



Calcium chloride pollution mitigates the negative effects of an invasive clam

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Abstract Invasive bivalves can drastically alter freshwater ecosystems and affect ecosystem services, but they can be influenced by external factors including calcium concentrations. When a common road salt, calcium chloride (CaCl_2), enters freshwater ecosystems, it may be toxic to organisms or facilitate bivalves by serving as a calcium source. Therefore, CaCl_2 could benefit invasive mollusks tolerant to chloride that require calcium to grow. We used mesocosms to investigate the impacts of CaCl_2

(35–187 mg Ca^{2+} L^{-1}) and invasive bivalves (Asian clams, *Corbicula fluminea*; zebra mussels, *Dreissena polymorpha*) on a native lake food web. We hypothesized that invasive bivalves facilitate benthic algae because they reduce phytoplankton and excrete waste. These changes in primary producers would subsequently impact consumers. We also hypothesized that low to moderate CaCl_2 concentrations promote the survival, growth, and reproduction of native and invasive mollusks, while causing few toxic effects. If so, we hypothesized that invaded communities exposed to CaCl_2 experience stronger impacts from the invasive bivalves. We found that invasive bivalves

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decreased phytoplankton, which led to decreases in periphyton, zooplankton, and native clams. They caused increases in filamentous algae and isopods. While zebra mussels survived poorly in all treatments, moderate concentrations of CaCl_2 substantially reduced Asian clams, which reduced their community effects. Our highest CaCl_2 treatments also reduced zooplankton densities. Thus, while freshwater salinization from road salts poses a concern, we observed no indication that CaCl_2 road salt will benefit Asian clams and zebra mussels. However, the community-wide consequences from Asian clams at low CaCl_2 emphasize the extensive effects that invasive bivalves can have on freshwater communities and the immense concern surrounding their invasions.

Keywords *Dreissena polymorpha* · *Corbicula fluminea* · Invasive species · Road deicing salt

Introduction

Increased connectivity has led to the movement of species around the world through a variety of avenues, including commercial (e.g., pet and food trades) and accidental transportation (e.g., shipping crates and ballast water; Crowl et al. 2008; Sousa et al. 2014; Gallardo et al. 2015; Moorhouse and Macdonald 2015). In the United States, there are approximately 50,000 non-native species and approximately 10% are categorized as invasive (i.e. causing some form of harm; Pimentel et al. 2005; Hoyer et al. 2015). Establishment of these species in non-native habitats has generated a wide array of impacts, which globally cost an estimated US\$1.4 trillion annually (Pimentel et al. 2001; Jeschke 2014). At the same time, humans continue to alter other physical and chemical aspects of the environment through activities such as deforestation, pollution, and climate change (McGill et al. 2015; Reid et al. 2019). To understand the full potential of invasive species to influence vital ecosystem processes, we must investigate the effects of these organisms in a variety of environmental contexts, both natural and anthropogenic.

Among the many species that invade freshwater ecosystems, invasive bivalves are well-known to affect native organisms and alter ecosystems because they possess many characteristics that native bivalves lack in European and American waters (Karatayev et al. 2007; Sousa et al. 2014). For example, their

elevated filtration rates and high densities can result in increased water clarity, superior competition against native filter feeders, and accidental consumption of native larvae (Karatayev et al. 2007; Sousa et al. 2014). Invasive bivalves also excrete large quantities of waste, which may alter nutrient cycling and provide resources to primary producers (Karatayev et al. 2007; Sousa et al. 2014). Furthermore, mass die-offs of these bivalves can litter lake bottoms with shells and cause local increases in nutrient concentrations, which can alter nutrient cycles, negatively affect water quality, and reduce oxygen (Iarri et al. 2011; Sousa et al. 2014; McDowell et al. 2017). Lastly, some invasive bivalves commonly attach to objects (i.e., biofoul), which can damage infrastructure and harm native species (McLaughlan and Aldridge 2013; Sousa et al. 2014). Because invasive bivalves have such great potential to alter the services of natural ecosystems, it is critical to understand the conditions that promote their invasion.

For bivalves, calcium is critical for reproduction, shell growth (made of calcium carbonate), and other biological processes (Machado and Lopes-Lima 2011; Ferreira-Rodríguez et al. 2017). Among common invasive bivalves, zebra mussels (*Dreissena polymorpha*) need relatively high calcium concentrations ($> 25 \text{ mg Ca}^{2+} \text{ L}^{-1}$) to have sustainable, reproducing populations in fresh water; although adults can persist at lower calcium concentrations (Cohen and Weinstein 2001; Karatayev et al. 2007; Whittier et al. 2008). In contrast, Asian clams (*Corbicula* spp.) can thrive at lower calcium concentrations ($3\text{--}10 \text{ mg Ca}^{2+} \text{ L}^{-1}$; Karatayev et al. 2007). As a result, calcium concentrations in freshwater systems can play a large role in determining the invasibility of a water body by particular bivalve species (Cohen and Weinstein 2001; Whittier et al. 2008; Ferreira-Rodríguez et al. 2017).

The calcium concentrations of freshwater ecosystems can be influenced by humans. For example, prior to the United States Clean Air Act of 1970, acid deposition caused calcium to leach from terrestrial soil into freshwater (Driscoll et al. 2003; Sullivan et al. 2018). The Clean Air Act reduced acid deposition, which reduced calcium leaching and therefore, the freshwater calcium concentrations in some areas are declining (Jeziorski et al. 2008; Reid et al. 2019). In other areas, calcium sources including the weathering of concrete, runoff after wildfires, and the application of road salts have the potential to increase the calcium

of a body of water (Ferreira et al. 2005; Davis et al. 2015; Kaushal et al. 2017, 2018). For example, calcium chloride (CaCl_2) now ranks among the top five chemicals used on roads in the USA for deicing and dust suppression (Transportation Research Board 2007; Kelting and Laxson 2010). In some countries (e.g., South Korea), CaCl_2 is the second most popular road salt used (Baek et al. 2014). Even in areas where CaCl_2 is not a primary deicer due to its higher costs, many agencies add small amounts of CaCl_2 to the most common salt, sodium chloride (NaCl), to create salt brines that further lower the freezing point (Transportation Research Board 2007). Additionally when non-calcium based salts (e.g., NaCl) wash off roads and into nearby soils, the sodium ions can displace calcium ions through cation exchange (Kaushal et al. 2018; Schuler and Relyea 2018). Snowmelt and rain events can wash CaCl_2 or displaced calcium ions into surface waters, which might increase the concentration of calcium and chloride ions in a body of water (Kaushal et al. 2018).

Large increases in salt ions may cause toxic effects to freshwater communities, which has been found for NaCl road salt at high concentrations (i.e., $> 400 \text{ mg Cl}^- \text{ L}^{-1}$; Stoler et al. 2016; Hintz et al. 2017; Jones et al. 2017). CaCl_2 is often more toxic to individual species than NaCl (Benoit and Stephan 1988; Evans and Frick 2001; Hintz and Relyea 2017; Coldsnow and Relyea 2018). Thus, CaCl_2 may affect communities at lower concentrations, but no study has investigated the effects of CaCl_2 at the community level. Low or moderate increases in salt ions, specifically CaCl_2 , may help some organisms. For example, low CaCl_2 concentrations ($< 100 \text{ mg Ca}^{2+} \text{ L}^{-1}$) in the laboratory increased the growth and survival of quagga mussels (*Dreissena bugensis*) and Asian clams (Davis et al. 2015; Ferreira-Rodríguez et al. 2017). Thus, it is reasonable to expect that CaCl_2 might act as a nutrient at lower concentrations—facilitating the establishment, persistence, and impact of invasive bivalves—but as a toxin at higher concentrations.

