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Assessment of quagga mussel (*Dreissena bugensis*) veliger survival under thermal, temporal and emersion conditions simulating overland transport

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Invasive quagga mussels *(Dreissena bugensis)* were first detected on the lower Colorado River at Lake Mead in January 2007 and have spread to a number of southern California reservoirs. Though it is highly likely that larval transportation along connected waters was the primary cause of these infestations, little is known about the potential for larval conveyance in association with trailered watercraft. We conducted laboratory experiments at the Lake Mead fish hatchery to determine the potential for larvae (veliger) survival under immersion and emersion conditions that simulate those potentially encountered on recreational watercraft when trailered from infested to uninfested waterbodies. Our results demonstrate that at or above an air temperature of 35°C there is no practical risk of conveyance under any condition of emersion or low volume immersion. For emersed veligers in high relative humidity microenvironments there is risk of conveyance over a 'next day' time frame at temperatures up to 25°C. For immersed veligers in volumes as small as 31 µl there is risk of conveyance for at least 20 hours at 30°C and for at least seven days at 25°C or lower. Larval densities in infested waters are low enough that a volume on the order of one droplet is unlikely to contain veligers, but low volume itself does not appear to be a significant impediment to survival. These studies support the development and implementation of robust decontamination methods for watercraft moving from infested or potentially infested waters to those assumed to be uninfested.

Key words: veliger, conveyance, *Dreissena bugensis*, emersion, immersion, invasive species, Lake Mead, quagga mussels, watercraft

The quagga mussel (*Dreissena bugensis,* Andrusov 1897) first appeared west of the Continental Divide in Lake Mead, Nevada, USA in January 2007 (Turner et al. 2011). Since that time numerous agencies and stakeholders have committed to stopping the spread of quagga mussels and limiting the damage these invasive, biofouling mollusks can cause (Turner et al. 2011). The quagga mussel life history is characterized by byssaceous attachment, planktotrophic larvae, and high fecundity, facilitating dispersal (Johnson and Carlton 1996). Byssally attached adults can pose an overland conveyance risk (Ricciardi 1995) and larval entrainment through contiguous waters is a primary dispersive mechanism in the geographic spread of dreissenid mussels (McMahon et. al. 1993). Larval entrainment is believed to be the cause of infestation in a number of southern California reservoirs, all of which received untreated Colorado River water known to harbor quagga mussel veligers (CDFW 2014). Few studies have focused on the potential overland conveyance of viable immersed veligers, and to our knowledge no other studies or publications address the survival and risks of transferring viable emersed veligers associated with overland conveyances. Immersion, as we apply it to dreissenid mussel larvae, means that veligers have not been removed from natal waters while emersion means veligers have been removed from water and are exposed to ambient air conditions in association with microhabitat. Craft and Myrick (2011) demonstrated that under experimental conditions immersed quagga veligers from the Willow Beach National Fish Hatchery, Willow Beach (Lake Mojave), Arizona, USA exhibited 100% mortality when exposed to a water temperature of 35°C for 26 hours. Their study also demonstrated that at experimental temperatures of 10°C, 15°C and 30°C, there was a negative correlation between veliger survival and temperature, with veligers surviving for 43.3 hours at 30°C, 163.3 hours at 15°C, and to the experimental endpoint of 210.9 hours when held at 10°C. In another immersion study conducted at Lake Mead, Choi et al. (2013) found that quagga veligers could survive up to five days during warm summer months and for 27 days during cooler autumn months. In this study summer air temperatures ranged from 25°C to 40°C and autumn air temperatures ranged from 6°C to 18°C, while summer water temperatures ranged from 27°C to 36°C and autumn water temperatures ranged from 8°C to16°C.

The purpose of our study was to further investigate the potential for quagga mussel veligers to survive conditions likely to be encountered on watercraft during overland transport. We focused on what we believed to be realistic scenarios for trailered watercraft transported between infested and uninfested waterbodies in California, examining survival potential at various air temperatures at four hours (same-day conveyance), 20 hours (overnight conveyance) and at moderate temperatures for up to seven days. We examined immersion survival in small droplets of water and emersion survival on moist substrate in order to simulate vessel microhabitat, such as small pockets of water or water-saturated materials like sponges or cloth.

