Using pressurized hot water spray to kill and remove dreissenid mussels on watercraft: Field testing on the efficacy of water temperature, high pressure, and duration of exposure[1]

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SUMMARY

The results of this study show that 3000 psi is the most effective pressure to remove dreissenid mussels if the watercraft is fresh out of the water. There is no difference in using pressurized water spray time between quagga mussels (*Dreissena rostriformis bugensis*) and zebra mussels (*D. polymorpha*). At the same time, 1500 psi will suffice to remove high density druses of dreissenids in 8.0 ± 2.3 s for quagga mussels and 15.6 ± 11 s for zebra mussels if the watercraft has been out of the water more than one week in the summer and 18.83 ± 9.32 s for quagga mussels if the watercraft has been out of the water for more than four weeks. This study also attempted to assess the shortest amount of time needed to attain 100% mortality of zebra mussels following exposure to hot-water spray. The results showed that zebra mussels exposed to 54°C (130°F) or higher for 10 s and 70°C (158°F) or higher for 5 s was sufficient to reach 100% mortality. From a previous study, it is also found that it takes 10 s to kill 100% quagga mussels when they are exposed to water with temperature 54°C (130°F) or higher. If a mussel fouled watercraft arrives at an inspection station, it is currently recommended by the federal and state agencies to decontaminate the vessel with 60^{o}C (140^{o}F) for 10 s. From the present project and results in previous studies, it is suggested that 54°C (130°F) for 10 s will be 100% effective for quagga and zebra mussels.

PROJECT OBJECTIVES

To collect field data on the lethal effect of hot-water spray on immersed zebra mussels attached to different areas of a watercraft at different combinations of water temperature and duration of exposure.

To validate the field data through tests on boats infested with adult zebra mussels to make sure decontamination programs with the identified standards will lead to 100% mussel mortality.

To collect field data on the efficacy of power wash on attached live and dead quagga/zebra mussels at different combinations of pressure and duration of exposure.

To provide field data on the minimal pressure and time required to reach and sustain 100% removal rate of live and dead quagga/zebra mussels attached to watercraft.

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MATERIALS AND METHODS

Mussel removal with high pressure water spray - quagga mussels

The effectiveness of high pressure water spray (1500 and 3000 psi) was evaluated for removing 100% of quagga mussels from watercraft in the winter season and then repeated in the summer season at Lake Mead NRA. A heavily encrusted Bayliner watercraft, which was slipped in Lake Mead for over four years, was pulled from a Las Vegas Boat Harbor slip on 28 January 2011 and brought to the maintenance yard (Figure 1). The mussels on the watercraft, pulled fresh out of the water, were presumed alive. That same watercraft remained in the maintenance yard where the experiment was repeated on days 14 (11 February 2011) and 30 (27 February 2011). Another Bayliner was pulled from a slip at Las Vegas Boat Harbor on 20 July 2011 for the summer season, high pressure experiment. It was brought back to the marina's maintenance yard where the experiment took place on day 0 (20 July 2011) and day 7 (27 July 2011).

Figure 1. Watercraft heavily encrusted with *D. rostriformis bugensis.*

Groups of mussels were divided into 24 treatment groups: 2 pressures (1500 and 3000 psi) x 2 densities (high and low) x 6 replicates (Table 1). The treated area was completely covered with mussels and was created by partitioning off areas (high or low density mussel groups) by scraping off the surrounding mussels (Figure 2). High mussel density groups consisted of approximately 23,220-46,440 mussels/m^2 (~75-150 individuals) and low density mussel groups consisted of approximately 7,772-10,363 mussels/m^2 (~15-20 individuals). Prior to using pressurized spray, each group of mussels was photographed for a more precise enumeration. A LANDA pressure washer (LANDA Cold Water Direct Drive Pressure Washer, Model # PD4-35324; American Pressure Inc.) was used for the high pressure experiments (Figure 3). The unit is capable of spraying at least 5 gallons/minute with a nozzle pressure of 3000 psi and greater, which is recommended by The Uniform Minimum Protocols and Standards for Watercraft Interception Programs for Dreissenid Mussels in the Western US (Zook and Phillips 2012). For this project, the 40 degree nozzle was used and the tip of the pressure washer wand remained 12 inches away from the watercraft. A sub-sample of mussels was removed from each replicate and the size of mussels was recorded. The shell length of the mussel is the distance measured from the posterior edge of the shell to the anterior tip of the umbos to the nearest 0.1 mm with digital calipers (VWR Digital 152 cm (6") Caliper - Stainless Steel, Model # 62379-531; VWR International, Inc.) (McMahon and Ussery, 2005).

