Management of Biological Invasions (2016) Volume 7, Issue 3: 257–268
DOI: http://dx.doi.org/10.3391/mbi.2016.7.3.05

Open Access

Research Article

# Toxicity of potassium chloride to veliger and byssal stage dreissenid mussels related to water quality

Christine M. Moffitt[1,*], Kelly A. Stockton-Fiti[2] and Renata Claudi[3]

[1]*US Geological Survey, Idaho Cooperative Fish and Wildlife Research Unit, University of Idaho, Moscow, ID 83844, USA*
[2]*KASF Consulting, Henderson, NV 89012, USA*
[3]*RNT Consulting Inc, Picton, Ontario, K0K 2T0, Canada*
**Corresponding author*
*E-mail: cmoffitt@uidaho.edu*

Received: 10 February 2016 / Accepted: 27 May 2016 / Published online: 23 June 2016

*Handling editor*: Catherine de Rivera

## Abstract

Natural resource managers are seeking appropriate chemical eradication and control protocols for infestations of zebra mussels, *Dreissena polymorpha* (Pallas, 1769), and quagga mussels. *D. rostiformis bugensis* (Andrusov, 1897) that have limited effect on non-target species. Applications of low concentrations of potassium salt (as potash) have shown promise for use where the infestation and treatment can be contained or isolated. To further our understanding of such applications and obtain data that could support a pesticide registration, we conducted studies of the acute and chronic toxicity of potassium chloride to dreissenid mussels in four different water sources from infested and non-infested locations (ground water from northern Idaho, surface water from the Snake River, Idaho, USA, surface water from Lake Ontario, Ontario, Canada, and surface water from the Colorado River, Arizona, USA). We found short term exposure of veligers (< 24 h) to concentrations of 960 mg/L KCl produced rapid mortality in water from three locations, but veligers tested in Colorado River water were resistant. We used probit models to compare the mortality responses, predicted median lethal times and 95% confidence intervals. In separate experiments, we explored the sensitivity of byssal stage mussels in chronic exposures (>29 d) at concentrations of 100 and 200 mg/L KCl. Rapid mortality occurred within 10 d of exposure to concentrations of 200 mg/L KCl, regardless of water source. Kaplan-Meier estimates of mean survival of byssal mussels in 100 mg/L KCl prepared in surface water from Idaho and Lake Ontario were 4.9 or 6.9 d, respectively; however, mean survival of mussels tested in the Colorado River water was > 23 d. The sodium content of the Colorado River water was nearly three times that measured in waters from the other locations, and we hypothesized sodium concentrations may affect mussel survival. To test our hypothesis, we supplemented Snake River and Lake Ontario water with NaCl to equivalent conductivity as the Colorado River, and found mussel survival increased to levels observed in tests of veliger and byssal mussels in Colorado River water. We recommend KCl disinfection and eradication protocols must be developed to carefully consider the water quality characteristics of treatment locations.

**Key words:** quagga and zebra mussels, potash, toxicity, invasive species, management

## Introduction

Dreissenid mussels were first identified in North America in the late 1980s and their infestations have posed great challenges to resource managers (Mackie and Claudi 2010; Nalepa and Schloesser 2013). Given potential range of expansion, many researchers have focused on modeling the risks of establishment of these mussels by examining the responses in existing and potential habitats (e.g. Ramcharan et al. 1992; Hinks and Mackie 1997; Allen and Ramcharan 2001; Jokela and Riccardi 2008; Whittier et al. 2008; Naddafi et al. 2011; Karatayev et al. 2015). Recently Davis et al. (2015) warned that interpreting data regarding risks of establishment of quagga mussels *D. rostriformis bugensis* (Andrusov, 1897) based on data from responses of zebra mussels *D. polymorpha* (Andrusov, 1897) in the Great Lakes and Europe may be inappropriate. Many of the models have not been confirmed by controlled investigations, and additional environmental variables not reported or monitored in theses retrospective evaluations may be critical and could be confounding interpretation of responses (Bourgeault et al. 2012).

Federal, state and provincial managers in North America have developed a suite of protocols for detection and have created planning documents to detail prevention protocols and response planning for potential infestations (e.g. National Park Service 2007; Smith and McMartin 2011; Draheim et al. 2013; Heimowitz and Phillips 2014; DeBruyckere et al. 2014; Glomski 2015). Among options in the protocols is

application of muriate of potash, an industrial grade chemical composed primarily of potassium chloride (KCl) in water-soluble form that can be introduced into contained areas of lakes or reservoirs. Such treatments have been suggested after reviewing a suite of chemicals including metallic salts, oxidizing biocides, and nonoxidizing biocides (Culver et al. 2013; Heimowitz and Phillips 2014; Glomski 2015), and conducting several tests of potash. A highly successful eradication of zebra mussels with potash was conducted at Millbrook Quarry, Virginia (Fernald and Watson 2014). The quarry was isolated, and an application of 100 mg/L KCl as potash resulted in eliminating the mussel population without detectable harm to any fish species. The target concentration chosen was based on concentrations from trials that validated the mortality to zebra mussels (Waller et al. 1993; Fernald and Watson 2014). Moreover these protocols were based on studies that indicated the target of 100 mg/L KCl was of low to no risk to non-molluscan aquatic species, vegetation, or humans (Fisher et al. 1991; Wildridge et al. 1998; Edwards et al. 2000).

With these data in mind, managers in the Pacific Northwest (North America), a region without known infestation of dreissenids, have considered applications of KCl as a potential emergency pesticide to respond to infestations of quagga or zebra mussels. The proposed response would be used if an infestation were to occur in an area that could be isolated and treated (Heimowitz and Phillips 2014). However, few if any controlled studies have been conducted to validate this tool as effective on quagga or zebra mussels in waters of the western region. Moreover, confidence in the efficacy of KCl as a toxicant for quagga mussels was lowered after studies by Sykes (2009) at Willow Beach National Fish Hatchery (WBNFH), Arizona (AZ) showed lack of mortality of quagga mussel veligers following a protocol of 1 h exposure to 750 mg/L KCl followed by treatment with 25 mg/L formalin for 2 h. Studies by Pucherelli et al. (2014) further challenged the applicability of this protocol in hatchery trials at Lake Meade hatchery. Previous laboratory studies by Edwards et al. (2000) in Lake Erie waters indicated the solutions of KCl and formalin killed zebra mussel veligers, were safe for the fish, and this protocol for hauling fish from hatcheries would eliminate the risk of carrying live zebra mussels. Sykes (2009) further studied the toxicity of KCl, with concentrations of up to 4,250 mg/L KCl combined with 100 mg/L formalin, but observed no significant mortality in veligers. Sykes (2009) speculated that water quality or species differences may have explained the lack of mortality she observed in exposures. In addition, potential interactions of sodium and calcium salts with the toxicity response were reported by Edwards et al. (2000, 2002) as they observed significantly reduced veliger mortality when 750 mg/L KCl was combined with 5,000 mg/L NaCl, or when KCl was combined with 800 mg/L $CaCl_2$. The mechanism of these responses and information regarding other factors of water quality have not been evaluated further. Since the $K^+$ ion relaxes the gill mussel activity (Medler et al. 1999) some speculated that because no recovery time was allowed, that some veligers may have been alive in this assessment.