Surviving an experimental condition is distinct from maintaining viability. Viability implies that a veliger is competent for settlement or can proceed with development to that point under certain conditions, while survival simply means that the organism is not dead at the point of examination. Given the complexities of determining viability, our aim was to use survival as a means of assessing conveyance risk. We use transportation and conveyance interchangeably to describe the potential to move live veligers between waterbodies within trailered watercraft. No assumptions were made about the events that would have to occur for the placement of veligers upon watercraft.

Materials and Methods

Location of study.—Experiments were conducted at the Nevada Division of Wildlife Lake Mead fish hatchery indoor laboratory facility (245 Lakeshore Road, Boulder City, NV 89005) from 11 June 2013 through 18 June 2013. Air temperature and relative humidity in the air-conditioned laboratory were measured each morning (between 0800 and 1100) and afternoon (between 1600 and 1630) and ranged from 23°C to 25°C and 26% to 39%, respectively, throughout the study period.

Veliger collection.—A plankton net (Aquatic Research Instruments, Hope, ID) was used to collect vertical plankton tows at the end of the Lake Mead Marina dock between 0830 and 0930 daily and the experiments set up each day used only veligers collected that morning. Net dimensions were 30 cm in diameter × 90 cm in length, with mesh of 64 µm. The cod end was two-piece with a 64 µm mesh. Tows were pulled from a depth of 25 m to the surface and decanted from the cod end into a single 250 ml wide-mouth Nalgene container. Four to six containers were collected each day with 2–3 tows per container. After collection the containers were placed in an 8 L cooler with several frozen gel packs to prevent overheating and transported to the Lake Mead fish hatchery where they were stored in a refrigerator at 4°C until veliger harvesting later that day.

Veliger harvesting.—Plankton tows were gently poured through stainless steel sieves (Fisher Scientific, U.S.A; Standard Test Sieves, ASTM E-11 Specifications) stacked in a column in order of descending mesh sizes of 1 mm, 300 µm, 150 µm, 106 µm, and 63 µm. The 150 µm sieve was back-flushed with unfiltered Lake Mead water into a finger bowl, while both the 106 µm and the 63 µm sieves were back-flushed with unfiltered Lake Mead water into a separate finger bowl. In this way, two size class populations of veligers were obtained: a large size class (i.e., those retained on the 150 µm mesh) and a small size class (i.e., those retained on the 63 and 106 µm meshes). The bowls were manually swirled in a gentle clockwise motion until the veligers were concentrated centrally, which facilitated harvest and reduced contamination with other material present in the tow, such as microalgae and crustacea. A stereomicroscope fitted with a cross-polarizing filter (Johnson, 1995) was used to identify veligers in finger bowls so they could be harvested with an Eppendorf pipettor fitted with 100 µl disposable tips. All experiments were conducted on each size class separately.

Determination of veliger survival.—Veliger survival in lake water was determined by observation using light microscopy at 100× magnification. Veligers were scored as live on the basis of any movement of the velum, cilia, or musculature, or any other movement that would indicate post-treatment survival.

Experiment 1: Acute immersed thermal tolerance.—A volume of 31 µl of lake water containing a target number of 30–50 veligers was pipetted from finger bowls into 200 µl PCR tubes (Eppendorf, Cat. No. 951010022). An MJ Research PTC-200 gradient thermal cycler was used to subject veligers to target temperatures for one-hour exposures. The cycler was programmed for a 10-minute, 25°C acclimation period prior to exposure and a 30-minute, 25°C recovery period after exposure. In a preliminary study, the thermal cyclers' gradient function was used to screen for veliger thermal tolerance at temperatures ranging from 30.0°C to 42.0°C. A double set of triplicate tubes was then run at target temperatures of 35.0°C, 36.0°C, and 37.0°C for one hour for each size class. After cycling, veligers from one triplicate set were immediately pipetted from the PCR tubes into wells on a ten-well glass microscope slide for microscopic observation. The second triplicate set

was held at 4°C overnight and then at room temperature for three hours prior to microscopic observation the following day, to potentially facilitate recovery.

