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Boat Decontamination with Hot Water Spray: Field Validation

Sean Comeau, Richard S. Ianniello, Wai Hing Wong, and Shawn L. Gerstenberger

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ABSTRACT The efficiency of hot water spray to decontaminate boats exposed to quagga mussels was evaluated in the field. The water temperature and exposure time needed to result in complete mortality were determined. The time needed for three types of boat areas (easy access, hard access, and specialized areas [i.e., bait and live wells]) to reach the lethal temperature for the required exposure time was determined. Field validation tests were conducted in the summer and winter for the easy access and hard access areas. Summer tests of both areas resulted in 100% mortality, while seven out of eight winter tests resulted in 100% mortality. For bait and live wells, the time needed to reach lethal temperature in winter was much longer than in summer time.

Introduction

The establishment and subsequent invasion of certain nonindigenous species has proven to have profound negative economic, environmental, and even human health impacts (Keller et al. 2007). In the United States alone, the cost and damages associated with aquatic invasive species (AIS) are estimated to be over $7 billion annually (Pimentel et al. 2005). Two AIS of particular importance are the zebra mussel (*Dreissena polymorpha*) and the quagga mussel (*Dreissena rostriformis bugensis*). They are commonly referred to as dreissenid mussels and are two of the most devastating AIS to invade North American freshwater systems (Western Regional Panel on Aquatic Nuisance Species 2010). Many authorities believe that the ongoing spread of these mussels to uncontaminated bodies of freshwater in North America can be ascribed to involuntary transportation of AIS from a contaminated body of water by watercraft (Bossenbroek et al. 2001; Johnson et al. 2001; Leung et al. 2006). While it is possible that some of the spread of the dreissenid mussels could be intentional, most cases of AIS translocation are most likely unintentional with the invasive organisms unknowingly present somewhere in or on the trailered vessel during overland transport (Johnson et al. 2001; Puth and Post 2005). There are many possible transport locations for AIS present on watercraft. These include undrained bait buckets, live wells, and bilge water, all of which provide favorable conditions for possible extended viability. They may also be present to some extent on the hull or entrained on boat exteriors, such as entangled on propellers and trailers (Rothelisberger et al. 2010) or attached to other entangled organisms (Johnson et al. 2001). Any watercraft, such as trailered vessel, kayaks, and canoes, that makes contact with an AIS-contaminated body of water, should be treated as a potential vector for AIS.

In order to prevent further infestations, new research and innovative approaches must be used to generate and revise uniform minimum protocols and standards for watercraft decontamination programs. Protocols regarding safe and inexpensive procedures, such as hot water sprays, which result in the 100% mortality of these dreissenid mussels, need to be established. This would increase the receptivity of the boating public to the established protocols and more likely to follow them.

Current Boat Contamination Protocols

The spread of dreissenid mussels to several previously uncontaminated inland bodies of water in the western United States (Benson 2011) has caused many federal, state, regional, and local agencies to initiate watercraft interception programs to prevent further infestations from occurring by implementing watercraft decontamination protocols (Zook and Phillips 2009). The objective of decontamination is to kill and remove all mussels (any stage of development) present on or in the watercraft. Many of these agencies have protocols that commonly decontaminate watercraft with a pressurized hot water spray exceeding 140°F/60°C (Zook and Phillips 2009; 2014 Chapter 14 in this book). This temperature is based on acute (short-term) upper thermal limit data generated for continuously immersed mussels (Morse 2009). The first data set on the use of hot water spray for mitigation of emersed zebra mussel fouling, which is closer to the field situation where sprays are applied to watercraft, was generated by Morse (2009). Morse found that the survivorship of mussels was affected by two major factors: spray water temperature and exposure duration. Water sprayed at greater than or equal to 140°F/60°C for 10 s or 176°F/80°C at greater than or equal to 5 s was 100% lethal to zebra mussels, which indicates that current decontamination recommendation of spray temperature of greater than or equal to 140°F/60°C may not result in 100% mortality of the mussels if the exposure duration is less than 10 s (Morse 2009).

The data from Morse (2009) can be potentially applied to watercraft areas where the spray directly contacts the fouled areas (Category I areas in Table 13.1). Concurrently, there are also areas on watercraft that hot water sprays cannot directly reach. These decontamination areas can be divided into three categories: (1) areas easy to access, (2) areas difficult to access, and (3) special areas (Table 13.1). These three categories of areas should each be treated differently in order to achieve 100% dreissenid mussel mortality for complete watercraft and equipment decontamination. It is important to evaluate the susceptibility of quagga mussels to hot water sprays to determine if they are more or less susceptible than zebra mussels. This information will be helpful in establishing standards for watercraft interception programs in areas susceptible to quagga mussel colonization (Zook and Phillips 2009).