We investigated the effects of invasive zebra mussels and Asian clams on native communities and whether an increase in the concentration of CaCl_2 road salt would promote or minimize their growth and ecological consequences. These species include some of the most well-known and densely populated invasive bivalves in the USA (Pimentel et al. 2007). We hypothesized that the invasive bivalves decrease

the abundance of zooplankton and native clams through competition for phytoplankton. We also hypothesized that invasive bivalves facilitate benthic algae through phytoplankton reduction and waste excretion, and subsequently increase the survival and abundance of native grazers (e.g., amphipods and snails). We also hypothesized that when native communities are exposed to increased CaCl_2 , native mollusks increase in biomass and density. We predicted that there would be no decreases in the biomass and density of organisms (i.e., negative consequences) in communities exposed to low CaCl_2 concentrations ($< 100 \text{ mg Ca}^{2+} \text{ L}^{-1}$ or $< 175 \text{ mg Cl}^- \text{ L}^{-1}$), and few negative consequences to communities exposed moderate CaCl_2 concentrations ($< 230 \text{ mg Ca}^{2+} \text{ L}^{-1}$ or $< 400 \text{ mg Cl}^- \text{ L}^{-1}$). Lastly, we hypothesized that the addition of CaCl_2 to invaded communities increases invasive bivalve growth and abundance, and this results in a greater negative impact on communities with invasive bivalves.

Methods

Experimental design and setup

We conducted the experiment outside at the Rensselaer Aquatic Laboratory (Troy, New York, USA) during summer 2016. We used a randomized, factorial design that consisted of two community treatments: (1) a community comprised entirely of native species (herein “native”) and (2) a community comprised of the same native species plus invasive Asian clams and zebra mussels (herein “invaded”). We crossed these two community treatments with five nominal CaCl_2 concentrations. Our nominal concentrations were originally 17 (control; no CaCl_2 added), 25, 50, 100, and $200 \text{ mg Ca}^{2+} \text{ L}^{-1}$. However, we later discovered that the CaCl_2 we used was 75% CaCl_2 and 25% water. As a result, our revised nominal concentrations based on the amount of CaCl_2 added to the tanks were 17, 23, 41, 79, and $154 \text{ mg Ca}^{2+} \text{ L}^{-1}$, which corresponded to 18, 29, 62, 128, and $261 \text{ mg Cl}^- \text{ L}^{-1}$. These nominal calcium concentrations are found in lakes in the USA (Fig. 1) and other water bodies around the world (Karatayev et al. 2007; Jokela and Ricciardi 2008). The nominal chloride concentrations occur in many lakes, streams, and wetlands (Hintz and Relyea 2019). Additionally, our highest nominal

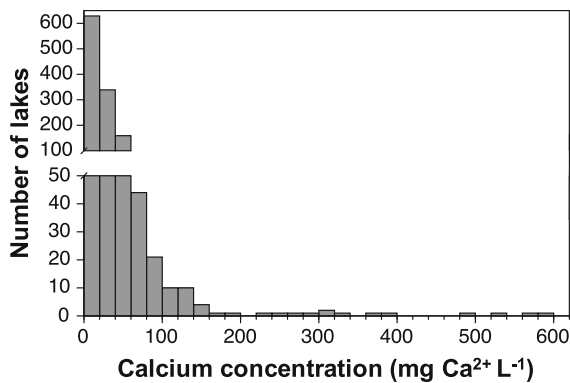


Fig. 1 The calcium concentrations found in nature based on the 2012 EPA NLA dataset (available online at <https://www.epa.gov/national-aquatic-resource-surveys/data-national-aquatic-resource-surveys>)

concentration ($261 \text{ mg Cl}^- \text{ L}^{-1}$) is above the US EPA chronic threshold of $230 \text{ mg Cl}^- \text{ L}^{-1}$ (Benoit and Stephan 1988). Actual calcium concentrations are described in the “Sampling abiotic variables” section. We replicated our ten treatment combinations four times, for a total of 40 experimental units.

Our experimental units were outdoor 800-L polyethylene experimental ponds (mesocosms), which we covered with 60% shade cloth to prevent organisms from colonizing or ovipositing (Howeth and Leibold 2010). On 9 May, we filled each mesocosm with 570 L of low-nutrient ($0.13 \text{ mg total N L}^{-1}$; $4.56 \text{ } \mu\text{g total P L}^{-1}$; $17 \text{ mg Ca}^{2+} \text{ L}^{-1}$) and low-salt ($18 \text{ mg Cl}^- \text{ L}^{-1}$) water that was collected from Lake George, New York ($43^\circ 33' 15.3'' \text{ N } 73^\circ 39' 09.0'' \text{ W}$). On 13 May, we added 100 L of sandy loam soil (average depth = 8 cm) to create a substrate for bivalves to bury. On 26 May, we added 125 g of dried oak leaf litter (*Quercus* spp.) to the mesocosms to add nutrients and substrate for the organisms. To provide a standardized substrate to sample periphyton during the experiment, we positioned two three cm by six cm ceramic tiles and one six cm by six cm ceramic tile vertically on top of the soil on the north-facing side of the mesocosm on 2 June.

We began adding native organisms on 27 May. To supplement the diversity of algae and zooplankton already in the lake water used to fill the mesocosms, we collected plankton from Lake George ($43^\circ 35' 24.0'' \text{ N } 73^\circ 37' 48.0'' \text{ W}$) using vertical plankton tows (64- μm mesh). We created a concentrated, homogenized slurry, removed any predators

from the slurry (e.g., fish and insects), and added a 450-mL aliquot to each mesocosm. From 31 May to 7 June, we added 15 amphipods (*Hyaella* sp.), 16 isopods (*Asellus aquaticus*), 10 pouch snails (*Physella acuta*), 10 ramshorn snails (*Planorbella trivolvis*), and 12 native fingernail clams (*Sphaerium occidentale*) to every mesocosm. For the invaded treatments, we added 100 Asian clams (10 to 19 mm long, anterior to posterior) and 100 zebra mussels (4 to 16 mm long, anterior to posterior) between 7 and 10 June. This number corresponds to a density of approximately $76 \text{ individuals (m}^2\text{)}^{-1}$ for each species, which is within the range of natural densities (Ramcharan et al. 1992; Lucy et al. 2012). We included both species in our tanks because they are commonly found together and the number of locations with more than one invasive bivalve is expected to increase (Karatayev et al. 2007). For all animals, we used a range of adult sizes determined via sieves and we provided each tank with the same assortment (i.e., the same size range per tank). We collected all animals from local lakes, ponds, and rivers.

We allowed all organisms to acclimate in the mesocosms together for an additional 4 d before applying the CaCl_2 treatments on 14 June. Our control concentrations, $17 \text{ mg Ca}^{2+} \text{ L}^{-1}$ and $18 \text{ mg Cl}^- \text{ L}^{-1}$, were the baseline concentrations of Lake George. We accounted for these when we calculated our nominal CaCl_2 concentrations. We used a commercial formulation of CaCl_2 (SafeStep[®] Ice Melter 7300, Overland Park, KS, USA), which was reported as “high purity,” 100% CaCl_2 and later found to be 75% CaCl_2 and 25% water. We added this to the tanks on 14 June to increase the calcium and chloride concentrations in the mesocosms, which constituted day 0 of the experiment.

Sampling abiotic variables

After adding the CaCl_2 , we let the experiment run for 92 d (14 June to 13 September). We assessed the calcium concentrations of our experiment, which we did by collecting water samples from the water column. We pooled water samples from the four replicates, providing one sample for each of the 10 treatments. We placed the pooled samples into acid-washed, 250-mL Nalgene bottles, added 0.5 mL of concentrated nitric acid to preserve the samples, and stored all samples at 4°C . All samples were measured

within 1 month of collection via flame atomic absorption spectroscopy (Greenberg et al. 1992a). To better understand the dynamics of CaCl_2 over time, we took calcium samples every week (Fig. 2). It took until the second week of the experiment (28 June) for the salt to stabilize and to be distributed throughout the water column. Therefore, we used the day 14 sample for our nominal vs actual comparison. Later in the experiment, on day 45, there was a large rainstorm, which diluted the calcium concentrations measured on day 49 and thereafter.