Table 1. Experimental design for pressurized water spray to remove *D. rostriformis bugensis* and *D. polymorpha* on watercraft.

Mussel Density	1500 PSI	3000 PSI
High	6 replicates	6 replicates
Low	6 replicates	6 replicates

Figure 2. A group of mussels segmented for high pressure testing.

The above experiment was repeated in the summer season at the Las Vegas Boat Harbor with one modification. The summer season experiment took place on days 0 (when the watercraft was pulled fresh out of the water) and 7. The shorter time range is because *D. rostriformis bugensis* byssal threads will dry out and decompose at a faster rate in the hot, arid days of summer in the Southwest compared to the winter months.

Figure 3. LANDA pressure washer used in the high pressure experiments.

Mussel removal with high pressure water spray - zebra mussels

The above study was repeated using zebra mussels *D. polymorpha* at Wilson Lake, Kansas during the summer season. Adult *D. polymorpha* colonies are not found in bodies of water in the southwest. Kansas is the closest place to Nevada where the reservoirs contain healthy populations of adult *D. polymorpha*. In August 2011, the LANDA pressure washer and all other equipment were transported by van to Wilson Lake, Kansas to conduct the experiment.

Arrangements were made with the marina staff at Wilson Lake to have a *D. polymorpha* encrusted watercraft removed from the reservoir to perform the project. Marina staff discovered they had a sunken boat lift that was encrusted with larger *D. polymorpha* compared to the watercrafts that were in the slips. The staff believes that the boat lift was in the water for over two years. It was removed from the reservoir and used for the high pressure experiment (Figure 4). On days 0 (1 August 2011) and 7 (8 August 2011), 1500 and 3000 psi was applied on high and low mussel densities to evaluate the duration it would take to remove 100% of the mussels.

Figure 4. Boat lift used for the summer season, Wilson Lake high pressure experiments.

Susceptibility of Zebra Mussels to Hot-water Spray

The susceptibility of quagga mussels to hot-water sprays at different temperatures and durations of spray contact at Lake Mead NRA was evaluated. Results showed that a spray temperature of 60°C for 5 s is recommended for mitigating fouling by *D. rostriformis bugensis* (Comeau et al. 2011). In their study, it was also found that 54^{o}C for 10 s can kill 100% of quagga mussels. We just repeated their experiment on zebra mussels. Zebra mussels have been tested in the lab before (Morse 2009), but the mussels in our experiment were collected and tested in situ. A Kansas Department of Wildlife permit was obtained to collect adult *D. polymorpha* from Wilson Lake. Specimens of healthy adult *D. polymorpha* (≥11 mm in length) were collected from the encrusted docks at the marina. The individuals were divided among 60 mesh spat bags (approximately 75 in each) and suspended in the lake, off the dock, and acclimated for ten days. After acclimation, adult mussels were randomly divided into 60 subsamples (n = 50) and placed into 60 identical pre-labeled, 3 mm spat bags (Aquatic Eco-Systems Inc., Apopka, FL) (Table 2). To avoid transporting the mussels, the experiment took place on the dock, close to an electrical outlet to plug in the equipment. Each bag was suspended over a PolyScience Programmable heated circulator wash bath with a 28 liter capacity during the thermal spray treatment (PolyScience, Model # 1137-2P; Niles, Illinois) (Figure 5). Treatment spray was applied to the samples at a flow rate of approximately 900 ml/min through a fan shaped nozzle (Comeau et al. 2011).

Table 2. Amount of *D. polymorpha* tested per treatment group (n = 50) (Comeau et al., 2011).

Temp °C/°F	Exposure Duration (s)							
	1	2	5	10	20	40	80	160
20/68	50	50	50	50	50	50	50	50
40/104	50	50	50	50	50	50	50	50
50/122	50	50	50	50	50	50	50	50
54/130	50	50	50	50	50	50	50	50
60/140	50	50	50	50	50	50	50	50
70/158	50	50	50	50	50	50	50	50
80/176	50	50	50	50	50	50	50	50

Figure 5. PolyScience Programmable heated circulator wash bath.