Protocols for boat decontamination using hot water spray are established mainly for dreissenid mussels in easily accessible areas of watercraft (i.e., surface areas) (Morse 2009; Comeau et al. 2011). At the same time, standards for inaccessible areas and areas with special temperature requirements of watercraft can also be created with proper research and evaluation. Watercraft decontamination areas can be divided into three categories: (I) areas easy to access (e.g., the hull), (II) areas difficult to access (e.g., gimbal areas), and (III) special heat-sensitive areas (e.g., ballast tanks/bladders) (Comeau et al. 2011). 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

TABLE 13.1

Accessibility Categories for Various Decontamination Areas

Category	Characteristics	Areas
I	Easy access surface areas	Hull, transducer, through hull fittings, trim tabs, zincs, centerboard box and keel (sailboats), foot-wells, lower unit, cavitation plate, cooling system intakes (external), prop, prop shaft, bolt heads, engine housing, jet intake, paddles and oars, storage areas, splash wells under floorboards, bilge areas, drain plug, anchor, anchor and mooring lines, PFDs, swim platform, inflatables, downriggers and planing boards, ice chests, fishing gear, bait buckets, stringers, trailer rollers and bunks, light brackets, cross-members, license plate bracket, fenders, spring hangers
II	Hard access areas	Gimbal areas, engine, generator, and AC cooling systems (internal)
III	Special areas that require water temperature ≤ 130°F for decontamination	Ballast tanks/bladders, wash-down systems, bait and live wells, internal water systems

to be conducted to determine how long hot water must be applied to these locations to ensure that they reach the determined lethal temperatures. It will likely take longer time 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 may be used to prevent heat-associated damage from occurring to these sensitive areas. These field data will also assist policy makers in developing minimal thresholds for associated decontamination and inspection parameters.

Hot Water Spray Decontamination of Quagga Mussels

The use of hot water spray as a method of watercraft decontamination for dreissenid mussels is widely accepted by many federal agencies. These agencies most commonly decontaminate watercraft with pressurized hot water spray temperatures exceeding 60°C. This temperature is based on acute (short-term) upper thermal limit data generated for spray for mitigation of immersed mussels. The first study regarding the use of hot water spray for mitigation of emersed zebra mussel fouling, which is closely related to a field situation where sprays are applied to watercraft, was by Morse (2009), whose results were previously highlighted. Morse's (2009) findings are particularly useful because it was the first study to test thermal spray treatments on emersed mussels and, as such, provides a solid starting point for determining effective field application for watercraft decontamination. There are, however, several important aspects that needed to be addressed regarding species-specific application. This is essential because some inland bodies of water may be infested with only zebra mussels, quagga mussels, or both. In the western United States, 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 2011). Previous studies have shown that there are differences between these two dreissenid species (Pathy and Makie 1993; Mills et al. 1996; Ricciardiand Rasmussen 1998; Peyer et al. 2009). It was important to determine if the quagga mussel is more or less susceptible than the zebra mussel to hot water spray. Studies have also 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 (Zhulidov et al. 2006), 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 biofouling by invasive quagga mussels in the western United States, hot water spray thresholds are needed to be evaluated specifically for quagga mussels. In order to accurately determine the temperatures and exposure times necessary to attain 100% mortality of specimens of quagga mussels following exposure to a hot water spray, the present study investigated the lethal effect of hot water sprays 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. The field data were then compared to existing data regarding zebra mussels to determine if there was any difference in susceptibility regarding the two dreissenid species. The data were also used in an evaluation of the necessary time needed to reach and sustain the lethal temperatures in inaccessible areas (Category II) and heat-sensitive areas (Category III), respectively (Comeau et al. 2011).

Field Tests on Emersed Quagga Mussels

Specimens of adult *D. rostriformis bugensis* (≥12 mm in length) were collected from the hull of an encrusted National Park Service boat that was stationed in Lake Mead, Nevada–Arizona, USA, in 2009. The individuals were then divided among 60 mesh spat bags (~75 in each) and 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.