To understand interactions and relationships of water quality parameters with the toxicity response of quagga mussels, we pursued laboratory-based toxicity testing of veligers and byssal stage quagga mussels with static renewal trials of KCl in several different water sources. Trials with veligers were conducted at elevated concentrations similar to those used by Edwards (2000) and Sykes (2009) to pursue the relation of water quality parameters on short-term survival. Byssal mussels were tested at two concentrations in longer-term trials (~1 month) at concentrations equal to or twice that currently recommended for rapid response field control measures.

## Methods

### *Location, timing of trials, chemical and water sources*

Tests were conducted at WBNFH (35º52′N, 114º39′W), in May–June and in August–September, 2015. Water sources used in testing included untreated WBNFH water from the Colorado River, AZ, specific pathogen free, dechlorinated well water from the University of Idaho fisheries wet laboratory, Moscow, ID (46º44′N; 117º00′W) and surface water collected from the Snake River, at C.J. Strike Reservoir, Elmore County, ID (42º57′N, 115º58′W) after filtration through a 35 µm net to remove other plankton. All test organisms used in trials conducted at WBNFH were obtained from hatchery inflow waters (veligers) or from dock structures in the river at the hatchery (byssal mussels).

Trials with mussels in water from Lake Ontario were conducted in October 2015 (veligers) and November–December 2015 (byssal mussels). All test organisms and water for Ontario trials were collected from Waupoos Marina, Picton, Prince Edward County, Ontario, Canada (44º0′N, 76º59′W). Water collected from Lake Ontario was filtered through a 35 µm net to remove other plankton. Although both quagga and zebra mussels are present in the lake nearly all (> 99%) of attached test organisms in the area were quagga mussels.

We used an analytical grade KCl (CAS 7447-40-7; FW 74.55, Macron Chemical Company, Center Valley, PA). Analytical grade sodium chloride (CAS 7647-14-5; FW 58.44, EMD Millipore, Billerica, MA) was used to supplement water from Lake Ontario and CJ Strike Reservoir to levels of conductivity and salinity similar to water from WBNFH.

*Test procedures*

Veliger trials – All tests of acute toxicity of KCl on veligers were conducted at room temperature (20–23°C) with one test concentration of 960 mg/L. For each trial, untreated water without KCl served as a control. Exposure times were selected to range from 1 to 24 h to compare the rate of morality of veligers in each of the water sources (Colorado River, University of Idaho, Snake River and Lake Ontario). Quagga mussel veligers in trials at WBNFH were collected from the Colorado River with 35 µm mesh plankton tow nets that were attached to two raceways inside the WBNFH at flows of 40 L/min. Collections of veligers lasted approximately 1 h (2,400 L filtered). In Lake Ontario, veligers were collected with 63 µm mesh plankton tow nets using vertical tows. For all trials, veligers from several collections were pooled for a trial.

Water and filtrate from the plankton net cod jar were filtered further through a 400 µm nylon mesh net to remove algae and larger particles. The contents from all collections were transferred into Nalgene or glass sample bottles and brought into the lab, and retained at room temperature (~20º C). Settled veligers were removed from the collection containers with a plastic serological pipette in aliquots of 1–2 mL to determine if collections contained an adequate number of live veligers. Each aliquot was evaluated for density of live veligers with a gridded Sedgewick-Rafter counting cell and compound microscope. We targeted a minimum of 500 live veligers per test container for trials at WBNFH, and at least 100 live veligers for each container in trials at Lake Ontario. The containers for all trials of KCl toxicity were acid-washed glass beakers (250 mL), with 2–3 replicate beakers with veligers assigned to each chemical treatment and control for an exposure time intervals, water source, and chemical constituents. The number of beakers for each trial ranged from 18 to 36.

To conduct a trial, we first added 4–10 mL of concentrated veligers to each beaker (test and control), and then brought the total volume of test organisms to 20 mL with the appropriate source water. We then added 80 mL of a 1.2 g/L KCl test solution mixed in each water source to the beaker. For controls at each treatment interval, we added 80 mL of the test water source to each beaker. At the end of a test interval, the contents of each beaker were drained over a 35 µm mesh filter, and the filter and retained contents were placed in a small glass Petri dish. We gently covered the filter with a 0.4% aqueous solution of fast green FCF (Harleton, Gibbstown, NJ, lot 4287G) for 20 min, and then washed the samples from the filter with fresh source water into a recovery beaker for 10–40 min until the sample was evaluated with a microscope. Staining with fast green allows for a rapid assessment of dead cells, and has been used as a tool evaluating viability of marine mussel spat (Webb and Heasman 2006) and juvenile salmon epithelial cells (Elliott et al. 2009) and recently for zebra mussel veligers (Whitledge et al. 2015). To evaluate survival, samples of ~ 200 settled veligers were removed from each recovery beaker with disposable pipets using a zig/zag motion, were visually evaluated with a gridded Sedgewick-Rafter counting cell and compound microscope (total magnification of 40 and 100$^{\times}$), and were scored for mortality. Scoring levels included live (non-stained with mantle intact), dead (stained mantle), and open shells (non-stained but with no mantle intact). In addition, we photographed retained samples of veligers using a compound microscope (Leitz Laborlux, Leica Microsystems, Buffalo Grove, IL) and Leica EC3 camera. Measures of the prodissoconch included shell length (umbo to ventral margin axis) and height (anterior to posterior axis) as described by Martel et al. (1995) of ~250 representative individuals from photographs using image analysis software (LAS EZ 1.8.0; Leica Microsystems, Buffalo Grove, IL).

To pursue the relationship of water quality and relative survival we supplemented source water from CJ Strike and Lake Ontario with additions of 0.1 M NaCl to reach an equivalent target salinity, conductivity and total dissolved solids (TDS) to that observed in the Colorado River water. In these trials the survival of veligers was evaluated for intervals of 12 and 24 h (Colorado River) and 24 h (Ontario).

