Salinity and pH effects on survival, growth, and reproduction of quagga mussels

Carina Seitz [Corresp., 1, 2, 3] , **Facundo Scordo** [1, 4, 5] , **Erin Suenaga** [1] , **Emily M. Carlson** [1] , **Shaye McMillen** [1] , **Logan Gregory** [1] , **Sudeep Chandra** [1]

[1] Department of Biology, Global Water Center and University of Nevada, Reno, Reno, NV, United State of America

[2] IPATEC, Centro Regional Universitario Bariloche (CRUB), (CONICET-UNCO), San Carlos de Bariloche, Rio Negro, Argentina

[3] Departmento de Geologia, Universidad Nacional del Sur, Bahia Blanca, Buenos Aires, Argentina

[4] Instituto Argentino de Oceanografia (IADO), Universidad Nacional del Sur (UNS)- CONICET, Bahía Blanca, Buenos Aires, Argentina

[5] Departamento de Geografía y Turismo, Universidad Nacional del Sur, Bahia Blanca, Buenos Aires, Argentina

Corresponding Author: Carina Seitz
Email address: cseitz@comahue-conicet.gob.ar

Background. In recent decades, invasive quagga mussels have expanded to the Western United States from the Great Lakes region of North America. Most studies that evaluate the invasion potential of quagga mussels in western water bodies have utilized physiological and life history information from zebra mussels, a related taxon. Few studies have assessed the potential for invasion using specific information from quagga mussel life history or experiments that test for their survival in the fresh and saline waters of the western United States. **Methods.** We investigated quagga mussel survival, growth, and reproduction using semi-natural experiments under temperature and light controlled conditions across a gradient of water salinity (fresh to brackish) and pH (8.4-11). Water from Lake Mead was used as a positive control in our experiment, and water from Pyramid Lake and the Truckee River was used as brackish and freshwater treatments, respectively. The mussels used in the experiments were collected from Lake Mead. **Results.** After 12 hours in brackish water (4 ppt, pH 9.3), we observed 100% mortality of adult mussels. The swelling and disintegration of body tissues and high mortality rates indicated that high potassium, sodium, and chloride concentrations were the likely causes of death in brackish water treatments. In contrast, mussels were able to survive, grow, and reach sexual maturity in freshwater (0.1 ppt) with a low calcium concentration (17 mg L^{-1}) after 57 days. Mussels died after two days at pH 11 and after 12 days at pH 10; during the 14-day monitoring period, no mortality was detected at pH 9.0, 9.3, or 9.5 and mussels did not exhibit any visual indications of stress. Understanding quagga mussel physiological and environmental tolerances appears to be essential for assessing their invasion potential in aquatic habitats.

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Carina Seitz[1,2,3], Facundo Scordo[1,4,5], Erin Suenaga[1], Emily Margaret Carlson[1], Shaye McMillen[1], Logan Gregory[1], Sudeep Chandra[1]

[1] Global Water Center and the Department of Biology, University of Nevada, Reno, NV, USA

[2] IPATEC, Centro Regional Universitario Bariloche (CRUB), (CONICET-UNCO), San Carlos de Bariloche, Argentina

[3] Departmento de Geología, Universidad Nacional del Sur, Bahía Blanca, Buenos Aires, Argentina

[4] Instituto Argentino de Oceanografía (IADO), Universidad Nacional del Sur (UNS)- CONICET, Bahía Blanca, Buenos Aires, Argentina

[5] Departamento de Geografía y Turismo, Universidad Nacional del Sur, Bahía Blanca, Buenos Aires, Argentina

Corresponding Author:
Carina Seitz[1]
Av De Los Pioneros 2350, 8400, San Carlos de Bariloche, Argentina
Email address: cseitz@comahue-conicet.gob.ar

Abstract

Background. In recent decades, invasive quagga mussels have expanded to the Western United States from the Great Lakes region of North America. Most studies that evaluate the invasion potential of quagga mussels in western water bodies have utilized physiological and life history information from zebra mussels, a related taxon. Few studies have assessed the potential for invasion using specific information from quagga mussel life history or experiments that test for their survival in the fresh and saline waters of the western United States.

Methods. We investigated quagga mussel survival, growth, and reproduction using semi-natural experiments under temperature and light controlled conditions across a gradient of water salinity (fresh to brackish) and pH (8.4-11). Water from Lake Mead was used as a positive control in our experiment, and water from Pyramid Lake and the Truckee River was used as brackish and

39 freshwater treatments, respectively. The mussels used in the experiments were collected from
40 Lake Mead.

41 **Results.** After 12 hours in brackish water (4 ppt, pH 9.3), we observed 100% mortality of adult
42 mussels. The swelling and disintegration of body tissues and high mortality rates indicated that
43 high potassium, sodium, and chloride concentrations were the likely causes of death in brackish
44 water treatments. In contrast, mussels were able to survive, grow, and reach sexual maturity in
45 freshwater (0.1 ppt) with a low calcium concentration (17 mg L^{-1}) after 57 days. Mussels died
46 after two days at pH 11 and after 12 days at pH 10; during the 14-day monitoring period, no
47 mortality was detected at pH 9.0, 9.3, or 9.5 and mussels did not exhibit any visual indications of
48 stress. Understanding quagga mussel physiological and environmental tolerances appears to be
49 essential for assessing their invasion potential in aquatic habitats.

50

Introduction

51

52

53 The proliferation of exotic species has been exacerbated by the globalization of economies
54 and trade (Karatayev et al., 2007). In the Northern Hemisphere, invasive zebra (*Dreissena*
55 *polymorpha* (Pallas, 1771) and quagga (*Dreissena rostriformis bugensis* (Andrusov, 1897))
56 mussels are one of the most aggressive freshwater invaders and have enormous economic and
57 ecological impacts (Karatayev, Burlakova & Padilla, 2015). These species alter the ecology of
58 lakes and rivers by decoupling pelagic and benthic trophic pathways, increasing offshore clarity,
59 stimulating benthic production, and altering biodiversity (Ricciardi, Neves & Rasmussen, 1998;
60 Makarewicz, Lewis & Bertram, 1999; Bially & MacIsaac, 2000). Mollusks, including zebra and
61 quagga mussels, are the fifth most costly invasive species in the United States with an annual
62 cost estimate of $4.8 billion (Fantle-Lepczyk et al., 2022). The cost is almost five times higher
63 than was predicted in 2005 for damages and associated control expenses in the United States
64 (Pimentel, Zuniga & Morrison, 2005). Invasive mussel species can be easily transported to new
65 water bodies, however, the physiochemical characteristics necessary for successful establishment
66 are still poorly understood.
67 The two mussel species colonized the mid-western and eastern regions of North America
68 around the same time; zebra mussels were discovered in the Great Lakes region in 1986 (Hebert,
69 Muncaster & Mackie, 1989) and quagga mussels were found in Lake Erie in 1989. However, the
70 quagga mussel was not recognized as a distinct species until 1991 (May & Mardsen, 1992; Mills
71 et al., 1996). In addition to invading the Great Lakes, both *Dreissena* spp. had colonized a total
72 of 772 water bodies in the United States and Canada by 2010, with zebra mussels occupying 17
73 times more water bodies than quagga mussels (Benson, 2014). In the early 2000s, a shift was
74 observed and quagga mussels became the predominant first colonizers, favored by small
75 differences in physiology and depth of reproduction (Ram et al., 2012). Quagga mussels first
76 appeared in the western U.S. in Lake Mead (AZ-NV USA) in early 2007 (Stokstad, 2007) and
77 rapidly spread across the Colorado River system colonizing 30 lakes and reservoirs in Arizona,
78 California, and Nevada by the end of 2008 (Nalepa, 2010). Recently, it was observed that zebra

79   mussels have infested Highline Lake in the state of Colorado (Colorado Parks & Wildlife, 2023).

80   These recent invasions have spurred efforts to determine the invasion risk posed by zebra and

81   quagga mussels in inland waters of the western United States.