After acclimation, the adult mussels were randomly divided into 60 subsamples (n = 50, Table 13.2) and placed into 60 identical pre-labeled 3.0 mm spat bags (Aquatic Eco-Systems Inc., Apopka, FL). Each bag was then suspended over one of two identical open Polyscience Programmable heated circulator wash baths with a 28 liter capacity during the thermal spray treatment (VWR International Inc.). The purpose of using two water baths was to increase the efficiency and speed at which the tests could be conducted by allowing limited water temperature variation. Each mesh spat bag containing a test subsample was held horizontally 20 cm above the heated water bath to prevent any difference in ambient air temperature, which may have resulted from the heated water in the open water baths. Treatment spray was then applied to the samples at a flow rate of approximately 900 mL/min through a fan-shaped nozzle. The distance above each sample at which the spray was applied was modified each time in order to maintain the constant test temperature used for each specific subset. This was done because the environmental field conditions, that is, wind, rain, and ambient air temperature, would affect the contact water temperature if there was a set distance. The specific distances prior to each spray were determined using a ruler and a fast-reacting remote water temperature probe (Pace Scientific Model XR440 Pocket Logger with four temperature probes). The distance between the spray nozzle and the contact point of the water at the necessary test temperature was then calculated. The Pace Scientific Model XR440 Pocket Logger was calibrated prior to use, and an NIST traceable certificate of validation was included from the manufacturer. Temperature readings obtained from the temperature probes were also verified by the use of a

TABLE 13.2

Amount of Adult Quagga Mussels Tested per Treatment Group (n = 50 per Group)

Temperature	1 s	2 s	5 s	10 s	20 s	40 s	80 s	160 s	
°F	°C								
68	20	50	50	50	50	50	50	50	50
104	40	50	50	50	50	50	50	50	50
122	50	50	50	50	50	50	50	50	50
130	54	50	50	50	50	50	50	50	50
140	60	50	50	50	50	50	50	50	50
158	70	50	50	50	50	50	50	50	50
176	80	50	50	50	50	50	50	50	50

Raytek MT4 noncontact mini infrared thermometer. The thermal spray was immediately applied to the specific subset at the specifically calculated distance. Each subset of mussels was positioned within the spat bag to form a horizontal line not exceeding 5 cm in width in order 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 would normally occur from direct exposure to the spray (Morse 2009). Each sample of mussels was separately exposed to thermal spray treatments at 20°C, 40°C, 50°C, 54°C, 60°C, 70°C, and 80°C and exposure durations of 1, 2, 5, 10, 20, 40, 80, and 160 s. Therefore, 56 combinations on temperature by exposure duration were treated (Table 13.2). Four bags that were not treated with hot water spray were used as controls.

Following treatment, each spat bag containing the treatment specimens was then attached to one of the seven 1 cm braided nylon lines (one for each temperature set) spanning the boat slip for over 10 days. These lines were attached to a grid composed of ABS pipe, which was positioned on either side of the slip to allow easy access to the samples. Each line holding the spat bags was approximately 1.5 m out of the water, and the mussels within the bags were kept at a depth of approximately 2 m.

Results from Field Tests on Emersed Mussels

After analysis of the data, it was found that there was a trend that indicated that the higher temperatures induced greater mortality following the same exposure duration (Figure 13.1, Table 13.3). Spray exposures of 1 or 2 s were not found to induce 100% mortality at any of the test temperatures (Table 13.3). However, a 5 s spray exposure did result 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, 2, and 5 s indicate that the temperature to kill 50% of the mussels was between 47.2°C and 47.9°C (Table 13.4), 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 13.4).

The continuously immersed control samples (11.86°C ± 1.60) and the samples exposed to the 20°C spray treatments exhibited high survival rates. The combined four groups of controls exhibited 97% survival (ranging from 94% to 100% (Figure 13.1a)), 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 13.1b). 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).

Evaluation of Category II Watercraft Areas

For areas of the watercraft that are not directly exposed to hot water sprays, the time necessary to preheat these locations to the lethal thermal temperature was evaluated because heat loss can occur during the water flow to these areas. The gimbal unit was tested on an uncontaminated boat, and the contact temperature (internal temperature) was monitored until it reached the lethal temperature. The temperature of the water exiting the gimbal unit was monitored by the use of a fast-reacting remote water temperature probe (Pace Scientific Model XR440 Pocket Logger with four temperature probes). Temperature readings from the contact water at the temperature probes were verified by the use of a Raytek MT4 noncontact mini infrared thermometer. The data from this test can be applied to Category II decontamination areas (Table 13.1). Since weather conditions, especially ambient temperature, could be a confounding factor affecting the surface temperature of these areas, the experiment was conducted twice, once in winter and again in the summer. The summer and winter evaluation experiments were conducted on September 1, 2010, and January 21, 2011, respectively. The longest durations determined from the evaluation of the Category II areas depending on the season were as follows (minimum of three replications): 43 s for summer and 2 min and 7 s for winter. There was a separate validation for both categories for summer and winter.