Byssal stage – Attached mussels were removed from dock structures at the WBNFH and Waupoos Marina, Ontario. Mussels were washed and sorted to remove dead organisms: empty shells, floating engorged mussels, broken shelled mussels. Following collection and sorting, 100 live mussels were enumerated and placed into each replicate plastic tote (280 mm wide, × 300 mm long and 15 cm deep). The totes were filled with 4 L of river or lake water. The mussels were placed into test containers (3 per each test concentration and control), and held in water at test temperatures overnight (~20°C).

Mussels collected from the Colorado River were tested in Colorado River water and Snake River

water, while mussels collected from Lake Ontario were tested in Lake Ontario water. All organisms tested at WBNFH were quagga mussels, and trials at Lake Ontario were conducted with a mixed population of quagga mussels and zebra mussels but < 1% of test organisms were identified as zebra mussels. Each trial lasted for up to 29–31 d.

At the start of each test, one half of the water in each tote with mussels was removed and replaced with a solution of KCl prepared at 2× the target concentration (i.e. 200 mg/L and 400 mg/L) to create triplicate treatments of 100 mg/L KCl and of 200 mg/L KCl. The same procedure was conducted for totes containing untreated controls. Each test solution was introduced slowly into a tote over approximately 2 min in a zigzag motion to simulate a field exposure to the chemical. Compressed air was provided to each tote with air stones to assure adequate mixing and dissolved $O_2$. All totes were covered with clear acrylic sheets and/or plastic wrap to limit evaporation and held at room temperature for the trials.

The mussels were observed each day and obvious mortalities removed. The test and control solutions were refreshed every 48 h by removing 2 L of the test water and replacing it with 2 L of fresh solution at the appropriate concentration. Midway through the experiment, all 4 L was replaced with freshly mixed solutions. To compare rates of mortality across the treatments and controls, we removed a random sample of 10 mussels from each replicate tote at selected pre-determined intervals throughout the trials. Both live and dead mussels were collected during a random selection of 10 mussels at each sample period, and those removed were placed into individual 500 mL jars filled with fresh source water and observed for up to 48 h for potential recovery. The number of live and dead mussels was then recorded for each sample and each interval to assess a measure of relative mortality and the potential for mussels to recover if toxicants were removed. After $\geq$ 29 d of continuous exposure to each test concentration, or when all individuals in containers were observed likely dead, the test solutions in all containers were drained, and individuals in each replicate were flushed three times with source water for a recovery in fresh water. Containers were filled with 4 L of water to retain until a final count of mortality after 48 h of recovery. Mussels were scored as dead if they had gaping shells with no closure when handled or probed. The samples from control and 100 mg/L KCl treatments were at 3, 5, 10, 15 or 18, 20, 25 d. For tests with 200 mg/L, since mortality was rapid, random samples were removed and placed into recovery at more frequent intervals (2, 5 and 9 d). Additional dead mussels were counted and removed between intervals in all tests when warranted. The test organisms were retained in 70% ethanol for later measurements of shell length, height and width (Beggel et al. 2015). For tests in Lake Ontario water we kept the live and dead mussels separated to test differences in size due to treatment.

To pursue the relationship of water quality and relative survival of byssal stages we supplemented source water from Lake Ontario with a 0.1 M solution of NaCl to reach an equivalent salinity, conductivity and TDS to that observed in the Colorado River water source. In these trials we added 100 mg/L KCl to the NaCl-supplemented water to compare responses with other treatments with and without supplemented NaCl, and included an additional treatment to test exposure to NaCl-supplemented water alone.

Characterization of source and treatment water – We measured conductivity, specific conductivity, pH, salinity, and TDS of all water sources with an YSI 556 multi-probe (YSI Yellow Springs, OH) to characterize test solutions. Water quality was measured at the end of trials with veligers, and at intervals during tests with byssal mussels. In tests with byssal mussels, we also monitored dissolved oxygen with a Hach LDO 101 probe (Hach, Loveland, CO) at daily intervals and recorded hourly temperatures with temperature loggers (HOBO, Onset Corporation, Bourne, MA) placed into at least one tote assigned to each treatment. For trials with byssal mussels in Ontario, temperature, conductivity, pH and dissolved oxygen were measured with a Hach HQ40d (Hach, Loveland, CO).

To further characterize the water from each source, we quantified the dissolved and total quantities of barium, cadmium, calcium, chromium, cobalt, copper, iron, magnesium, manganese, molybdenum, nickel, potassium, sodium, vanadium, zinc and phosphorus of representative samples using inductively coupled plasma (ICP) protocols at the University of Idaho Analytical Sciences Laboratory. In addition we examined a profile of the metals in samples of all water sources supplemented with 100 mg/L KCl. In trials with NaCl supplemented conductivity and salinity (Lake Ontario and Snake River), we analyzed the ion fraction of our supplemented source water in addition to a metals screen.

### *Statistical analysis*

To analyze veliger responses, the proportions of live and dead veligers were counted in each replicate treatment by test interval and were summarized. The proportion of mortality over time of exposure in the

**Table 1.** Wald chi-square values, probability of fit, and estimated $LT_{50}$ and $LT_{99}$ of duration of exposure in probit models of veliger mortality by treatment for veligers tested in different water sources.

| Water source | Month of test | Treatment | Wald Chi-Square | P | $LT_{50}$ | $LT_{99}$ |
|---|---|---|---|---|---|---|
| Colorado | May | 960 KCl | 0.013 | 0.911 | not est. | |
| Colorado | May | Control | 4.156 | 0.042 | not est. | |
| Colorado | Aug | 960 KCl | 0.000 | 0.986 | not est. | |
| Colorado | Aug | Control | 1.700 | 0.192 | not est. | |
| U of Idaho | May | 960 KCl | 335.659 | <.0001 | 2.737 | 5.721 |
| U of Idaho | May | Control | 1.177 | 0.278 | not est. | |
| Snake River | Aug | 960 KCl | 64.510 | <0.0001 | 5.816 | 11.050 |
| Snake River | Aug | Control | 1.029 | 0.311 | not est. | |
| Snake River | Sept | NaCl KCl | 6.249 | 0.012 | 53.689 | 102.249 |
| Snake River | Sept | NaCon | 2.828 | 0.093 | not est. | |
| Ontario | Oct | 960 KCl | 248.076 | <.0001 | 3.658 | 9.615 |
| Ontario | Oct | Control | 0.784 | 0.376 | not est. | |

treatment and control solutions was modeled with a probit regression to determine the best model fit and model prediction of 95% confidence intervals for estimates of lethal time of exposure (median $LT_{50}$), and 99% mortality ($LT_{99}$). We tested the significance of the test replicates as a covariate in models, and collapsed the model when test replicate was not significant to yield one model fit to time of exposure and predicted mortality. We found an inverse standard normal distribution provided the best fit for all models by comparing log likelihood values. We reported the probit model fits with Wald chi-square values and for estimates of the 95% confidence intervals for $LT_{50}$ and $LT_{99}$ distributions.