Our observed dissolved concentrations—averaged across both community types—were 35, 38, 58, 91, and 187 $\text{mg Ca}^{2+} \text{L}^{-1}$, respectively on day 14. The control did not receive CaCl_2 , so the discrepancy between the actual and revised nominal concentrations (ranging from 12 to 33 $\text{mg Ca}^{2+} \text{L}^{-1}$) is likely due to calcium leaching from the soil in the mesocosms. Because all mesocosms received the same amount of soil, the soil would have had a similar contribution to all tanks (i.e., a mean increase ± 1 SE of $19 \pm 4 \text{ mg Ca}^{2+} \text{L}^{-1}$). Because we have actual, measured concentrations of calcium, but they differ from our nominal, we report all results and figures using the observed calcium concentrations with the understanding that the associated chloride ions were present.

Therefore, any changes to our response variables could be due to either ion or both ions in combination.

We measured temperature ($^{\circ}\text{C}$), dissolved oxygen ($\text{mg O}_2 \text{L}^{-1}$), conductivity ($\mu\text{S cm}^{-1}$), and pH on days 7, 29, 52, 63, and 87. We conducted these measurements using a calibrated YSI ProPlus Multi-parameter Instrument (YSI, Yellow Springs, OH, USA). We performed all measurements midway in the water column and at midday to limit diel variation. Because conductivity (C) varies with temperature, we converted conductivity to specific conductance (SC) to standardize it using the equation:

$$SC = \frac{C}{1 + r(T - 25)}$$

where r is a constant temperature correction coefficient (0.0191) and T is water temperature ($^{\circ}\text{C}$; Greenberg et al. 1992b). Complete results for the abiotic response variables (including figures and statistical tables) can be found in the “Appendix”.

Sampling biotic variables

We sampled phytoplankton on days 17, 51, and 86. To do this, we collected two, 400-mL samples from the middle of the water column: one from the north side

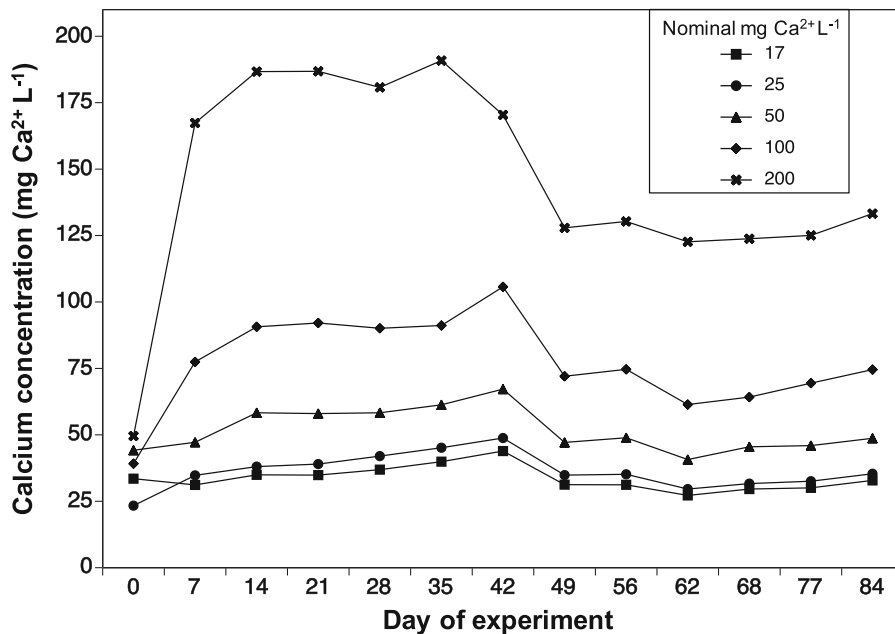


Fig. 2 The measured calcium concentrations averaged across both communities throughout the experiment. On day 45, there was a large rain storm, which diluted the concentrations measured on day 49 and thereafter

and one from the center of each mesocosm. We then combined these two samples and filtered them through a glass microfiber filter (1.2- μm pore size; Grade GF/C; Whatman, Inc.). We immediately froze the filters until later analysis via fluorometry (TD-700 Fluorometer, Turner Designs, San Jose, CA, USA) with acid correction to analyze chlorophyll *a* concentrations (Arar and Collins 1997).

To quantify filamentous algae, we took photos midday on day 87 of the experiment (for an example of the tank pictures, see Fig. 4). We used the ellipse select tool in GIMP version 2.8.16 to estimate the number of pixels in each circular tank bottom. Next, we used the free select tool (or “lasso”) to select the area that contained algae and estimated the pixels in that selection. Lastly, we divided the algae pixels by the tank bottom pixels to obtain the proportion of the tank covered in filamentous algae. In order to be blind to the treatments, we performed these measurements in the original randomized order of the tanks.

We commonly take our first periphyton sample after the first phytoplankton and zooplankton sample. A change in zooplankton, and any subsequent changes in phytoplankton, can occur quickly. However, consequential changes in periphyton from phytoplankton changes often lag behind. Therefore, we sampled periphyton on days 30, 51, and 86 of the experiment. On each date, we removed one tile that we placed in the tanks on 2 June and then scrubbed and rinsed the periphyton off the front surface. We then filtered the resulting periphyton slurry through glass microfiber filters (1.2- μm pore size; Grade GF/C; Whatman, Inc.) that had been dried and pre-weighed. After this, we dried the periphyton-covered filters at 60 °C for 48 h and then reweighed the filters. We divided the periphyton mass by the surface area of the tiles to obtain a measurement in $\text{g (cm}^2\text{)}^{-1}$.

We sampled zooplankton on days 17, 50, and 86 of the experiment. We collected 500 mL from the middle of the water column at the four cardinal directions and the center of each mesocosm. We then filtered the 2.5 L of water through a 64- μm mesh and preserved all samples with Lugol’s iodine solution. Our initial investigation indicated no species-level differences in response to the treatments. Therefore, we later classified the zooplankton into four major taxonomic groups, including cladocerans, copepods (i.e., nauplii, copepodites, and adult copepods), rotifers, and

ostracods. Rotifers were rare among samples, so we did not include them in our final analyses.

On day 92 of the experiment, we sampled macroinvertebrates by scraping 50% of the side area of the mesocosm with an aquarium net (250- μm mesh). We then scraped 50% of the bottom area of the mesocosm with a D-frame net (500- μm mesh). We combined these two samples, washed and removed large pieces of leaf litter, and preserved all organisms in 70% ethanol. Due to the size of the samples, we later homogenized the samples and subsampled one-fourth of each. After subsampling, we sorted all organisms under a light microscope (Olympus SZ51, Olympus America, Center Valley, PA). We recorded the density of amphipods, isopods, fingernail clams, pouch snails, and ramshorn snails. For both snail species, we counted juveniles and adults that were > 2 mm and also recorded total wet-mass data for the snails. During this sampling effort, we collected all zebra mussels and Asian clams that were in the macroinvertebrate sample. We preserved these individuals in 70% ethanol and set them aside to be added with the remaining individuals collected the following day.

The following day, we drained every mesocosm assigned to the invaded treatment through a 7-mm sieve. This sieve size was sufficient to capture the smallest Asian clams and zebra mussels (there was no evidence of either species reproducing). As with the other invertebrates, we preserved them in 70% ethanol and later combined them with the invasive bivalves collected the day prior in the macroinvertebrate net sweeps. We recorded density and total dry-biomass data for Asian clams and zebra mussels.

