Management of Biological Invasions (2015) Volume 6, Issue 3: 277–286
doi: http://dx.doi.org/10.3391/mbi.2015.6.3.06

Open Access

Research Article

Evaluation of watercraft quagga mussel decontamination in saltwater

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Received: 29 August 2014 / Accepted: 7 January 2015 / Published online: 3 February 2015

Handling editor: Amy Fowler

Abstract

Restricting the spread of invasive quagga and zebra mussels by decontaminating recreational watercraft is an important management practice in Europe and the United States. These mussels impact freshwater ecosystems and leave massive economic costs of restoration for ecological services and human infrastructure for each water body they invade. The authors investigated, at the request of the California Department of Fish and Game, whether decontaminating watercraft by placement into saline waters after coming into contact with freshwater bodies infested with invasive mussels eliminates the risk of spreading the species. Evaluating whether or not this is a viable management practice is critical where zebra and quagga mussel freshwater habitats are directly connected to brackish and saline water bodies, like the Sacramento-San Joaquin River Delta (Delta), California United States of American (USA). In this study, quagga mussels were exposed to water collected from different locations within the Delta, with salinities ranging from 4 parts per thousand (ppt) to 33.4 ppt for up to fifteen days. We observed a mortality gradient correlating to salinity levels. The water with the highest salinity content killed 100% of the quagga mussels within 40 hours. One hundred percent mortality was not observed until 70 hours of exposure for quagga mussels exposed to lower salinity concentrations of 21.3 ppt and 15.3 ppt. However, 99% of mussels exposed to 4.0 ppt salinity brackish water remained alive for the 16 day duration of the study. These results are similar to mussels held in water with low salinity content similar to most freshwaters. In the Delta, salinity fluctuates naturally and travel time by boat between saline and freshwaters is a few hours or less. Therefore, it would be challenging if not impossible for watercraft operators to ensure the proper exposure time and salinity exposure. Based on these results, the authors do not recommend launching boats into salt water or brackish water prior to entry into freshwater as a decontamination option for watercraft or as a management tool for preventing invasive mussel infestations.

Key words: *Dreissena polymorpha, Dreissena bugensis*, zebra mussels, invasive mussels, salinity tolerance

Introduction

Zebra mussels, *Dreissena polymorpha* (Pallas, 1771) and quagga mussels, *Dreissena bugensis* (Andrusov, 1897) are invasive bivalves that are impacting freshwater ecosystems (Caraco et al. 1997; Strayer 2008) and causing significant economic damage (Richerson 2013) throughout Europe and North America. Both species are native to Ukraine, the Azov and Black Seas, and the Dnieper River drainage basin (Mills et al. 1996; Mackie and Schloesser 1996) however the invasion timeline of each species is very different. In Europe between 1800 and 1900 zebra mussels more than doubled in their infestation area with the help of anthropogenic assisted dispersion (Mackie and Schloesser 1996; van der Velde 2010). By the late 1900's to early 2000's they had made their way to Western Europe (Molloy et al. 2007, Son 2007). Zebra mussels arrived in North America in the 1980's in the Great Lakes, spread through the Hudson River and Mississippi River systems (Mills et al. 1993; Spidle et al. 1994). The quagga mussel invasion of Europe did not begin until the middle 1900's when they expanded

into Russia (Molloy et al. 2007; Son 2007). In 2006, quagga mussels were discovered in The Netherlands while scientists tracked zebra mussel populations marking the first record of quaggas in Western Europe (Molloy et al. 2007). In 1991, the quagga mussel was discovered in the Great Lakes (Mills et al. 1993; Spidle et al. 1994) and have since slowly made their way westward, arriving in Lake Mead, Nevada in 2007 (Labounty and Roefer 2007; Choi et al. 2013).

Once a mussel population is growing and reproducing in a water body, options to eradicate them are limited and present numerous challenges (Smith 1999). Therefore, both in Europe and North America there has been a focus on preventing the spread of invasive dreissenid mussels (Padilla et al. 1996). It is believed that the most common route of infestation is through recreational watercraft and ballast water from commercial shipping vessels (Padilla et al. 1996; Schneider et al. 1998; Mackie and Claudi 2010). Particularly in North America, many state, federal, and local authorities focus on watercraft decontamination and limiting access to water bodies to boats that could be carrying adult zebra and quagga mussels or their early life stages, namely veligers (Mackie and Claudi 2010; Richerson 2013).