Each sample of mussels was exposed to thermal-spray treatments from a distance of 15 cm horizontally above the mussel-containing mesh bag (Morse 2009) at 20, 40, 50, 54, 60, 70, and 80°C and exposure durations of 1, 2, 5, 10, 20, 40, 80, and 160 s. The control was 20°C. Therefore, 56 combinations of temperature by exposure duration were tested (Table 2). The water temperature, on contact with the treatment group, was constantly monitored by the PolyScience Programmable heated circulator wash bath. Four bags containing *D. polymorpha* were treated with hot-water spray as they were used as controls and were left suspended in Wilson Lake. Following treatment, each spat bag containing the treatment specimens was tied to a line hanging from the dock. Mortality was assessed at the time of treatment and every day thereafter for ten days. To test for mortality, gaping mussels were gently prodded on their shell valves. Individual mussels that did respond by immediate shell closure were stimulated in the area of their siphons. Mussels that did not respond to siphon stimulation had their shell valves forcible closed with forceps. Mussels were considered dead if the shell immediately reopened upon release of the forceps (Harrington et al. 1997; Morse 2009; Comeau et al. 2011). Dead mussels were removed and measured, then recorded and placed into a different labeled mesh bag. The control groups remained in Wilson Lake for ten days and survivorship was assessed as described previously.

Hot-water Spray Watercraft Validation

After the minimum time to kill 100% *D. polymorpha* was identified from the above experiment, the protocol was field validated using a zebra mussel encrusted Crestliner pontoon vessel from Wilson Lake, Kansas (Figure 6). The watercraft was pulled from the reservoir on 3 August 2011. Five groups of mussels were segmented on the watercraft (as discussed previously) and served as the treatment groups. An additional group was segmented and served as the control, and those mussels were gently scraped off the watercraft, transferred to a mesh bag and placed in the lake for ten days to assess survivorship. A PolyScience Programmable heated circulator wash bath was set at 54°C, which was the temperature that was discovered to kill 100% of *D. polymorpha* in the previous laboratory experiment. The spray nozzle was placed 15 cm from the treatment groups. The hot-water spray was applied to each group for 10 s. Once the *D. polymorpha* were treated with the hot-water spray, the mussels were gently scraped off the watercraft, transferred into a labeled mesh bag, and placed back into the lake to confirm mortality after 24 h and for ten days thereafter.

Figure 6. Encrusted pontoon boat from Wilson Lake, Kansas.

RESULTS AND DISCUSSION

Pressurized Water Spray to Remove Dreissenids on Watercraft

When the boat was just out of water (at week 0), it takes an average of 430 s to remove a cluster of high density (11,246 ± 1,152*)* *D. rostriformis bugensis* with 1500 psi (low pressure) in the summer season at Lake Mead, and it takes an average of 472 s to remove a cluster of high density (8,091 ± 327) *D. polymorpha* with 1500 psi in the summer season at Wilson Lake (Table 3). Conversely, when 3000 psi is used in the summer season on a high density group of *D. rostriformis bugensis*, the time is greatly reduced to 48 s and 52 s for *D. polymorpha* (Table 3). To remove *D. rostriformis bugensis* from a watercraft in the winter season, using high or low psi, it takes on average 297 s (range = 202-390 s). Decontamination of *D. rostriformis bugensis* will take longer when the watercraft is pulled fresh from the water body in the winter season. It takes an average of 346 s (5.77 min) to remove 15,615 m^2 of *D. rostriformis bugensis* from a watercraft. Comparing it to the data from watercraft being out of the water for 2 and 4 weeks, decontamination times are reduced to 3.33 s to remove 21,084 m^2 and 1.83 s to remove 12,540 m^2, respectively (Tables 4 and 5). When the watercraft was just out of the water (week 0), the time to remove *D. rostriformis bugensis* and *D. polymorpha* from watercraft was shorter when mussel density was low (~1,754 m^2 ± 1,003) and the pressure was set to 3000 psi (high) (Table 3). In winter or summer seasons, it was easier to remove mussels from the watercraft when it had been out of the water for at least two weeks or one week, respectively, when compared to being at week 0, or fresh out of the water. The time to remove mussels from watercraft was shorter when mussel density was low and the pressure was high (Table 3). The results show that it takes more time to remove mussels in winter than summer (Analysis of variance (ANOVA), $F_{43, 44} = p < 0.0001$). A general linear model was used to test if the amount of time a watercraft was out of the water (0, 1, 2, or 4 weeks) would have an effect on time to remove dreissenids. Statistical results show that the time a watercraft is out of the water, the density and pressure are significant contributing factors in removing mussels. There is an interaction between the time the watercraft has been out of the water and density as well (Analysis of covariance (ANCOVA), $F_{4, 115} = 13.30$, $p < 0.001$). The time the watercraft is out of the water is the most significant factor affecting removal time ($F_1 = 36.24$, $p < 0.0001$).