Experiment 2: Immersion tolerance.—Short-term (i.e., 20-hour) and long-term (i.e., 5-day and 7-day) immersion experiments were conducted, each consisting of incubations of veligers in 31 µl of lake water. Plastic inserts from disposable pipet tip boxes were used to hold the PCR tubes (short-term experiment and long-term 7-day experiment) or slides (long-term 5-day experiment), which were then placed in a 4-liter Rubbermaid storage container lined with tap water-saturated paper towels to maintain high humidity. Temperature and relative humidity were measured inside the container at 15-minute intervals using a data logger (Onset HOBO Pro v2 Data Logger, Part No. U23-001).

The short-term experiment had a 20-hour end-point and constant temperatures of 5°C, 20°C, 25°C, 30°C, 35°C or 40°C using a digitally controlled incubator, applying one of the six temperatures during each 20-hour incubation. Each sample consisted of a targeted number of 30-50 veligers in 31 µl of lake water within capped 200 µl PCR tubes in triplicate for each size class.

Two long-term experiments were conducted for five and seven days at ambient laboratory temperatures ranging from 21°C to 25°C. Samples were removed and examined on a daily basis. Each daily sample consisted of twenty replicate tubes or slide wells with a targeted number of 1 to 10 veligers per replicate for each of the two size classes. The 5-day experiment utilized 31 µl droplets with veligers on 10-well microscope slide wells. On days three, four, and five of the 5-day experiment, 10 – 20 µl of unfiltered Lake Mead water was added to each droplet immediately after removal from the Rubbermaid container to prevent desiccation during observation. For the 7-day experiment 31 µl droplets were placed in capped PCR tubes, as was done for the short-term experiment. These two methods were used for the long-term studies because we reasoned that each had distinct advantages and disadvantages that could influence survival. After incubation, survival was determined by direct microscopic observation of veligers at 100× on slides (i.e., 5-day experiment) or observation after transferring veligers from tubes to slides using an Eppendorf pipettor (i.e., short-term and 7-day experiments). Daily samples were discarded after examination.

Experiment 3: Emersion thermal tolerance.—Emersion experiments were run at 4- and 20-hour end-points and constant temperatures of 5°C, 20°C, 25°C, 30°C, 35°C and 40°C in triplicate, separately for each quagga veliger size class. Emersion experimental protocol was based on the ISO-GRID membrane filtration methodology used in food microbiology applications (Entis and Lerner 1996). Veligers were pipetted in 31 µl volumes from finger bowls onto pre-moistened nylon filter paper (MAGNA, 1.2 µm, 25 mm, GE Water and Processing Technologies, Cat. No. R12SP02500) situated on a glass microanalysis vacuum filter holder (Fisherbrand 09-753E) connected to a 125 ml filtration flask. A targeted delivery of 30 – 50 veligers was delivered onto nylon filter paper in each replicate droplet. Rubber tubing was attached to the flask tube and a pipet bulb was used to gently create a vacuum, drawing veligers onto the filter paper with no visible damage. Nylon filter papers with emersed veligers were removed from the vacuum frit with tweezers and placed on top of cellulose filter paper (Whatman No. 1, 42.5 mm) moistened with 500 µl of unfiltered Lake Mead water and placed in disposable Petri dishes. Petri dishes, with the top lids removed, were placed in 4 L Rubbermaid storage containers lined with tap water-saturated paper towels. Two dishes were placed in each container, each containing three filter papers representing triplicate replication of each of the two size classes of quagga veligers. A HOBO data recorder was included in each container to monitor temperature and relative humidity at

15-minute intervals. Containers were incubated in a digitally controlled incubator for the targeted experimental times and temperatures. After incubation, filter papers were removed from the Petri dishes with tweezers and the contents rinsed into a 1.0 ml gridded Sedgewick Rafter counting chamber; each filter paper was rinsed six times with 100 µl unfiltered Lake Mead water using an Eppendorf pipet. Subsequently, microscopic observations of veligers were made at 100× to determine survival. The time interval between rinsing veligers from filter papers and microscopic observation ranged from 5 to 60 minutes due to the logistics of running simultaneous experiments.

Data analysis.—Obtaining data that could be used to statistically compare the effects of temperatures, size class, or temporal trends was beyond the scope of this investigation. Cursory examination of the survival data indicated high variation among triplicate replication and presenting arithmetic means was deemed inappropriate. As a result most of the data are presented graphically to reflect raw triplicate values on the proportion of veligers that showed any evidence of survival (% survival). The long-term immersion experiments used 20 replicates per treatment and for these data the means and standard errors of the means were calculated and displayed. The range and mean number of veligers examined in each experiment are noted in the figure legends.