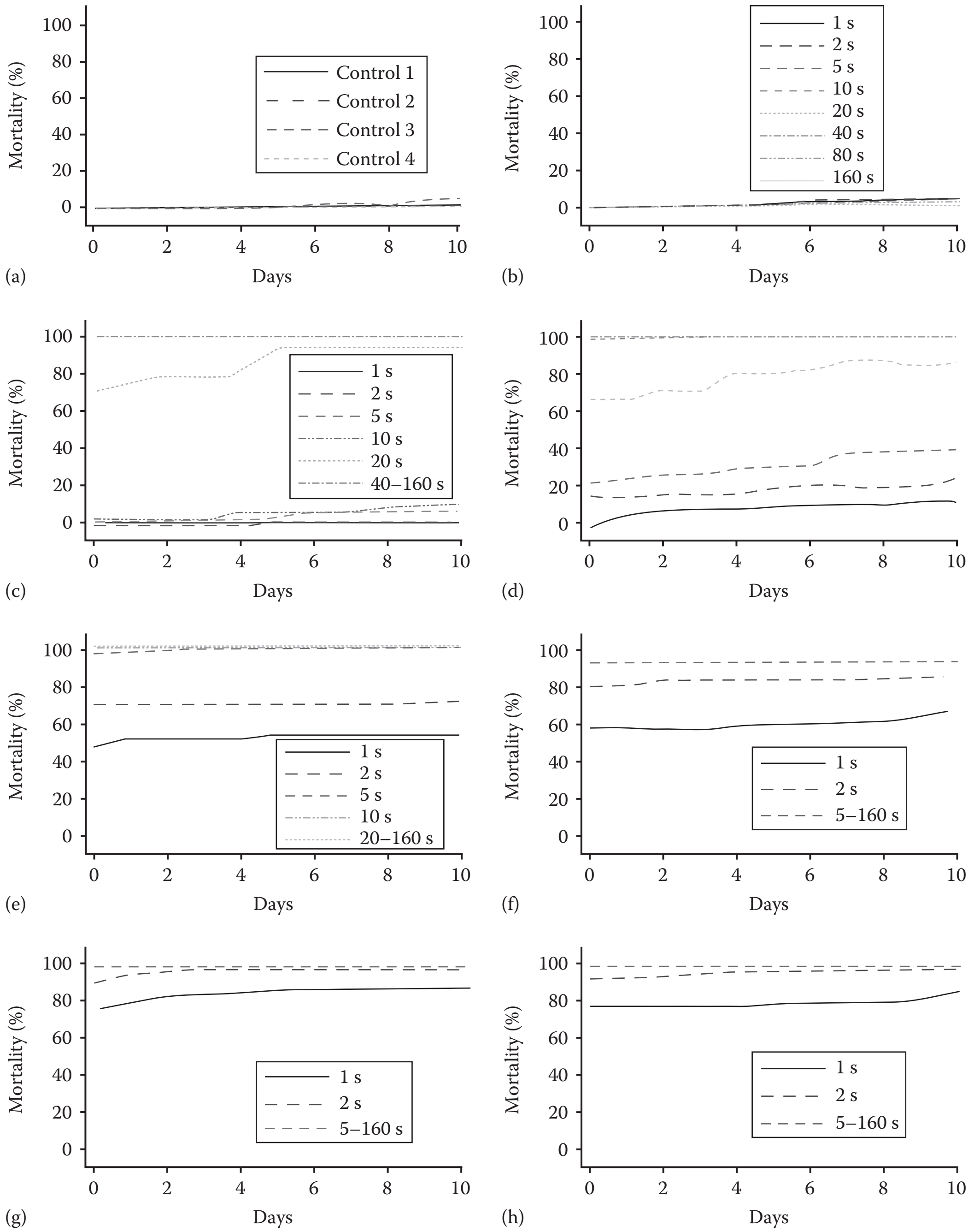

FIGURE 13.1 Mortality (%) of quagga mussels in Lake Mead after hot water spray treatment. (a) Control (11.86°C), (b) 20°C, (c) 40°C, (d) 50°C, (e) 54°C, (f) 60°C, (g) 70°C, (h) 80°C. Note that (c) and (d) share the same symbol and line styles. (Modified from Comeau, S. et al., *Biofouling*, 27(3), 267, 2011.)

TABLE 13.3

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

Note: The mortality of control (n = 4) was 3%.

TABLE 13.4

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 (n = 350 for Each Duration)

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.