82   Although there has been an increase in the number of publications about *Dreissena* spp. since

83   their first appearance in North America, only 30 % of the articles are related to quagga mussels

84   (Passamaneck, 2018; Karatayev & Burlakova, 2022). Consequently, the information available on

85   their life history and environmental conditions that could promote establishment may not be

86   sufficient to predict their potential spread and the ecological implications of invasion (Nalepa,

87   2010; Karatayev, Burlakova & Padilla, 2015). The most commonly used variables for assessing

88   potential mussel invasion are pH and calcium (Ca) concentrations, but quagga mussel tolerance

89   to these variables is still poorly understood. Several studies indicated that quagga mussels may

90   be less tolerant to low Ca concentrations (>12 mg L^{-1}) relative to zebra mussels (>8 mg L^{-1}) since

91   they are usually found in environments with higher Ca concentrations (>12 mg L^{-1}). However,

92   quagga mussels could establish in Lake Tahoe, which has low Ca concentrations (9 mg L^{-1})

93   (Davis et al., 2015), but would require higher concentrations (>18 mg L^{-1}) for the establishment

94   of permanent populations (Baldwin et al., 2012). Permanent populations of adult quagga mussels

95   in water with low Ca concentrations have been observed in places that are hydrologically

96   connected to waters where veligers can develop (Cohen, 2007). Zebra mussel pH tolerance has

97   also been used to evaluate the potential for quagga mussel invasion (Mackie & Claudi, 2010).

98   Only two studies have assessed quagga mussel tolerance to different pH gradients, and they have

99   only focused on the use of very high (pH>12) or low pH (< 7.5) to prevent or control mussel

100   invasions (Claudi et al., 2012, 2013) (Table 1). These studies did not have conclusive results

101   regarding the upper pH limits tolerated by quagga mussels due to technical issues during the

102   experiments (Claudi et al., 2013). Similar to pH, upper limits for salinity have been more

103   intensively examined for zebra mussels over quagga mussels. Salinity tolerances determined by

104   laboratory experiments and presence-absence data are highly variable, but suggest that adults can

105   tolerate up to 6-8 ppt when first acclimated to a lower salinity concentration (Garton, McMahon

106   & Stoeckmann, 2014). Quagga mussels are considered a euryhaline species, living in both

107   freshwater and brackish water in their native ranges (Orlova et al., 2005), and have shown rapid

108   adaptation to new environmental conditions after only a few generations (Mills et al., 1996).

109   Studies of *Dresseina* spp. salinity tolerance have not been done since the mid-1990s, so

110   analyzing the salinity tolerance of the new generations of *Dreissena* spp. in North America are

111   warranted despite their absence from brackish waters to date.

112   We conducted site specific studies to assess the potential for adult quagga mussels to establish

113   in fresh to saline water ecosystems, including terminal (endorheic) lakes, that are present in the

114   western United States. Site specific studies suggested the potential for adult quagga mussel

115   establishment in the freshwater of Lake Tahoe (Davis et al., 2015), which is connected to the

116   more brackish waters of Pyramid Lake through the Truckee River. In this study, we investigated

117   quagga mussel survival, growth, and reproduction in an ecosystem with a salinity (fresh to

118   brackish) and pH (>8) gradient, as well as their tolerance to high pH concentrations. Specifically,

119 our objectives were to 1) determine adult quagga mussel survival, growth, and potential for
120 producing viable gametes that could result in the establishment and spread of mussels in Pyramid
121 Lake (brackish water) and the Truckee River (fresh water); 2) explore which factors (pH,
122 salinity, or both) are causing the adult quagga mortality in Pyramid lake water; and 3) analyze
123 the role of pH on adult quagga mussel survival.
124
125
126
127
128 **Study area**
129 We implemented seminatural laboratory experiments to determine adult quagga mussel survival
130 and growth in water from two different locations within Pyramid Lake (brackish waters of
131 Warrior Point and Popcorn Rock) and one location in the lower basin of the Truckee River
132 (freshwater below Marble Bluff Dam, 1.6 km above Pyramid Lake). These locations were
133 selected to capture the salinity and pH gradients of the Truckee River-Pyramid Lake ecosystem.
134 Currently, there are no established populations of quagga mussels at these sites. Pyramid Lake is
135 a large (487 km^2, maximum depth 105 m) terminal desert lake located within the Pyramid Lake
136 Paiute Tribe Reservation in western Nevada, approximately 50 km northeast of Reno (Fig. 1).
137 Pyramid Lake receives about 85% of its annual water input from the Truckee River, which
138 originates in the upper watershed of the Lake Tahoe Basin in the Sierra Nevada Mountains (Fig.
139 1). As a positive control for the experiments, we utilized water from the Lake Mead Fish
140 Hatchery which draws water from Lake Mead, the largest reservoir in the United States. Quagga
141 mussels have established populations throughout the reservoir and Colorado River since 2007
142 (Fig. 1).
143
144 **Materials & Methods**
145
146
147 **Collection, handling of mussels, and assessment of survival in the experiments.**
148
149 We assessed survival and growth of adult quagga mussels of different sizes and under different
150 water quality conditions through a series of seminatural experiments. We collected adult quagga
151 mussels (> 9mm) from the Lake Mead Marina via a special permit provided by the US State of
152 Nevada. The mussels were carefully removed from ropes hanging from the dock of the marina.
153 The mussels were then transported to our laboratory in containers with Lake Mead water and
154 bubblers to keep the water well oxygenated. We let the mussels acclimatize in Lake Mead water
155 for 48 hours at 20 ºC before placing them in the different water treatments. Length and wet-
156 weight were measured pre- and post- experiments. The length of individual mussels was
157 measured using a digital caliper (General Ultratech®). We also measured change in wet mass,
158 including live soft tissue and shell mass, to assess total mussel growth throughout the

159   experiment. Individual live mussels were dried externally with a tissue and weighed on a digital
160   scale (Mettler Toledo AB104-S®). Each day, we noted the behavior of the mussels (e.g.,
161   opening and extraction of the siphon, attachment to container surfaces, and motility) and the
162   status of the mussels' survival (live or dead) by noting gape and response to physical contact.
163   Water from each ecosystem was stored near the laboratory in 2,273 L opaque tanks. We carried
164   out the experiments in a temperature-controlled room using a 12-hour day-night cycle to
165   simulate natural conditions and optimize the filtering activity of the mussels. At the end of the
166   experiment, the mussels were dried at 95°C to ensure complete death before being discarded. To
167   avoid contaminating local water systems with quagga mussels or veliger larvae, we treated all
168   water and containers with 6% sodium hypochlorite bleach solution for 24 h before being
169   discarded according to the Guide to Preventing Aquatic Invasive Species Transport by Wildland
170   Fire Operations (National Wildfire Coordinating Group, 2017).
171

172   **Adult mussels' survival, growth, and reproduction in brackish to freshwater ecosystems**

173

174   Experiment 1: Individual mussels of similar size (18 ± 2 mm length) were placed in 13 L
175   containers in a climatized room at 20 ºC (1 mussel per container with 30 replicates per treatment)
176   (Fig 1e). Treatments included water from the following locations: Lake Mead (control where
177   mussels have established since 2007), Pyramid Lake from Popcorn Rock (brackish water), and
178   Pyramid Lake from Warrior Point (brackish water).

179

180   Experiment 2: To determine if the size of the quagga mussel affected their survival and mortality
181   rate, we implemented another experiment using the same water treatments as Experiment 1 but
182   with mussels of three different sizes: large (16-26 mm length, N=30), medium (8-16 mm length,
183   N=30), and small (3-8 mm length, N=20). The individual mussels were monitored for survival
184   hourly for 12 hours. The number of replicates was lower in the small size class due to a lack of
185   mussels within this range from our source population.

186

187   Experiment 3: To determine if adult quagga mussels were able to survive and grow in Truckee
188   River water, the primary inflow into Pyramid Lake and the outflow for Lake Tahoe, we
189   implemented another experiment with Lake Mead water as our positive control and Truckee
190   River water (freshwater) as our treatment. Thirty adult mussels of similar size (15±4 mm) were
191   placed in individual containers with the treatment water being exchanged every 48 h to avoid the
192   depletion of nutrients and food sources for the mussels. We checked physical parameters
193   (temperature, salinity, conductivity, and dissolved oxygen) and mussel survival daily over the
194   58-day experiment. At the end of the experiment we weighed, measured, and extracted the
195   gonads of all the mussels. We calculated the growth rate using the differences between the initial
196   and final mass and length measurements divided by the number of weeks that the experiment
197   lasted. To determine sex and reproductive viability, we dissected the mussel under a microscope
198   to remove the gonad and placed the corresponding tissue into a histological cassette and

199 preserved it in 10% formaldehyde solution. The samples were sent to the North Bay Histology
200 Lab where permanent slides were produced. Each slide contained six tissue sections, 5 μm thick,
201 stained with hematoxylin and eosin. We analyzed the slides using a compound light microscope
202 (Meiji) with 100X magnification and a Lumenera Infinity II microscope camera to determine the
203 sex and gonadal status. We differentiated three gonadal statuses according to the categories
204 proposed by Guillou et al. (1990): (1) recovering (non-discriminable sex), (2) premature
205 (discriminable sex), and (3) mature gonads (discriminable sex). We classified each mussel by
206 gonad status, and when possible, by its sex. The percentage of reproductively viable individuals
207 was determined following Davis et al. (2015) as:

209 % reproductively viable = (male count + female count) / (male count + female count +
210 indeterminate count).

