into deionized water (US Environmental Protection Agency 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) was used to prepare NaCl concentrations. Seven NaCl concentrations (50% serial dilution; nominal concentrations 160, 320, 630, 1250, 2500, 5000, and 10 000 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 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 the influence of hardness on NaCl toxicity to juvenile mussels. The 4 test waters were prepared as described in the previous section for the glochidia tests. The other 4 NaCl tests were conducted in the 50 hard water with 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, $CaCl_2$, Na_2SO_4, or $NaHCO_3$ into the 50 hard water to match the level of

K, Ca, SO_4, or HCO_3 in the 200 hard water. In each case, the counterion for the manipulated ion was either Na or Cl, addition of which was negligible compared with 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, 5 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 ($30 \times 18 \times 10$ 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. Approximately 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-min observation period).

Water quality and chemical analyses

Water quality (dissolved oxygen, pH, conductivity, hardness, and alkalinity) was 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 SO_4) 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). The ICP-MS methods were similar to USEPA method 6020B (US Environmental Protection Agency 2014). Water samples for major anion analyses were stored at 4 °C for up to 28 d before analysis and analyzed by ion chromatography (ICS-1100; Dionex) using a method similar to USEPA method 9056A (US Environmental Protection Agency 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, multiparameter meter. 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 ICP-MS as described previously.

Analyses of tested chemicals were performed by the CERC chemistry laboratory, following internal standard operating procedures and quality assurance/quality control protocols developed based on the USEPA documents (US Environmental Protection Agency 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 International 2017) using the Toxicity Relationship Analysis Program (Ver 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-Karber or trimmed Spearman-Karber method was used to determine the EC50s following the flowchart recommended by the USEPA (US Environmental Protection Agency 2002) using TOXSTAT® software (Ver 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%; Supplemental Data, Table S2). Mean viability of the glochidia in the controls was 93% at the beginning of the acute test and 94% at the end of the test (Supplemental Data, Table S2). The adjusted control viability at the end of the test was 100% and met the test acceptability criterion of $\geq$90% control viability in acute 24-h exposures with glochidia (ASTM International 2017).

Low viability (<50%) was observed at the 2 high concentrations at the end of the test (Supplemental Data, 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

TABLE 1: Mean measured water quality characteristics[a] (standard deviation in parentheses) and EC50s (95% confidence interval in parentheses) 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 (mg/L as $CaCO_3$; $n=6$)	Alkalinity (mg/L as $CaCO_3$; $n=6$)	Major cation and anion (mg/L; $n=1$)						EC50 (mg K/L)
				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)

[a]Water quality was measured in the control, medium-, and high-exposure concentrations at the beginning and end of tests. Major ions were measured in the control water at the beginning of the test.
CERC = Columbia Environmental Research Center; EC50 = 50% effect concentration.

a previous 96-h KCl exposure (46 mg K/L; Wang et al. 2017) and an EC50 for 2-mo-old juvenile fatmucket in the first 4 d of a chronic 28-d KCl exposure in the companion study (37 mg K/L; N. Wang et al., unpublished manuscript). All of 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 present 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, unpublished data) 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 (R. 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 (US Environmental Protection Agency 2002), and mean measured hardness values 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 (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 test acceptability criterion of ≥90% control viability (ASTM International 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 1597 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 moderately hard reconstituted water of the present study (Table 2) was between the EC50s of 168 and 1430 mg Cl/L from 2 previous acute NaCl tests with fatmucket glochidia in a moderately hard reconstituted water (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 present study, though the diluted well water in the present study showed a much stronger correlation between water hardness and NaCl toxicity (Figure 1) than the Gillis (2011) study, which used reconstituted waters based on USEPA formulas (US Environmental Protection Agency 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

TABLE 2: Mean measured water quality characteristics[a] (standard deviation in parentheses) of different base waters and EC50s (95% confidence intervals in parentheses) 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 $CaCO_3$; $n=6$)	Alkalinity (mg/L as $CaCO_3$; $n=6$)	Major cation and anion (mg/L; $n=6$)						EC50 (mg Cl/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)

[a]Water quality was measured in the control, medium-, and high-exposure concentrations at the beginning and end of tests. Major ions were measured in the control water at the beginning of the test.
[b]The EC50 was 603 (564–645) when glochidia viability was determined using a saturated KCl solution (rather than NaCl solution).
CERC = Columbia Environmental Research Center; EC50 = 50% effect concentration; MHRW = moderately hard reconstituted water.