Evaluation of Category III Watercraft Areas

In some areas of watercrafts, the temperature cannot exceed 130°F, such as ballast tanks and bladders (Zook and Phillips 2009; Comeau et al. 2011). Therefore, the necessary time it takes to reach and maintain a lethal water temperature needed to be evaluated for these areas. The contact temperature (internal temperature) was monitored on the live wells and bait wells of an uncontaminated boat until it reached the lethal temperature. The temperature of the water exiting these areas was monitored by the use of a fast-reacting remote water temperature probe (Pace Scientific Model XR440 Pocket Logger with four temperature probes). Temperature readings from the contact water at the temperature probes were verified by the use of a Raytek MT4 noncontact mini infrared thermometer. Since weather conditions, especially ambient temperature, could be a confounding factor, this experiment was conducted twice: once in winter and again in the summer. The time data from this test would be applied to Category III (Table 13.1) decontamination areas.

For areas on watercrafts that cannot withstand temperatures above 130°F/54°C, flush tests with 54°C water were ran on the live and bait wells of a Cobia 296 boat. Just as in the evaluation of Category II areas, there were tests conducted during the winter and summer, which showed a difference between the necessary application times in the differing ambient conditions (Tables 13.5 through 13.8).

TABLE 13.5

Evaluation of Lethal Temperature Recommendations on the Bait Wells during Summer Weather Conditions

Hot Water Temperature (°F)	Hot Water Temperature (°C)	Air Temperature (°F)	Air Temperature (°C)	Target Temperature (°F)	Target Temperature (°C)	Attempt	Time to Reach Target Temperature (min)
130	54	98	36.7	130	54	1	00:33.1
130	54	98	36.7	130	54	2	00:34.4
130	54	98	36.7	130	54	3	00:33.9

TABLE 13.6

Evaluation of Lethal Temperature Recommendations on the Bait Wells during Winter Weather Conditions

Hot Water Temperature (°F)	Hot Water Temperature (°C)	Air Temperature (°F)	Air Temperature (°C)	Target Temperature (°F)	Target Temperature (°C)	Attempt	Time to Reach Target Temperature (min)
130	54	38	3.3	130	54	1	01:06.9
130	54	38	3.3	130	54	2	01:05.4
130	54	39	3.9	130	54	3	01:03.1

TABLE 13.7

Evaluation of Lethal Temperature Recommendations on the Live Wells during Summer Weather Conditions

Hot Water Temperature (°F)	Hot Water Temperature (°C)	Air Temperature (°F)	Air Temperature (°C)	Target Temperature (°F)	Target Temperature (°C)	Attempt	Time to Reach Target Temperature (min)
130	54	98	36.7	130	54	1	01:10.1
130	54	98	36.7	130	54	2	00:58.2
130	54	98	36.7	130	54	3	01:06.9

TABLE 13.8

Evaluation of Lethal Temperature Recommendations on the Live Wells during Winter Weather Conditions

Hot Water Temperature (°F)	Hot Water Temperature (°C)	Air Temperature (°F)	Air Temperature (°C)	Target Temperature (°F)	Target Temperature (°C)	Attempt	Time to Reach Target Temperature (min)
130	54	38	3.3	130	54	1	01:51.4
130	54	38	3.3	130	54	2	01:41.9
130	54	38	3.3	130	54	3	01:20.5

Boat Decontamination Validation Results

In order to accurately determine if the results regarding the susceptibility of quagga mussels to hot water spray from the previous experiments was applicable as a means of watercraft decontamination, it was necessary to conduct actual field experiments on watercrafts that were encrusted with quagga mussels. Category I areas were sprayed with hot water at a temperature of 60°C for a duration of 5 s because it was the lowest temperature capable of 100% mortality at 5 s, and higher temperatures could be seen a threat to human health (Morse 2009). Category II areas were also sprayed with hot water at a temperature of 60°C, but used the longest duration determined from the evaluation of the areas depending on the season: 43 s for summer and 2 min and 7 s for winter. There was a separate validation for both categories for summer and winter.

Summer Validation

The summer validation experiment took place on September 28, 2010, with an ambient air temperature averaging 95°F/35°C. There were seven replicates for the Category I assessment and three controls. These were located on various freely accessible areas on the boat and were sprayed with 60°C water for a duration of 5 s. For each of the replicates, 100% mortality was achieved immediately after testing (Table 13.9).