212 **Survival of mussels exposed to Pyramid Lake brackish water with lower pH (short-term**

213 **experiment)**

215 Experiment 4: To test if the high pH of the Pyramid Lake water affected the survival of quagga
216 mussels, we used water from Warrior Point (Pyramid Lake) with its natural pH values of 9.31
217 along with water titrated with phosphoric acid to reduce pH to the following concentrations 8.53,
218 8.83, 9.06, 9.21. Lake Mead water without pH alteration (8.6) was used as a control. Twenty-five
219 adult mussels with a shell length range between 10-26 mm were selected for each water
220 treatment, with all mussels from each treatment placed together in 4 L glass containers. When we
221 observed a dead mussel, it was retrieved from the experiment and the time of death was
222 recorded. The temperature during the experiment was reduced to 15 ºC to decrease the metabolic
223 rate and increase the mussels' tolerance to higher salinity concentrations based on the
224 observation made by Mills et al. (1996). We measured the pH using a handheld pH meter (Ohaus
225 starter 300) with a measurement resolution of 0.01 pH. We checked mussel survival hourly for
226 12 hours, and after 24 hours.

228 **Survival of mussels exposed to Lake Mead and Truckee River freshwater with higher pH**

229 **concentration (short-term experiment)**

231 Experiment 5: To test if increasing pH reduced the survival of quagga mussels in water where
232 they survive in natural conditions, we amended Lake Mead and Truckee River water with
233 sodium hydroxide to increase the pH until reaching levels 9.0, 9.3, 9.5, 10, and 11. We used
234 Lake Mead (pH 8.6) and Truckee River (pH 8.42) water without modifying the pH as control
235 treatments. Thirty adult mussels with a shell length range between 12-24 mm were selected for
236 each of the water treatments and mussels for each treatment were placed together in 4 L glass
237 containers. The temperature in the chamber was set to 20 ºC to assure high metabolic rates. We

238 measured the pH three times a day using a handheld pH meter (Ohaus starter 300) with a
239 measurement resolution of 0.01 pH. We monitored the experiments continuously for 11 days.
240
241 **Water quality monitoring and characterization**
242
243 We routinely monitored the water quality during the experiments to determine the conditions
244 under which quagga mussels can survive and grow. We measured temperature, pH, conductivity,
245 salinity, and dissolved oxygen daily using a Y.S.I. digital handheld meter ProDSS. We analyzed
246 the different treatments every other week for total hardness and alkalinity (Phenolphthalein and
247 total alkalinity) using a handheld titrator with EDTA 0.800 M and Sulfuric Acid Titration
248 Cartridge 1.6 N, respectively (Hach Co). Samples were filtered using glass fiber filters
249 (Whatman GF/F filters with a pore size of 0.7 µm) and analyzed for dissolved anion and cations
250 at the University of California, Davis Analytical Laboratory following standard operating
251 procedures (https://anlab.ucdavis.edu/). Total Nitrogen (TN) was analyzed at the University of
252 California, Davis Analytical Laboratory by combustion following standard procedures with a
253 detection limit of 0.1 mg L^{-1}. Total Phosphorus (TP) was analyzed at the High Sierra Water Lab
254 following the EPA 365.3 method with a detection limit of 1 µg L^{-1}. We determined chlorophyll *a*
255 concentrations by filtering 150 ml of water through a Whatman GF/F filter and then measuring
256 pheophytin-corrected chlorophyll *a* on a Turner 10-AU fluorometer following methanol
257 extraction (Marker, 1972).
258
259 **Statistical Analysis**
260
261 The data were expressed as means ±1 standard error. All statistical analyses were completed
262 using R software (R Core Team, 2020) and graphics were plotted using the ggplot package
263 (Wickham, 2016). We used linear regression to analyze mortality and growth rates in relation to
264 mussel size. We quantified differences in growth (length and weight) between Truckee River and
265 Lake Mead treatments using linear mixed-effects models (LMM), with site modeled as a fixed
266 effect. To assess differences in sexual maturity between Truckee and Mead water treatments, we
267 compared the percentage of each sex and gonad status for each water treatment. Differences in
268 mortality rates between Truckee River and Lake Mead mussels under different pH treatments
269 were tested using a LMM with site and pH modeled as fixed effects. All models met the
270 assumption of homoscedasticity. For all the models, we considered a significance level (alpha) of
271 0.05. Tests for significance of the fixed effects and their interactions in the models were
272 performed via the Wald statistic (Zuur et al., 2009) using the gls and lme functions in the nlme R
273 package (Pinheiro et al., 2020). Multiple comparisons among sites and pH were performed with
274 Tukey's HSD post hoc test (emmeans R package; (Lenth, 2021)).
275
276
277 # Results

278

279 **Water quality monitoring and characterization**

280

281 The two Pyramid Lake locations (Popcorn Rock and Warrior Point) were similar with higher
282 salinity, pH, total alkalinity and hardness compared to the Truckee River and Lake Mead (Table
283 1). The quality of the water in the storage tanks for each of the lakes and treatments remained
284 stable throughout the duration of the experiments (Table 1). The highest chlorophyll *a*
285 concentrations, that were used as an estimate of algal biomass, were found at Warrior Point (0.9±
286 1.3 µg L^{-1}) and Popcorn Rock (0.8 ±1.1 µg L^{-1}). However, for all treatments, we observed a stark
287 increase in algal growth when water was transferred from the tanks to the experimental chamber
288 with increased light and regulated temperature. There was slightly higher salinity at Warrior Point
289 (4.3 ±0.1ppt) compared to Popcorn Rock (4.1 ±0.1 ppt), which is closer to the Truckee River
290 inflow (Table 1), and the pH values were 9.3 at both sites (Table 1). Truckee River water had lower
291 pH (8.1) and salinity (0.2±0.0) and Lake Mead water had lower pH (8.3) and ten times lower
292 salinity (0.4±0.0 ppt) compared to Pyramid Lake. Additional parameters such as total alkalinity,
293 hardness, dissolved oxygen, calcium, and others can be found in Table 1.

294

295 **Survival, growth, and reproduction of mussels in Pyramid Lake brackish water**

296

297 In Experiment 1, with Warrior point and Popcorn Rock treatments, all but one of the adult
298 mussels died after 19 hours, whereas all mussels survived in the positive control treatment (Lake
299 Mead water). Similar results were found in the Experiment 2 when analyzing the effect of mussel
300 size on survival. Approximately 90% of the medium-sized mussels died after 4 hours in Popcorn
301 Rock and Warrior Point water, and approximately 90% of all mussels were dead within 9 hours
302 (Fig 2). After 12 hours, almost all the mussels in the three different size classes died in Warrior
303 point and Popcorn Rock treatments, with a 90% mortality in large sized mussels after 12 hours.
304 Only one mussel of medium size survived for more than 24 hours in the Popcorn Rock water.
305 Conversely, in Lake Mead water treatment (Experiment 2), we recorded only one mussel death
306 after 12 hours. The relationship between size (weight and length) and time showed high
307 dispersion with mussels of different sizes dying indistinctly every hour. The body tissue of the
308 dead mussels retrieved after 19 hours had a bloated appearance with some exhibiting tissue
309 disintegration (Fig. 1f). The mussels' shell gape closure time in response to touch was very slow
310 before death.