To analyze the survival of byssal stage mussels, we used two approaches. First we compared the proportion of live and dead mussels in replicate treatments at each of the sampling intervals in a trial with chi-square tests of independence. These tests helped to determine when significant differences occurred between treatments and controls over exposure time. Secondly, we used nonparametric models of survivorship using the product-limit Kaplan-Meier method for each test group after censoring the samples removed at selected intervals above. The survival distribution for each treatment (100, 200 mg/L and control) and water source was modeled and comparisons between and across treatments were made using censored data linear rank statistics based on the exponential scores (log-rank test) and Wilcoxon chi-square scores. We compared models for each treatment across water sources. The mean, median and range of size for mussels tested in Colorado River water May–June. We tested for differences between the shell sizes of mussels in two water sources in August–September with a generalized linear model (GLM). For all mussels retained from trials at Lake Ontario, we tested for differences in size of mussels between those that died or survived across treatments with a GLM.

We summarized mean specific conductivity, pH, salinity, TDS, temperature and dissolved oxygen by time of trial and water source. To characterize metals and ions of source waters, after finding no difference between the dissolved and total fractions, we used a GLM to test for differences across the suite of parameters (except Na, Cl and K), by water source, and reported least squared means and significant differences for all variables. All statistical tests in the study were conducted using SAS 9.3 (SAS Institute, Cary, NC), and reported results as significant if $P < 0.05$.

## Results

### *Veliger trials*

We observed little mortality in veligers exposed to 960 mg/L KCl prepared in Colorado River water. Because of the limited mortality, no significant probit model could be fit with duration of exposure, even after 24 h. (Table 1). We detected significant mortality in veligers exposed to KCl in the other water sources tested, and fit significant probit models with 95% confidence intervals estimates (Figure 1). The mortality of veligers in U of Idaho water showed the most rapid response to time of exposure (Wald $\chi^2 = 344.6$; $P < 0.0001$). Test replicates were not significantly different, thus we predicted a median lethal time of 2.7 h (95% CI = 2.6–2.9 h). The veligers exposed to 960 mg/L in Snake River water showed a significant mortality response to time of exposure (Wald $\chi^2$ =64.5; $P < 0.0001$), with no significant effect of test replicate. The predicted median lethal time to death was 5.8 h (95% CI = 5.25–6.49 h). Veligers collected from Lake Ontario and exposed in source water with 960 mg/L KCl

**Table 2.** Summary of mean temperature, pH, specific conductivity, TDS, salinity and dissolved oxygen measured in trials of veligers exposed to tests of toxicity of 960 mg/L KCl in different water sources by time of trials.

| Month | Source | Treatment | Temp (°C) | pH | Specific conductivity (ms/cm) | TDS (mg/L) | Salinity ppt | DO (mg/L) |
|---|---|---|---|---|---|---|---|---|
| May | U Idaho | Control | 22.20 | 8.07 | 0.37 | 0.25 | 0.18 | 8.69 |
| | U Idaho | 960 KCl | 22.16 | 7.93 | 2.13 | 1.37 | 1.08 | 8.69 |
| | Colorado River | Control | 22.24 | 7.90 | 1.08 | 0.67 | 0.51 | 8.77 |
| | Colorado River | 960 KCl | 22.25 | 7.78 | 2.78 | 1.79 | 1.43 | 8.76 |
| Aug-Sep | Snake River | Control | 23.23 | 8.15 | 0.47 | 0.31 | 0.23 | 8.15 |
| | Snake River | 960 KCl | 22.80 | 8.05 | 2.30 | 1.50 | 1.18 | 8.49 |
| | Snake River | NaCl | 20.47 | 8.17 | 1.04 | 0.68 | 0.52 | 8.41 |
| | Snake River | NaCl & 960 KCl | 20.58 | 8.10 | 2.80 | 1.82 | 1.46 | 8.45 |
| | Colorado River | Control | 22.40 | 8.21 | 1.02 | 0.67 | 0.51 | 8.13 |
| | Colorado River | 960 KCl | 22.27 | 8.01 | 2.79 | 1.82 | 1.45 | 8.54 |
| Oct | Lake Ontario | Control | 19.95 | 8.36 | 0.33 | 0.212 | 0.15 | |
| | Lake Ontario | 960 KCl | 19.97 | 8.11 | 2.18 | 1.41 | 1.12 | |
| | Lake Ontario | NaCl | 19.29 | 8.97 | 0.91 | 0.59 | 0.45 | |
| | Lake Ontario | NaCl & 960 KCl | 19.29 | 8.58 | 2.64 | 1.72 | 1.37 | |

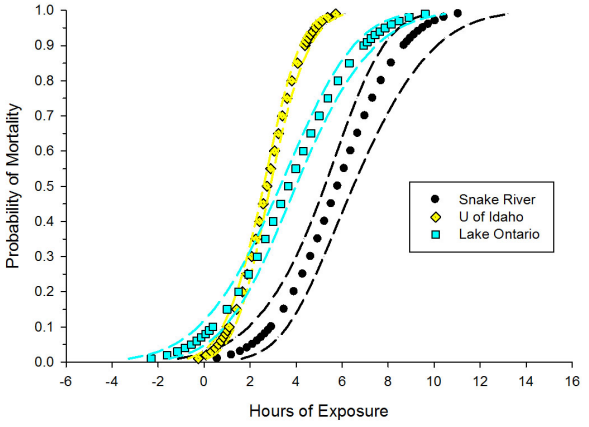


**Figure 1.** Probit model predictions with 95% CI for veligers exposed to 960 mg/L KCl prepared in each of three water sources.