Results

Immersed veligers: acute thermal tolerance (Experiment 1).—After one hour at 35°C without a recovery period, survival of quagga mussel veligers was 87–100% and 96–100% for the small- and large-size-classes respectively (Figure 1A). At 36°C survival percentages were reduced to 50–73% and 47–60% for small and large size classes, respectively, and at 37°C there was no survival in either size class. A recovery period consisting of overnight incubation at 4°C followed by three hours at room temperature did not result in increased survival (Figure 1B).

Immersed veligers: thermal tolerance of 20-hour small-volume incubations (Experiment 2).—After 20 hours immersion in 31 µl droplets of water in closed tubes, small size class veligers exhibited survival rates of 62% or better at 5°C, 20°C, 25°C, and 30°C and no survival at 35°C or greater (Figure 2). The large-size-class veligers appeared more sensitive to high temperature, with 0–26% survival at 25°C and 0–3% at 30°C compared to 97–100% survival at 25°C and 63–91% at 30°C for small-size-class veligers (Figure 2).

Immersed veligers: long-term survival in small volumes (Experiment 2).—Experiments were conducted to examine multiple-day survival of veligers in 31 µl volumes using two methods: droplets on slides and droplets in closed tubes. Veligers in the small size class immersed in droplets of lake water on a microscope slide, incubated at ambient lab temperature with a relative humidity >95%, exhibited mean survival rates of ≥14% upon examination daily over five days (Figure 3A). Veligers in the large size class under identical conditions exhibited ≥23% mean survival rates over the five-day observation period (Figure 3A).

Veligers in the small size class immersed in 31 µl droplets of water in closed tubes incubated at ambient lab temperature exhibited mean survival rates of at least 45% over seven days (Figure 3B). Under the same conditions the large size class veligers exhibited mean survival rates of ≥7% over seven days including 16% on day seven (Figure 3B).

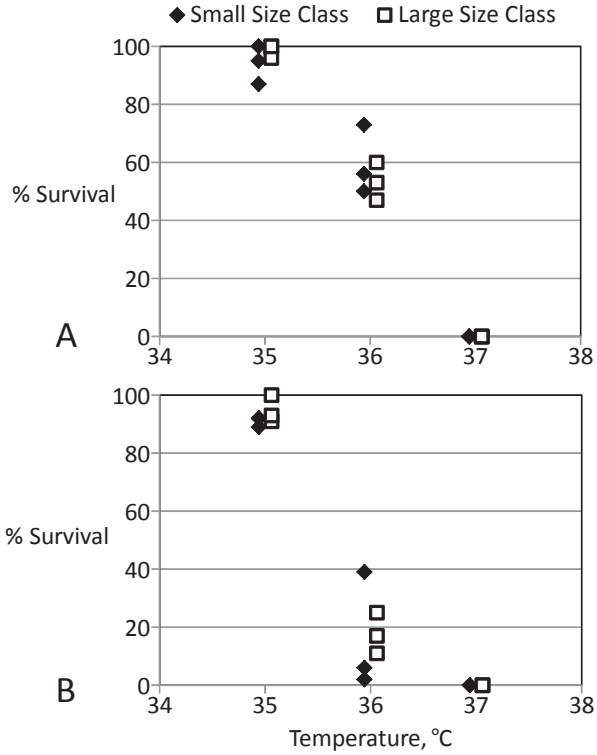

Figure 1.—Acute thermal tolerance of small and large size class quagga mussel (*Dreissena bugensis*) veligers in 1-hour lake water immersion exposures. Data are the percentages of survival in three replicate tubes at each temperature shown. Observations were made immediately after exposure (A) and after a recovery period ranging from 14.5 to 18.5 hours at 4°C followed by 2.5–3 hours at ambient laboratory temperature (23°C–25°C). (B). The number of veligers per replicate immediately after exposure (A) ranged from 38 to 87, with an average of 58 for the small size class and a range of 9 to 25 with an average of 16 for the large size class. The number of veligers per replicate after overnight recovery (B) ranged from 27 to 106 with an average of 56 for the small size class and a range of 9 to 28, with an average of 18 for the large size class.