311

312

313 **Survival, growth and reproduction of quagga mussels in Truckee River fresh water**

314

315 Quagga mussels were able to survive, grow, and reach reproductive maturity in the freshwater of
316 the Truckee River. All 30 mussels survived in the Truckee River water after 58 days of the
317 experiment, while two individuals died in the Lake Mead treatment (positive control). The
318 increase in weight (mean difference: 0.02) and length (mean difference: 0.27) of the mussels was

319   double in the Truckee River treatment compared to the Lake Mead treatment (mean differences:
320   0.01 and 0.11 for weight and length, respectively) (Figure 3a and 3b). The mean growth rate of
321   adult mussels was 0.0402 g/week (p<0.001, R^2= 0.53, linear regression) for Truckee River water
322   treatment (Figure 3b), and -0.0026 g/week (p=0.94, R^2< 0.01, linear regression) for Lake Mead
323   treatment (Figure 3a). Most of the mussels increased their size and weight in the Truckee River
324   treatment. There was a positive and significant correlation between the differences in mussel
325   weight and length between the start and the end of the experiments (Truckee: p<0.001, R^2= 0.53;
326   Mead: p=0.94, R^2< 0.01, lineal regression; Figure 3a and 3b). The mussels in the Lake Mead
327   treatment showed only slight changes in length and weight between the start and end of the
328   experiment, and there was no correlation between the increase in these variables (p=0.94, R^2<
329   0.01, linear regression; Figure 3a). The differences in length and weight between the Mead and
330   Truckee water treatments were not significant (p= 0.12 and p=0.11, respectively, linear mixed-
331   effects models)
332   Surviving adults showed high reproductive viability at the two sites (Figure 3c and 3d), but
333   we observed higher reproductive viability in the Truckee River treatment (93.3%) compared to
334   the Lake Mead (78.5%) treatment (Figure 3c). In the Truckee River treatment, 80% of the adult
335   mussels were in a reproductively mature state, ~13% in a premature state, and ~7% were
336   immature or in a recovering state (Figure 3d). In the Lake Mead treatment, ~32% of the adult
337   mussels were in a reproductively mature state, ~46% in a premature state, and ~21% were
338   immature or in a recovering state (Figure 3c).
339
340
341   **Survival of quagga mussels exposed to Pyramid Lake brackish water with lower pH**
342
343   Lowering the pH of Pyramid Lake water to values similar to Lake Mead, where they have
344   established a permanent population, did not result in higher mussel survival. Between 90-100%
345   of the mussels exposed to different pH concentrations at Warrior Point water treatment were
346   dead after 24 hours (Figure 4), while all mussels survived in the control treatment. The mussels'
347   survival rate at pH levels below 9.0 was lower than at pH levels higher than 9.0. Two of the
348   twenty-five mussels survived in each of the experiments with pH 8.53 and 8.83 after 24 hours,
349   one of twenty-five mussels survived at pH 9.31 after 24 hours, and all the mussels died at pH
350   9.21 and 9.06 after 24 hours. In all of the treatments more than 60% of the mussels died after ten
351   hours, except for pH 8.53, where 40% of the mussels died during that period. During the
352   experiment, the mussels in Warrior Point treatment appeared more stressed (partially opened
353   valves and did not extend the siphon for filtering) compared to those in Lake Mead control water
354   treatment.
355
356   **Survival of mussels exposed to Lake Mead and Truckee River freshwater with higher pH**
357   **concentration**
358

359   Increasing the pH of freshwater resulted in higher mussel mortality rates at very high pH
360   (pH>10), however, the mortality rates at any pH in freshwater were very low compared to those
361   found in the brackish Pyramid Lake water. All mussels were dead after 6 days (Truckee River)
362   or 9 days (Lake Mead) in the treatments where we increased the pH to 11 (Figure 5). After ten
363   days, only 15% of the mussels died at pH 10 in Mead and Truckee water treatments, but after 14
364   days, all the mussels died at pH 10 (Figure 5). We also observed no significant differences
365   between Lake Mead and Truckee River at pH 10 and 11 (p= 0.50, linear mixed-effects models),
366   but there were significant differences between the pH 10 and 11 (p<2e-16, linear mixed-effects
367   models) in each of the water treatments. We also noted low mortality in other pH levels. Only
368   one mussel died after 14 days at pH 9.0 in Lake Mead and at pH 9.0 and 9.3 in Truckee River
369   water (Figure 5). No mortality was recorded in the controls, pH 9.5, and pH 9.3 in Lake Mead
370   water (Figure 5). In Lake Mead water treatments of pH 9.5, 10, and 11, we observed carbonate
371   precipitation, but this was not observed in Truckee River water at any pH. The mussels had no
372   visual signals of stress in either Mead or Truckee water. In our daily observations, we observed
373   that the mussels exhibited less stress in these treatments with freshwater compared to the
374   experiments where we used the brackish water of Pyramid Lake.
375
376

377   **Discussion**

378

379   Adult quagga mussels can survive, grow, and reach sexual maturity in the freshwater of the
380   Truckee River, but not in the brackish waters of Pyramid Lake (Fig. 6). A reduction in the pH of
381   the Pyramid Lake brackish water did not improve survival of the mussels (Fig. 6). Similarly,
382   increasing the pH of the Truckee River water did not result in higher mortality rates (Fig. 4 and
383   6). These results suggest that the chemical composition of Pyramid Lake's water (particularly
384   salinity and ion concentrations) could be the primary reason that quagga mussels cannot survive
385   in this lake with the relationships between growth and shell length are indicative of stressful
386   conditions for mussels (Claudi et al., 2013). In our experiments, we observed increased mussel
387   growth with no evidence of stressed conditions in Truckee River water compared to Lake Mead
388   water. Furthermore, our results align with Davis et al. (2015) who found that adult quagga
389   mussels can settle, grow, and survive in the upper basin of the Truckee River. These combined
390   results indicate that adult quagga mussels may have a chance of establishing in the lower
391   Truckee River if they were to be introduced and given optimal conditions for growth. We did not
392   assess the influence of physical turbulence to the maintenance of mussels which could be
393   important during the winter and spring snow melt conditions of the region. In addition, our study
394   analyzed the reproductive potential and not the capacity of quagga mussel veligers to complete
395   the life cycle in the Lower Truckee River, which would be needed to establish a permanent
396   population. Thus, the invasion potential could be restricted by other environmental variables that
397   prevent quagga mussel veliger survival and settlement (such as flow velocity, water temperature,
398   and substrate composition) that were not analyzed in this study (Chen et al., 2011).

399   The Truckee River presents a high risk for quagga mussel infestation by adults if the veligers
400   are able to survive in the calcium (Ca), alkalinity, and total hardness conditions of the water.
401   Results from Pyramid Lake water's Ca, alkalinity, and total hardness values were not conclusive
402   enough to use as exclusive predicters of potential quagga mussel invasion. Calcium tolerance for
403   quagga mussels has been based on presence/absence and abundance distribution data, suggesting
404   that quagga mussels are less tolerant to lower Ca concentrations than zebra mussels, but the
405   tolerances of both species mostly overlap (Jones & Ricciardi, 2005; Mackie & Claudi, 2010;
406   Karatayev & Burlakova, 2022). The concentration of Ca in Pyramid Lake is low (8.2-8.8 mg L^{-}
407   ^{1}), indicating a low risk of invasion given the limits proposed by Mackie and Claudi (2010).
408   However, one experimental-based analysis showed that quagga mussels have the potential to
409   establish in Lake Tahoe, which has low concentrations of Ca at 9 mg L^{-1} (Davis et al., 2015). In
410   addition, sink populations of quagga mussels on the St. Lawrence River below the Ottawa River
411   confluence have been favored by its hydrological connectivity with the Great Lakes that have
412   higher Ca concentrations (Cohen, 2007). Alkalinity and hardness values in Pyramid Lake are
413   also higher than the limits proposed by Mackie and Claudi (2010) for systems considered at high
414   risk of invasion, but the optimal ranges in their study were based on zebra mussels. Although pH,
415   Ca, hardness, and alkalinity are correlated, the relationship is not strong, and Ca can originate
416   from other compounds that do not contribute to total alkalinity (Mackie and Claudi, 2010). Thus,
417   limits of hardness and alkalinity tolerances could differ slightly from those found for zebra
418   mussels since calcium and pH limiting values have been poorly studied for quagga mussels
419   specifically.
420   Salinity concentrations in Pyramid Lake are in the upper ranges of tolerance observed for
421   quagga mussels (Mackie & Claudi, 2010; Garton, McMahon & Stoeckmann, 2014). Therefore,
422   we suggest that the current global method for estimating the potential invasion of quagga
423   mussels based on chalk variables (Ca, pH, Total alkalinity, and Total hardness) should also
424   include additional variables such as salinity and Na and K ion concentrations, especially in the
425   case of fresh-brackish waters or terminal lakes. Having specific case studies of survival across
426   mussel life history will be valuable for waters with distinctly different ionic characteristics.
427   Quagga mussels of all sizes died after 12 h of exposure to brackish Pyramid Lake water, even
428   when we reduced the temperature to 15 ºC to decrease the metabolic rate. Such a rapid mortality
429   rate has not been previously reported. The swelling and disintegration of body tissues suggest
430   salinity and pH as probable causes of mortality (Claudi et al., 2013). The osmoregulation system
431   of quagga mussels is adversely affected by high salinity and pH (Claudi et al., 2013). The
432   salinity (4 - 4.3 ppt) and pH (9.31) in both Pyramid Lake treatments could be sufficiently
433   elevated to cause high mortality in mussels (Aleksenko, 1991; Spidle, Mills & May, 1995;
434   McMahon, 1996). Other experiments with mussels in North America indicate that the maximum
435   salinity tolerance is in between 4-5 ppt with a mean survival time of 9-14 days at 15 ºC and five
436   days at 20 ºC in waters with salinities of 5 ppt without acclimation (mussels transferred from 0 to
437   5 ppt) (Aleksenko, 1991; Spidle, Mills & May, 1995; McMahon, 1996; Hofius, Mandella &
438   Rackl, 2015). Acclimatization to a prior salinity of 2 ppt could increase tolerance to higher