Many state, federal, and local authorities have developed and enforced specific protocols for boat decontamination; including the California Department of Fish and Wildlife (CADFG) (California Department of Fish and Wildlife 2013). However, in California (CA) USA, many recreational boat owners wanted to place their boats into saline water or brackish water with the assumption that this would kill any invasive mussels on their watercraft prior to navigating their boats into freshwater (personal correspondence with Catherine Mandella, CADFG 2013). One region this debate is prominent is in the Sacramento–San Joaquin River Delta where boats can launch in the San Francisco Bay, or more brackish environments, and within hours move into the freshwaters of the Delta (personal correspondence with Catherine Mandella, CADFG 2013).

The salinity of freshwater is usually less than 0.5 parts per thousand (ppt). Brackish water occurs where fresh and salt water meet, such as a delta or estuary, and results in a mixture that has salinity between freshwater and sea water (Horne and Goldman 1994; Remane and Schlieper 1971) which ranges between 0.5 ppt and 17 ppt. The average ocean salinity is 35 ppt but can vary from about 32 ppt and 37 ppt (Office of Naval Research 2014). Both species of mussels are usually found in freshwater in salinities up to one ppt. However, upper limit of tolerance to salinity depends on species location and acclimatization (Spidle et al. 1994; Cohen 2005). Adult zebra and quagga mussels acclimated to river saline levels die when exposed to a range of 5 ppt to 8 ppt (Spidle et al. 1995; Setzler-Hamilton et al. 1997). Alternatively, zebra and quagga mussels have been found in estuarine environments in both Europe (Wolff 1969; Strayer and Smith 1993) as well as the United States (Baker et al. 1993).

While both species are found in fresh and brackish waters, there is debate about whether the different species have different salinity tolerance levels (Mackie and Claudi 2010). Some studies show zebra mussels to be slightly more saline resistant than quagga mussels with tolerances between 4 ppt and 7 ppt (Mills et al. 1996; Setzler-Hamilton et al. 1997). The zebra mussel larvae also showed higher resistance to salinity than quagga larvae in one study where salinity tolerance in embryos increased with larval age up to 8 ppt, in which they survived and grew for 5 months (Wright et al. 1996). However, in one study, both zebra and quagga mussel populations from estuarine environments survived in salinities up to 10 ppt due to being acclimatized to higher saline ranges (Cohen 2007). Additional studies have shown no evidence of differences between the species and attribute salinity tolerance differences to rates of exposure, acclimation speed, water temperature, and stage of mussel development (Strayer and Smith 1993; Baker et al. 1993; Spidle et al. 1994; Spidle et al. 1995).

The objective of this study was to evaluate the impact of varying concentrations of salinity on quagga mussels to determine at what concentration and duration exposure to salinity kills adult mussels. In this study, we looked at quagga mussels because, due to proximity, they are more likely to be transported to the Delta via recreational watercraft from the recently invaded Colorado River (Labounty and Roefer 2007; Cohen 2007). The organism selection and the study design was selected to help determine if placing boats in saline or brackish water is an effective tool for decontamination. Selected salinity concentrations and exposures replicated potential real world situations relevant to the San Francisco Bay and Delta areas. These locations' specific salinities are approximate as delta temperature and salinity gradients vary often due to the nature of deltas and the influence of outside forces, such as salt water intrusion or increased runoff, which cause fluctuation in salinity values.

Table 1. Water source locations and average salinity at the time of collection.

Studies	Classification	Site	Location	Average salinity (parts per thousand, ppt)
1, 2	saline	Vincent Park, Richmond, CA	37°54'28" N, 122°20'59" W	33.4
3	saline	C&H Plant, Benicia, CA	38°02'16" N, 122°07'51" W	21.3
3	saline	Preston Point, Bay Point, CA	38°02'59" N, 121°56'19" W	15.3
1	brackish	Pittsburg Marina, Pittsburg, CA	38°02'08" N, 121°52'56" W	4.0
1	fresh	Made in-house, MBI, Davis, CA	n/a	0.4

Methods

Experimental conditions

We conducted the tests in controlled temperature conditions, targeted at 20 degrees Celsius (°C), and used United States Environmental Protection Agency (USEPA) hard synthetic freshwater as a control freshwater (USEPA 1993). Marrone Bio Innovations (MBI) scientists previously evaluated the use of the USEPA hard synthetic freshwater water for supporting mussel health and determined that the salt balance was sufficient to support healthy mussels in the laboratory for a number of months (data not included). The saline and brackish waters were collected in the field as described below. For the saline and brackish water test conditions we had two test chambers each and one freshwater control chamber.

