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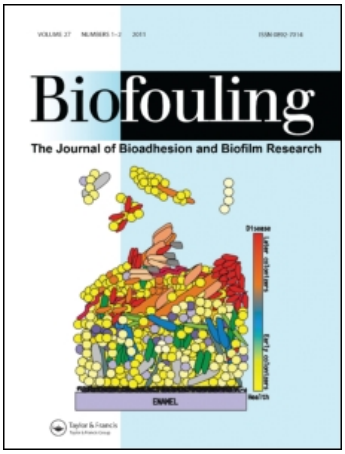

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Susceptibility of quagga mussels (*Dreissena rostriformis bugensis*) to hot-water sprays as a means of watercraft decontamination

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The recent spread of dreissenid mussels to various bodies of water in the western US has sparked interest by many state and federal agencies to develop protocols to stop further expansion. Quagga mussels *(Dreissena rostriformis bugensis)* are of particular importance as they are currently the most widespread dreissenid species in the region. This project examined the susceptibility of quagga mussels to hot-water sprays at different temperatures and durations of spray contact at Lake Mead (Nevada-Arizona, USA). Emersed adult quagga mussels were exposed to hot-water sprays at 20, 40, 50, 54, 60, 70, and 80°C for 1, 2, 5, 10, 20, 40, 80, and 160 s. Sprays at ≥60°C for 5 s were shown to be 100% lethal. Sprays of 54°C for 10 s, 50°C for 20 s, and 40°C for 40 s also resulted in 100% mortality. A spray temperature of 60°C for 5 s is recommended for mitigating fouling by quagga mussels.

Keywords: *Dreissena bugensis*; quagga mussel; aquatic invasive species; dreissenid; hot-water spray; recreational boats; thermal tolerance; watercraft decontamination; zebra mussel

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Introduction

The introduction and establishment of non-indigenous species has proved to be one of the top causes of global diversity loss and ecologic change; further, it can be financially costly (Leung et al. 2006). The costs and damages associated with the control of aquatic invasive species (AIS) in the US alone are estimated to be >$7 billion annually (Pimentel et al. 2005). Although it is possible that some of the spread of AIS could be intentional (Johnson et al. 2001; Puth and Post 2005), the spread of AIS to the inland water bodies of North America can most likely be attributed to the unintentional overland transport of trailered boats contaminated with the invasive organisms into an uninfested body of water (Bossenbroek et al. 2001; Johnson et al. 2001; Leung et al. 2006). Possible transport locations for AIS include undrained bait buckets, live wells, and bilge water which may provide favorable conditions for extended survival. Mussels may also be present on the hull or entrained on boat exteriors, ie entangled on propellers and trailers (Rothelisberger et al. 2010), or attached to other entangled organisms (Johnson et al. 2001). Exterior/hull fouling has already been established as a serious problem for marine vessels, and in regions such as Australia, North America, and Hawaii, it is estimated that between 55% and 85% of recorded marine non-indigenous species are introduced this way (Piola et al. 2009 and references therein). Although various methods have been established to reduce hull fouling (eg biocide-containing antifouling paints, biocide-free fouling-release coatings, physical removal by rotating brush systems), these methods are not 100% effective in mitigating the transfer of AIS consistently (Piola et al. 2009; Hopkins et al. 2010). For these reasons, any trailered vessel that makes contact with an AIS contaminated body of water should be treated as a possible vector for AIS. Therefore, decontamination procedures need to be established and followed to prevent the additional spread of AIS to uncontaminated inland water bodies.

Two AIS of particular importance that are being involuntarily introduced into uncontaminated inland bodies of water are the zebra mussel (*Dreissena polymorpha*) and the quagga mussel *(Dreissena rostriformis bugensis)*. The small size and resilience of both species enables them to avoid detection during boat inspection and to remain viable for several days during overland transport from a contaminated body of water (Ricciardi and Rasmussen 1998). These mussels have arguably become the most serious non-indigenous biofouling pests introduced into North