In the summer season, *D. rostriformis bugensis* and *D. polymorpha* are removed from watercraft using high pressure spray in less time because the byssal threads have a higher chance of drying out. However, the longer the vessel is out of the water, the quicker the mussels will be removed. For instance, to remove *D. rostriformis bugensis* and *D. polymorpha* from watercraft on week 0 using the recommended 3000 psi, it took on average 48 s to remove 13,350 m^2 individuals and 52 s to remove 8,091 m^2 individuals, respectively (Table 3). When the watercraft sat out of the water for 1 week prior to decontamination, the time to remove *D. rostriformis bugensis* and *D. polymorpha* was greatly reduced to 3.5 s to remove 1,844 m^2 individuals and 4.5 s to remove 7,767 m^2 individuals, respectively (Table 6). Kappel (2012) found that after just 4 hours of exposure to 30ºC ambient temperature, *D. rostriformis bugensis* achieved 30% mortality which agreed with the data suggesting the *D. rostriformis bugensis* has a lower acute thermal tolerance than *D. polymorpha*, thus explaining the ease in removing the mussels (Spidle et al., 1995; Mills et al., 1996).

There are no data for removal times of *D. polymorpha* in the winter season. However, based on the results with *D. rostriformis bugensis* and *D. polymorpha* having similar removal times in the summer, the data suggests the results from removing *D. rostriformis bugensis* from watercraft in the winter season may be applicable to removal times of *D. polymorpha* from watercraft in the winter season. There was no significant difference between *D. rostriformis bugensis* and *D. polymorpha* in removal times (ANOVA and Student-Newman- Keuls multiple comparison, P = 0.81). This recommendation would need to be field tested.

Table 3. Average time to remove high or low densities of *D. rostriformis bugensis* and *D. polymorpha* from watercraft in summer and winter seasons using 1500 or 3000 psi on week 0 (n = 6).

Species	**Season**	**Pressure (psi)**	**Density (m^2)**	**Time (s)**
D. rostriformis bugensis	Summer	1500	High (11,246 ± 1,152)	430 ± 370
D. rostriformis bugensis	Summer	1500	Low (1,909 ± 312)	37 ± 15
D. rostriformis bugensis	Summer	3000	High (13,350 ± 1,136)	48 ± 14
D. rostriformis bugensis	Summer	3000	Low (3,317 ± 335)	43 ± 87
D. rostriformis bugensis	Winter	1500	High (16,586 ± 5,509)	390 ± 140
D. rostriformis bugensis	Winter	1500	Low (1,326 ± 224)	249 ± 174
D. rostriformis bugensis	Winter	3000	High (15,615 ± 258)	346 ± 155
D. rostriformis bugensis	Winter	3000	Low (1,068 ± 1,091)	202 ± 52
D. polymorpha	Summer	1500	High (6,068 ± 530)	472 ± 178
D. polymorpha	Summer	1500	Low (1,262 ± 220)	41 ± 14
D. polymorpha	Summer	3000	High (8,091 ± 327)	52 ± 24
D. polymorpha	Summer	3000	Low (1,246 ± 1,434)	32 ± 32

Table 4. Average time to remove high or low densities of *D. rostriformis bugensis* from watercraft in the winter seasons using 1500 or 3000 psi on week 2 (n = 6).