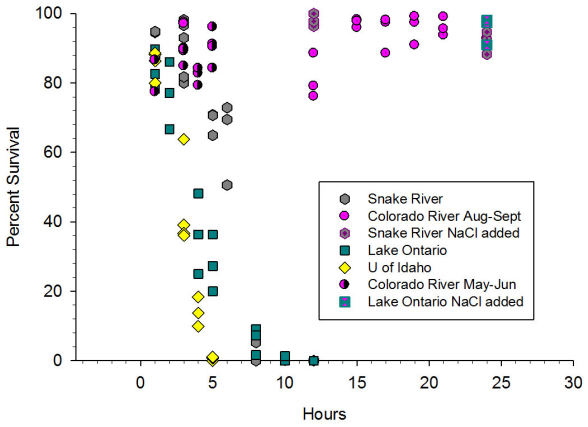


**Figure 2.** Summary of survival of veligers in multiple trials of toxicity of 960 mg/L KCl. Survival of source water supplemented with NaCl is provided for trails with Snake River and Lake Ontario water sources. Survival of veligers held in controls (with and without KCl ranged from 88–100% for all trials.

showed a significant model fit with no significant differences among replicates (Wald $\chi^2$ =248.08; $P < 0.0001$). We estimated a median lethal time for mortality of 3.7 h (95% CI 3.31–3.99; Table 1).

Veligers in water from Snake River and Lake Ontario supplemented with NaCl at equivalent salinities to levels observed in Colorado River water (Table 2) showed a reduced rate of mortality to KCl. We fit a significant probit model (Wald $\chi^2 = 6.245$; $P < 0.012$) to the response in Snake River water, and estimated the $LT_{50}$ for the NaCl supplemented water as 53.69 h, with exceptionally wide 95% CI, 37.86–181.56 h. The tests with NaCl-supplemented water from Lake Ontario were not sufficient to estimate an $LT_{50}$, but survival time of veligers was > 90% after 24 h exposure to 960 mg/L KCl (Figure 2). Little to no mortality was observed in veligers held in controls, and percent survival ranged from 91–100% in all replicates. Most veligers tested in all trials of toxicity were umbonal stage (Table 3), with few pediveligers observed. The median shell height of veligers across all trials ranged from 124.6 to 161.4 µm (Table 4).

*Byssal stage*

We observed little mortality in mussels held as controls in water from the Colorado River (Figure 3). We found no significant differences in survival of controls in repeated trials (May–June and August–

**Table 3.** Percentage of straight-hinge, umbonal and pediveliger stages observed in toxicity testing of veligers over four months of testing by location of testing and mussel source. Trials of veligers in Lake Ontario likely included < 3% zebra mussels, all others were quagga mussels.

| Month of test | Percent of sample in life stage | | |
|---|---|---|---|
| | Straight-hinge | Umbonal | Pediveliger |
| WBNFH, Colorado River | | | |
| May | 22 | 76.1 | 1.9 |
| August | 41.2 | 57.2 | 1.6 |
| September | 50.3 | 45 | 4.7 |
| Picton, Lake Ontario | | | |
| October | 38.6 | 65.3 | 5.1 |

**Table 4.** Dimensions of veligers measured in retained samples from toxicity tests by water sources and month of trial. Measures of length and height were made following criteria of Martel et al. (1995) for veligers with length measured as the distance from the developing shell umbo or mid hinge to the ventral margin, and height measured from the antero-posterior axis.

| Water source | Month | Measurement | N | Mean (um) | Median | SD | Range min-max (um) | |
|---|---|---|---|---|---|---|---|---|
| Colorado River (WBNFH) | May | Length | 24 | 146.1 | 139.8 | 42.8 | 87.0 | 222.9 |
| | | Height | 24 | 159.6 | 154.8 | 41.6 | 97.3 | 235.7 |
| University Idaho | May | Length | 43 | 152.8 | 154.7 | 40.8 | 78.5 | 253.2 |
| | | Height | 43 | 166.6 | 161.6 | 39.2 | 96.8 | 265.0 |
| Colorado River (WBNFH) | Aug | Length | 74 | 123.9 | 110.0 | 40.1 | 68.6 | 231.8 |
| | | Height | 74 | 139.5 | 126.7 | 38.4 | 79.1 | 237.9 |
| Snake River (CJ Strike) | Sep | Length | 50 | 125.5 | 109.3 | 44.7 | 70.7 | 223.2 |
| | | Height | 50 | 142.0 | 126.3 | 43.5 | 90.2 | 239.8 |
| Colorado River (WBNFH) | Sep | Length | 14 | 127.1 | 108.2 | 51.2 | 62.2 | 234.7 |
| | | Height | 14 | 138.9 | 124.6 | 44.9 | 81.7 | 235.7 |
| Lake Ontario (Picton) | Oct | Length | 43 | 148.6 | 143.4 | 65.0 | 77.3 | 460.9 |
| | | Height | 43 | 165.0 | 161.4 | 49.6 | 93.3 | 353.4 |

September) Wilcoxon $\chi^2$ = 1.92, DF = 1, $P$ = 0.17. Kaplan-Meier estimated mean survivals for the May–June and August–September trials were 30.32 d, SE = 0.26; and 24.83 d, SE = 0.12 respectively. We found no significant differences in the proportion of live and dead removed at selected intervals across replicates within controls from any water source. However, the quagga mussels held in untreated control water from Snake River showed reduced survival (Kaplan-Meier estimated mean = 22.99 d; SE = 0.44; Figure 3). Comparisons among controls including controls from Snake River with others showed significant differences attributed to water source (Wilcoxon $\chi^2$ = 217.86; DF = 3; $P$ < 0.001). In Lake Ontario water, models for survival of controls estimated a mean of 28 d but lack of mortality prohibited estimates of SE. Mean shell lengths of mussels tested in all trials at WBNFH did not differ significantly (Table 4); however, mussels tested in Lake Ontario water comprised a larger range of sizes as smaller mussels were included in these samples (Table 5). Across all treatments in Lake Ontario we found no difference between the size of mussels that survived versus those that died (all $P$ > 0.70).