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American freshwater systems (LaBounty and Roefer 2007), and two of the world's most economically and ecologically important pests (Aldridge et al. 2006). The recent discovery and ensuing spread of these dreissenids to several previously uncontaminated inland bodies of water in the western US (Benson 2010) has caused many government agencies to initiate watercraft interception programs to prevent further infestations (Zook and Phillips 2009). There are several accepted methods of watercraft decontamination which are currently approved by the US Bureau of Reclamation (USBR). These include chemical decontamination (eg acetic acid, bleach), heat (eg live steam), hot-water/high-pressure washing, freezing, physical removal, and desiccation (USBR 2010). Of these procedures, most agencies commonly decontaminate watercraft using pressurized hot-water spray at temperatures exceeding 60°C. This temperature is based on acute (short-term) upper-thermal-limit data determined from studies of immersed mussels (Morse 2009; Zook and Phillips 2009). The first study regarding the use of hot-water spray for the treatment of emersed zebra mussels, which is closely related to the field situation where sprays are applied to watercraft, was by Morse (2009). Morse found that the survival of mussels was affected by two major factors, viz. spray water temperature and exposure duration. Water sprayed at $\geq$60°C for 10 s or 80°C at $\geq$5 s was shown to be 100% lethal to zebra mussels. This suggests that the decontamination recommendation of spray temperatures of $\geq$60°C may not result in 100% mortality if the exposure duration is $<$10 s.

As the first study to test thermal spray treatments on emersed mussels, Morse's (2009) findings are helpful in providing a solid starting point for generating and revising uniform minimum protocols and standards for watercraft decontamination programs (Zook and Phillips 2009). There are, however, several important aspects that need to be addressed regarding species-specific application. This is a key component because some inland bodies of water may be infested with only zebra mussels, only quagga mussels, or a combination of both. In the western US, quagga mussels are of particular importance, as they are currently the most widespread dreissenid species, whereas only one water body in California is infested by zebra mussels (Benson 2010). Previous studies have shown some differences between these two dreissenid species (Pathy and Mackie 1993; Ricciardi et al. 1995; Mills et al. 1996; Baldwin et al. 2002; Peyer et al. 2009), and it is important to determine if the quagga mussel is more or less susceptible than the zebra mussel to hot-water spray. Studies have shown that the upper thermal limit of the quagga mussel is lower than that of the zebra mussel (Mills et al. 1996). Zebra mussels survive indefinitely at 30°C, but quagga mussels show rapid mortality at 30°C (Spidle et al. 1995; McMahon 1996). Quagga mussels are also reported to have thinner shells (Zhulidov et al. 2006), less tightly sealing shell valves (Claxton et al. 1997), and lower byssal thread synthesis rates in higher flows (Peyer et al. 2009). Therefore, quagga mussels may be more susceptible to death by hot-water sprays at a lower temperature than zebra mussels, and the application of hot-water spray to these two dreissenid species may be different. To be effective and efficient in mitigating the transfer of invasive quagga mussels in the western US, a hot-water spray technique needs to be evaluated specifically for quagga mussels. In order to accurately predict if the quagga mussel is more susceptible to hot-water than zebra mussels owing to its physiological differences (ie thinner shell and less tightly sealing shell valve), the present study investigated the lethal effect of hot-water spray on emersed specimens of quagga mussels at water temperatures ranging from 20°C to 80°C and exposure durations of 1, 2, 5, 10, 20, 40, 80, and 160 s.

Materials and methods

Specimen collection and holding conditions

In February 2010, specimens of *D. rostriformis bugensis* were collected from a quagga mussel-colonized boat in Lake Mead, Nevada-Arizona, USA. The individuals were then divided into 60 groups with approximately 75 mussels in each group. The groups were then placed into individual identical pre-labeled mesh spat bags (3-mm mesh size, Aquatic Eco-Systems Inc., Apopka, FL), and were acclimated to the lake water in a boat slip within the Las Vegas Bay Marina (N 36°01.764, W 114°46.400) for 2 weeks prior to experimentation (temperature 11.85°C $\pm$1.61°C).

Thermal spray treatments

Subsequent to acclimation, the surviving adult mussels were randomly divided into 60 groups ($n = 50$), consisting of four control groups and eight exposure duration groups (1, 2, 5, 10, 20, 40, 80, and 160 s) for each of the seven temperatures to be tested (20, 40, 50, 54, 60, 70, and 80°C). Each group was placed into a pre-labeled 3.0 mm spat bag. During treatment, each bag was suspended over one of two identical open Polyscience Programmable heated circulator wash baths with a 28 l capacity during the thermal spray treatment (VWR International Inc.). Water obtained from Lake Mead was heated within the water baths and used for treatment. Open water baths were used for the study because treatment water applied to the suspended mussels could reenter the reservoir bath at a

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much higher temperature than if the water bath was continuously refilled with new lake water. This allowed the reservoir water to be reheated at a much faster rate, and increased the speed at which thermal spray tests could be conducted. The two water baths were set to the same target temperature, and were alternately used between the treatments of the subsamples tested at the same temperature. Both water baths were necessary because the temperature of the reservoir water rapidly decreased when the treatment spray reentered the open water bath. By alternating between the baths, one bath would be reheating while the other would be ready at the specific test temperature. The use of two baths also allowed for greater efficiency and speed at which the tests could be conducted by limiting the water temperature variation between subsamples.