Statistical analysis

Our statistical analyses examined the effects of CaCl_2 , community type, and their interaction. For response variables collected multiple times during the experiment (abiotic measurements, phytoplankton, periphyton, and all zooplankton groups), we conducted two-way repeated-measures analyses of variance (rm-ANOVA). If there were any significant ($p < 0.05$) or marginally significant ($p < 0.10$) time-by-treatment interactions, we subsequently analyzed each response variable within a sample date using a two-way ANOVA. For ANOVAs, we increased our α a priori to $p < 0.10$ for several reasons including: (1) p values are continuous, (2) 0.05 is arbitrary and patterns can

still exist beyond 0.05, (3) these experiments often have high variation, and (4) we wanted to protect against Type II error (Murtaugh 2014; Hintz et al. 2017). For response variables collected once (i.e., at the end of the experiment), we conducted a two-way ANOVA for the native macroinvertebrate response variables and filamentous algae and a one-way ANOVA for the invasive bivalves. We checked all ANOVA assumptions and when the assumptions were not met, we transformed data. If a transformation was necessary, we indicate what transformation was performed within the y-axis label and figure caption.

When the ANOVAs were significant or marginally significant, we used post-hoc tests to investigate what concentrations differed from the control (Dunnett's; indicated by stars in figures), when communities differed (Tukey's; indicated by daggers), and what timepoints differed (Tukey's; indicated by letters). For example, when there was a main effect of CaCl_2 , we used Dunnett's post-hoc test to compare each of the elevated CaCl_2 treatments against the low- CaCl_2 control (indicated by gray stars). If there was a significant or marginally significant CaCl_2 -by-community interaction, we performed a Dunnett's post-hoc test to compare the CaCl_2 treatments *within each community* to the low- CaCl_2 control (native—white stars; invaded—black stars). When there was a CaCl_2 -by-community interaction, we also compared the two community types at each CaCl_2 concentration with Tukey's post-hoc test to see at which CaCl_2 concentration the communities differed (indicated by daggers). If time was the only significant effect, we used Tukey's post-hoc test to compare the multiple sample times (indicated by letters). ANOVA tables can be found in the "Appendix" for all variables. All analyses were completed in RStudio version 1.1.383 (R Development Core Team 2015). All figures were made using DeltaGraph version 7.1.3.

Results

Primary producers

Our analysis of phytoplankton indicated effects of CaCl_2 , community, time, and several time-by-treatment interactions, including a three-way interaction (Table S2). On day 17, we found no effects of CaCl_2 , community, or their interaction ($p \geq 0.284$; Fig. 3a).

On day 51, we found main effects of CaCl_2 ($p = 0.022$), community ($p < 0.001$) and a marginal interaction ($p = 0.099$; Fig. 3b). Within community treatments (indicated by stars), the native community showed no response to increased CaCl_2 ($p \geq 0.350$), whereas the invaded communities exhibited a 456% increase in chlorophyll *a* at the highest CaCl_2 concentration relative to $35 \text{ mg Ca}^{2+} \text{ L}^{-1}$, the control ($p < 0.001$). Within CaCl_2 treatments (indicated by daggers), the invaded communities had 73–89% less chlorophyll *a* than the native communities at the three intermediate concentrations ($p \leq 0.024$), but there was no difference between community types at the lowest ($35 \text{ mg Ca}^{2+} \text{ L}^{-1}$) or highest ($187 \text{ mg Ca}^{2+} \text{ L}^{-1}$) CaCl_2 concentrations ($p \geq 0.151$). On day 86, we found a significant effect of community ($p < 0.001$), but not CaCl_2 ($p = 0.216$) or their interaction ($p = 0.446$; Fig. 3c). On this day, across all CaCl_2 concentrations, invaded communities had 57% less chlorophyll *a* than native communities.

Our analysis of filamentous algal abundance revealed only a main effect of community (Table S2; Fig. 4), with invaded communities having more filamentous algae than native communities. Because the data suggested a pattern of declining differences between communities with increased CaCl_2 concentrations, we also examined the effects of community type within each CaCl_2 concentration. In doing so, we found that the community effect was driven by invaded communities having 553% and 656% more filamentous algae in the two lowest CaCl_2 concentrations (both $p \leq 0.05$), but not in the higher CaCl_2 concentrations.

Our analysis of periphyton abundance (i.e. attached to the tiles along the bottom of the mesocosms) revealed main effects of CaCl_2 , community, and time (Table S2). Among CaCl_2 concentrations, we found a 56% increase at $38 \text{ mg Ca}^{2+} \text{ L}^{-1}$ compared to the control ($p = 0.058$), but the higher concentrations did not differ from the control ($p \geq 0.721$; Fig. 5a). Invaded communities had 18% less periphyton than native communities ($p = 0.018$; Fig. 5b). The time effect occurred because there was more periphyton on days 51 and 86 than on day 30 ($p < 0.001$; Fig. 5c).

Zooplankton

Our analysis of cladoceran density revealed a three-way interaction ($p = 0.003$; Table S3). On day 17, we

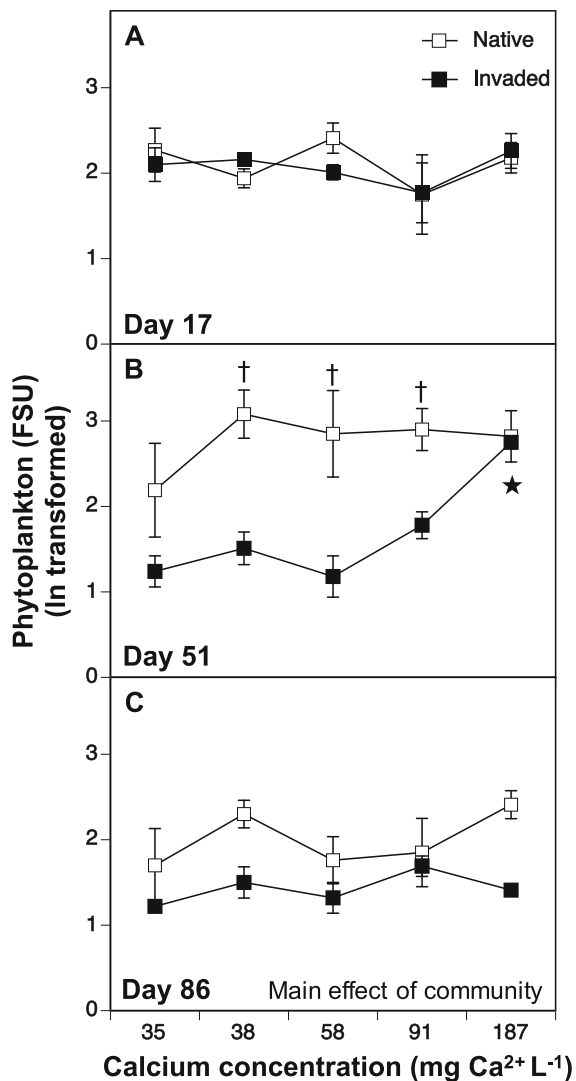


Fig. 3 The effects of invasive bivalves and CaCl₂ salt on phytoplankton abundance measured as chlorophyll *a* fluorescence units (FSU) across time. To meet the ANOVA assumptions, we natural log transformed the data. Error bars represent standard errors. All points have error bars, but some are masked by the data points. Stars indicate significant differences ($p < 0.05$) horizontally from the lowest concentration within a community (black = invaded community, white = native community). Dagggers indicate significant differences ($p < 0.05$) vertically between communities within a calcium concentration

found a CaCl₂-by-community interaction ($p = 0.026$; Fig. 6a). Native communities at 91 mg Ca²⁺ L⁻¹ and 187 mg Ca²⁺ L⁻¹ had 70–87% fewer individuals compared to the control ($p \leq 0.010$); invaded communities at 187 mg Ca²⁺ L⁻¹ had 92% fewer

individuals ($p = 0.006$). There were also community differences at 35 and 187 mg Ca²⁺ L⁻¹, with fewer cladocerans in the invaded communities ($p \leq 0.04$). On day 50, we only found an effect of CaCl₂ ($p = 0.023$; Fig. 6b), which was driven by 76% fewer individuals in 91 mg Ca²⁺ L⁻¹ compared to the control ($p = 0.065$). On day 86, we found a CaCl₂-by-community interaction, which was driven by fewer individuals in the native 187 mg Ca²⁺ L⁻¹ treatment ($p = 0.052$; Fig. 6c) and the invasive 58 mg Ca²⁺ L⁻¹ than the controls within those communities ($p = 0.019$).

