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Assessing the effects of application time and temperature on the efficacy of hot-water sprays to mitigate fouling by *Dreissena polymorpha* (zebra mussels Pallas)

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Dreissenid mussel (*Dreissena polymorpha, Dreissena rostriformis bugensis*) expansion into the Western US has renewed interest in hot-water spray mitigation of mussel fouling on boat hulls, trailers, and other equipment. However, the efficacy of hot-water sprays to mitigate dreissenid fouling has not been experimentally assessed. Emersed, adult *D. polymorpha* were exposed to low-pressure, hot-water sprays at 40, 50, 60, 70, and 80°C for 1, 5, or 10 s. Sprays at ≥60°C for 10 s or 80°C at ≥5 s were 100% lethal. In contrast, 1–10 s exposures did not induce 100% mortality at ≤50°C. The results indicate that mitigation of *D. polymorpha* fouling, especially in areas protected from the hydraulic impacts of high-pressure sprays requires spray temperatures of > 80°C applied for >5 s or no less than 60°C applied for >10 s. Thus, presently recommended spray temperatures of ≥60°C may not be 100% effective unless applied for >10 s.

Keywords: *Dreissena polymorpha*; zebra mussel; thermal treatment; hot-water spray; trailered boat; acute thermal tolerance

Introduction

The transport of dreissenid mussels, *Dreissena polymorpha* (Pallas 1771) and *Dreissena rostriformis bugensis* (Andrusov 1897) by trailered boats is widely accepted as an important vector for their overland dispersal into uninfested water bodies (Padilla et al. 1996; Buchan and Padilla 1999; Johnson et al. 2001; Britton and McMahon 2005). The recent discovery of dreissenids and their subsequent rapid spread in water bodies of the Western US (Table 1) has renewed interest in mitigation of mussel fouling on trailered boats and other transportable submerged equipment. Many state and federal agencies have proposed that watercraft and trailers be cleaned with a pressurized hot-water spray exceeding 60°C (140°F) to kill and remove mussels from attached surfaces (Charlebois 2001; Wisconsin Department of Natural Resources 2004; Idaho Invasive Species Council Technical Committee 2007; New Hampshire Department of Environmental Services 2007; Utah Division of Wildlife Resources 2007; Lake Champlain Sea Grant 2008; California Department of Fish and Game 2008; Kansas Department of Wildlife and Parks 2009; Protect your waters 2009). This recommendation has been based on acute (short-term) upper-thermal-limit data generated for continuously immersed mussels (Iwansky and McCauley 1993; McMahon and Ussery 1995; Spidle et al. 1995). However, there have been no published data regarding the use of hot-water spray for fouling mitigation of emersed mussels, the condition in which such sprays are applied to boat hulls and other marine equipment.

Immersion testing of acute thermal tolerance, where treatment typically consists of exposures to temperatures <45°C and for durations of minutes to hours cannot accurately predict survival of mussels exposed to hot-water sprays at durations much less than 1 min. Immersion testing of acute thermal tolerance allows internal mussel tissues to become completely equilibrated with the test temperature. In contrast, spray application durations may not be long enough to allow internal mussel tissues to be completely equilibrated to the spray temperature. In addition, on cessation of a thermal spray, mussel tissues cool rapidly on emersion in air of lower temperature and with subsequent evaporative cooling. Thus, mussel survivorship from exposure to thermal sprays is very likely to be greater than predicted by standard immersion testing of acute thermal tolerance.

The acute, upper-thermal limits of submerged specimens of *D. polymorpha* and *D. rostriformis bugensis* are dependent on acclimation temperature and rate of temperature increase (McMahon and Ussery 1995; Spidle et al. 1995). Specimens of

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Table 1. Water bodies in the Western United States invaded by dreissenid mussels.

Water body	Date of discovery
Arizona	
Lake Havasu	January 2007
Central Arizona Project Canal	August 2007
Lake Pleasant	December 2007
Imperial Dam	February 2008
Salt River	October 2008
California	
Parker Dam	January 2007
Colorado River Aqueduct	March 2007
Lake Mathews	August 2007
Lake Skinner	August 2007
Dixon Reservoir	August 2007
Lower Otay Reservoir	August 2007
San Vicente Reservoir	August 2007
Murray Reservoir	September 2007
Lake Miramar	December 2007
Sweetwater Reservoir	December 2007
San Justo Lake	January 2008
El Capitan Reservoir	January 2008
Olivenhain Reservoir	March 2008
Lake Jennings	April 2008
Irvine Lake	April 2008
Rattlesnake Reservoir	May 2008
Colorado	
Pueblo Reservoir	January 2008
Lake Granby	July 2008
Grand Lake	September 2008
Willow Creek Reservoir	September 2008
Shadow Mountain Reservoir	September 2008
Jumbo Lake	October 2008
Tarryall Reservoir	October 2008
Nevada	
Lake Mead	January 2007
Lake Mohave	January 2007
Utah	
Electric Lake	November 2008
Red Fleet Reservoir	February 2009

United States Geological Survey 2009.