TABLE 13.9

Number of Mussels, Percent Mortality, and Average Shell Length of Experimental Groups and Controls for Category I Areas (Summer)

Group	Number of Mussels Present	Number of Mussels Dead	Mortality (%)	Average Shell Length (mm)
1	121	121	100	7.28 ± 0.99
2	163	163	100	8.07 ± 1.47
3	271	271	100	8.04 ± 1.48
4	30	30	100	6.77 ± 1.13
5	39	39	100	4.99 ± 1.50
6	77	77	100	5.62 ± 1.80
7	35	39	100	5.25 ± 1.37
Control 1	126	4	3	8.60 ± 1.84
Control 2	111	19	17	8.15 ± 1.97
Control 3	146	8	6	8.64 ± 1.85

TABLE 13.10

Number of Mussels, Percent Mortality, and Average Shell Length of Experimental Groups and Controls for Category II Areas (Summer)

Group	Number of Mussels Present	Number of Mussels Dead	Mortality (%)	Average Shell Length (mm)
1	109	109	100	7.12 ± 1.18
2	94	94	100	7.92 ± 2.31
Control	57	18	32	10.79 ± 2.83

There were two replicates and one control for the Category II assessment, which evaluated the encrusted gimbal unit of the watercraft. The gimbal unit was flushed with 60°C water for a duration of 48 s (including 5 s of duration to ensure the predetermined lethal duration was met). For each of the replicates, 100% mortality was achieved immediately after testing (Table 13.10). There was a significant difference in the percent mortality of the experimental groups and the controls for each of the tested categories.

Winter Validation

The winter validation experiment took place on January 27, 2011, with an ambient air temperature averaging 50°F/10°C. There were six replicates for the Category I assessment and three controls. These were located on various freely accessible areas on the boat and were sprayed with 60°C water for a duration of 5 s. For each of the replicates, mortality was assessed after 10 days of immersion in Lake Mead after treatment, and 100% mortality was achieved for each replicate (Table 13.11). There were two replicates

TABLE 13.11

Number of Mussels, Percent Mortality, and Average Shell Length of Experimental Groups and Controls for Category I Areas (Winter)

Group	Number of Mussels Present	Number of Mussels Dead	Mortality (%)	Average Shell Length (mm)
1	55	55	100	16.04 ± 4.02
2	43	43	100	18.73 ± 3.85
3	48	48	100	14.78 ± 3.55
4	34	34	100	15.24 ± 4.93
5	64	64	100	13.57 ± 3.25
6	37	37	100	17.03 ± 4.58
Control 1	134	2	2	12.59 ± 4.54
Control 2	107	0	0	12.35 ± 4.78
Control 3	83	0	0	12.35 ± 3.31

TABLE 13.12

Number of Mussels, Percent Mortality, and Average Shell Length of Experimental Groups and Controls for Category II Areas (Winter)

Group	Number of Mussels Present	Number of Mussels Dead	Mortality (%)	Average Shell Length (mm)
1	77	77	100	13.70 ± 3.07
2	55	53	96	13.70 ± 3.73
Control	125	2	2	13.12 ± 4.78

and one control for the Category II assessment, which evaluated the encrusted gimbal unit of the watercraft. The gimbal unit was flushed with 60°C water for a duration of 2 min and 12 s (adding 5 s of duration to ensure that the predetermined lethal duration was met). Only one replicate from the gimbal unit had a resulting 100% mortality, while the other exhibited 96% mortality (Table 13.12). There was a significant difference in the percent mortality of the experimental groups and the controls for each of the tested categories.

Discussion

The data obtained from the evaluation of hot water sprays as a method of decontamination for quagga mussels mirror the reported species-specific characteristic of the upper thermal limit of quagga mussels being lower than that of zebra mussels (Spidle et al. 1995; McMahon 1996; Mills et al. 1996). This vulnerability could be exploited by management agencies in regard to developing a more adaptable and efficient boat decontamination protocol, which may be more apt for recreational boaters to follow due to the less time needed to apply hot water sprays greater than or equal to 60°C to ensure 100% quagga mussel mortality when compared to zebra mussels. There are many areas of boats and other various watercrafts that are capable of being subjected to direct thermal spray (i.e., hull, trim tabs). These areas would require only hot water application of greater than or equal to 5 s at temperatures of greater than or equal to 60°C, instead of the greater than or equal to 10 s contact duration necessary to kill zebra mussels at the same temperature. Though this may not seem like a tremendous difference in application time, a vast majority of the boat area (i.e., hull, deck) would have the treatment time regarding boat decontamination reduced by half. This would appeal to both recreational boaters and government agencies because less money would be spent on the necessary time of labor required to conduct the entire decontamination procedure, and it would allow boaters to leave freshwater recreation areas more quickly. The use of species-specific guidelines for boat decontamination procedures would be more agreeable to monitoring agencies in the western United States, where water bodies are heavily infested specifically by quagga mussels (Benson 2011). In cases where the water body is infested by only zebra mussels or both zebra and quagga mussels could possibly be involved in fouling a boat, a duration of greater than or equal to 10 s at temperature greater than or equal to 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 particular invasive mussel population.