Each mesh spat bag containing 50 mussels was tested at ambient air temperature (range of 5–10°C) at the Las Vegas Bay Marina. Treatment spray was applied to samples at a flow rate of 910 ml min^{-1} through a fan shaped nozzle (Rainbird 15A-C1, Rain Bird Corporation, Tucson, AZ 85706). The spray pressure was 14.07 kPa (2.04 PSI). Using the nozzle described above, the temperature of each treatment group was made constant by predetermining the distance between the spray nozzle and the contact point of the treatment water on the mussels at the necessary test temperature. This was achieved by adjusting the distance between the nozzle and the contact point using a ruler, and determined by the use of a fast-reacting remote water temperature probe (Pace Scientific Model XR440 Pocket Logger with 4 temperature probes). This procedure was necessary because the environmental field conditions (ie wind, rain, ambient air temperature) would have affected the contact water temperature if there was a set distance. The thermal spray was immediately applied to the specific treatment group based on temperature at the predetermined distance. Each subset of mussels was positioned within the spat bag to form a horizontal line not exceeding 5 cm in width to allow the hot-water spray to be equally distributed over all of the mussels. The polyethylene mesh of the spat bags allowed the water spray to pass over them without additional pooling or heat transfer beyond that which would normally occur from direct exposure to the spray (Morse 2009). Each sample of mussels was separately exposed to thermal-spray treatments at 20, 40, 50, 54, 60, 70, and 80°C and exposure durations of 1, 2, 5, 10, 20, 40, 80, and 160 s. Therefore, 56 combinations of temperatures and exposure durations were administered to the quagga mussels contained in the spat bags. Four subsamples ($n = 50$) were left untreated and remained in the slip to be used as controls.

Following treatment, the spat bag containing the treatment specimens was attached to one of seven 1 cm braided nylon lines (one for each temperature set) which spanned the boat slip and returned to the water (in the Lake Mead boat Harbor) to determine subsequent mortality. Lines were attached to a grid composed of 6.4 cm ABS pipe that was positioned on either side of the slip to allow easy access to the samples. Mussels within the bags were then suspended at a depth of approximately 2 m. Sample mortality was recorded immediately after testing and daily thereafter for 10 days. Viability was tested by inspecting post-treatment samples for specimens with widely gaping valves, which is similar to the mortality assessment test conducted by Morse (2009). Bags containing specimens were removed from the water and the mussels were examined on a plastic table. Individuals were gently prodded on their shell valves with a pair of blunt-end forceps. Specimens that failed to respond by immediate shell valve closure were then gently stimulated in the area of their inhalant and exhalent siphons using slight pressure from fingertips. Those which did not respond to this latter stimulus by immediate valve closure had their shell valves forcibly closed by pressure from the fingertips. If their valves immediately re-opened following release from the fingers, specimens were considered to be dead (Morse 2009). Dead mussels were then completely opened using pressure from fingers to ensure that they would continue to be counted as dead. At the end of the 10 day period, total mortality for each of the groups was determined and documented. The shell length of each mussel was also recorded to the nearest 0.01 mm using digital calipers by the same standard as Morse (2009), which measures the greatest distance from the anterior tip of the umbos to the posterior shell valve margins. The control group samples ($n = 50$ for each of the four groups) were continuously immersed in the lake over the 10 day period and recovery period. Their survivorship was assessed as described above.

It should be noted that only calculations for LT_{50} and LT_{99} at 1, 2, and 5 s were provided. There are two reasons for this. First, 100% mortality in the mussels may occur at even modest temperatures for long durations of exposure, and second, as a pragmatic application, exposure times > 5 s over a large area may not represent a feasible management strategy.