For copepod density, a time-by-CaCl₂ interaction occurred (Table S3). On day 17, we found an effect of CaCl₂ ($p = 0.008$; Fig. 6d). There were 122% more individuals in 91 mg Ca²⁺ L⁻¹ ($p = 0.051$) and 174% more individuals in 187 mg Ca²⁺ L⁻¹ compared to the control ($p = 0.009$). On day 50, we also found an effect of CaCl₂ ($p = 0.020$; Fig. 6e). There were 58% fewer individuals in 187 mg Ca²⁺ L⁻¹ relative to the control ($p = 0.014$). We found no effect of CaCl₂ on day 86 ($p = 0.236$; Fig. 6f). Additional details on zooplankton results can be found in the “Appendix”.

Native macroinvertebrates

For native macroinvertebrates, we did not find widespread, similar effects across the taxonomic groups. Our ANOVA analysis of amphipod abundance revealed an effect of CaCl₂ but when we completed the post-hoc analysis, we did not find any CaCl₂ treatment differences (Table S4; Fig. 7a). For isopods abundance, we only found an effect of community, which was 62% more isopods in invaded communities ($p = 0.036$; Table S4; Fig. 7b). Native clam abundance showed a CaCl₂-by-community interaction (Table S4; Fig. 7c). We found 76–90% fewer clams in the invaded communities at 35, 38, and 58 mg Ca²⁺ L⁻¹ ($p \leq 0.022$), but no difference among CaCl₂ treatments within each community ($p \geq 0.159$). For the two species of snails, we found an effect of community on ramshorn snail individual mass, but we observed no effects on ramshorn snail abundance or on pouch snail abundance and mass (Table S4; Fig. 7d). Ramshorn snail mass was reduced by 58% in invaded communities compared to native communities. Additional details on macroinvertebrate results can be found in the “Appendix”.

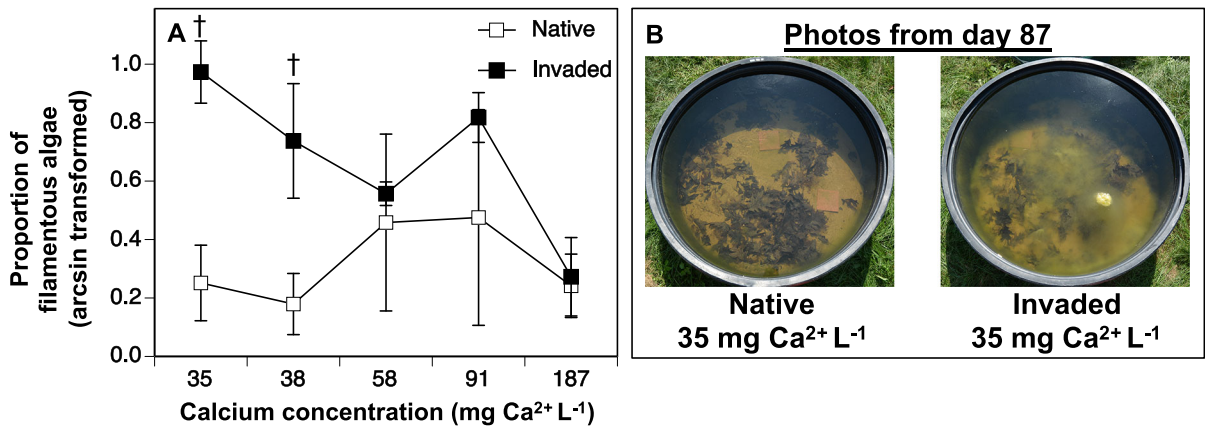


Fig. 4 Filamentous algae abundance on day 87 measured via pixel count of photos (a) and examples of the photos showing the filamentous algae in the physical tanks (b). To meet the ANOVA assumptions, we arcsine transformed the data. Error

bars represent standard errors. Daggers indicate significant differences ($p < 0.05$) between communities within a calcium concentration

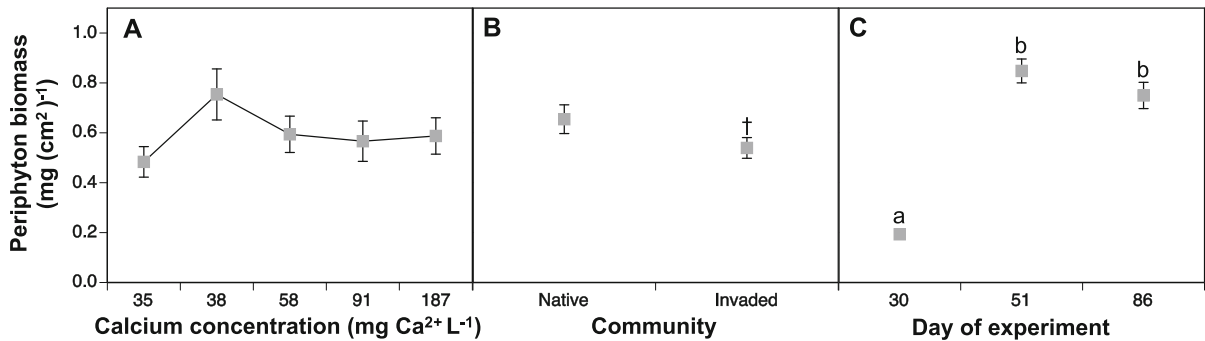


Fig. 5 The main effects of **a** increased CaCl₂ (averaged across communities and time), **b** invasive bivalves (averaged across calcium concentrations and time), and **c** time (averaged across communities and calcium concentrations) on periphyton abundance measured via ceramic tiles. Error bars represent standard

errors. All points have error bars, but some are masked by the data points. Daggers indicate significant differences ($p < 0.05$) between communities. Letters indicate significant differences between time points

Invasive bivalves

Our analysis of Asian clam survival revealed an effect of CaCl₂ (Table S5; Fig. 8a). The 91 mg Ca²⁺ L⁻¹ and 187 mg Ca²⁺ L⁻¹ treatments had 34% and 80% fewer surviving Asian clams, respectively, than the control ($p \leq 0.002$). We found no effect on the individual mass of the clams (Table S5). The average number (± 1 SE) of Asian clams recovered (alive and dead) was 99 ± 0.4 , indicating no reproduction.

For zebra mussels, we found no effects of CaCl₂ on survival or individual mass (Table S5; Fig. 8b). Zebra mussel survival was poor across all treatments with an average survival of 13%. The average number (± 1 SE) recovered (alive and dead) was 51 ± 3.3 . The

remaining 49% likely died and their shells broke down during the experiment, which prevented us from recovering them. Therefore, an average of 87% of the zebra mussels died across the treatments, with no significant effect of the CaCl₂ treatments. Zebra mussels likely did not reproduce in our mesocosms, which was also supported by the lack of small, juvenile individuals.