Water Collection

We collected water from various locations in the Delta and measured the salinity with a Hydrolab multimeter model YSI 85. The selected locations included saline and brackish water qualities (Table 1, Figure 1).Collections occurred three times in the first study, approximately every seven days on 10/04/2013, 10/10/2013, and 10/16/2013 and water stored at 4 °C to limit water quality degradation. For the second and third studies water collections occurred once per study on 10/21/2013 and 01/29/2014 respectively.

Mussel Collection and Handling

We collected quagga mussels from Lake Havasu on 09/19/2013 and 11/19/2013. The mussels were acclimatized to lab conditions for two weeks in USEPA hard synthetic freshwater as a control freshwater (USEPA 1993). While the exact mussels used in this study were not weighed and measured, the overall average size and length of the mussels used in our laboratory are 16.19 mm ±2.62 mm and 0.58 g ±0.12 g. Once acclimatized, we placed 50 healthy mussels each in an enclosure made of hard mesh (12” × 6” × 8.5”). The hard mesh enclosures contained the mussels yet still allowed water to diffuse evenly through the enclosure. We defined unhealthy mussels as mussels that were dead or gaping open, unresponsive (meaning there is no movement when sound, light, or water flow changes around the mussel), damaged, empty, or cracked. We then placed two enclosures into each test chamber.

Monitoring

Mussel mortality monitoring, water quality monitoring, water changes, and tested salinity conditions for each of the studies were as follows.

Mussel Mortality Monitoring

We determined mortality by removing each enclosure from the testing chamber and inspecting the 50 mussels for mortality based on the criteria stated above. Other visual criteria to determine mussel health included: siphon activity, closure response time, byssal thread strength, and amount of foam present upon completion of the study. Foam is a visual indicator of mussel stress that forms when mussels excrete protein based waste which clumps and binds together on air bubbles (Sharpe 2014).

Water Quality Monitoring

The measured water quality parameters included; ammonia, temperature, pH, and conductivity. Water quality readings were collected prior to placing mussels in the test chambers and 30 minutes after placing the mussels in the test chambers.

Study 1- We tested 33.4 ppt salinity water and 4.0 ppt brackish water compared to the laboratory