Statistics and data interpretation

A two-way analysis of variance (ANOVA) was used to examine if there was any significant difference in mussel size (ie shell length) among different temperatures/exposure duration treatment groups, while an independent samples *t*-test was used to determine if

there was any significant difference in shell length between mussels in the treatment group (pooled data) and the control group (Zar 1996). The significance criterion was set at $\alpha = 0.05$. A binary logistic regression model was used to estimate mortality (a binary response) as a function of exposure time and water spray temperature. Model parameters and their associated standard errors (SEs) were used to produce estimates and confidence intervals of the LT_{50} and LT_{99} values, and these estimates were further used to compare the corresponding LT_{50} and LT_{99} values for zebra mussels generated by Morse (2009). LT_{50} and LT_{99} estimates were defined as the temperatures required to induce mussel mortalities of 50% and 99%, respectively. All the statistics and model estimation were performed using SAS® (Version 9.2, SAS Institute Inc. Cary, NC).

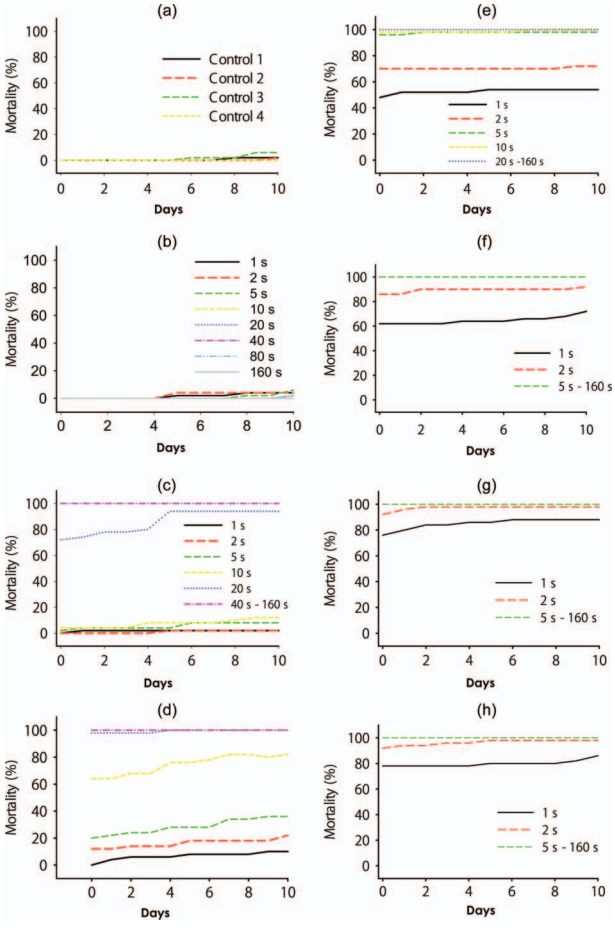

Figure 1. Mortality (%) of quagga mussels in Lake Mead after hot-water spray treatment. (a) Control (11.86°C); (b) 20°C; (c) 40°C; (d) 50°C; (e) 54°C; (f) 60°C; (g)70°C; (h) 80°C. Note that (c) and (d) share the same symbol and line styles.

Results

As expected, there was a trend indicating that higher temperatures induced greater mortality following the same exposure duration (Figure 1b–h, Table 1). Spray exposures of 1 s or 2 s did not induce 100% mortality at any of the test temperatures (Table 1). However, a 5 s spray exposure resulted in 100% mortality (≥60°C). The other temperature and time combinations that resulted in 100% mortality were 54°C for 10 s, 50°C for 20 s, and 40°C for 40 s. Estimated LT_{50} values for 1 s, 2 s, and 5 s indicate that the temperature to kill 50% of the mussels was between 47.2°C to 47.9°C (Table 2), while the estimated LT_{99} with these exposure durations varied significantly from >80°C at 1 s and 2 s to 58.8°C at 5 s (Table 2).

The continuously immersed control samples (11.86°C ± 1.60) and the samples exposed to the 20°C spray treatments exhibited high survival rates over the 10 day period. The combined four groups of controls exhibited 97% survival (ranging from 94% to 100% (Figure 1a)), and the eight 20°C spray treatment subsamples displayed a mean 98% survival rate (ranging from 94% to 100%) with no apparent correlation to duration time (Figure 1b). Survival was also high for 40°C at spray exposures of 1 s (98% survival), 2 s (98% survival), 5 s (92% survival), 10 s (88% survival), and for 50°C at 1 s (90% survival).

The average shell length for the hot-water spray treated mussels ranged from 18.65 mm to 20.00 mm (Table 3). Shell length of mussels in the 56 treatment groups (mean = 19.2 mm, range = 18.7–20.0 mm, Table 1) did not differ significantly between temperature and exposure duration combinations (Two-way ANOVA, DF = 13, F = 1.5, P = 0.1), and was comparable

Table 1. Quagga mussel mortality (%) under different treatments at day 10.