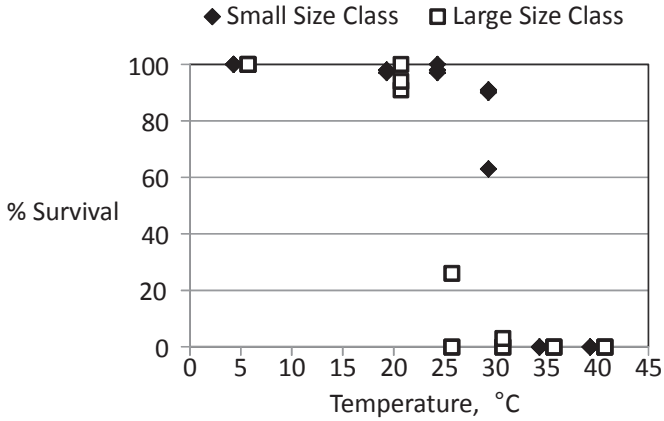

Figure 2.—Survival of small- and large-size class quagga mussel (*Dreissena bugensis*) veligers following 20-hour immersion in 31 µl lake water at various temperatures. Data are the percentages of survival in three replicate tubes at each temperature shown. The number of veligers per replicate ranged from 33 to 78 with an average of 51 for the small size class, and from 7 to 47 with an average of 21 for the large size class.

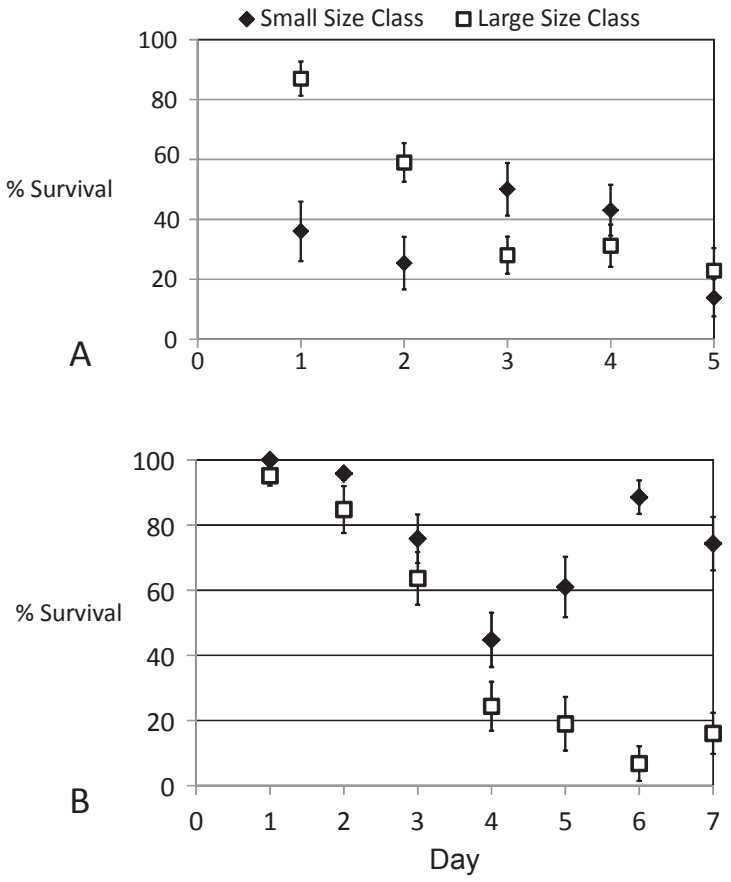

Figure 3.—Survival of small- and large-size class quagga mussel (*Dreissena bugensis*) veligers following immersion in 31 µl lake water droplets on glass slides (A) and in closed 200 µl PCR tubes (B) at ambient laboratory temperature ranging from 21°C to 25°C. Each data point represents the average survival in 20 replicate droplets. Bars indicate standard error of the mean. The average number of veligers per replicate in the small-size class ranged from 4.5 to 9.8 in (A) and from 7.4 to 18.6 in (B). Average numbers of veligers per replicate in the large class ranged from 3.9 to 6.2 in (A) and from 2.2 to 8.6 in (B).