439   salinities. However, both acclimatization and non-acclimatization experiments showed values of
440   salinity above 4 ppt are lethal for mussels (Kilgour et al., 1994). The high rate of mortality we
441   observed in our experiments compared to previous studies suggests that factors other than
442   salinity may affect survival.
443   The high pH of Pyramid Lake water was not the cause of high mortality of quagga mussels.
444   Claudi et al. (2013) observed tissue damage in adult quagga mussels exposed to high pH
445   concentration and showed higher sensitivity to pH relative to zebra mussels (Claudi et al., 2013).
446   Quagga mussels presented 100% mortality in pH 12 in less than 15 h (Claudi et al., 2013).
447   However, in the experiment where the pH of brackish water was decreased to 8.4, we still
448   observed 100% mortality after 24 h. Like Claudi et al. (2013), we did not observe mortality after
449   14 days of exposure to freshwater at pH ≤ 9.1, 9.3, and 9.5, but there was a 50% mortality at pH
450   11 after 24 h in fresh water (Truckee River) where mussels were able to survive for 6 days.
451   Therefore, we concluded that quagga mussels are sensitive to high pH in fresh waters, but high
452   mortality of this species in brackish water might be attributed to other chemical and ionic
453   characteristics.
454   *Dreissena* species are sensitive to ionic concentrations such as chloride, sodium, and
455   potassium. The survival of zebra mussels in higher salinities depends on the balance of K and Na
456   that allows them to eliminate water gained over an elevated osmotic gradient (Dietz & Byrne,
457   1997). Low concentration of K can kill zebra mussels within 6-24 h by inhibiting respiration and
458   reducing filtration rates (Fisher et al., 1991; Horohov et al., 1992). Zebra mussel veligers cannot
459   settle, adults cannot attach to substrates at concentrations of K as low as 10-30 mg L^{-1} (Fisher et
460   al., 1991), and their valve closure is inhibited by high concentrations of K around 70-80 mg L^{-1}
461   (Wildridge et al., 1998). The concentration of K at Pyramid Lake is about 126 mg L^{-1} (Reddy &
462   Hoch, 2012), so the slow reaction to touch exhibited by quagga mussels in our experiments when
463   monitoring for survival could be the result of stress induced by high concentrations of K. In a
464   study by Horohov et al. (1992), zebra mussels died after one week of exposure to low
465   concentrations of NaCl (40 meq L^{-1}). At Pyramid Lake, the concentration of NaCl is ~50 meq L^{-1}.
466   ^{1}. The survival of a few individuals for more extended periods in the different experiments can
467   be explained by the mussels' ability to sense and respond to toxins by closing their shell valves
468   for periods greater than 48 h (Rusznak, Mincar & Smolik, 1994).
469   Salinity and specific ion (K, Na, and Cl) concentrations may explain the survival of quagga
470   mussels in Truckee River water, and their high mortality in Pyramid Lake water. The historical
471   record of evolution and dispersal of dreissenid mussels provide evidence of their high potential
472   to adapt to extreme environments; for example, Mills et al. (1996) showed that after a few
473   generations these mussels evolved to be more tolerant to salinity in Europe. Given the so-called
474   rapid evolution, or perhaps genetic drift, of quagga mussels a question still remains: if *Dreissena*
475   spp. establish in the Truckee River with hydrologic connections to the Pyramid Lake ecosystem,
476   can there be adaption and colonization of quagga or zebra mussels to new habitats outside of
477   their existing distribution? Yokomizo and Takahashi (2020) found that in systems with different
478   environmental conditions next to each other at relatively small spatial scales, individuals that can

479   adapt to the environment could expand and colonize new habitats. A clear example of this
480   potential situation is the case of the Colorado River system, where indirectly connected sites
481   favored unprecedented high rates of quagga mussel spread in the western U.S. This connectivity
482   facilitated establishment of quagga mussel populations under variable environmental conditions
483   (Wong & Gerstenberger, 2011; Pucherelli et al., 2016). Invasive species, such as the *Mytilopsis*
484   *spp* (recognized as the brackish water equivalent of zebra mussels), have shown differences in
485   their biological traits, environmental conditions, and substrate utilization between their native
486   and non-native habitats, which has been attributed to their wide plasticity and ability to adapt to
487   new conditions in the invaded system (Rodrigues et al., 2022). As alternatives to species
488   plasticity or broad physiological tolerances, other studies have suggested that invasive species
489   are genetically dynamic populations over short periods of space and time (Lee, 2022). After
490   invasions, genetic drift and natural selection to local environmental gradients could alter the
491   genetic structure of the invading populations, which could favor establishment success through
492   modifications to their tolerances and/or behaviors, resulting in rapid evolutionary events (Lee,
493   2022). Thus, it well could be that connected ecosystems with contrasting environmental
494   characteristics could favor genetic adaptation that leads to a shift into novel niches in the invaded
495   ecosystem. Future studies should consider exploring genomic characteristics of invasive species
496   in addition to ecological preferences in native and non-native species.
497   Most of the studies analyzing the ranges of water quality parameters that favor the invasion of
498   dreissenid mussels are based on the tolerances of zebra mussels (Mackie & Claudi, 2010;
499   Karatayev & Burlakova, 2022). Furthermore, those studies found different limits of tolerances of
500   Dreissenid mussels for parameters such as salinity, temperature, substrate, and dissolved oxygen
501   (Mackie & Claudi, 2010). However, the environmental conditions such as pH, Ca concentration
502   and salinity in locations where quagga mussels can establish a permanent population are still
503   scarce (Karatayev & Burlakova, 2022). Studies of zebra and quagga mussel tolerances for
504   salinity were made in the eastern region of the U.S. in the 1990s, and after 30 years of invasion, a
505   reassessment of the environmental conditions, in particular salinity, is needed as we should not
506   dismiss the possibility of an invasion of quagga mussels in waterbodies with higher salinity than
507   previously described. Therefore, we recommend that additional studies assess the environmental
508   conditions that favor quagga mussels' invasion and their tolerance to physical and chemical
509   conditions. Perhaps one or a few parameters may not be enough to determine the potential for
510   quagga mussel invasions and specific site studies are needed.
511
512

Conclusions

513
514   Adult quagga mussels can survive, grow, and reproduce in the Truckee River's freshwater
515   conditions, but not in the brackish waters of Pyramid Lake. In Pyramid Lake, decreases in pH
516   and temperature did not reduce their mortality rate and we observed mussel mortality in all size
517   ranges. Our results suggest the chemical condition (especially salinity and ion concentrations) of
518   Pyramid Lake water may be the main reason quagga mussels cannot survive in this lake. Mussels