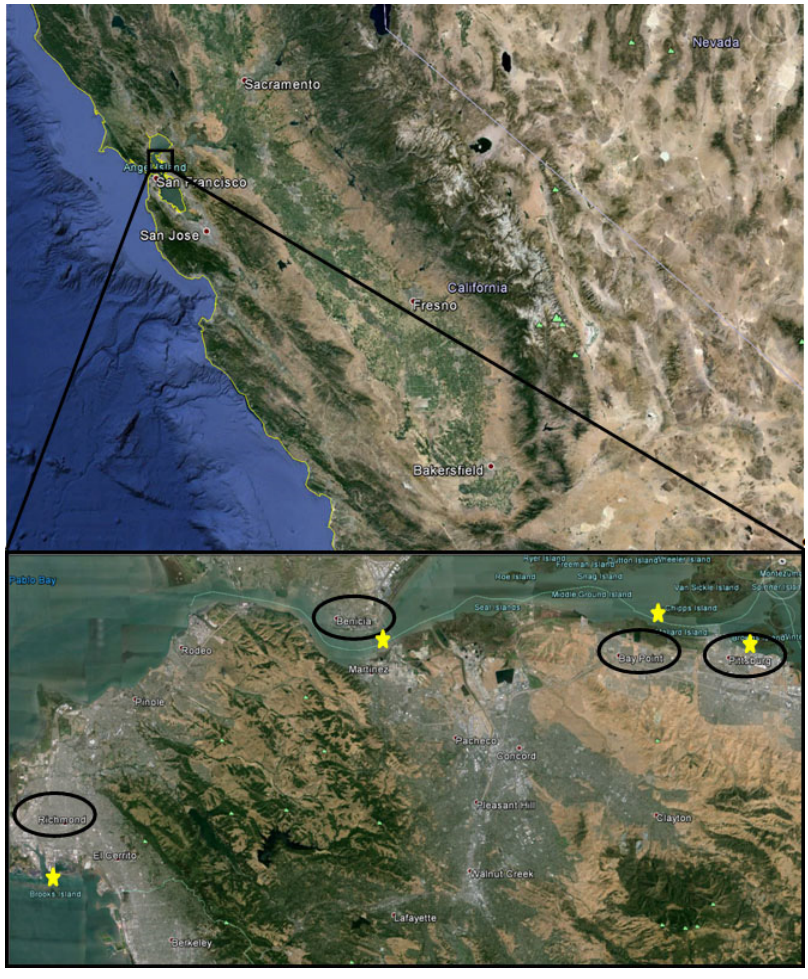

Figure 1. Map of location of saline and brackish water collection in the Bay-Delta, California.

freshwater control. We monitored water quality and mussel mortality every third day for 15 days or until mussel mortality reached 100% to ensure we were capturing any long term exposure impacts in addition to acute exposure results. After these initial water quality readings and mussel mortality results, we changed half of the water from each test chamber and replaced it with new water that we stored at 4 °C. We recorded the water quality parameters again 30 minutes after the water change.

Study 2- Only 33.4 ppt salinity water collected on 10/21/2013 was paired with the laboratory freshwater control to compare mortalities. We recorded mortality and water quality every morning and evening until all the mussels died and did not change water during any of the mortality or water quality monitoring to obtain a more accurate time point of when acute saline toxicity affected quagga mussel mortality.

Study 3- In the third study, we tested lower salinity concentrations of 21.3 ppt and 15.3 ppt collected on 01/29/2014 compared to the laboratory freshwater control. We recorded mortality and water quality three times a day (morning, mid-day, and evening) until all the mussels died. We did not change water during any of the mortality checks to distinguish differences in time to mortality more accurately for these lower salinity waters.

Results

Study 1

For saline water, 33.4 ppt, on the first mortality monitoring on day 3, all the mussels were dead (Figure 2). Figure 3 shows one of the saline enclosures with the gaping dead shells. After 16 days, mortality was low for the mussels in freshwater and brackish water chambers, 2±2 %

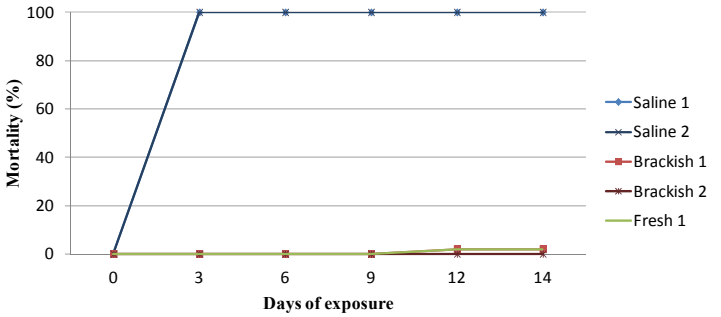

Figure 2. Mussel mortality over time in study 1; saline- 33.4 ppt salinity, brackish- 4.0 ppt salinity, fresh- 0.4 ppt salinity.

Figure 3. Dead mussels exposed to 33.4 ppt salinity in hard mesh enclosure; study 1. Photograph by Jessica Hofius.

and 1±2 % respectively (Figure 2). We determined that the remaining mussels in both the brackish water and the freshwater were healthy based on our definition of healthy mussels as stated in the methods.

The pH ranged between pH 7.8 and 8.1 for the duration of the test for all water (Table 2). The pH of the saline water decreased as the ammonia concentrations increased. The ammonia concentration in both the brackish and saline waters increased from 0 mg/L to 3.7 mg/L when we first placed the mussels in the test chamber (Figure 2). After three days, the brackish water ammonia concentrations had decreased to 0.3 mg/L. The ammonia concentrations in the saline water increased at day 3 corresponding to the mussel mortality.

The first temperature reading of each day was consistent at ~ 20 °C, our target temperature (Table 2). Since water was stored at 4 °C, the water temperature in each chamber decreased to 17 °C when we added the new water. The conductivity of each type of water remained consistent through multiple batches of each type of water: 4.5 to 7.3 in brackish water, 51.3 to 53.6 in saline water, and 729.5 to 801.0 in the fresh water as a result of the solutes used to make the synthetic hard water (Table 2).