Discussion

Our study is the first to examine the effects of CaCl₂ road salt on invasive bivalves and their combined effects on freshwater communities (summarized in

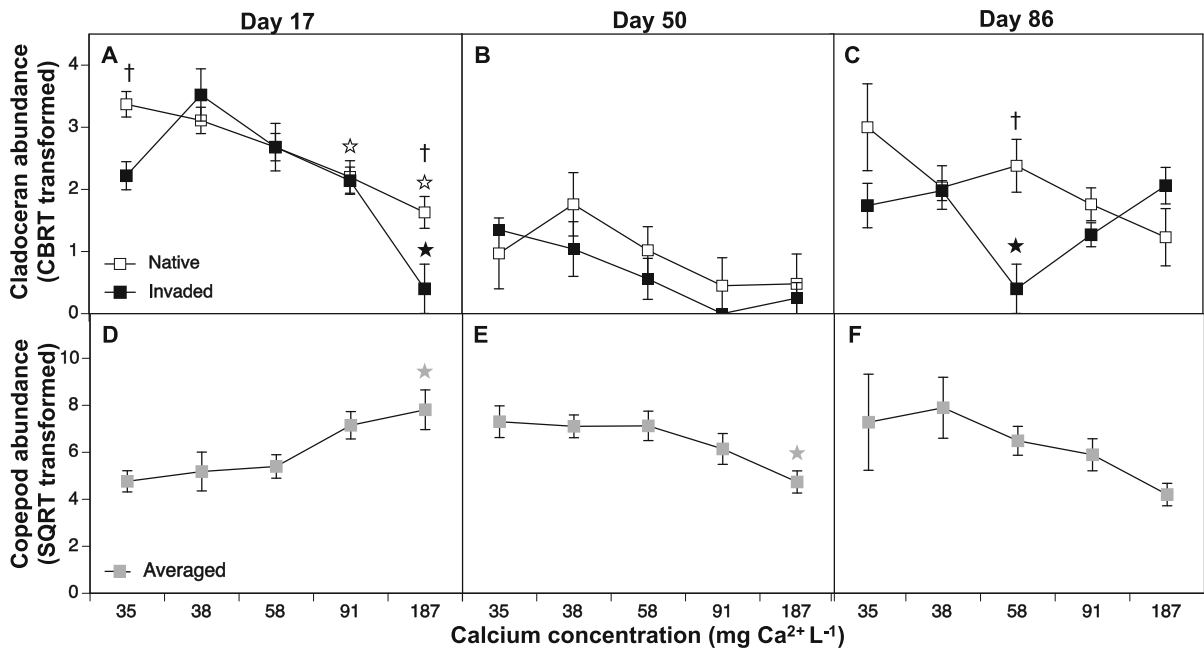


Fig. 6 The effects of invasive bivalves and CaCl_2 salt on cladoceran abundance (**a, b, c**) and the main effects of CaCl_2 salt on copepod abundance (averaged across communities; **d, e, f**). To meet the assumptions of the ANOVA, we cube-root transformed the cladoceran data and square-root transformed the copepod data. Error bars represent standard errors. Stars

indicate significant differences ($p < 0.05$) horizontally from the lowest concentration within a community (black = invaded community, white = native community, gray = both communities averaged). Daggers indicate significant differences ($p < 0.05$) between communities within a calcium concentration

Fig. 9). We hypothesized that increases in calcium from CaCl_2 would promote the growth and abundance of invasive bivalves, while resulting in few toxic effects from the associated chloride ion. We found no positive effects of calcium increases at low CaCl_2 concentrations (38–58 $\text{mg Ca}^{2+} \text{L}^{-1}$) and substantial Asian clam mortality at high CaCl_2 concentrations (91–187 $\text{mg Ca}^{2+} \text{L}^{-1}$). Thus, in our experiment, elevated calcium concentration from CaCl_2 road salt did not facilitate successful growth and reproduction of invasive bivalves.

At the lowest CaCl_2 concentrations, the invasive bivalves had major effects on native communities. Invaded communities had a lower abundance of phytoplankton, native clams, and periphyton, but more filamentous algae. Given that zebra mussel survival was low, but similar, across all calcium concentrations, it is likely that the effects of the invasive species were largely caused by the Asian clams. Further evidence for the strong effect of Asian clams, rather than zebra mussels, on the community can be inferred from the lack of differences in most of the community responses between native and invaded

communities when exposed to the highest CaCl_2 concentration, in which Asian clams exhibited low survival. However, across all CaCl_2 concentrations, nutrient increases from zebra mussel death could have contributed to some of the effects observed.

Primary producers

The CaCl_2 effect we observed on phytoplankton in only the invasive community was an indirect effect of the Asian clam mortality. At low concentrations, invaded communities had reduced phytoplankton and at the high concentration where substantial death occurred, the phytoplankton concentrations did not differ from the native community. Asian clams filter feed quickly, filtering up to $347 \text{ mL hr}^{-1} \text{ clam}^{-1}$ (Buttner and Heidinger 1981), which means they could filter the entire water volume in our mesocosms in approximately 16 h. Through filtering, invasive bivalves remove phytoplankton, chemicals, and other particles in the water, which may improve water quality (Strayer 2010; Ismail et al. 2014; Sousa et al. 2014). This can also reduce the abundance of other

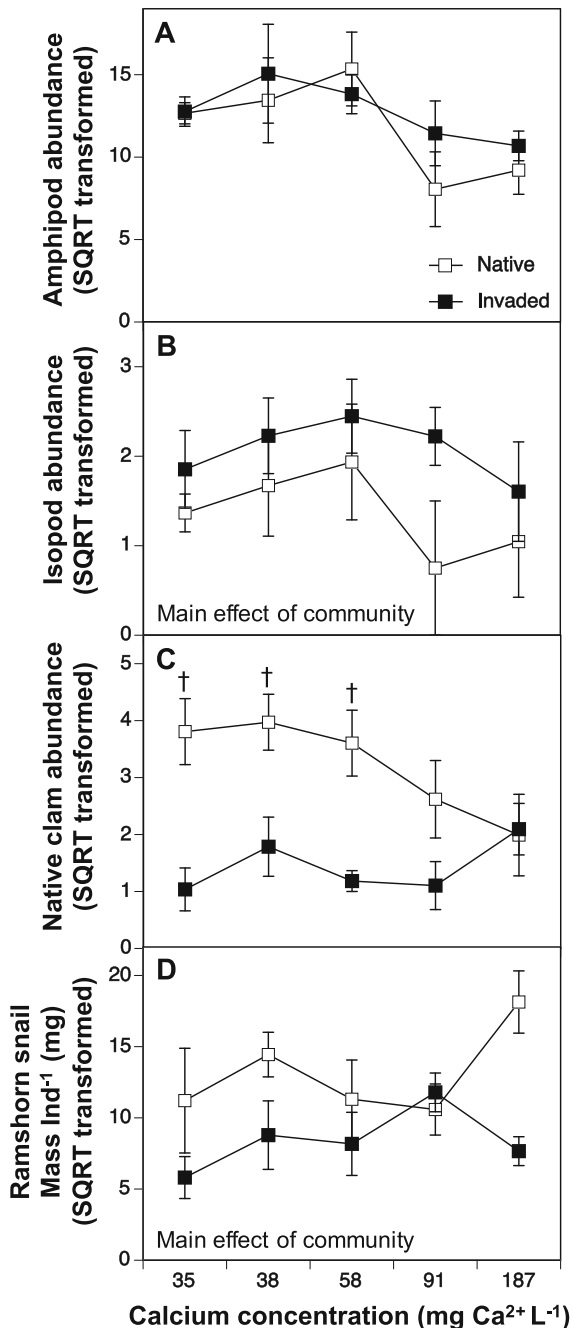


Fig. 7 The impacts of invasive bivalves and CaCl_2 salt on native macroinvertebrates including **a** amphipod abundance, **b** isopods abundance, **c** native clam abundance, and **d** ramshorn snail mass per individual. To meet the assumptions of the ANOVA, we square-root transformed all of the data. Error bars represent standard errors. Dagggers indicate significant differences ($p < 0.05$) between communities within a calcium concentration

organisms that consume phytoplankton (i.e., native bivalves and zooplankton; Karatayev et al. 2007; Lucy et al. 2012; Sousa et al. 2014). We found evidence of this indirect effect on native clams, but we did not find any evidence that it occurred with zooplankton in our study. For example, decreases in phytoplankton on days 51 at 38, 58, and 91 $\text{mg Ca}^{2+} \text{L}^{-1}$ (Fig. 3), did not correspond to decreases in zooplankton at these CaCl_2 treatments within any time point (Fig. 6). However, more frequent, staggered sampling might reveal differences in zooplankton abundance that are not concurrent, but rather lag behind the decreases in phytoplankton and thus should be investigated further.