D. polymorpha acclimated to 20°C and subjected to a 0.2°C min^{-1} increase in water temperature (the fastest rate studied) experienced 100% mortality when water temperatures reached 39.26°C (McMahon and Ussery 1995). As the rate of temperature increase was accelerated, thereby decreasing the exposure duration to any one temperature, the temperature required to induce 100% morality of samples of *D. polymorpha* increased exponentially (McMahon and Ussery 1995). As such, the model of McMahon and Ussery (1995) cannot accurately predict the temperature required to induce 100% mortality in dreissenids subjected to thermal spray, where mussels are instantaneously exposed to elevated temperatures for extremely short durations not tested by these authors. Iwansky and McCauley (1993) found that the survival time of *D. polymorpha* specimens instantaneously exposed to temperatures ≤ 40°C by submersion decreased exponentially with increasing treatment temperature. Submersion of tested samples and lack of testing at temperatures above 40°C by Iwansky and McCauley (1993) prevent their data from being used to estimate survivorship of aerially exposed mussels treated with a thermal spray. In order to more accurately predict the temperatures and exposure times needed to attain 100% mortality of specimens of *D. polymorpha* following exposure to a thermal spray, the present study investigated the lethal effect of hot-water spray on emersed specimens of *D. polymorpha* at water temperatures ranging from 40 to 80°C and exposure durations of 1, 5 and 10 s.

Materials and methods

Specimen collection and holding conditions

Specimens of *D. polymorpha* were collected from Hedges Lake, New York in early summer 2007 and shipped to The University of Texas at Arlington. Individuals were acclimated to 20 $\pm$ 1°C in 34 l food-grade plastic containers for 2 weeks prior to experimentation. All holdings and treatments utilized dechlorinated, City-of-Arlington tap water (DCATW), which has been shown to support mussels for extended periods (Chase and McMahon 1995).

Thermal spray treatments

After acclimation, adult mussels (shell length >5.0 mm) were randomly divided into 57 subsamples ($n = 20$). Samples were aerially exposed at room temperature (20 $\pm$ 1°C) for 10 min prior to hot-water treatment on a plastic 3 mm mesh suspended over a 34 l tank similar to that used for prior mussel acclimation. The 10 min aerial exposure prior to spray treatment was intended to mimic the conditions that mussels fouling a boat hull might experience during its hauling from a mussel-infested water body for onsite mussel removal with a thermal spray. Treatment spray was applied to samples from a distance of 15 cm above them at a flow rate of 900 ml min^{-1} (0.23 gpm) through a fan-shaped nozzle producing a pressure of 103.4 kPa (15 psi). Maintenance of mussels on the plastic mesh 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. Three samples of mussels were separately exposed to thermal-spray treatments at 20 (control), 40, 50, 60, 70, and 80°C and exposure durations of 1, 5, and 10 s (ie 18 temperature by exposure duration combinations). The water temperature, on contact with test samples, was experimentally assessed with a fast-reacting thermocouple probe. The nozzle-to-sample

temperature declined by no more than 1.2% of nozzle temperature. The 20°C test temperature was utilized as a control as it was well below the 30°C incipient upper-thermal limit for *D. polymorpha* (McMahon et al. 1995).