Emersed veligers: thermal tolerance (Experiment 3).—After four hours emersion at a relative humidity >95%, the small-size-class veligers exhibited survival rates of ≥24% at temperatures of 5°C, 20°C, 25°C, and 30°C and no survival at 35°C or 40°C (Figure 4A). The large-size-class veligers exhibited survival rates of ≥67% at 5°C, 20°C, 25°C, and 30°C but, again, no survival at 35°C or 40°C (Figure 4A). After 20 hours of emersion the small-size-class veligers exhibited much lower and more variable survival rates than at four hours; survivors were present at 5°C, 20°C and 25°C, but not at 30°C or higher (Figure 4B). The large-size-class had survival at 5°C and in two of the three replicates at 20°C, but not at 25°C or higher (Figure 4B).

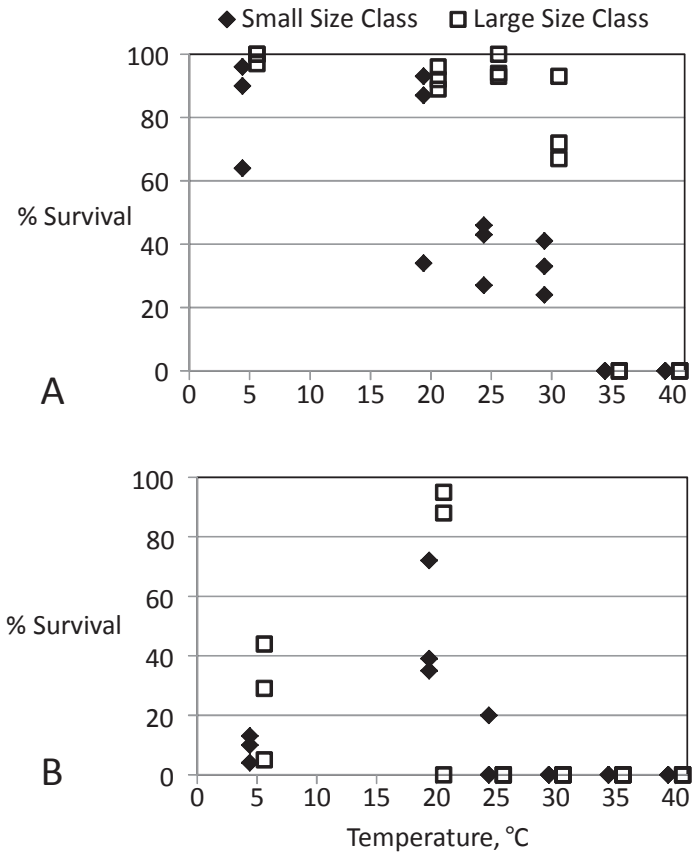

Figure 4.—Survival of small and large size class quagga mussel (*Dreissena bugensis*) veligers following emersion for 4 hours (A) and 20 hours (B) at various temperatures. Data points represent the percent survival for each replicate with three replicates per size class at each temperature. The number of veligers per replicate for 4-hour emersions (A) ranged from 23 to 262 with an average of 78 for the small-size class and a range of 14 to 56 with an average of 28 for the large-size class. The number of veligers per replicate following emersion for 20 hours (B) ranged from 13 to 89 with an average of 37 for the small size class and a range of 3 to 49 with an average of 13 for the large-size class. Data for 30C° are represented by only two replicates because the third replicate, with only one veliger present, was excluded.

Discussion

The first phase of this study involved developing methods to determine veliger survival. Using proxy native oyster (*Ostrea lurida,* Carpenter 1864*)* veligers from the Bodega Marine Laboratory, Bodega Bay, California and quagga mussel veligers from Lower Otay Reservoir, California, neutral red (a living-cell inclusion stain) and trypan blue (a living-cell exclusion stain) were employed to develop a means of determining survival that is independent of behavior (Crippen and Perrier 1974, Tolnai 1975). Unfortunately, the use of stains proved to be unreliable in that results were inconsistent and difficult to

interpret. Our results were in agreement with those of Sykes (2009), who reported neutral red staining too ambiguous and variable to determine veliger survival. Ultimately, any movement of the musculature, cilia or velum was used as an indication of post-treatment survival, with the recognition that some unknown percentage of those showing movement would not be viable until and completing metamorphosis. The same approach was used in earlier studies (Sykes 2009, Craft and Myrick 2011, Choi et al. 2013). Observations of 9,700 veligers were made during eight days at Lake Mead in the study presented here, and in all but a few cases the determination of survival, as we defined it, was unambiguous. Extensive observations were made to carefully examine each veliger for movement in cases where survival was not obvious. Nevertheless, mortality could have been overestimated.