519 exposed to Truckee River water exhibited positive growth and presented no visual evidence of
520 stress. Mortality was not observed in increased pH of the fresh, Truckee River water and Lake
521 Mead water, except at pH>10. Adult quagga mussels can tolerate pH as high as 10 for 14 days,
522 while pH 11 caused 100% mortality after 6 or 9 days, and no mortality was observed at pH<9.5
523 during the 14 day experiment. The results of the high pH experiment from this work are an
524 important contribution to fill the gaps in knowledge about the upper tolerance limits of adult
525 quagga mussels.
526   Hydrological connectivity between fresh and brackish inland ecosystems can favor the
527 adaptation and colonization of quagga or zebra mussels to new habitats outside of their existing
528 ranges. After 30 years of invasion and evolution, the quagga mussels' environmental tolerances
529 are still uncertain; this fact, contrasted with the possibility of quagga mussel invasion of higher
530 salinity waterbodies, suggests the need for further investigations of the impact of water physical
531 and chemical conditions on their invasion potential, especially regarding salinity. Also, our
532 results suggest that one or a few parameters may not be enough to determine the potential for
533 quagga mussel invasion, and specific site studies are needed.
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Water quality variables of the water treatment from field locations stored in the tanks and containers holding the mussels.

Parameter	Lake Mead	Popcorn Rock	Warrior Point	Truckee River
Chalk variables				
Calcium (mg L^{-1} Ca)	54.8±6.0	8.8±1.0	8.2±0.8	16±1.2
pH	8.3 ± 0.1	9.3±0.1	9.3±0.1	8.1±0.1
Total Alkalinity (mg L^{-1} Ca CO3)	135.0 ± 6.0	1012.0 ± 25.0	1068.0 ± 25.0	86.0 ± 8.0
Bicarbonate Alkalinity (mg L^{-1}Ca CO3)	135.0 ± 6.0	793.0 ± 36.0	800.0 ± 69.0	86.0 ± 8.0
Carbonate Alkalinity (mg L^{-1} Ca CO3)	-	219.0 ± 21.0	263.0 ± 55.0	-
Total hardness (mg L^{-1} Ca CO3)	278.0 ±17.0	455.0 ±8.0	475.0 ±14.0	83.5 ± 8.0
Nutrient variables				
Dissolved Oxygen (%)	82.5±2.6	77.4±0.4	74.5±0.5	81.9±4.0
Chlorophyll a (µg L^{-1})	0.1± 0.1	0.8 ± 1.1	0.9± 1.3	0.1± 0.1
Total Phosphorus (µg L^{-1})	11.0 ± 4.0	206.0 ± 15.0	114.0 ± 38.0	68.2 ± 22.0
Total Nitrogen (mg L^{-1})	0.5± 0.2	0.5± 0.1	0.6± 0.1	0.5± 0.2
Physical variables				
Temperature	20.6± 1.2	19.7±0.4	19.6±0.1	20.8±1.4
Conductivity (uS cm^{-1})	9.3±0.20	75.1±5.00	75.2±6.00	3.6±0.20
Salinity (ppt)	0.4±0.1	4.07±0.1	4.34±0.1	0.17±0.1
Other chemical variables				
SAR	2.4±0.1	2.2±0.1	2.3±0.1	2.0±0.1
Na (Soluble) (mg L^{-1})	87.1±3.7	99.5±2.1	101.8±6.9	40.00±3.4
Mg (Soluble) (mg L^{-1})	24.9±1.1	83.8±4.5	89.7±13.2	7.9±0.6
B (Soluble) (mg L^{-1})	0.1±0.0	9.7±0.3	9.8±1.5	0.4±0.0
Cl (mg L^{-1})	87. 8 ± 3.5	1748.4 ± 61.3	1558.0 ± 442.0	40.7 ± 2.5

3

Figure 1

Location of the study area

A) Location of the study area within the United States. B) Location of Pyramid Lake and Lake Mead in the western United States; C) Location of the study sites where water was collected for the experiments in Pyramid Lake (Warrior Point; Popcorn Rock) and the Truckee River (Marble Bluff Dam); D) Location of the study sites where water (Fish Hatchery) and Quagga mussels (Lake Mead Marine) were collected for the experiment in Lake Mead; E) Experimental set-up using 13 L containers with mussels under different water treatments in a climatized room; F) Dead mussels retrieved after 19 h in brackish water with a bloated appearance and tissue disintegration; G) Mussel growth in Truckee River water treatment after 58 days.
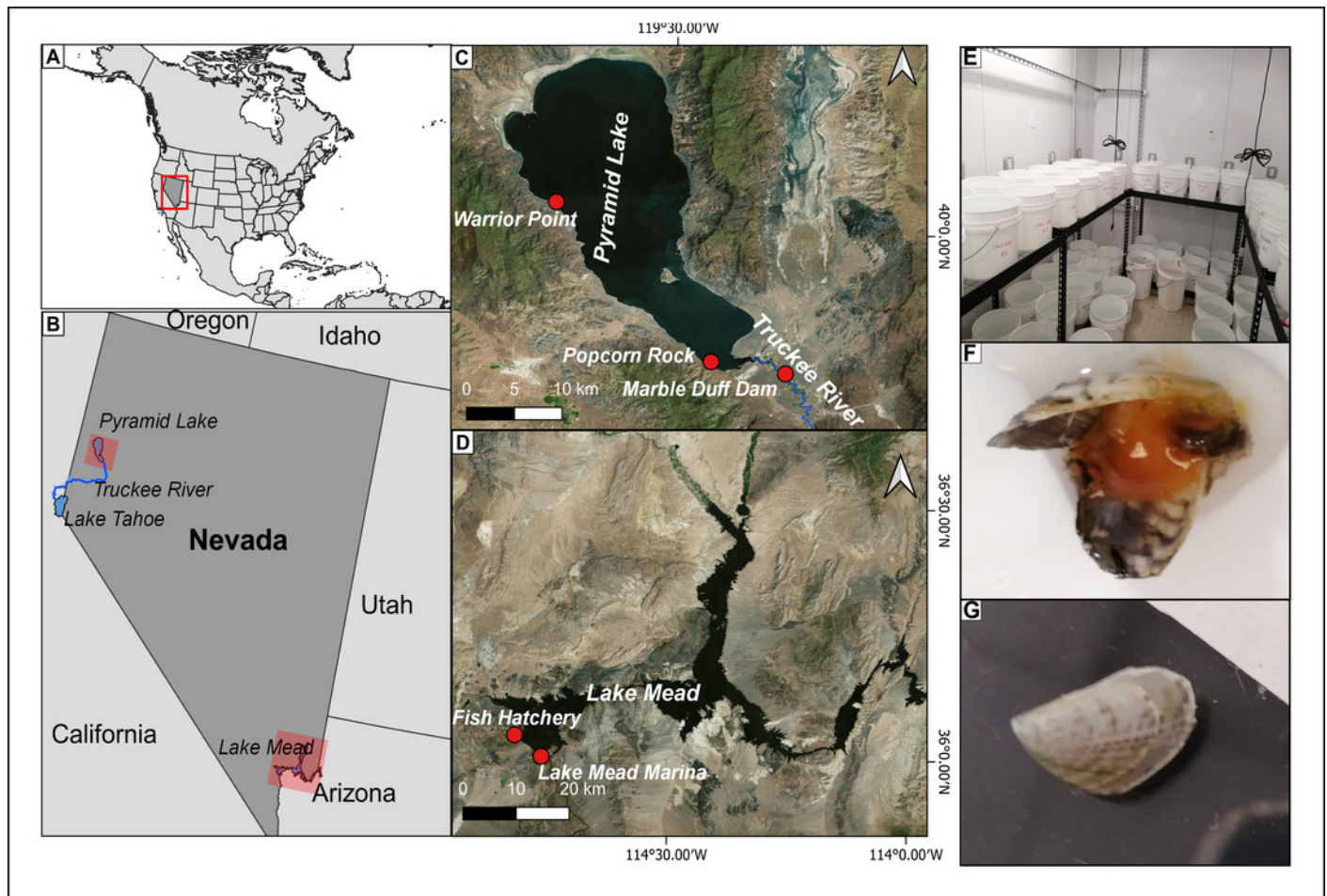
Figure 2