Following treatment, specimens were placed in 300 ml glass dishes, covered with 3.5 mm nylon mesh to prevent escape, and randomly submerged in 34 l holding tanks filled with continuously aerated and filtered DCATW maintained at a constant temperature of 20°C. Sample mortality was recorded immediately after testing and daily thereafter for 10 days. Viability testing involved inspecting post-treatment samples for specimens with widely gaping valves. Containers with gaping individuals were removed from the holding tank while still retaining water. While still immersed in the container, gaping mussels were gently prodded on their shell valves with a pair of blunt-end forceps. Specimens not responding by immediate shell valve closure were then gently stimulated in the area of their inhalant and exhalent siphons with the tips of the forceps. Individuals not responding to this latter stimulus by immediate valve closure had their shell valves forcibly closed with the forceps. If their valves immediately re-opened after release from the forceps, specimens were considered to be dead. Previous testing by McMahon and Ussery (1995) indicated that non-responsive, widely gaping mussels exposed to thermal stress were dead and did not recover on return to 20°C. Dead individuals were removed from the container and their shell lengths (ie the greatest distance from the anterior tip of the umbos to the posterior shell valve margins measured to the nearest 0.1 mm with dial calipers) recorded. If an individual's shell valves remained adducted or did not widely gape after being forcibly closed, the specimen was considered to be viable and was re-immersed into the holding tank with the remaining sample. An additional control group involved three samples ($n = 20$ each) of mussels continuously immersed at 20°C over the 10 day testing and recovery period. Their survivorship was assessed as described above.

Statistical analysis of the data

The experimental outcome (ie mortality) from the three samples for each temperature × duration combination (eg 40°C for 1 s, three samples of $n = 20$ each) were very similar and, hence, were combined into a single data set. The three replicates were primarily used to separate mussels into three groups of 20 (rather than 1 group of 60) for holding purposes, to ensure that treated mussels were not cramped during post-treatment holding. Thus, the dataset contained 60 individuals treated at every temperature × duration exposure combination, which were all modeled and analyzed together to increase statistical power. Logistic regression using SAS® Proc LOGISTIC was used to analyze the data. A saturated linear model for the binary response variable (mortality) was fitted with two continuous predictors, treatment temperature and shell length, and a categorical predictor, treatment duration. The effects of duration, temperature and shell length were determined using SAS® TEST statements to simultaneously test the general linear hypotheses for all model terms containing the desired effect. Model-estimated survival functions were used to produce estimates of the LT_{50} and LT_{99} values for each treatment duration standardized to a median shell length of 15 mm, which was also the mean shell length of the tested specimens (15.0 ± 4.1 mm; range: 5.1–27.4 mm). LT_{50} and LT_{99} estimates were defined as the temperatures required to induce sample mortalities of 50% and 99%, respectively. Model fit was assessed by a Hosmer and Lemeshow Chi-squared goodness-of-fit test (Hosmer et al. 1997; Hosmer and Lameshow 2000) and a maximum rescaled R^2 approximation (Nagelkerke 1991) using the SAS® LACKFIT and RSQUARE modeling options, respectively.

Results

The saturated linear model ($n = 1075$) adequately fit the data ($\chi^2 = 8.6781$, df = 8, $p = 0.3702$) and sufficiently explained a majority of data variance ($R^2 = 0.9293$). Sample survivorship decreased with both increased exposure duration and test temperature (Wald $\chi^2 = 98.5$, df = 8, $p < 0.0001$; Wald $\chi^2 = 84.3$, df = 6, $p < 0.0001$, respectively). However, individual shell length did not affect survivorship (Wald $\chi^2 = 10.0$, df = 6, $p = 0.13$).

The 20°C continuously immersed control samples and samples exposed to the 20°C spray treatments exhibited 100% survival. Survival was also high in samples exposed to 1-s spray durations with 97% of mussels surviving 80°C, 98% at 70°C and 100% at temperatures ≤60°C, making calculation of LT_{50} and LT_{99} values impossible. Spray exposures of 5 s or 10 s did not induce 100% mortality at ≤50°C. In contrast, 5 s and 10 s spray exposure induced 100% sample mortality at 80°C and 60°C, respectively (Table 2, Figure 1). Estimated LT_{99} values suggested that 99% mortality could be achieved with a 5-s spray at 69.1°C (95% confidence limit (CL) = 65.5–75.5°C) and 10-s spray at 53.9°C (95% CL = 52.0–60.2°C) (Table 2, Figure 1). In contrast, estimated LT_{50} values for 5-s and 10-s spray durations were considerably lower at 54.6°C (95% CL = 52.8–56.5°C) and 46.9°C (95% CL = 43.1–58.3°C), respectively (Table 2, Figure 1).

Table 2. Estimated LT_{50} and LT_{99} values (in bold) and their 95% CLs for hot-water spray treatments of *D. polymorpha* at 1-, 5-, and 10-s application durations ($n = 1075$).