In contrast, we found that filamentous algae was more abundant in invaded communities, specifically at the lower CaCl_2 concentrations that did not affect Asian clam survival. Other studies, such as those completed on Lake Tahoe, have also noted that Asian clams are associated with increases in filamentous algae (Forrest et al. 2012; Wittmann and Chandra 2015). Asian clams excrete nutrients at the benthos and this can provide benthic algae a competitive advantage (Hakenkamp et al. 2001; Lucy et al. 2012; Xiao et al. 2014). The removal of phytoplankton by Asian clams should also increase light at the benthos, further promoting benthic algae growth. However, because the growth of filamentous algae can occur throughout the water column, as well as at the benthos, an increase in filamentous algae can limit/impede light penetrance and consume nutrients. This might prevent increases in attached periphyton, which is what we observed in our experiment. Lastly, the death of zebra mussels across all CaCl_2 treatments could have altered nutrients concentrations and resulted in subsequent changes on all algal types. Changes in the competitive dynamics of resources could have bottom-up effects on higher trophic levels.

Zooplankton

While prior research suggests invasive bivalves can directly compete with zooplankton for resources (Karatayev et al. 2007; Lucy et al. 2012; Sousa et al. 2014), we did not observe a concurrent decrease in phytoplankton and zooplankton densities. However, we did observe a direct effect of CaCl_2 treatments on zooplankton. Cladoceran and copepod densities decreased with increasing CaCl_2 , regardless of community type. Initially, however, copepods increased,

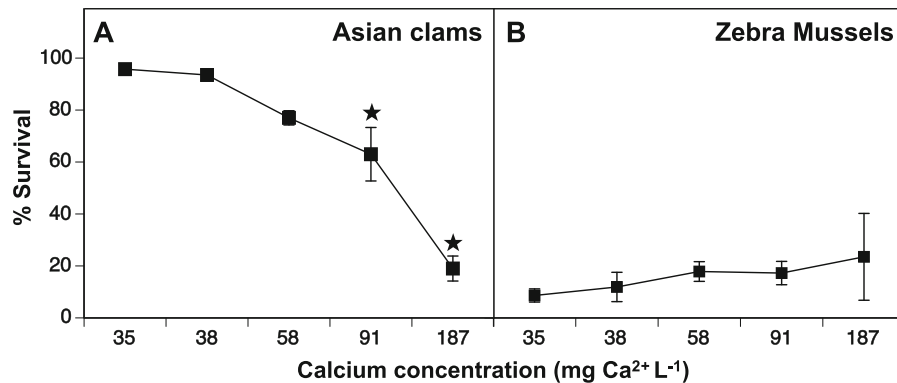


Fig. 8 The impacts of CaCl_2 salt on the survival of the invasive **a** Asian clam and **b** zebra mussel. Error bars represent standard errors. All points have error bars, but some are masked by the

data points. Stars indicate significant differences ($p < 0.05$) from the lowest concentration

which was likely due to reduced competition with large-bodied cladocerans and a delay in CaCl_2 toxicity. Many other road salt community studies show decreases in zooplankton density at chloride concentrations above what we used in our study, although most of these studies use NaCl (Van Meter et al. 2011; Dalinsky et al. 2014; Van Meter and Swan 2014; Jones et al. 2017). However, it is well established in laboratory studies on aquatic organisms, including zooplankton, benthic invertebrates, and fish, that CaCl_2 can be more toxic than NaCl (Benoit and Stephan 1988; Evans and Frick 2001). Additionally, at our highest CaCl_2 concentration, the nominal chloride concentration ($261 \text{ mg Cl}^- \text{ L}^{-1}$) did exceed the EPA chronic threshold of $230 \text{ mg Cl}^- \text{ L}^{-1}$. This threshold is based on experiments that used the less toxic NaCl (Benoit and Stephan 1988). Thus, further investigation into the toxic effects of CaCl_2 on zooplankton are needed, especially if we wish to use this salt more in the future.

The effects of CaCl_2 on zooplankton did not last until the end of the experiment. This could be a result of evolved tolerance to CaCl_2 . *Daphnia pulex*, a common cladoceran and model organism, has been shown to evolve tolerance to NaCl road salt in 2.5 months, or 5–10 generations, in a similar mesocosm setting (Coldsnow et al. 2017). Increased salt tolerance also occurred in *Daphnia* in a number of other circumstances, including seawater intrusion (Weider and Hebert 1987; Latta et al. 2012; Loureiro et al. 2012; Liao et al. 2015; Venâncio et al. 2019). Evolved salt tolerance remains largely unknown for other zooplankton species. Nevertheless, the potential

recovery of zooplankton from evolved tolerance might help buffer aquatic communities experiencing road salt contamination. However, we do not know the maximum concentration zooplankton can survive and evolve tolerance to or what the sublethal effects are to the populations.

Native macroinvertebrates

We found no effects of increased CaCl_2 on macroinvertebrate abundance or snail mass, despite the importance of calcium in macroinvertebrate metabolism and exoskeletons (Greenaway 1985). In amphipods, for example, calcium carbonate makes up approximately 40% of their cuticles during the third stage of their molt cycle (Hyne 2011). As for the accompanying chloride, studies have shown that macroinvertebrate survival is not affected until prolonged exposure reaches approximately $500 \text{ mg Cl}^- \text{ L}^{-1}$ (Bartlett et al. 2012; Hintz et al. 2017), whereas our highest chloride concentration was approximately $261 \text{ mg Cl}^- \text{ L}^{-1}$. Furthermore, some snail species appear quite tolerant to salt. For example, in some community studies they increase in abundance with increasing NaCl road salt (Evans and Frick 2001; Stoler et al. 2016; Hintz et al. 2017).

We found that invasive bivalves increased isopod abundance and decreased ramshorn snail mass. We hypothesize that the increase in isopods was from the large increase in filamentous algae, which is a food source for isopods (Marcus et al. 1978). The decrease in ramshorn snail mass likely occurred from the decrease in periphyton resources. However, pouch