Study 2

After rapid mussel mortality in the first study, we again tested the highest salinity water, 33.4 ppt,

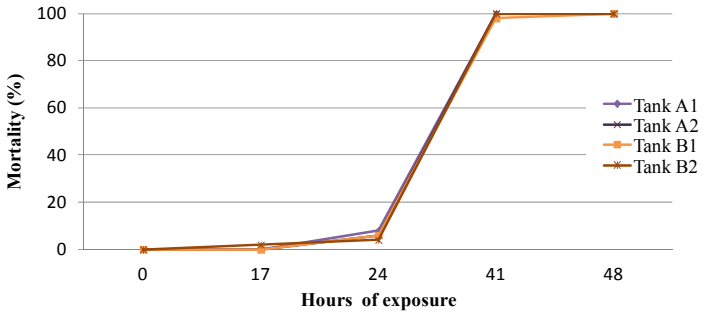

Figure 4. Percent mussel mortality over time in study 2; 33.4 ppt salinity water.

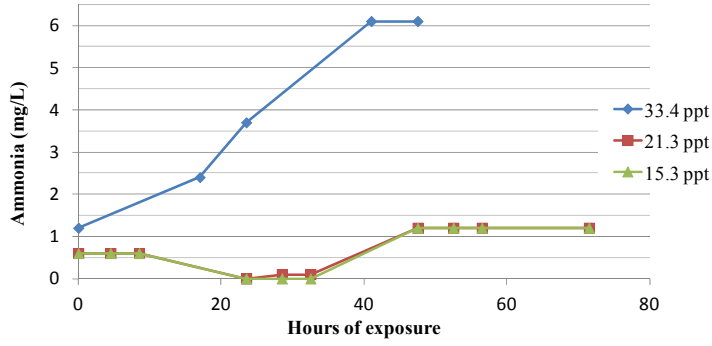

Figure 5. Ammonia concentration over time in studies 2 (33.4 ppt salinity) and 3 (21.3 ppt and 15.3 ppt salinity).

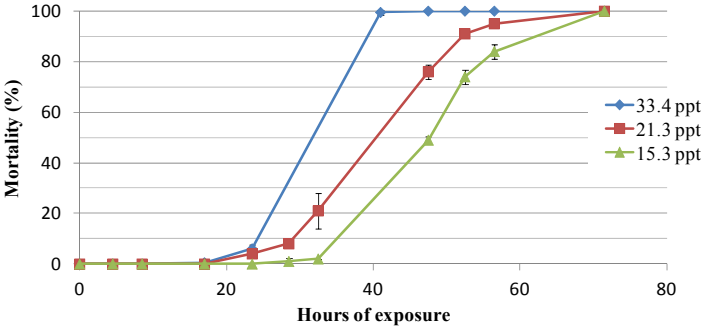

Figure 6. Mussel mortality over time in studies 2 and 3; 33.4 ppt, 21.3 ppt, and 15.3 ppt salinity water.

conducting mortality checks and water quality monitoring twice daily to determine when the mortality occurred. We found that at 24 hours, 6±1.6% of the mussels were dead; at ~ 40 hours there was 100% mortality (Figure 4). We found little change in most of the water quality parameters. There was a slight rise in ammonia levels, and corresponding drop in pH, as the mussels began to die (Table 3).

Study 3

In the third study, we tested additional saline concentrations, 15.3 ppt and 21.3 ppt, conducting mortality checks and water quality monitoring three times a day to distinguish different responses of mortality with time. At 24 hours we observed, 4±1.6% mortality in mussels exposed to 21.3 ppt salinity and 0% mortality in mussels exposed to 15.3 ppt salinity (Figure 6). At ~ 47 hours there was 76±2.8% mortality in the 21.3 ppt mussels and 49±1.4% mortality in the 15.3 ppt mussels. By 71 hours, we observed 100% mussel mortality for both salinity values. There was little change in the water quality parameters, except ammonia (Table 4). There was a slight rise in ammonia levels to 0.6 mg/L when the mussels were first placed into the test chambers.

Table 2. Average water quality for study 1.