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, 2 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 (EC50 = 0.76 mM/L) was more than 20-fold higher than the toxicity of NaCl to glochidia (EC50 = 20.5 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).

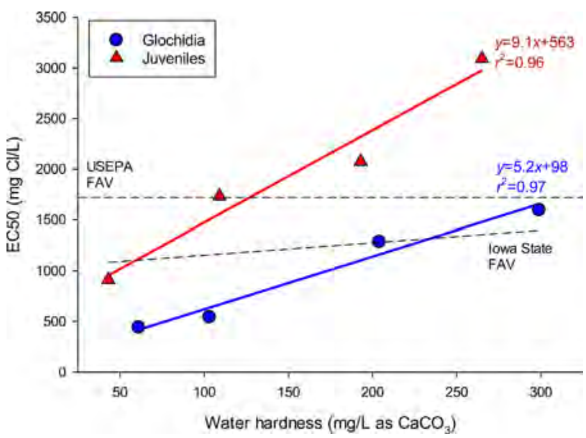

FIGURE 1: Relationships between acute 50% effect concentrations (EC50s) and water hardness obtained in a 24-h NaCl toxicity test with glochidia of fatmucket (*Lampsilis siliquoidea*) and a 96-h NaCl toxicity test with juvenile fatmucket. Dashed line indicates final acute value (FAV) in the US Environmental Protection Agency (USEPA) water quality criteria (US Environmental Protection Agency 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.

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 HCO_3 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 (differences typically within 10%; Supplemental Data, Table S4).

Mean control survival in all 8 tests was 100% (Supplemental Data, Table S4) and met the test acceptability criterion of ≥90% control survival (ASTM International 2017). The EC50s ranged from 911 to 3092 mg Cl/L in the 4 hardness waters of 50 to 300 mg/L (tests 1–4 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, SO_4, or HCO_3 (tests 5, 7, and 8 in Table 3), the EC50s ranged from 937 to 1164 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 (2106 mg Cl/L; test 6 in Table 3) increased by a factor of 2 in comparison with the EC50 from the 50 hard water (test 1; with no overlapping of the 95% confidence limits) and was approximately equal to the EC50 from the 200 hard water (2075 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 covary 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 that for *C. dubia*, the latter showing only an approximately 50% increase in acute EC50 over a similar range in Ca (Erickson et al. 2018). The results

TABLE 3: Mean measured water quality characteristics[a] (standard deviation in parentheses) and EC50s (95% confidence intervals in parentheses) in acute 96-h NaCl toxicity tests with juvenile fatmucket (*Lampsilis siliquoidea*) conducted in CERC 50, 100, 200, and 300 hard well water, and in the 50 hard well water with addition of KCl, $CaCl_2$, Na_2SO_4, or $NaHCO_3$ to match the level of K, Ca, Na, or HCO_3 in the 200 hard water

					Major cation and anion (mg/L; $n=1$)							
Test	Dissolved oxygen (mg/L; $n=6$)	pH ($n=6$)	Hardness (mg/L as $CaCO_3$; $n=6$)	Alkalinity (mg/L as CaCO3; $n=6$)	Ca	K	Mg	Na	Cl	SO_4	HCO_3	EC50 (mg Cl/L)
1. 50 hard	7.90 (0.26)	7.8 (0.2)	43 (0.6)	36 (0.8)	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)	94 (3.7)	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)	158 (4.5)	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)	221 (2.1)	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)	38 (2.0)	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)	38 (3.1)	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)	36 (2.0)	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)	164 (3.0)	9.2	0.4	3.7	63	4.7	8.5	200	937 (877–1000)

[a]Water quality was measured in the control, medium-, and high-exposure concentrations at the beginning and end of tests. Major ions were measured in the control water at the beginning of the test.
[b]An EC50 could not be calculated because of no partial mortality (Supplemental Data, 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%].
CERC = Columbia Environmental Research Center; EC50 = 50% effect concentration.