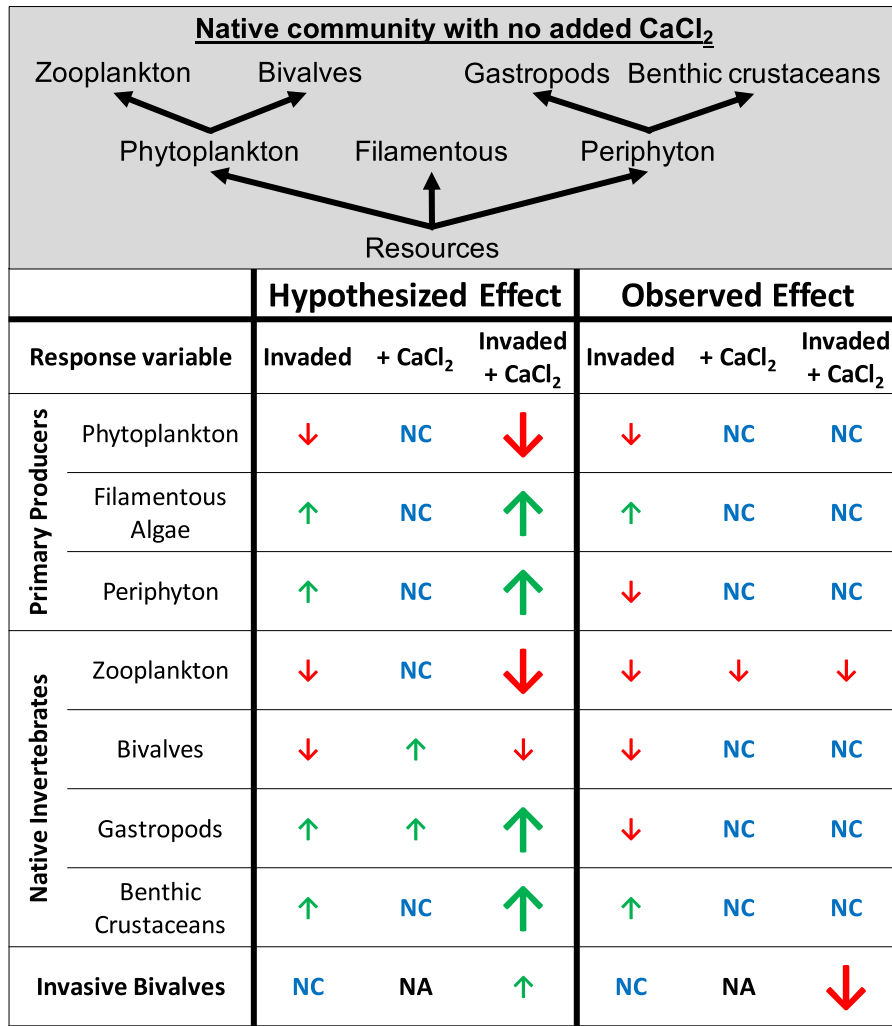


Fig. 9 A conceptual figure illustrating the hypothesized and observed effects. The top of the figure shows a basic, control (no invasive species, no CaCl₂ addition) food web for reference. The first main column (“Response Variable”) lists each variable in the above food web. The “Hypothesized Effect” columns indicate the direction and magnitude of the hypothesized change for each response variable when exposed to invasive bivalves (“Invaded”), CaCl₂ (“+ CaCl₂”), and both (“Invaded + CaCl₂”). This can also be found in the last paragraph of the

introduction. The “Observed Effect” columns indicate the direction and magnitude of the observed change for the same variables and conditions. *Down, red arrows* indicate a decrease in that response variable, while *up, green arrows* indicate an increase. The *size of the arrow* indicates the magnitude of the change. The blue “NC” indicate no change hypothesized or observed. Lastly, “NA” for the “Invasive Bivalve” row under the “+ CaCl₂” column is because there were no invasive bivalves present in that treatment

snails also consume periphyton, but they did not exhibit a similar response. Ramshorn snails employ different feeding strategies, they consumer different food types and sizes, and their larger teeth might have affected their ability to consume the limited periphyton, all which may have affected their ability to gain enough food for their larger bodies (Lombardo and Cooke 2002, 2004; Zimmermann et al. 2014). Further research should investigate whether invasive mollusks

decrease all components of periphyton, or if certain components (i.e., algae versus bacteria) or certain sizes (i.e., large versus small-bodied algae) are more affected.

The reduction in phytoplankton by invasive bivalves likely caused the observed decrease in native clams. Higher densities (2000 individuals (m²)⁻¹) of Asian clams can negatively affect the growth, physiological condition, and activity of native mussels in

Europe (Ferreira-Rodríguez et al. 2018); while lower densities (< 50 individuals $(\text{m}^2)^{-1}$) were not correlated with native mussel abundance in the USA (Karatayev et al. 2003). In addition, some native bivalve populations recovered 8 years after zebra mussels invaded the Hudson River (Strayer 2010). Therefore, the effects on native bivalves depends on the recency of invasion and invasive density, as well as environmental conditions. The impacts of invasive bivalves on native bivalves should be investigated further, particularly since the status of many native bivalves is currently of conservation concern (Lopes-Lima et al. 2018).

Invasive bivalves

We did not see an effect of CaCl_2 on zebra mussels, but overall survival was poor across all CaCl_2 concentrations. Zebra mussels are far more sensitive to low calcium (Cohen and Weinstein 2001; Karatayev et al. 2007; Whittier et al. 2008), which they experienced in husbandry and before CaCl_2 treatments were added. Additionally, the presence of Asian clams, which reduced native clams, may have also affected zebra mussel survival. When the zebra mussels died, there was likely a release of nutrients into the water, which could have affected other abiotic and biotic responses. Zebra mussel survival did not vary across CaCl_2 concentrations, while some response variables did vary across concentrations and for some, only in invaded communities. The only other variable that did vary was Asian clam survival.

We found that our higher concentrations of CaCl_2 (91 to 187 $\text{mg Ca}^{2+} \text{L}^{-1}$) had a negative effect on Asian clams. Other studies have used CaCl_2 to increase calcium in a laboratory setting (up to 74 $\text{mg Ca}^{2+} \text{L}^{-1}$) and saw increases in the growth and survival of Asian clams and quagga mussels (Davis et al. 2015; Ferreira-Rodríguez et al. 2017). Additionally, Asian clams raised in the lab are very tolerant to CaCl_2 , with an $\text{LC}_{50_{8-d}}$ of 2235 $\text{mg Cl}^- \text{L}^{-1}$ (which corresponds to 1262 $\text{mg Ca}^{2+} \text{L}^{-1}$; Coldsnow and Relyea 2018). This LC_{50} is relevant because we observed a large amount of death in the first week of this outside community study.

A number of hypotheses might explain the high mortality of Asian clams in our outdoor mesocosm experiment. The most likely explanation is that the CaCl_2 road salt settled during the first week, making

the benthos concentration higher than in the water column. When road salt first enters a system, the difference in density compared to fresh water cause density gradients and increasing concentrations with increasing depth (Hintz and Relyea 2019). However, eventually the layers mix and the salt is distributed throughout the water column. We saw that it took between 7 and 14 days for the concentrations to stabilize and distribute through the tank (Fig. 2). Another explanation could be that CaCl_2 forms calcium oxide (CaO) and hydrochloric acid (HCl) in water, which can alter pH (Brown et al. 2011). However, we did not detect a significant change in pH on day 7. In fact, only the highest CaCl_2 treatment reduced pH across all dates, and the pH in those tanks still remained above 7.8. Lastly, road salts can form toxic complexes with metals (Schuler and Relyea 2018). While we do not have a complete analysis of the soil, metals might have come in with the soil. In all hypotheses, the higher reproduction rates of the other native organisms, as well as occurring mainly at the benthos, likely made the toxicity unnoticeable by the end of the experiment for native organisms. These results and uncertainty stress the importance of further research on road salts and invasive species.

Conclusions

The Asian clam and zebra mussel are some of the most prominent invasive species, costing the USA approximately US\$6 billion annually to control (Pimentel et al. 2007). Thus, understanding the factors, including those anthropogenically influenced, that promote or enhance their invasion is vital. While the salinization of freshwater from road salts is an ecological concern (Cañedo-Argüelles et al. 2013; Kaushal et al. 2018), we observed no indication that the use of CaCl_2 road salt will benefit Asian clams and zebra mussels. At our highest concentrations, CaCl_2 caused mortality in Asian clams. In addition, our highest concentration affected zooplankton abundance, which are vital to aquatic ecosystems. Other taxa were not impacted, despite our highest concentration being above the EPA chronic threshold (230 $\text{mg Cl}^- \text{L}^{-1}$). Higher concentrations of CaCl_2 might result in larger impacts and should be investigated further if municipalities and state agencies plan to use CaCl_2 road salt more.

The community-wide consequences caused by Asian clams (in the absence of high CaCl_2) emphasize the effects that invasive bivalves can have on freshwater communities. Furthermore, since we saw differing effects on primary producers, it is important that algal changes from invasive bivalves in bodies of water are accurately described, as this may affect management strategies. An “algal increase” from Asian clams would likely correspond to an increase in filamentous algae, not phytoplankton. Lastly, despite the large amount known about invasive mollusks at the individual level, little is known at the community level. Thus, invasive species research and management should be a top priority because they may affect vital ecosystem services, such as water quality and recreational use (Walsh et al. 2016).

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Authors' contribution KC, WH, MS, AS, and RR formulated the idea. KC and RR designed the experiment. KC, WH, MS, and AS performed the experiment. KC analyzed the data and wrote the manuscript. WH, MS, AS, and RR provided editorial advice.

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Availability of data and materials Data are available via request from K. D. Coldsnow (corresponding author).

Code availability Relevant code is available via request from K. D. Coldsnow (corresponding author).

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval Institutional and national guidelines for the care and use of animals were followed.

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