We found significant differences between the survival model responses of mussels tested in 100 mg/L KCl across water sources (Wilcoxon $\chi^2$ = 1,199.9; DF = 3; P <.0001). The mortality of quagga mussels tested in 100 mg/L KCl in Snake River water was rapid with an estimated mean survival of 4.91 d (SE = 0.08). Survival in Lake Ontario water was somewhat longer at 6.90 d (SE = 0.08). For quagga

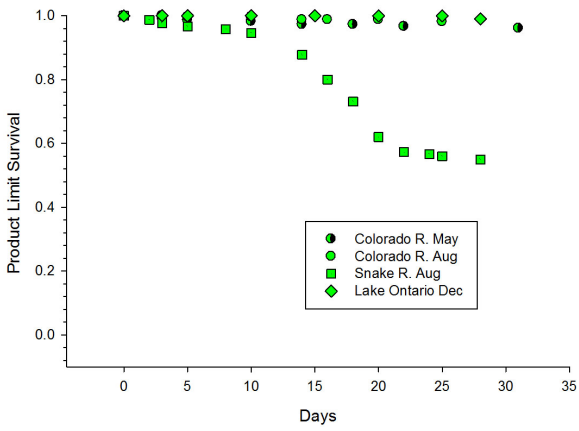


**Figure 3.** Summary of Kaplan-Meyer product limit survival estimate for replicated trials of quagga byssal mussels by source water. Test organisms were from source water except for trials in Snake River water from CJ Strike Reservoir where the source of test quagga mussels was from the Colorado River at WBNFH.

**Table 5.** Summary of shell length (maximum distance from umbo to posterior margin) of byssal mussels measured in trials with KCl, separated by water source and time of test. All retained mussels were measured in trials at Lake Ontario, including smaller organisms attached to larger adults.

| | | Shell length mm | | | | |
|---|---|---|---|---|---|---|
| | N | Mean | SD | Median | Min | Max |
| Colorado River (WBNFH) May-Jun | 874 | 20.85 | 2.24 | 21 | 7 | 31.2 |
| Snake River (CJ Strike) Aug-Sep | 84 | 18.6 | 3.21 | 18.1 | 11.7 | 26.2 |
| Colorado River (WBNFH) Aug-Sep | 225 | 18.16 | 3.03 | 18.1 | 8.7 | 27.9 |
| Lake Ontario (Picton) Nov-Dec | 1565 | 14.59 | 6.14 | 14.5 | 1.2 | 51.0 |

**Table 6.** Summary of least squared means and indications of significant differences from means comparisons among water sources for metals and ions not including K, $Na^+$ and Cl. ND = parameter not tested for this source. Significant differences ($P < 0.05$) among means for each variable across locations are indicated with different superscript letters. BDL indicates below detection limits. SR = Snake River at CJ Strike Reservoir, ON = Lake Ontario at Picton, UI = well water at U of Idaho; CO = Colorado River at WBNFH.

| Location | Fluoride | Nitrate-N | Sulfate | Barium | Calcium | Cadmium | Magnesium | Manganese | Molybdenum | Nickel | Zinc |
|---|---|---|---|---|---|---|---|---|---|---|---|
| SR | $0.311^a$ | $0.828^a$ | $43.732^a$ | $0.032^a$ | $36.049^a$ | 0.002 | $18.369^a$ | 0.003 | $0.025^a$ | 0.006 | 0.018 |
| ON | $0.175^b$ | $0.21^b$ | $23.00^b$ | $0.024^a$ | $33.00^a$ | BDL | $8.025^b$ | 0.005 | BDL | BDL | BDL |
| UI | ND | ND | ND | $0.167^b$ | $23.667^b$ | BDL | $11.833^c$ | 0.006 | BDL | BDL | 0.091 |
| CO | $0.049^c$ | $0.401^c$ | $234.418^c$ | $0.126^c$ | $79.047^c$ | 0.004 | $27.588^d$ | 0.005 | $0.054^b$ | 0.009 | 0.204 |

**Table 7.** Summary of means from analysis of sodium ($Na^+$), potassium ($K^+$), and chloride ($Cl^-$) in water sources before and after supplementation. Replicated samples allowed for statistical comparisons of $Na^+$ and $K^+$ in water sources before supplementation, and separation of least squared means is provided by different superscripts showing significant differences. Inadequate replication of water samples after NaCl and KCl additions limited any statistical analysis. ND = not determined in the assay procedure. SR = Snake River at CJ Strike Reservoir, ON = Lake Ontario at Picton, UI = well water at U of Idaho; CO = Colorado River at WBNFH.

| Source | Addition | Sodium | Potassium | Chloride |
|---|---|---|---|---|
| SR | none | $27.35^a$ | $4.31^a$ | 24.01 |
| ON | none | $14.00^b$ | $1.60^b$ | 23.00 |
| UI | none | $24.75^{ab}$ | $5.98^a$ | ND |
| CO | none | $100.12^c$ | $4.59^c$ | 88.43 |
| SR | NaCl | 140.40 | 4.47 | 193.35 |
| ON | NaCl | 150.00 | 2.80 | 250.00 |
| ON | KCl | 14.00 | 72.00 | 89.00 |
| UI | KCl | 22.00 | 61.00 | ND |
| CO | KCl | 100.88 | 62.05 | 137.93 |
| SR | NaCl & KCl | 136.30 | 57.69 | 246.49 |
| ON | NaCl & KCl | 150.00 | 71.00 | 300.00 |

mussels tested in Colorado River water, we failed to see complete mortality at the end of the month: mean survival was 25.86 d (0.48 SE) in the May–June study, and 23.79 d (SE = 0.44) in August.

The proportion of live and dead in the samples removed at selected intervals of 3, 5, and 10 d in the trials of Colorado River water were not significantly different between tests of untreated control and 100 mg/L KCl (all $P > 0.30$). However, increased mortality over controls was observed in 100 mg/L exposures in samples removed after 16–18 d exposure (all $P < 0.01$). These results support that 100 mg/L KCl in Colorado River water source became irreversibly toxic only after a consecutive exposure of more than 10 d.

All tests of 200 mg/L KCl showed rapid and irreversible mortality even in Colorado River water. We found significant differences in survival models compared across all water sources (Wilcoxon $\chi^2 = 332.96$; DF = 3; $P < 0.001$). The estimated mean survival of quagga mussels in Colorado River water was different between the May–June and August–September trials at WBNFH (Wilcoxon $\chi^2 = 251.59$; DF = 1; $P < 0.001$). Quagga mussels tested in Colorado River water showed reduced survival in August–September 3.42 d (SE = 0.04) over that in May–June of 7.48 d (SE = 0.15); (Figure 4). Estimated mean survival in 200 mg/L KCl in Snake River water was 3.63 (SE = 0.04), and in the Lake Ontario water was 3.33 d (SE = 0.12).