Cumulative frequency of survival in the Lake Mead, Popcorn Rock, and Warrior Point treatments in three different class sizes: Large (16-26 mm), Medium (6-16 mm), and Small (3-6 mm).
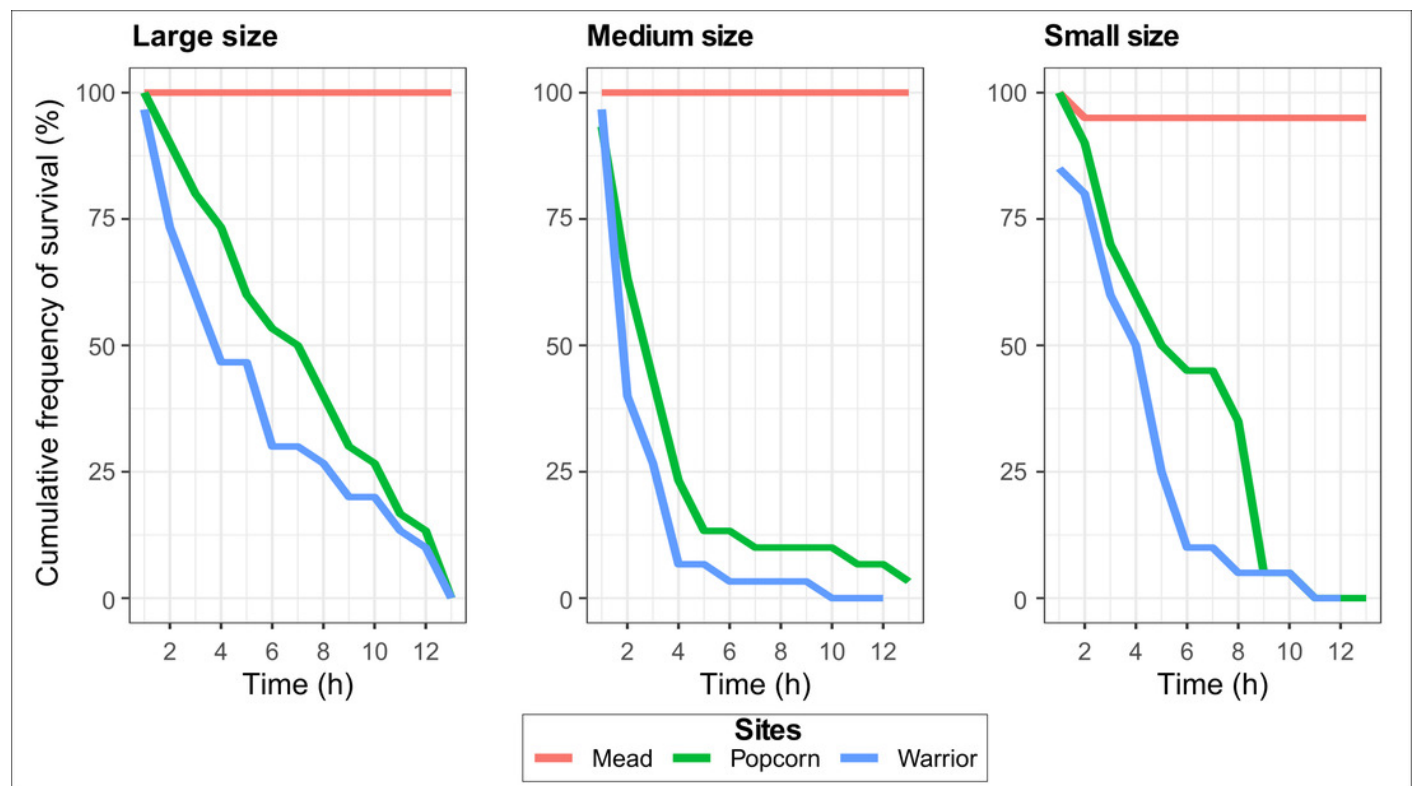
Figure 3

Linear regression and barplot for analysing growth and reproductive potential

Linear regression between the difference in length and weight between the start and the end of the experiments in Lake Mead with numbers indicating individual mussels in the experiment (A) and Truckee River (B) treatments. Barplot showing the sex and gonads status from adult mussels after 58 days of being in Lake Mead (C) and Truckee River (D) water. n.d: no determined sex.

A **Mead**

$y = 0.0134 - 0.0026\,x$ $R^2 < 0.01$

$P = 0.940$

B **Truckee**

$y = 0.0163 + 0.0402\,x$ $R^2 = 0.53$

$P < 0.001$

C **Mead**

D **Truckee**

State

■ Mature ■ Premature ■ Recovering
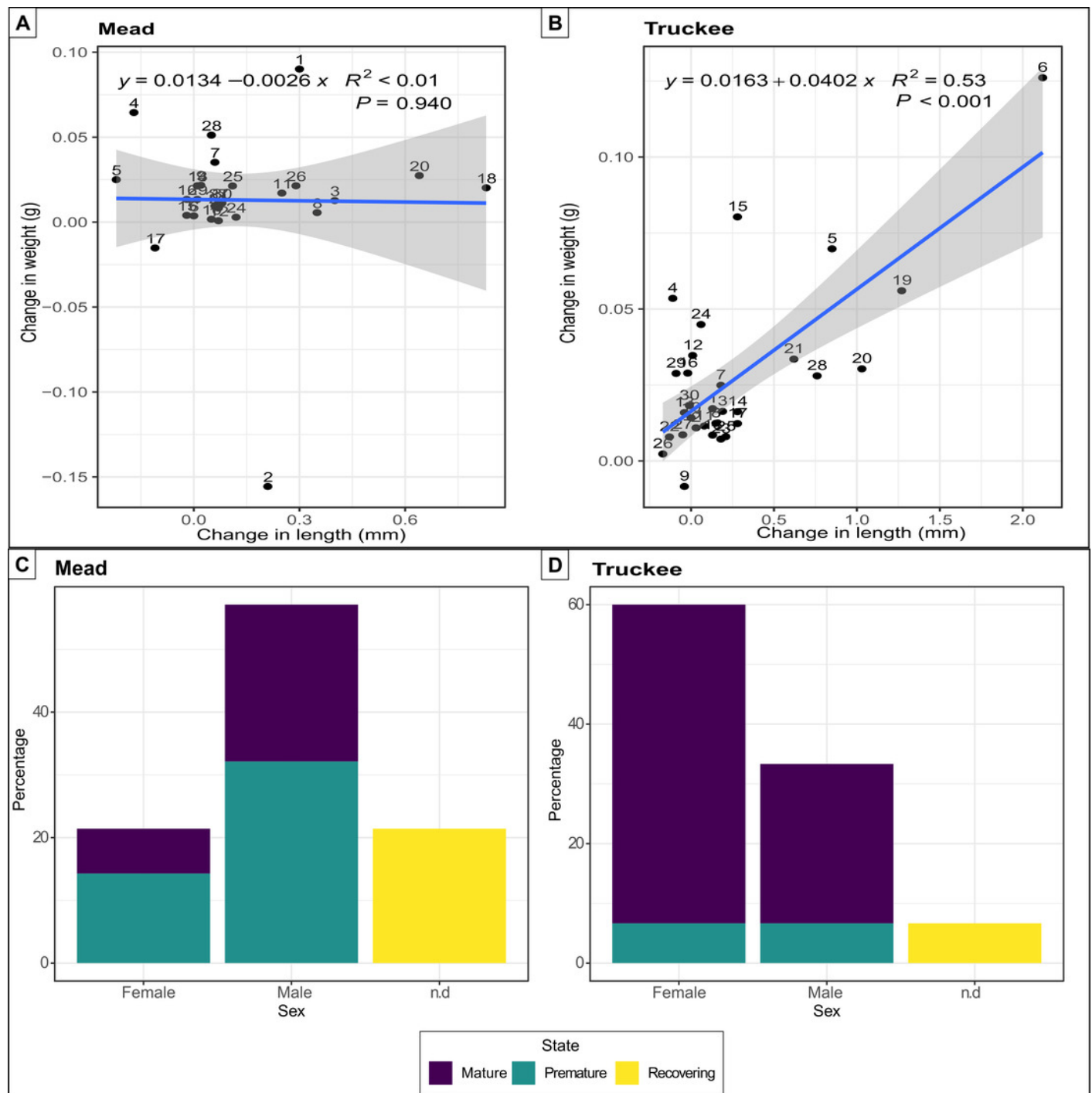
Figure 4