Temperature (°C)	1 s (%)	2 s (%)	5 s (%)	10 s (%)	20 s (%)	40 s (%)	80 s (%)	160 s (%)
20	4	4	6	0	0	2	2	0
40	2	2	8	12	94	**100**	**100**	**100**
50	10	22	36	82	**100**	**100**	**100**	**100**
54	54	72	98	**100**	**100**	**100**	**100**	**100**
60	72	92	**100**	**100**	**100**	**100**	**100**	**100**
70	88	98	**100**	**100**	**100**	**100**	**100**	**100**
80	86	98	**100**	**100**	**100**	**100**	**100**	**100**

Control mortality ($n = 4$) was 3%. Bold values emphasize the mortality rate of 100%.

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Table 2. Estimated LT_{50} and LT_{99} values (in bold) and their 95% confidence limit for hot-water spray treatments on quagga mussels at 1-, 2-, and 5-s application durations.

Duration (s)	LT_{50} (°C)	LT_{99} (°C)	SM_{100} (°C)[a]
1	44.1 < **47.9** < 52.5	> 80	> 80
2	44.0 < **47.8** < 52.3	> 80	> 80
5	43.5 < **47.2** < 51.7	54.1 < **58.8** < 64.4	60

[a]The SM_{100} is the temperature observed in the experiment that induced 100% mortality. $n = 350$ for each duration.

(t-test, DF = 2998, $t = -0.29$, $P = 0.77$) to the controls (mean = 19.0 mm).

Discussion

Containing existing infestations of zebra and quagga mussels within already contaminated inland bodies of water seems to be the primary technique for preventing their spread (Western Regional Panel on Aquatic Nuisance Species 2010). Methods which allow effective, eco-friendly, and economical means of control are also likely to be implemented as primary means of prevention. Using thermal water at temperatures ≥60°C to decontaminate mussel-fouled surfaces is widely disseminated as recommended boat cleaning protocol (Morse 2009). It was found that spray temperatures of ≥60°C may not be 100% effective in killing zebra mussels unless applied for > 10 s (Morse 2009). In the present study, it was found that 5 s was sufficient to induce 100% mortality in quagga mussels. For zebra mussels (Morse 2009), LT_{50} and LT_{99} at 1 s duration were both > 80°C while they were 47.9°C and > 80°C for quagga mussels in the present study (Table 2). At 5 s duration, LT_{50} and LT_{99} for zebra mussels were 54.6°C and 69.1°C while they were 47.2°C and 58.8°C for quagga mussels. Therefore, these results suggest that quagga mussels are more susceptible to hot-water sprays than zebra mussels. This is probably due to the fact that quagga mussels have thinner shells (Zhulidov et al. 2006) and less tightly sealing shell valves (Claxton et al. 1997), which may allow the heating of the soft tissues of the quagga mussel to occur more rapidly than that of the zebra mussel. Another potential reason for this difference is the impact of ambient temperature and seasonal productivity variations on the acute thermal tolerance of dreissenid mussels (Elderkin and Klerks 2005). These factors may account for Morse's (2009) longer application time at 60°C for 100% kill in zebra mussels, as dreissenid mussels tend to have higher thermal elevated acute thermal tolerance temperature if they are acclimated in warmer waters before treatment (McMahon and Ussery 1995). The specimens of zebra mussels from Hedges Lake (New York) used in Morse's study (2009) were acclimated to 20 ± 1°C water for 2 weeks already prior to experimentation. The mussels in the present study, which experienced winter water conditions (ie lower temperature) before treatment, may require higher temperature or longer application times to achieve 100% mortality if the mussels were taken from Lake Mead during summer time. When the surface water temperature ranges are higher (ranging from 25°C and 30°C) in summer time, mussels could have had elevated acute thermal tolerances (Robert McMahon, personal communication). There is also a chance that the results may have been slightly different had the two mussel species tested by the same authors under the same experimental conditions. It should be noted that in Morse's study the immediate mortality of the mussels after hot-water application was recorded, whilst in the present study it was found that some mussels did not die immediately after treatment (Figure 1). This could also be a potential reason that a longer application time was required to kill all zebra mussels at 60°C. A future study on quagga and zebra mussels acclimated to summer time temperatures is needed to determine the role of ambient water temperature on mussel mortality. There was a noticeable jump in mortality within the present experiment that occurred between the 20°C group and 40°C group (Figure 1). This was mainly due to the fact that the upper thermal tolerance temperature for dreissenid mussels is around 30°C (McMahon and Ussery 1995; Karatayev et al. 1998).