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 SO_4 from 13 to 40 mg/L on the NaCl toxicity to the mussels in the 50 mg/L hardness water (Table 3) and confirm 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 SO_4 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 Na_2SO_4 mixtures to juvenile fatmucket in the CERC 100 hard water showed that the acute toxicity of NaCl significantly increased with increasing concentrations of SO_4 from 350 to 1800 mg/L (C.D. Ivey, unpublished data). The additive toxicity of the NaCl and Na_2SO_4 mixtures has 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 SO_4 might be underprotective if there are substantial co-occurring concentrations of either ion.

The EC50 of 1733 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 (1897–2246 mg Cl/L; $n=5$) for newly transformed fatmucket tested in the 100 hard water in a previous study (Wang et al. 2017) and close to the EC50 of 1500 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 WQC and WQS in the United States

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 (N. Wang et al., unpublished manuscript), fatmucket would be the second most sensitive species among tested freshwater organisms to K, and the 4 most sensitive genera would all be mussels. No USEPA WQC or state WQS for K have 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 used for deriving the 1988 USEPA acute WQC for Cl (i.e., one-half final acute value; US Environmental Protection Agency 1988) and equal to or 2-fold below the hardness- and sulfate-dependent final acute values 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 final acute values 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).

Supplemental Data—The Supplemental Data are available on the Wiley Online Library at DOI: 10.1002/etc.4206.

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Data availability—Data, associated metadata, and calculation tools are available from the corresponding author (nwang@usgs.gov).

REFERENCES

ASTM International. 2017. Standard guide for conducting laboratory toxicity tests with freshwater mussels. E2455-06 (2013). In *Annual Book of ASTM Standards*, Vol 11.06. Philadelphia, PA, USA.

Augspurger 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.

Barnhart MC. 2006. Bucket of muckets: 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.

Canadian Council of Ministers of the Environment. 2011. *Canadian water quality guidelines for the protection of aquatic life: Chloride*. Winnipeg, Canada.

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 North 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. American Public Health Association, Water Environment Federation, American Water Works Association, Washington, DC.

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.

Erickson RJ. 2015. *Toxicity Relationship Analysis Program (TRAP)*, Ver 1.30a. EPA/600/C-11/002. US 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.

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.

Gillis PL. 2011. Assessing the toxicity of sodium chloride to the glochidia of freshwater mussels: Implications for salinization of surface waters. *Environ Pollut* 159:1702–1708.

Haag WR. 2012. *North American Freshwater Mussels: Natural History, Ecology, and Conservation*. Cambridge University Press, New York, NY, USA.

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* 31:1611–1620.

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, Bespalaya 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.

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.

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 siliquoidea*) in acute water exposure to ammonia. *Environ Toxicol Chem* 29:2112–2116.

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.

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 siliquoidea*). *Sci Total Environ* 532:309–315.

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.

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.

US Environmental Protection Agency. 1988. Ambient water quality criteria for chloride. EPA-440-5-88-001. 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.

US 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. 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.

Wang N, Dorman RA, Ingersoll CG, Hardest 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.

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.

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.

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.

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CERTIFICATE OF SERVICE

I, the undersigned, certify that on December 28, 2018, I served electronically the attached PRE-FILED TESTIMONY OF LAURA BARGHUSEN, OPENLANDS to the participants listed on the attached SERVICE LIST.

Stacy Meyers

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Stacy Meyers