Date	Saline Chamber - 33.4 ppt salinity				Brackish Chamber - 4.0 ppt salinity				Freshwater Chamber - 0.4 ppt salinity			
	pH	Temp (°C)	Conduct (mS/cm)	Ammonia (mg/L)	pH	Temp (°C)	Conduct (mS/cm)	Ammonia (mg/L)	pH	Temp (°C)	Conduct (mS/cm)	Ammonia (mg/L)
10/07/2013	7.99	19.2	51.3	2.5	7.96	18.5	4.5	2.5	8.05	19.0	729.50	0.0
10/11/2013	7.87	19.0	53.6	4.9	7.90	18.5	4.8	0.3	8.01	18.1	768.50	0.0
10/14/2013	n/a	n/a	n/a	n/a	7.89	19.1	5.6	0.3	7.99	19.2	773.00	0.0
10/17/2013	n/a	n/a	n/a	n/a	7.94	18.9	5.1	0.3	8.07	19.4	770.50	0.0
10/20/2013	n/a	n/a	n/a	n/a	7.91	18.3	6.9	0.5	8.11	19.5	796.50	0.0
10/23/2013	n/a	n/a	n/a	n/a	7.77	19.9	7.3	0.1	7.92	19.4	801.00	0.0

Table 3. Water quality for study 2.

Date	Time of Day	Salinity Chamber A					Salinity Chamber B				
		pH	Temp (°C)	Conductivity (mS/cm)	Ammonia (mg/L)	Salinity (ppt)	pH	Temp (°C)	Conductivity (mS/cm)	Ammonia (mg/L)	Salinity (ppt)
10/21/2013	PM	8.09	10.3	47.90	1.2	n/a[2]	8.13	11.1	50.2	1.2	n/a[2]
10/22/2013	AM	7.95	21.0	44.86	1.2	31.7	8.00	20.6	50.5	2.4	33.2
	PM	7.81	20.8	51	1.2	n/a[2]	7.85	20.7	53.1	3.7	33.5
10/23/2013	AM	7.78	20.2	45.5	1.2	32.7	7.82	20.1	47.2	6.1	34.3
	PM	n/a[1]	n/a[1]	n/a[1]	n/a[1]	n/a[1]	7.71	20.2	50.4	6.1	34.2

n/a[1]- data point not taken; no mussels left in tank
n/a[2]- data point not taken; equipment malfunction

Table 4. Water quality for study 3.

Date	Time of Day	21.3 ppt Salinity Chamber				15.3 ppt Salinity Chamber			
		pH	Temperature (°C)	Conductivity (mS/cm)	Ammonia (mg/L)	pH	Temperature (°C)	Conductivity (mS/cm)	Ammonia (mg/L)
	8:30 AM	8.27	19.2	31.8	0.6	8.23	19.2	24.8	0.6
1/30/2014	1:00 PM	8.21	20.5	31.9	0.6	8.26	20.7	25.0	0.6
	5:00 PM	8.19	2.6	31.9	0.6	8.21	20.6	25.1	0.6
	8:00 AM	8.30	19.8	32.2	0	8.37	20.0	25.1	0.0
1/31/2014	1:00 PM	8.15	19.2	32.5	0.1	8.13	19.3	25.1	0.0
	5:00 PM	8.11	19.7	32.4	0.1	8.20	19.8	25.2	0.0
	8:00 AM	8.20	14.4	33.0	1.2	8.32	17.7	25.4	1.2
2/1/2014	1:00 PM	8.14	17.7	32.8	1.2	8.17	18.0	25.7	1.2
	5:00 PM	8.14	18.9	32.8	1.2	8.21	19.1	25.6	1.2
2/2/2014	8:00 AM	8.35	17.1	33.3	1.2	8.35	17.6	26.1	1.2

The ammonia concentrations then dropped to 0 mg/L for ~ 24 hours while there was little mussel mortality. Between 32 hours and 47 hours after exposure began, the ammonia levels rose to 1.2 mg/L, corresponding to a rise in mussel mortality (Figure 5).

Discussion

The final mortality result from the brackish water, 4.0 ppt, was statistically equal to the freshwater control demonstrating that placing watercraft in brackish waters is not an effective means of control to prevent the spread of invasive mussels. According to this study, quagga mussels from inland lakes can remain healthy in brackish water with 4 ppt salinity for a minimum of two weeks. Based on the health of the mussels when we concluded the first study, we believe the mussels could have thrived in the brackish water for a significantly longer period of time. Saline water was shown to be toxic to adult quagga mussels. However, it took 40 hours at a 33.4 ppt salinity water to kill 100% of the adult quagga mussels. It took 70 hours for lower salinity water, 21.3 ppt and 15.3 ppt, to kill 100% of the adult quagga mussels. The observed ammonia spike in the saline waters, we hypothesize, is

most likely a shock response due to being introduced to the higher salinity water.