Nonetheless, the data from the present experiment are in line with the report that the upper thermal limit of the quagga mussel is lower than that of the zebra mussel (Spidle et al. 1995; McMahon 1996; Mills et al. 1996). Since effective hot-water sprays ≥60°C may be applied for a shorter period of time to ensure 100% quagga mussel mortality compared to zebra mussels, a more adaptable and efficient boat decontamination protocol which boaters may be more apt to follow could be developed. Many areas of the boat which are capable of receiving direct thermal spray (ie hull, deck) would only require hot-water application for ≥5 s at temperatures ≥60°C, instead of 10 s to kill zebra mussels at the same temperature. The shorter application time in these regions would reduce the total time of their decontamination by half. This would appeal to both recreational boaters and government agencies because less money would be spent on labor needed to

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Table 3. Average (±1 SD) shell length (mm) of quagga mussels for the hot-water spray experiment.

Temperature (°C)	1 s	2 s	5 s	10 s	20 s	40 s	80 s	160 s
20	19.64 ± 2.44	19.44 ± 2.30	19.00 ± 2.01	19.53 ± 2.24	19.38 ± 2.10	19.32 ± 2.23	19.52 ± 2.22	19.69 ± 2.26
40	19.82 ± 2.50	19.29 ± 2.18	18.94 ± 2.26	19.23 ± 2.49	19.30 ± 2.48	19.21 ± 2.17	19.41 ± 1.94	19.02 ± 1.90
50	19.71 ± 2.14	19.93 ± 2.25	19.84 ± 2.04	19.22 ± 2.03	19.14 ± 2.20	19.64 ± 2.47	20.00 ± 2.54	19.30 ± 2.00
54	18.77 ± 2.18	18.77 ± 2.31	19.27 ± 2.50	19.89 ± 1.88	19.40 ± 2.03	19.56 ± 2.55	18.90 ± 2.56	19.20 ± 2.08
60	19.21 ± 2.30	18.84 ± 2.41	19.33 ± 2.52	19.03 ± 2.03	19.01 ± 2.47	19.37 ± 2.04	19.60 ± 1.98	19.64 ± 2.28
70	19.69 ± 2.90	19.53 ± 2.52	18.57 ± 2.97	18.91 ± 2.38	19.34 ± 2.17	18.86 ± 2.36	19.33 ± 2.08	18.65 ± 2.43
80	19.71 ± 2.71	18.86 ± 2.38	18.68 ± 2.34	19.15 ± 2.45	19.24 ± 2.08	19.35 ± 2.48	19.72 ± 2.05	19.88 ± 2.56

The shell length (mm) of the four control groups are 19.50 ± 2.13, 19.08 ± 1.95, 19.19 ± 1.81, and 19.32 ± 2.13, respectively. $n = 50$ for each combination of temperature and exposure duration.

conduct the decontamination procedure and it would allow boaters to leave the freshwater region/park more quickly. The use of species-specific guidelines for boat decontamination procedures should be more useful in the western United States where water bodies are heavily infested by quagga mussels (Benson 2010). Whereas, if zebra mussels or both zebra and quagga mussels are fouling a boat, the 10 s or more at temperature ≥60°C should be implemented. Freshwater regions with active surveillance of their specific dreissenid populations will be able to employ species-specific decontamination procedures most effectively as they can determine and use the hot-water decontamination standard most applicable toward their respective AIS population.