We found survival of mussels in Lake Ontario water supplemented with NaCl was similar to that observed in tests with Colorado River water. Mortality was minimal and estimated mean survival 23.25 d

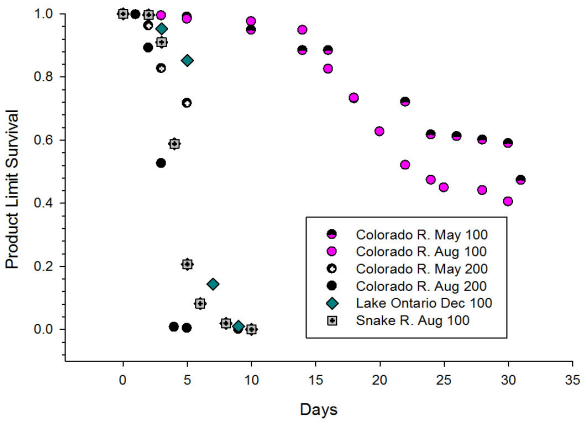


**Figure 4.** Comparison of Kaplan-Meyer product limit survival function estimates for multiple trials of byssal stage mussels exposed in different water sources in KCl for up to 31 consecutive days. Each replicate contained approximately 100 mussels, and exposures were at room temperature with partial solution renewal every 48 h. Because of rapid mortality, survival curves for tests of 200 mg/L KCl in Lake Ontario water were not plotted.

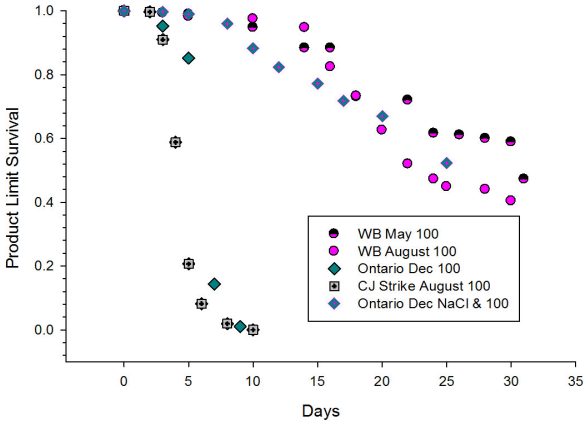


**Figure 5.** Summary of Kaplan-Meyer product limit survival estimates for studies of toxicity of 100 mg/L KCl in mussels from various water sources. In tests of Lake Ontario water, survival in water supplemented with NaCl with KCl is presented.

(SE = 0.48) (Figure 5). In Lake Ontario trials, tests with NaCl supplemented lake water, and no KCl, and resulted in nearly 100% survival. Estimated mean survival was 30.31 d (SE = 0.255) and 28.8 d (SE = 0.17, for control and NaCl supplemented water, respectively. We found no significant differences between mean survival between these groups (Wilcoxon $\chi^2 = 2.30$; DF = 1; $P = 0.14$).

Metals and ion concentrations of test waters showed significant differences (Tables 6 and 7). Fluorides and nitrate-N were significantly higher in Snake River water compared to Colorado River and Lake Ontario. Calcium levels in Snake River and Lake Ontario were similar, and about half that measured in Colorado River. Magnesium levels were lowest in Lake Ontario water, and highest in Colorado River, but were significantly different across all locations. The water sources before supplementation with NaCl and KCl showed significant variation, and $Na^+$ was nearly four times higher in the Colorado River (Table 7). Baseline $K^+$ was low, ranging from 1.6 to 4.6 mg/L. Chloride levels from the test water were not quantified for the U of Idaho source. The chlorides in water supplementation with KCl and NaCl were consistently elevated (Table 7).

## Discussion

Our study provides important insight into factors of water quality that may influence the toxicity of KCl to quagga and zebra mussels. We observed very little mortality in repeated trials of veligers held in Colorado River water dosed with 960 mg/L KCl for up to 24 h. We also observed limited mortality in byssal stage mussels after a 30 d exposure to 100 mg/L KCl. The lowered mortality in Colorado River water was likely related to the cations in the source water. Dietz et al. (1994) determined that zebra mussels were unable to survive beyond 5 d in deionized water and required minimal concentrations of $Na^+$, $Cl^-$, $K^+$, and $Mg^{2+}$ for prolonged holding (>51 days) under laboratory conditions. Potassium chloride disassociates in water into chloride and potassium ions. The Colorado River water at WBNFH had elevated sodium concentrations (Table 7), and our data suggest the higher salinity affected the responses we observed, as water sources supplemented with small quantities of NaCl, significantly reduced the toxicity of KCl to veliger and byssal stage mussels (Figures 2 and 5). For Snake River water from CJ Strike Reservoir, supplemented with NaCl, estimated mean mortality was 53.69 h, more than 9 times the mean survival observed in un-supplemented water. Nearly 100% of the veligers tested in Lake Ontario water supplemented with NaCl survived for 24 h. Byssal stage mussels exposed to 100 mg/L KCl in Lake Ontario water supplemented with NaCl had mean survival of more than 23 d, similar to mussel survival in 100 mg/L KCl in Colorado River water.

Fisher et al. (1991) tested the efficacy of several formulations of potassium on byssal zebra mussels in static 24 h tests. They reported an $LC_{50}$ of 138 mg/L KCl (95% CI = 123–161). This response was rapid compared with trials we conducted in 100 mg/L of

KCl. However, Fisher et al. (1991) did not provide details of the water quality in their tests except to report a standard reference water pH of 7.0 at 20° C. Unfortunately no further details of the specific chemical constituents of the test water could be determined, as the EPA has guidance protocols for standard reference water that range from soft to hard water. Fisher et al. (1991) pursued their studies of the action of $K^+$ on samples of mussel gill tissue isolated in vitro and reported the frequency of cilia beating. They found cessation of ciliary activity occurred after 173 and 550 min. of exposure in 320 mg/L $K^+$ and 160 mg/L $K^+$, respectively. They reported widespread vacuolation of the ciliated brachial epithelium, and concluded that the pathology was likely related to loss of fluid and/or electrolyte balance in the epithelial cells due to functional or structural changes in the plasma membrane. O'Donnell et al. (1996) pursued these studies to examine nuclear magnetic resonance spectroscopy of gills, and indicated that recovery following exposure to KCl was possible at concentrations < 320 mg/L, if exposures were limited to 24 h. They speculated that elevated extracellular potassium was rapidly taken up by zebra mussel tissues and the cell volumes could not be regulated.

We believe elevated $Na^+$ in the supplemented test solutions (or in the water source from the Colorado River) in our trials was responsible for reversing the electrolyte imbalance, as the $Na^+$ $K^+$ ion exchange pumps were not constrained by a limited supply of $Na^+$, thus retaining the electrolytes needed for gill homeostasis. This mechanism was proposed by Fisher et al. (1991) in studies of zebra mussels, who also postulated that excess $K^+$ likely altered the membrane polarization and interfered with the $Na^+K^+$ ATPases. Data from our studies validated most of these speculations, and were confirmed through analysis of water metals and ions of the test waters. The increased survival after NaCl supplementation supports that the mortality response to a given a challenge of potassium was driven by low sodium levels.