These results demonstrate that there is a mortality gradient based on salinity levels and are consistent with previous work published in relation to adult quagga and zebra mussels and saline tolerance (Spidle et al. 1995; Mills et al. 1996; Setzler-Hamilton et al. 1997). Due to the documented similarities of salinity tolerances, we would expect zebra mussel mortality upon exposure to the same salinity levels to be similar to, or just slightly lower than, the observed quagga mussel mortality (Strayer and Smith 1993; Baker et al. 1993; Spidle et al. 1994). While the environmental tolerances of the quagga mussel are less documented than the zebra mussel (Nalepa and Schloesser 1993) there are documented similarities and differences (Cohen 2007; Mills et al. 1993). The zebra mussel may have a higher temperature threshold than the quagga mussel but, like salinity, this is dependent on which ranges the population has been acclimated (Domm et al. 1993; Spidle et all. 1995).

Since the threshold for salinity tolerance is also dependent on temperature (Baker et al. 1993; Cohen 2007; Mackie and Claudi 2010) there is more uncertainty in the ability to use salinity as a decontamination technique. Additionally, fluctuating salinity from rainfall, inlets, water relocation, and salt water intrusions in the Delta make it challenging for boat owners to determine the salinity of a specific location at any given point in time (Padilla et al. 1996; California Department of Water Resources 2014). It is even more challenging to determine the conditions of the water in the interior of the boat, which may be a mix of residual freshwater from a previous location and local water quality. These uncertainties make it difficult for a boat owner to leave their watercraft in saline waters for any period of time long enough to ensure total mussel mortality. In addition, the Pittsburg Marina in CA, the source for our brackish water, is approximately 60 miles from the Richmond, CA the site of our most saline water collection. Travelling at 30 knots, 34.6 miles per hour, it would take only approximately 120 minutes to travel between the two sites. For these reasons the authors do not recommend placing watercraft in saline waters as a method for decontamination.

While the majority of the research on controlling zebra and quagga mussels has been completed for in-pipe treatments using chemicals, biologicals, filtration, temperature (EPRI 1993; Mackie and Claudi 2010), some acceptable options for decontamination of watercraft have been documented. Spraying all areas of a boat that comes into contact with infested water, including areas inside the boat, with at least 60°C water for five to ten seconds will kill veligers and adult mussels (Morse 2009; Comeau et al. 2011). For boaters who have more time or do not have access to hot sprays, desiccation also kills all life stages of mussels (McMahon et al. 1992; Collas et al. 2014). Desiccation time depends on the ambient temperature and humidity where the vessel is stored. For example, adult zebra mussels have been shown to survive in cool (15 °C) and humid conditions for over ten days while conversely only surviving for 6 days at 25 °C or less than 2 days at 40 °C (McMahon et al. 1992). Size was also a factor in the speed of desiccation as larger quagga mussels survived longer than smaller quaggas when exposed to the same temperature and relative humidity (Collas et al. 2014). While there are many chemical molluscicides available for use in industrial facilities and cooling systems, toxicity risks and lack of watercraft feasibility studies limit their use in watercraft decontamination (EPRI 1993). Antifouling coatings for boats have also been developed but should be used with caution as they only protect against the outside attachment of mussels and provide no defense for the rest of the boat or against microscopic free floating veligers (Mackie and Claudi 2010).

Because it is believed that the most common route of infestation is through recreational watercraft and ballast water in commercial ships (Padilla et al. 1996; Schneider et al. 1998; Mackie and Claudi 2010), additional tools and research would further support those agencies attempting to limit the spread. Further research and documenting the impacts of salinity on the spread of invasive mussels would also further support water resource managers. We recommend two options to further investigate the impact of salinity for boat decontamination; (1) investigate the juvenile dreissenid response to various salinity levels in the context of boat transport and; (2) evaluate the inverse relationship between water temperature and salinity tolerance determined in this study.

Acknowledgements

Our thanks to the other scientists and technicians who assisted with this research: Jillian Slingsby, Tyler Goodearly, Jason Robards, and George Zaragoza. Thank you to Caitlin Rippner, Elizabeth Faulkner, Tamara Nicholson, and the reviewers for help preparing the manuscript.

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