In addition to settling on easily accessible areas of watercraft, it has been reported that dreissenid mussels tend to settle in particularly well-sheltered areas on watercraft such as motors, anchors, intakes and outlets, trim tabs, and centerboard slots (Morse 2009). These are areas where the mussels would not receive a direct thermal spray and/or may come in contact with sprayed water as runoff from other surfaces where it may have cooled to an ineffective temperature. For ease of understanding, watercraft decontamination areas can be divided into three categories: (I) areas easy to access eg the hull, (II) areas difficult to access eg gimbal areas, and (III) special areas eg ballast tanks/bladders. These three categories of areas should be treated differently to achieve 100% mussel mortality for legitimate watercraft and equipment decontamination. For category II areas, tests need to be conducted to determine how long hot-water must be applied to these locations to ensure that they reach the determined lethal temperatures. This will likely take significantly longer than the predetermined 5 s duration because heat would be lost to conduction across metal and other materials, and will probably vary depending on ambient outside air temperatures. For category III areas, the determined 100% mortality rates for temperatures ≤54°C may be used to prevent heat-associated damage from occurring to these sensitive areas. The temperature 54°C (130°F) was used because it is the highest temperature at which the system components of heat sensitive areas (ie ballast tanks and bladder) are designed to withstand (Zook and Phillips 2009). Temperatures >54°C can possibly result in permanent heat-related damage to these areas of the boat. For this reason, the temperatures of 54°C and 40°C were evaluated to determine the necessary application times needed to achieve 100% quagga mussel mortality. These areas will also require additional testing to determine the approximate amount of time they each need to reach the predetermined lower lethal temperatures. The

aforementioned times needed to achieve and sustain lethal temperatures at different locations may also vary significantly with different weather/climate conditions. Therefore, more experiments are needed to determine the durations necessary to reach the targeted lethal temperatures in temperature-sensitive areas under different environmental conditions. It is necessary to ensure that each region of the watercraft receives the required combination of high temperature and adequate exposure time to each specific area of the boat in order to obtain 100% mussel mortality. If this is not achieved, then water bodies are still susceptible to dreissenid mussel introduction by means of recreational boating.

A combination of hot-water sprays and aerial exposure/desiccation procedures may present a more efficient and effective way to ensure that 100% quagga mussel mortality is achieved. However, with varying environmental conditions depending on the season and location, developing such a time standard for aerial exposure would be difficult to enforce. Under different conditions (climate/weather), the longest time to reach lethal temperature and maintain 100% mortality with 60°C hot-water spray will be implemented as the standard protocol for boat decontamination if the western states adopt a uniform protocol (Zook and Phillips 2009).

According to the data obtained from this study, it is recommended that hot-water sprays at 60°C for a duration of 5 s can be utilized to ensure 100% quagga mussel mortality under experimental conditions. If the water temperature is lower, 100% mortality cannot be achieved; if the temperature is higher, it can be dangerous to human health (Morse 2009). In addition, 60°C/5 s is only to be used for readily accessible areas of the watercraft. For other special areas, more research is needed to determine the best conditions which result in 100% quagga mussel mortality.

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References

Aldridge DC, Elliott P, Moggridge GD. 2006. Microencapsulated BioBullets for the control of biofouling zebra mussels. Environ Sci Technol 40:975–979.

Baldwin B, Mayer MS, Dayton J, Pau N, Mendilla J, Sullivan M, Moore A, Ma A, Mills ML. 2002. Comparative growth and feeding in zebra and quagga mussels (*Dreissena polymorpha* and *Dreissena bugensis*): implications for North American lakes. Can J Fish Aquat Sci 59:680–694.

Benson AJ. 2010. Quagga mussel sightings distribution. USGS [Internet]. [cited 2010 Aug 7]. Available from: http://nas.er.usgs.gov/taxgroup/mollusks/zebramussel/quaggamusseldistribution.aspx

Bossenbroek JM, Kraft CE, Nekola JC. 2001. Prediction of long-distance dispersal using gravity models: zebra mussel invasion of inland lakes. Ecol Appl 10:1778–1788.

Claxton WT, Martel A, Dermott RM, Boulding EG. 1997. Discrimination of field-collected juveniles of two introduced dreissenids (*Dreissena polymorpha* and *Dreissena bugenisis*) using mitochondrial DNA and shell morphology. Can J Fish Aquat Sci 54:1280–1288.

Elderkin CL, Klerks PL. 2005. Variation in thermal tolerance among three Mississippi river populations of the zebra mussel, *Dreissena polymorpha*. J Shellfish Res 24:221–226.

Hopkins GA, Forrest BM, Coutts ADM. 2010. The effectiveness of rotating brush devices for management of vessel hull fouling. Biofouling 26:555–566.

Johnson LE, Ricciardi A, Carlton JT. 2001. Overland dispersal of aquatic invasive species: a risk assessment of transient recreational boating. Ecol Appl 11:1789–1799.

Karatayev AY, Burlakova LE, Padilla DK. 1998. Physical factors that limit the distribution and abundance of *Dreissena polymorpha* (Pall.). J Shellfish Res 17:1219–1235.

LaBounty JF, Roefer P. 2007. Quagga mussels invade Lake Mead. LakeLine 27:17–22.