Despite sieve fractionation of plankton tows, there were unavoidable phytoplankton and zooplankton contaminants of sizes similar to those of the veligers in both size classes. These contaminants could have confounded results of the immersion trials in two distinct ways. First, these contaminants could increase biological oxygen demand and potentially result in hypoxia or other adverse environmental conditions, particularly in closed tubes. Survival of veligers in closed tubes for at least seven days suggests that this was not a critical factor, although it may have contributed to the variation observed. Second, copepods were a common contaminant in our immersion treatments and are known to prey upon the veligers of a quagga mussel congener, the zebra mussel (*Dreissena polymorpha*, Pallas 1771) (Liebig and Vanderploeg 1995). We observed copepod-veliger interactions that may have indicated a predator-prey relationship. In addition, the occurrence of serrated veliger shell fragments suggested the presence of copepod predation (J. P. Snider, California Department of Fish and Wildlife (CDFW), unpublished observations). Empty veliger shells and shell fragments that may have resulted from predation were not included in counts.

Scavenging, ciliated protozoans were observed occasionally within the valves of dead veligers. The movements of these protozoans could have been mistaken for veliger ciliary movement but careful observation was employed to account for this potentially confounding factor.

Our results demonstrate that there is a risk of transporting live immersed quagga veligers within 31 µl droplets of water at ambient air temperatures of up to at least 25°C for at least seven days and likely longer, considering the rates of survival at the experimental endpoint. The individual water droplets in our five-day and seven-day treatments averaged between 2.2 and 18.6 veligers per replicate, which would be concentrations far exceeding those realistically encountered under field conditions given reported densities of dreissinid larvae in infested waterbody samples. For example, Gerstenberger et al. (2011) reported a density of 28.6 veligers/L in September of 2008 in the Boulder Basin of Lake Mead. At that density, the likelihood of one veliger being in a single 31 µl droplet of water would be one (veliger) in 1,128 (droplets of water). Nevertheless it is reasonable to assume conveyance risk if standing water is present on a watercraft leaving an infested waterbody. As previously noted, Choi et al. (2013) reported that Lake Mead quagga veligers held in shaded, 15-L containers of lake water survived up to about five days under summer conditions (summer air and water temperatures ranged from 25°C to 40°C and 27°C to 36°C respectively) and 27 days under autumn conditions (autumn air and water temperatures ranged from 6°C to 18°C and 8°C to 16°C respectively). Together with the results of our studies, which included more defined, repeatable conditions, as well as exceedingly smaller water volumes, it is clear that residual water on trailered watercraft leaving infested waterbodies can pose significant risk of live veliger conveyance. Short-term immersion survival data suggest a risk for next-

day conveyance up to a temperature of 30°C. There was no evidence of immersed veligers suffering cold-related mortality, in agreement with our unpublished observations that veligers typically survive well for at least eleven days in a standard laboratory refrigerator (4° C). Our acute thermal tolerance data suggest that veligers are able to tolerate temperatures of 36°C for one hour; however, given 100% mortality at 35°C in all treatments greater than one hour, there would be no practical conveyance risk if microhabitat conditions reach this temperature for one hour or longer.

The pattern of immersed veliger survival under closed environmental conditions (e.g., 200 µl PCR tubes) was very different from that of emersed veligers or veligers in open droplets, for which the larger veligers appeared to demonstrate a higher survival rates than the smaller veligers. Higher metabolism of the larger size-class or the presence of more predators, or both, may have accounted for the lower survival rates in closed tubes. One reason we used closed tubes in addition to open microscope slides for the long-term experiments was the risk of the open slides evaporating over the multiple-day period despite our efforts to maintain high humidity. While we were able to maintain live veligers in droplets on slides for five days at high humidity, some evaporative loss on slides was observed and recorded on days three, four, and five of the five-day immersion study. Consequently, the addition of 10–20 µl of water was required to prevent desiccation during observation.