Duration (s)	LT_{50} (°C)	LT_{99} (°C)	SM_{100} (°C)
1	≫80	≫80	≫80
5	52.8 < **54.6** < 56.5	65.5 < **69.1** < 75.5	80
10	43.1 < **46.9** < 48.3	52.0 < **53.9** < 60.2	60

The SM_{100} is the temperature (from raw data) that was required to induce 100% mortality.

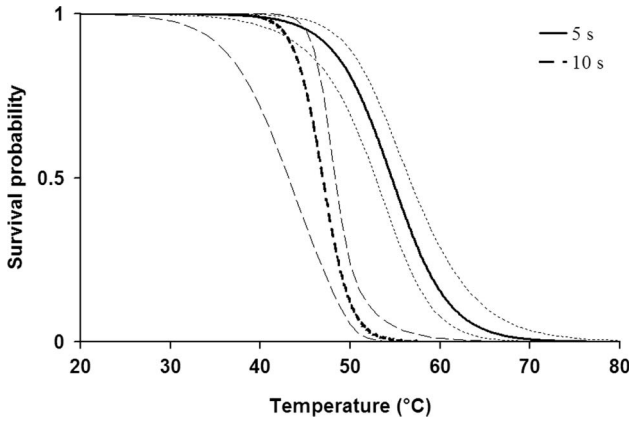

Figure 1. Survival probability curves for specimens of *D. polymorpha* exposed to hot-water sprays for 5 s (solid thickened black line) and 10 s (dashed thickened line) as determined from logistic regression analysis. The finely dashed lines represent the 95% CL for the 5 s treatment while the more coarsely dashed lines represent the 95% CL for the 10 s treatment.

Discussion

The data presented suggest that hot-water sprays can be utilized to mitigate adult zebra mussel fouling on boat hulls and trailers under specific application conditions. While widely disseminated boat washing instructions typically recommend spraying mussel-fouled surfaces with water ≥60°C (140°F) (Charlebois 2001; Wisconsin Department of Natural Resources 2004; Idaho Invasive Species Council Technical Committee 2007; New Hampshire Department of Environmental Services 2007; Utah Division of Wildlife Resources 2007; California Department of Fish and Game 2008; Lake Champlain Sea Grant 2008; Kansas Department of Wildlife and Parks 2009; Protect your waters 2009), none indicate the duration of application required to ensure 100% mussel mortality.

In this study, treatment at a spray temperature of 60°C for 10 s resulted in 100% sample mortality. Reducing the 60°C treatment time to 5 s resulted in 87% mortality while a 1-s spray resulted in 100% survivorship. To be effective at preventing introduction of dreissenid mussels to a water body, mussel-fouling mitigation techniques for boats and other equipment being moved between infested and uninfested water bodies must eliminate 100% of mussel fouling. Thus, it is imperative that heated-pressure washing be conducted in a manner that will mitigate 100% of dreissenid-mussel fouling. Based on these results, it is likely that hot-water sprays, as presently utilized, will not guarantee successful eradication of all encrusted mussels. Specifically, hot-water sprays are probably not applied at high enough temperatures (>60°C) for long enough (>10 s) to all infested surfaces to ensure 100% mussel mortality before launching. In addition, mussels tend to settle in particularly well-sheltered areas of boats and trailers such as anchors, motors, intakes and outlets, trim tabs, and centerboard slots where they will not receive a direct thermal spray or may come in contact with sprayed water only after it has run off other surfaces and cooled below effective temperatures.

Mussel fouling of accessible surfaces is more likely to be mitigated as a result of removal from a surface by direct contact with a high-pressure spray than by exposure to a lethal spray temperature; note the inability of a 1-s spray exposure to induce significant mussel mortality at a spray temperature of 80°C in this study. When mechanically disturbed, as occurs with exposure to a high-pressure spray, dreissenid mussels, like all bivalves, close the shell valves tightly. This offers protection for their soft tissues with a possible exception of the ventral byssal groove that is sheltered by the attachment surface. Valve closure during a thermal wash prevents direct penetration of the sprayed water to the soft tissues, slowing the rate at which those tissues reach a lethal temperature. Specimens of *D. polymorpha* were observed gaping while emersed prior to thermal-spray treatment, but closed their valves immediately on contact with the water spray. Thus, mussel mortality was assumed to result from heating of soft tissues to a lethal temperature by heat conduction across the shell valves. The relatively few mussels that were observed to continue gaping when contacted with thermal sprays of 70°C and 80°C were killed almost immediately as the heated water came into contact with their soft tissues. This result suggests that inducing mussels to gape prior to

application and/or delaying the valve closure response by exposing mussels to air for several days could greatly increase the efficacy of thermal-spray mitigation of mussel fouling. It is of interest that quagga mussels (*D. rostriformis bugensis*) are reported to have thinner shells (Zhulidov et al. 2006) and less tightly sealing shell valves than zebra mussels (Claxton et al. 1997) which may make them more susceptible to hot-water sprays. However, this supposition requires experimental confirmation.