Horohov et al. (1992) provided further evidence of the osmotic regulatory constraints of zebra mussels. They reported blood solutes in zebra mussels were among the lowest recorded for freshwater mussels. They observed a high turnover rate in $Na^+Cl^-$ flux. Deaton and Greenberg (1991) reported a correlation between osmoregulatory capability of bivalves and mobilization of calcium. The calcium contents of the waters we tested were also different, but all concentrations were above limiting levels of 12–15 mg/L proposed by Wu et al. (2010). Moreover a cation such as $Ca^{2+}$ may not be that useful in an osmotic challenge from elevated $K^+$. Dietz et al. (1994) conducted careful ion permutation experiments with zebra mussels and determined that two salts (NaCl and $MgSO_4$) were the most important to promote survival in the ion deficient media.

There were likely stress factors associated with moving mussels into water from novel locations, given the differences in water quality parameters of conductivity, metals and ions. We did not measure hemolymph ionic concentrations, but we would expect osmotic stress to occur when mussels that were acclimated to higher conductivity were placed into lower conductivity waters such as U of Idaho, and Snake River. Martem'yanov (2000) reported sodium and magnesium concentration in the hemolymph of the zebra mussels decreased by 25.1 and 25.6%, respectively, after catching and transportation. He found that acclimation of 18 d was needed to restore the hemolymph homeostasis. In these studies, he observed no change from stress in the $K^+$ content of hemolymph. We believe this stress likely contributed to the mortality observed in control treatments of byssal mussels placed in water from Snake River (Figure 3). Such observations call into question the likely survival success of mussels transported from high conductivity and salinity environments such as the Colorado River to lower conductivity locations such as the Snake River. Perhaps the stress of this transport and resulting electrolytes would reduce the risks of establishment post transport.

We observed normal filtering behavior in the controls and 100 mg/L test replicates for all trials with byssal mussels, indicating the mussels were being exposed to solutions of the toxicant. We believe our approach to leave a reserve of water in the containers so that individual mussels were not disturbed or closed before introducing the test chemical was helpful to simulate a natural exposure to the chemicals. This approach helped to reduce the probability of mussels closing up, and reduced variation in responses measured. The mortality response is affected by temperature, and a lower test temperature would likely have increased the time to mortality. Tests conducted in larger and deeper test containers or tests with substrates may better simulate a field exposure. Lewis et al. (1997) performed tests with zebra mussels in Lake Erie water near Dunkirk, New York, and estimated $LT_{95}$ for 100 mg/L KCl as 56 h for trials at room temperature, and 165 h for tests at 12–14°C.

Our study used reagent grade KCl in contrast to industrial sources used in field trials such as those conducted in Millbrook Quarry, Virginia (Fernald and Watson 2014). The reagent grade chemicals could have impurities that might affect the activity

of the KCl. Future efforts to support registration of this as a pesticide will have to specify appropriate vendors and grades. Moreover, previous field trials have introduced a highly concentrated solution that is dispersed, rather than using at most a 0.4% concentration, as in our trials. Fernald and Watson (2014) applied a 12% potassium solution with muriate of potash (MOP 98%).

Samples of test water analyzed by ICP verified the $K^+$ levels of the 100 mg/L KCl solutions to range from 57–72 mg/L $K^+$ (Table 7). A pure salt of KCl would constitute approximately 77% $K^+$, thus these concentrations were within the range expected, although some were lower than the estimated target, given that the background concentrations of $K^+$ ranged from 1.6 to 5.98 mg/L. Although we were unable to obtain sufficient test organisms for any comparisons of survival between zebra and quagga mussels in trials with 100 mg/L KCl, the 10 identified zebra mussels in trials with the test solutions showed no outlying effects. From insight into the physiology of both species, we observed similar responses between the species.

We recommend further testing of the influence of water quality parameters on the toxicity response of KCl to quagga and zebra mussels. We believe our approach of testing veliger survival in elevated concentrations (~ 10×) may be a way to model the responses related to water quality in short-term tests. We also propose further testing is needed to understand the relative survival of mussels acclimated to water of low conductivity, such as those in Lake Ontario, then transported into waters of low conductivity such as the Snake River. We believe laboratory exposures of mussels in different water sources may help us to better understand the relative risks of successful introduction from different locations, and assist managers in prioritizing monitoring and check stations. Recent studies by Früh et al. (2012) used a multivariate approach to explore what combination of chemical variables best characterized the habitat of invasive species. Davis et al. (2015) also emphasized the risks of using a single parameter such as calcium in assigning establishment risk because of the interactions and complexity of aquatic systems and their organisms. Bourgeault et al. (2012) found that the uptake rate constant of Ni by zebra mussels was significantly affected by $Ca^{2+}$, $Zn^{2+}$, and dissolved organics in the test systems. They found uptake was inhibited by $Ca^{2+}$ and enhanced by $Zn^{2+}$ and they hypothesized that $Ni^{3+}$ uptake is facilitated by Zn-dependent transport sites. We suggest further tests with multiple factors should be conducted to examine synergy and interactive components that will help managers refine their approach to control and eradication of these species. In the interim, while further studies are conducted on refining the mechanisms of water quality effects, our findings suggest that managers considering application of potash as an eradication tool must consider the local water quality, especially cations such as $Na^+$ in their response plans.

## Acknowledgements

E. Hays provided critical field and laboratory assistance and data entry. J. Browning and T. LaCroix provided for assistance with sample analysis. M. Olson, T. Frew, S. Karpowicz, G. Cappellii, A. Flaten, J. Saccomanno and S. Peterson at Willow Beach National Fish Hatchery provided essential facilities, support and assistance. We thank D. Parrish, Idaho Department of Fish and Game, and B. Kibler U.S. Fish and Wildlife Service for field logistics and assistance with sampling and shipping water from the Snake River. J. Nielson, Aquatic Invasive Species Coordinator at Utah Division of Wildlife Resources, and S. Phillips Pacific States Marine Fisheries Commission provided funding this project. We are grateful to P. Heimowitz, and three anonymous reviewers for their thoughtful critiques of earlier drafts of this manuscript. Any use of trade, firm, or product names is for descriptive purposes only and does not imply endorsement by the U.S. Government.

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