Cumulative frequency of survival in Warrior Point (Pyramid Lake) water treatment amended with phosphoric acid to obtain different pH concentrations. Lake Mead water treatment was the control treatment.
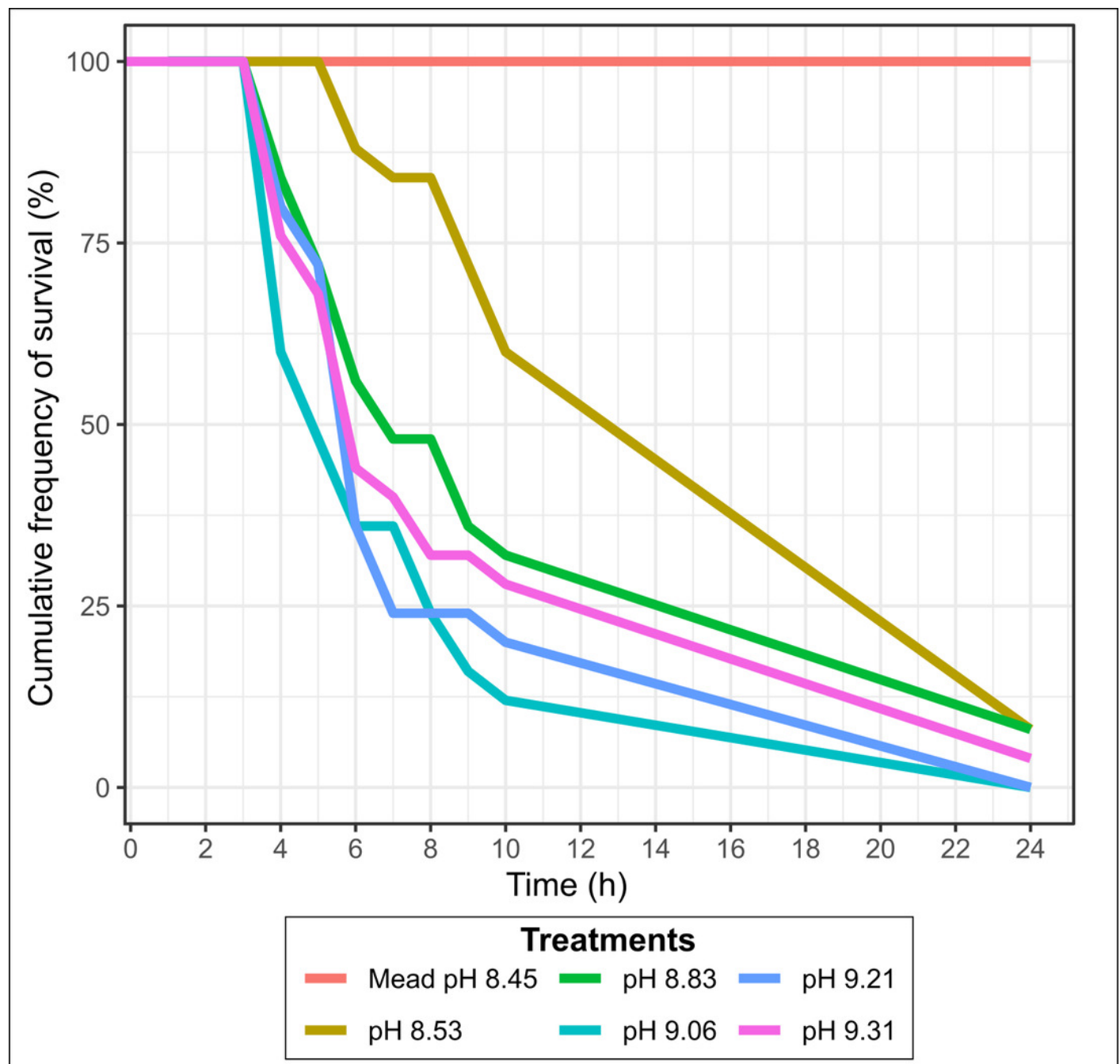
Figure 5

Cumulative frequency of survival in Lake Mead and Truckee River freshwater treatments amended with sodium hydroxide to obtain higher pH concentrations. Lake Mead and Truckee River water treatment without amendments were used as a control.
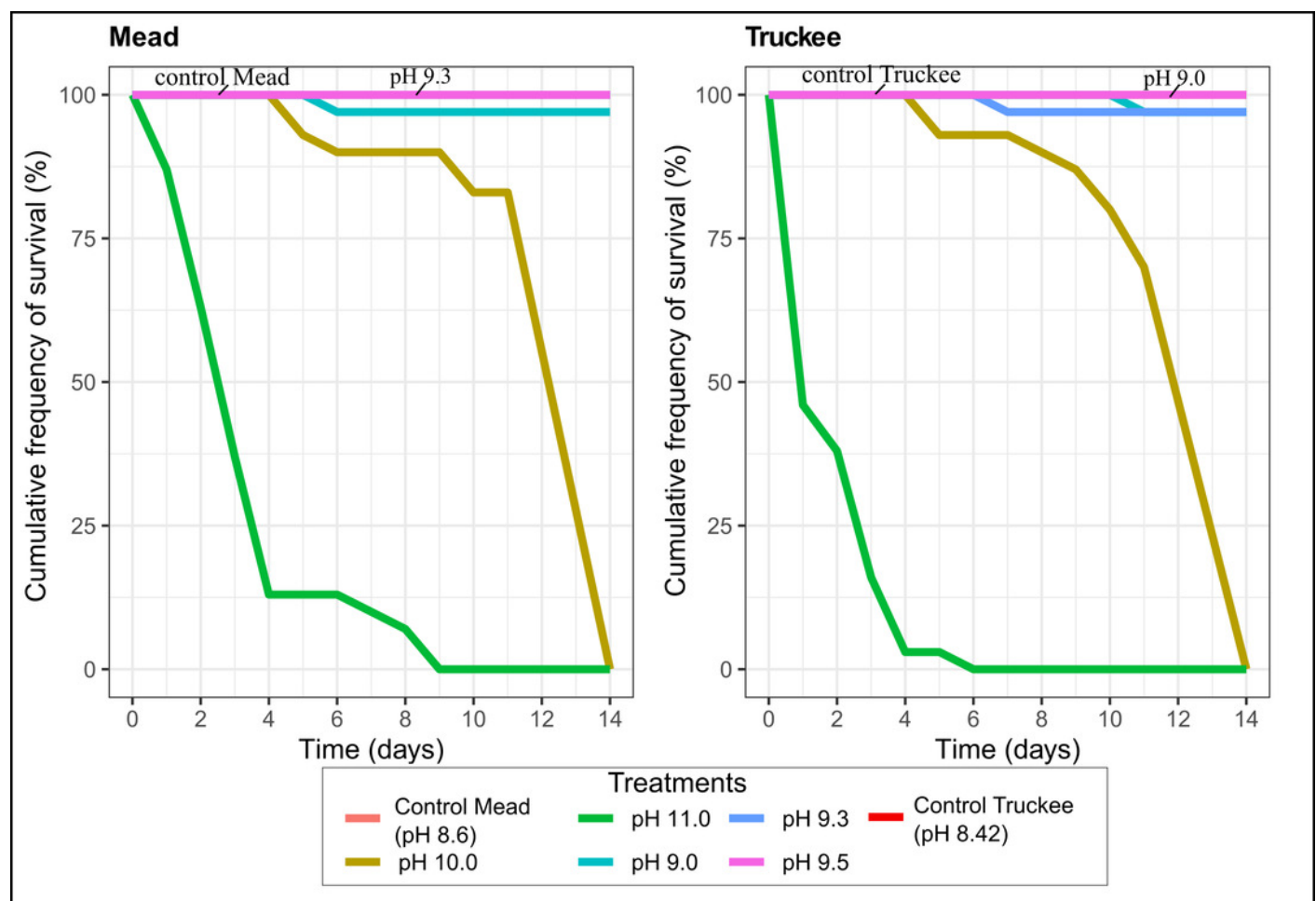
Figure 6

Conceptual model with the main results from this work and rising concern.