High-pressure washers are commonly used to remove dreissenid mussels from boats and trailers (California Fish and Game 2008) and have been used by power industry to mitigate mussel macrofouling of intake embayments and large-diameter water conduits (Claudi and Mackie 1994). As described above, pressurized sprays are effective in mechanically removing adult dreissenid mussels from exposed surfaces but do not remove them from refugia inaccessible to direct spray contact. For mussels in such refugia, the temperature and duration of the thermal spray treatment is far more critically important for successful mitigation than is water pressure. As shown in this study, high-pressure thermal spray mitigation of mussel fouling at insufficient temperatures and/or durations to kill mussels is likely to leave live mussels in protected areas not directly exposed to the hydraulic pressure of the spray. In addition, utilization of spray-water temperatures high enough for near instantaneous kills of mussels ($>80^{\circ}$C) will also induce mortality in mechanically spray-dislodged live mussels released into the containment systems of the launch site wash station, thus preventing their release into an uninfested water body with the wash station effluent. Otherwise, wash station effluent should be subjected to further treatment to ensure that it contains no live mussels. Nevertheless and regardless of effluent containment, if high-pressure boat cleaning stations do not do not utilize sufficiently high water temperatures and exposure durations to ensure 100% mortality, water bodies will still be susceptible to dreissenid mussel introduction via recreational boating.

The water temperatures and exposure times elucidated by this study for 100% mitigation of dreissenid mussel fouling on recreational boats and trailers may be open to a number of concerns regarding their application. Exposure to the minimum spray temperature of 60°C for zebra mussel eradication could cause a second degree burn to children after 0.7 s, and for adults, after 2.8 s (Bynum et al. 1998, personal communication). A third degree burn could be caused by 60°C exposures of 1.5 s and 5.4 s in children and adults, respectively (Bynum et al. 1998, personal communication). Thus, the critical threshold for water spray mitigation of dreissenid macrofouling at 60°C for 10 s presents a significant burn risk to individuals near to, or operating, a high-pressure washer, especially if not wearing protective clothing. Operation risks are further increased by the requirement of initial water temperature to be maintained well above 60°C to overcome the rapid cooling of thermal spray after leaving the nozzle. Clearly, use of higher water temperatures (ie 80°C) to reduce the time for mitigation of mussel fouling and water usage will present an even greater threat to wash station operators, boat owners and bystanders.

The combination of high temperatures and long exposure durations needed to ensure mussel mitigation by high-pressure water sprays, the probability that 100% mitigation of mussel fouling will not be achieved, and the potential for personal injury in spray-wash operations at effective temperatures warrants examination of safer and more effective mitigation alternatives. To date, the safest and most predictably effective method for complete eradication of dreissenid mussel fouling on boats and trailers remains aerial exposure and desiccation, although under certain weather conditions the time required could be considerable (McMahon et al. 1993). Under hot, dry conditions (30°C and 0% relative humidity) specimens of *D. polymorpha* suffer 100% mortality within 2 days; while under cooler, humid conditions (10°C and 80% relative humidity) 100% mortality may require 17 days (McMahon et al. 1993). Zebra mussels can survive more than 1 month when emersed in cold, humid climates (5°C and 100% relative humidity) (McMahon et al. 1993). Nevertheless, mitigation of mussel fouling by emersion requires little effort by boat operators and is highly effective. The primary drawback of boat drying is that launch operators cannot readily ascertain if a boat has been sufficiently dried before launching in an uninfested water body. Another alternative would be enclosed areas on the shore or in the vicinity of the launch site containing water heated to $\geq 40^{\circ}$C into which trailers with attached boats could be immersed for several minutes after being spray washed. Immersion at $\geq 40^{\circ}$C would induce 100% mortality among mussels not removed by spray washing (Iwansky and McCauley 1993; McMahon et al. 1995).

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