Field Validation of Category I and Category II Watercraft Areas

Although the new information regarding the increased susceptibility of quagga mussels to thermal spray compared to zebra mussels will be quite useful in revising and developing watercraft decontamination standards and procedures where applicable, the direct application of these data can be used only to readily accessible areas of the watercraft capable of receiving the contact spray directly (Category I areas in Table 13.1). Dreissenid mussels do display a tendency to settle in particularly well-sheltered areas of watercraft such as motors, anchors, intake and outlets, trim tabs, and centerboard slots (Morse 2009), where they may not be able to receive a direct hot water spray and/or may come in contact with sprayed water as runoff from other surfaces where it may have cooled below the lethal temperatures. For these reasons, it was necessary to conduct experiments to evaluate the amount of time necessary for hot water to be applied to these inaccessible areas (Category II in Table 13.1) in order to reach the most efficient and safe temperature resulting in 100% quagga mussel mortality. The inboard/outboard motor gimbal units of two separate boats were evaluated for this experiment, one in the summer and one in the winter. This was done because depending on the ambient temperature and conditions, the surface temperature of the gimbal units may vary, meaning the amount of time necessary to reach the lethal temperature in differing conditions may also vary. As expected, it took significantly longer than the Category I recommended duration of 5 s with 60°C hot water for the top flush of the gimbal unit to reach the target lethal temperature at the bottom of the gimbal unit. The amount of time needed to achieve the target lethal temperature also varied with the specific season: a maximum of 43 s for the summer flush and a maximum of 2 min and 7 s for the winter flush. This was probably due to the different surface area temperatures present between the two seasons.

In addition to areas that are inaccessible to hot water sprays on watercraft, there are also areas that are not capable of withstanding temperatures in excess of 54°C. These areas may be made of materials that could be susceptible to heat-associated damage such as thick plastics or tubing. For these areas (Category III on Table 13.1), the determined 100% mortality rates for temperatures less than or equal to 54°C may be used to prevent such damage from occurring. For the evaluation of Category III areas, the live and bait wells of the same recreational boat were tested in both summer and winter. The temperature of 54°C was used because it would require the least amount of additional contact duration to ensure 100% mussel mortality. As in the evaluation of the gimbal unit, the amount of time necessary to flush the live wells and bait wells was significantly longer than the necessary time regarding Category I areas.

In order for the information obtained from these experiments to be put to practical use in the real world, it was necessary to conduct field tests on actual boats encrusted with quagga mussels. The data regarding the most effective and least hazardous lethal temperature and duration regarding Category I areas could be applied directly to any area of the boat encrusted with mussels that could receive a direct spray. This was determined to be hot water at a temperature of 60°C for a duration of 5 s. The same standard was used for both the winter and summer experiments because this spray would be directly contacting the mussels allowing the lethal temperature to heat the soft tissues of the mussels completely through heat conduction across the shell valves without conduction from outside materials that may be inferring and protecting the mussels. For both winter and summer experiments, all Category I groups tested at this specific temperature and time combination had a 100% quagga mussel mortality result (Tables 13.6 and 13.8). The density of the experimental groups did vary between winter and summer, allowing many more smaller mussels to be killed per experimental group in the summer (mean n = 105) than the winter (mean n = 47). The average shell size between the summer and winter experimental groups also varied at 6.57 ± 2.48 mm and 15.70 ± 4.27 mm, respectively. The 100% mortality within the larger winter group offered confirmation that this specific standard can be used to ensure 100% mortality among some of the hardiest of quagga mussels.

The validation experiments concerning the Category II areas, specifically the quagga encrusted gimbal units of the contaminated boats that were tested, were treated differently than the Category I areas because the hot water spray could not directly contact the mussels colonized deep within the gimbal unit. For these experiments, a combination of the data obtained from the field test on emersed mussels and the evaluation of time needed to reach and sustain lethal temperatures in Category II areas was used.