This is the only study to date that examines and documents veliger immersion survival in a small water volume and emersion survival on moist microhabitat. In preliminary, unpublished observations, we found no difference in survival between incubation volumes ranging from 31 µl to 4 ml. In conjunction with the results reported here, we hypothesize that veliger survival in un-concentrated lake water samples is independent of water volumes 31 µl or greater. Survival in such small volumes of water for at least seven days was an unanticipated result, but provided for efficient microscopic sample assessment without further concentration, sample splitting, or other manipulations.

The emersion periods we examined, 4 hours and 20 hours, were chosen to represent same-day and next-day conveyance of watercraft from infested to uninfested waterbodies. Our results suggest that there is a risk of emersed quagga veliger survival on trailered watercraft under microhabitat conditions of high relative humidity (>95%) for four hours at up to 30°C and for 20 hours at up to 25°C. A number of other observations can be gleaned from the emersion data, with the caveat that the numbers of veligers contributing to the survival data was relatively low (Figure 4A, 4B). Four-hour emersion followed a pattern where survival decreased as temperatures increased (Figure 4A). The apparent greater emersion tolerance of large veligers as compared to small veligers followed the same pattern that Ricciardi et al. (1995) described for emersion tolerance of adult quagga mussels. An unexpectedly low survival was observed at 5°C after 20 hours of emersion (Figure 4B). Evaporative stress could be a factor given that at 95% relative humidity with a standard barometric pressure of 760 mm Hg, absolute humidity at 5°C is 6 g/m^3, while at 20°C the absolute humidity is 16 g/m^3 (PlanetCalc Online Calculator version 2.0.1533.0). This suggests that moderately warm temperatures may actually facilitate emersion survival. There was large inter-replicate variation in 20-hour emersion survival at 5°C, 20°C and 25°C. We speculate that this may have been due to temperature and humidity variation within the holding containers, underscoring the importance of microhabitat conditions in transport risk. After 20 hours of emersion, some replicate filter papers at both 20°C and 25°C were qualitatively dry while others remained moist. This observation provides evidence of such

variation, although survival data did not seem directly related to filter moisture (S. N. Byron and J. P. Snider, CDFW, unpublished observations).

Generalizations can be made regarding watercraft access policies as they relate to veliger conveyance. If exposure temperatures reach 35°C there is no practical risk of conveying emersed veligers and at exposure temperatures ≥25°C the risk is likely no more than one day. If immersed veligers are in water that reaches a temperature of 35°C, there is no practical risk of conveyance. This assumption can be made if the ambient temperature exceeds 35°C and the water volume is small (e.g., on the order of the 31 µl droplets we used). However for larger volumes or thermally protected microhabitat such as a hull or live well, it would be very difficult to accurately determine actual exposure temperature in a field setting. Comparisons between air temperature and relative humidity inside and outside of several different types of watercraft demonstrated variable temperatures under different exposures, and that temperatures inside watercraft cannot be reliably predicted by ambient air temperature alone (L. Corvington, CDFW, unpublished observations). At temperatures ≤25°C, any volume of water might harbor live veligers for at least one week. Therefore, under essentially any realistic thermal regime, a policy of complete draining and drying of watercraft is recommended. In comparing our data on emersed veligers with that for emersed adult quagga and zebra mussels (McMahon et al. 1993, Ricciardi et al. 1995), veligers are far less resistant to aerial exposure than adults. Thus, any evaluation standard applied to emersed adults would prevent the conveyance of emersed veligers as well.

Additional studies are required to more accurately resolve thermal immersion sensitivities between 25°C and 35°C as a function of time. A time-series study assessing survival of single veligers in droplets of water to 100% mortality (or settlement) with realistic temperature resolution would be informative. Future 'next-day' and long-term survival studies could employ thermal regimes with diel changes rather than constant temperatures, as were used in our investigations. Also, determination of emersion conveyance risk for time intervals at temperatures <30°C will require additional studies, and further development of emersion study techniques should be explored. Lastly, studies of veliger viability through successful metamorphosis and settlement under various conveyance scenarios are needed to assess fully the actual risk of mussel establishment into new water bodies.

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