Pressure (psi)	**Density (m^2)**	**Time (s)**
1500	High (19,660 ± 8,395)	39.5 ± 15.44
1500	Low (10,784 ± 10,869)	2.33 ± 1.37
3000	High (21,084 ± 4,740)	3.33 ± 1.51
3000	Low (1,553 ± 122.8)	15.5 ± 5.05

Table 5. Average time to remove high or low densities of *D. rostriformis bugensis* from watercraft in the winter seasons using 1500 or 3000 psi on week 4 (n = 6).

Pressure (psi)	**Density (m^2)**	**Time (s)**
1500	High (12,459 ± 7,256)	18.83 ± 9.32
1500	Low (1,504 ± 419.8)	1.17 ± 0.41
3000	High (12,540 ± 5,820)	1.83 ± 0.98
3000	Low (1,537 ± 444.5)	1 ± 0

Table 6. Average time to remove high or low densities of *D. rostriformis bugensis* and *D. polymorpha* from watercraft in the summer season using 1500 or 3000 psi on week 1 (n = 6).

Species	**Season**	**Pressure (psi)**	**Density (m^2)**	**Time (s)**
D. rostriformis bugensis	Summer	1500	High (12,216 ± 4,760)	8.0 ± 2.3
D. rostriformis bugensis	Summer	1500	Low (1,779 ± 396.3)	15.8 ± 13
D. rostriformis bugensis	Summer	3000	High (15,776 ± 2,972)	3.5 ± 3.8
D. rostriformis bugensis	Summer	3000	Low (1,844 ± 363.3)	8.67 ± 5.2
D. polymorpha	Summer	1500	High (8,576 ± 3,316)	15.6 ± 11
D. polymorpha	Summer	1500	Low (1,375 ± 365)	12.3 ± 15
D. polymorpha	Summer	3000	High (7,767 ± 1,816)	4.5 ± 1.8
D. polymorpha	Summer	3000	Low (1,165 ± 237.8)	6.33 ± 3.8

The data show there was not a significant difference between *D. rostriformis bugensis* and *D. polymorpha* when using pressurized water spray to remove them from watercraft in the summer season (ANOVA, $F_1 = 0.03$, P = 0.81). However, the pressure applied (ANOVA, $F_1 = 25.27$, $p < 0.0001$) and the density of mussels was significant (ANOVA, $F_1 = 26.13$, $p < 0.0001$).

Depending on the size of the watercraft and the amount of biofouling present, decontamination using high pressure spray will take a considerable amount of time. It is recommended to use 3000 psi on the hull, centerboard box and keel (sailboats), lower unit, cavitation plate, and prop. These external areas can handle 3000 psi on most watercraft without causing damage and usually have the most amount of mussel fouling. For internal and other sensitive areas of watercraft, manual removal, using brushes and scrapers, of mussels may be necessary. At the same time, 1500 psi will suffice to remove high density druses of dreissenids if the watercraft has been out of the water more than one week. For personal safety, only trained personnel should use high pressure spray to remove dreissenids from watercraft. If the vessel is left out of the water for at least one week in the summer or two to four weeks in the winter, the decontamination time can be significantly reduced.

Susceptibility of Zebra Mussels to Hot-water Spray

There is a trend which shows that as the treatment temperatures increase, greater mortality in *D. polymorpha* following the same exposure duration also increases (Table 7). *Dreissena polymorpha* reached 100% mortality within 10 s at 54°C and 60°C and 5 s at 70°C and 80°C treatments (Table 7). Spray exposures of 1 s and 2 s were not found to induce 100% mortality at any of the test temperatures. Treatments of 20°C were ineffective, as only 4% mortality was seen when *D. polymorpha* were exposed to treatment for 80 s (Table 7).

Table 7. Mortality rate (%) of *Dreissena polymorpha* under different treatments by day 10.

Temp °C/°F	**Exposure Duration (s)**							
	1	**2**	**5**	**10**	**20**	**40**	**80**	**160**
20/68	0	0	0	0	0	4	4	0
40/104	2	2	4	4	90	100	100	100
50/122	10	24	38	80	96	100	100	100
54/130	52	72	96	100	100	100	100	100
60/140	84	84	96	100	100	100	100	100
70/158	84	96	100	100	100	100	100	100
80/176	84	96	100	100	100	100	100	100

Estimated LD_{50} values for 1 s, 2 s, 5 s, and 10 s indicate that the temperature to kill 50% of *D. polymorpha* was between 49.8°C to 60.3°C (Table 8). The estimated LD_{99} with these exposure durations varied from >102.8°C at 1 s to 51.1°C at 10 s (Table 8).