For the summer validation, a hot water flush (60°C) was applied to the top of the gimbal unit for a total of 48 s: 43 s required to heat the entire unit to the necessary lethal temperature and an additional 5 s to ensure 100% quagga mussel mortality. The results of the experiment showed that the application of hot water to the gimbal unit for this amount of time did ensure 100% quagga mussel mortality in the two experimental groups. For the winter validation, the same technique was used on the gimbal unit regarding the flush, but the amount of time was increased in order to make sure the unit was heated to the necessary lethal temperature in the colder conditions. The hot water flush (60°C) lasted for a duration of 2 min and 12 s: 2 min and 7 s to ensure the unit would be heated to the lethal temperature and 5 s to ensure 100% quagga mussel mortality. Of the two experimental groups, only one displayed 100% mortality, while the other displayed 96% mortality. Since 100% mortality was not achieved in the second experimental group, this current combination of duration and lethal temperature should not be used for Category II areas in winter conditions. One aspect to examine why this specific combination of duration and temperature did not work is in regard to the structure of the gimbal unit. Applying a hot water to only the top of the unit was shown not to be effective. A hot water flush applied only to the top of the gimbal unit may not reach all of the settled quagga mussels within the sides of the hollow cylindrical structure. Therefore, a 2 min and 12 s rinse should have been conducted at both the top and the sides of the gimbal unit in order to make certain that all of the parts are heated to the necessary lethal temperature. For Category III areas (which may vary in size and volume depending on the watercraft), it is recommended that the temperature of the hot water flush be monitored until a temperature of 54°C is reached. After this target temperature is reached, it is necessary to maintain a constant flush of that temperature for at least 10 s in order to ensure 100% quagga mussel mortality.

Conclusion

According to the data obtained from the study testing the susceptibility of quagga mussels to hot water spray as a means of watercraft decontamination, 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 and differing field conditions (winter and summer). If the water temperature is lower than this, 100% mortality cannot be achieved for that specific duration. A temperature of 60°C rather than a higher temperature is recommended because it is reported to have the same efficacy at the same durations, and higher temperatures may be hazardous to human health (Morse 2009). The 60°C/5 s standard is to be used only for readily accessible areas of the watercraft and only used for mitigation of the quagga mussel. For other areas of watercraft (Category II and Category III), it is necessary to verify all surface areas are heated to the correct predetermined lethal temperature for the required amount of time to ensure 100% quagga mussel mortality. The results of the study attempting to validate a time standard for a specific watercraft area (i.e., the gimbal unit) show that developing a specific time standard may not be entirely effective for larger parts and under different weather conditions. Further research needs to be conducted regarding different areas on specific watercraft so that decontamination procedures can be developed depending on the type and model of boat contaminated with quagga mussels.

REFERENCES

Benson AJ. 2011. Quagga mussel sightings distribution. USGS. Retrieved June 20, 2013 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. *Ecological Applications* 10:1778–1788.

Comeau S, Rainville S, Baldwin W, Austin E, Gerstenberger S, Cross C, Wong WH. 2011. Susceptibility of quagga mussels (*Dreissena rostriformis bugensis*) to hot-water sprays as a means of watercraft decontamination. *Biofouling* 27(3):267–274.

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

Keller RP, Drake JM, Lodge DM. 2007. Fecundity as a basis for risk assessment of nonindigenous freshwater molluscs. *Conservation Biology* 21:191–200.

Leung B, Bossenbroek JM, Lodge DM. 2006. Boats, pathways, and aquatic biological invasions: Estimating dispersal potential with gravity models. *Biological Invasions* 8:241–254.

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

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. *American Zoologist* 36(3):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, Makie GL. 1993. Comparative shell morphology of *Dreissena polymorpha*, *Mytilopsis leucophaeata*, and the "quagga" mussel (Bivalvia: Dreissenidae) in North America. *Canadian Journal of Zoology* 73:1012–1023.

Peyer SM, McCarthy AJ, Lee CE. 2009. Zebra mussels anchor byssal threads faster and tighter than quagga mussels in flow. *Journal of Experimental Biology* 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. *Ecological Economics* 52:273–288.

Puth LM, Post DM. 2005. Studying invasion: Have we missed the boat? *Ecology Letters* 8:715–721.

Ricciardi A, Rasmussen JB. 1998. Predicting the identity and impact of future biological invaders: A priority for aquatic resource management. *Canadian Journal of Fisheries and Aquatic Sciences* 55:1759–1765.

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*). *Canadian Journal of Fisheries and Aquatic Sciences* 52:2108–2119.

Western Regional Panel on Aquatic Nuisance Species 2010. http://www.fws.gov/answest/documents.html (accessed on April 20, 2011).

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. *International Review of Hydrobiology* 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]. [Accessed June 20, 2013]. Available from: http://www.aquaticnuisance.org/wordpress/wp-content/uploads/2009/01/Recommended-Protocols-and-Standards-for-Watercraft-Interception-Programs-for-Dreissenid-Mussels-in-the-Western-United-States-September-8.pdf.