Leung B, Bossenbroek JM, Lodge DM. 2006. Boats, pathways, and aquatic biological invasions: estimating dispersal potential with gravity models. Biol Invas 8:241–254.

McMahon RF. 1996. The physiological ecology of the zebra mussel, *Dreissena polymorpha*, in North America and Europe. Am Zool 36:339–363.

McMahon RF, Ussery TA. 1995. Thermal tolerance of zebra mussels (*Dreissena polymorpha*) relative to rate of temperature increase and acclimation temperature. Vicksburg (MS): US Army Corps of Engineers, Waterways Experiment Station. p. 1–21.

Mills EL, Rosenberg G, Spidle AP, Ludyanskiy M, Pligin Y, May B. 1996. A review of the biology and ecology of the quagga mussel (*Dreissena bugensis*), a second species of freshwater dreissenid introduced to North America. Am Zool 36:271–286.

Morse JT. 2009. Assessing the effects of application time and temperature on the efficacy of hot-water sprays to mitigate fouling *by Dreissena polymorpha* (zebra mussels Pallas). Biofouling 23:605–610.

Pathy DA, Mackie GL. 1993. Comparative shell morphology of *Dreissena polymarpha, Mytilopisi leucophaeata*, and the "quagga" mussel (Bivalvia: Dreissenidae) in North America. Can J Zool 73:1012–1023.

Peyer SM, McCarthy AJ, Lee CE. 2009. Zebra mussels anchor byssal threads faster and tighter than quagga mussels in flow. J Exp Biol 212:2027–2036.

Pimentel D, Zuniga R, Morrison D. 2005. Update on the environmental and economic costs associated with alien-invasive species in the United States. Ecol Econ 52:273–288.

Piola RF, Dafforn KA, Johnston EL. 2009. The influence of antifouling practices on marine invasions. Biofouling 25:633–644.

Puth LM, Post DM. 2005. Studying invasion: have we missed the boat? Ecol Lett 8:715–721.

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Ricciardi A, Rasmussen JB. 1998. Predicting the identity and impact of future biological invaders: a priority for aquatic resource management. Can J Fish Aquat Sci 55:1759–1765.

Ricciardi A, Serrouya R, Whoriskey FG. 1995. Aerial exposure tolerance of zebra and quagga mussels (Bivalvia, Dreissenidae) - Implications for overland dispersal. Can J Fish Aquat Sci 52:470–477.

Rothelisberger JD, Chadderton LW, McNulty J, Lodge DM. 2010. Aquatic invasive species transport via trailered boats: what is being moved, who is moving it, and what can be done. Fisheries 35:121–132.

Spidle AP, Mills EL, May B. 1995. Limits to tolerance of temperature and salinity in the quagga mussel (*Dreissena bugensis*) and the zebra mussel (*Dreissena polymorpha*). Can J Fish Aquat Sci 52:2108–2119.

United States Bureau of Reclamation (USBR). 2010. Inspection and cleaning manual for equipment and vehicles to prevent the spread of invasive species [Internet]. [cited 2010 Dec 14]. Available from: http://www.usbr.gov/mussels/prevention/docs/EquipmentInspectionandCleaning Manual2010.pdf

Western Regional Panel on Aquatic Nuisance Species. 2010. Quagga-Zebra Mussel Action Plan for Western US Waters. ANS Taskforce [Internet]. [cited 2010 Aug 7]. Available from: www.anstaskforce.gov/QZAP/QZAP_FINAL_Feb2010.pdf

Zar JH. 1996. Biostatistical analysis. 3rd ed. Upper Saddle River (NJ): Prentice Hall, Inc.

Zhulidov AV, Pavlov DF, Nalepa TF, Scherbina GH, Zhulidov DA, Gurtovaya TY. 2006. Relative distribution of *Dreissena bugenisis* and *Dreissena polymorpha* in the Lower Don River System, Russia. Int Rev Hydrobiol 89:326–333.

Zook B, Phillips S. 2009. Recommended uniform minimum protocols and standards for watercraft interception programs for dreissenid mussels in the western United States. Western Regional Panel on Aquatic Nuisance Species [Internet]. [cited 2010 Jun 9]. Available from: http://www.aquaticnuisance.org/wordpress/wp-content/uploads/2009/01/Recommended-Protocols-and-Standards-for-Water craft-Interception-Programs-for-Dreissenid-Mussels-in-the-Western-United-States-September-8.pdf

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