Table 8. Estimated LD_{50} and LD_{99} values (in bold) and their 95% confidence limit for hot-water spray treatments on *Dreissena polymorpha* at 1 s, 2 s, 5 s, and 10 s application durations (n = 400 for each duration).

Duration (s)	LD_{50}(°C)	LD_{99}(°C)	SM_{100}(°C)*
1	53.6 < **60.3** < 68.5	> 102.8	> 80
2	44.6 < **56.8** < 69.9	> 86.5	> 80
5	50.7 < **51.2** < 51.7	> 55.0	> 60
10	49.6 < **49.8** < 50.0	50.9 < **51.1** < 51.4	54

*The SM_{100} is the temperature observed in the experiment that induced 100% mortality in *Dreissena polymorpha*

The mussels in the control groups (n = 200) (mean = 16.02 mm, range = 11.01-21.24 mm) remained in the spat bags immersed in Wilson Lake for ten days. The water temperature of the lake averaged 26.79°C ± 1.7 for the duration of the project. The control groups and the mussels exposed to 20°C spray treatments exhibited high survival rates. Figure 7a shows the combined four groups of controls exhibited a 97% survival rate with a range from 96%-98%; Figure 7b the shows the eight 20°C spray treatment subsample displayed a mean 98.5% survival rate with a range of 96% to 100% with no apparent correlation to duration of exposure. Figures 7c-7h similarly display % mortality for spray temperatures of 40°C, 50°C, 54°C, 60°C, 70°C and 80°C, respectively. For all of Figure 7, mortality was evaluated daily for 10 days following exposure to the spray water.

Of *D. polymorpha* exposed to 40°C, 67% survived treatment. Those mussels that were exposed to that treatment for 1 s and 2 s exhibited a 98% survival rate, those exposed for 5 s exhibited a 96% survival rate and when exposed for 10 s, the mussels had a 94% survival rate (Figure 7c). Mussels exposed to 40°C and 50°C for 1 s exhibited a 98% and 90% survival rate, respectively. The average shell length of mussels in the 56 treatment groups (n = 2,800) is 16.65 mm (range = 11.02-28.06 mm).

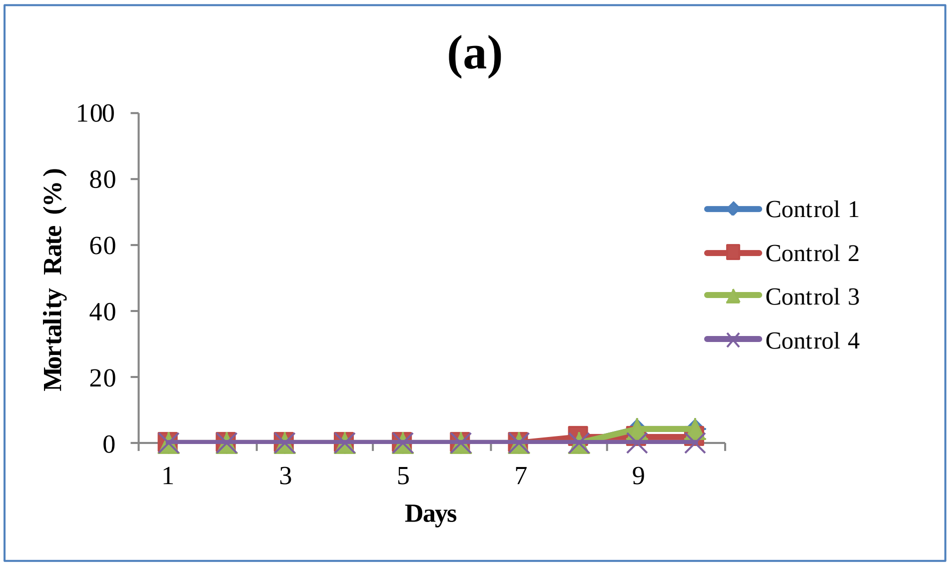

Figure 7a. Mortality rates (%) *of Dreissena polymorpha* in Wilson Lake after hot-water spray treatment: Control (26.79°C).

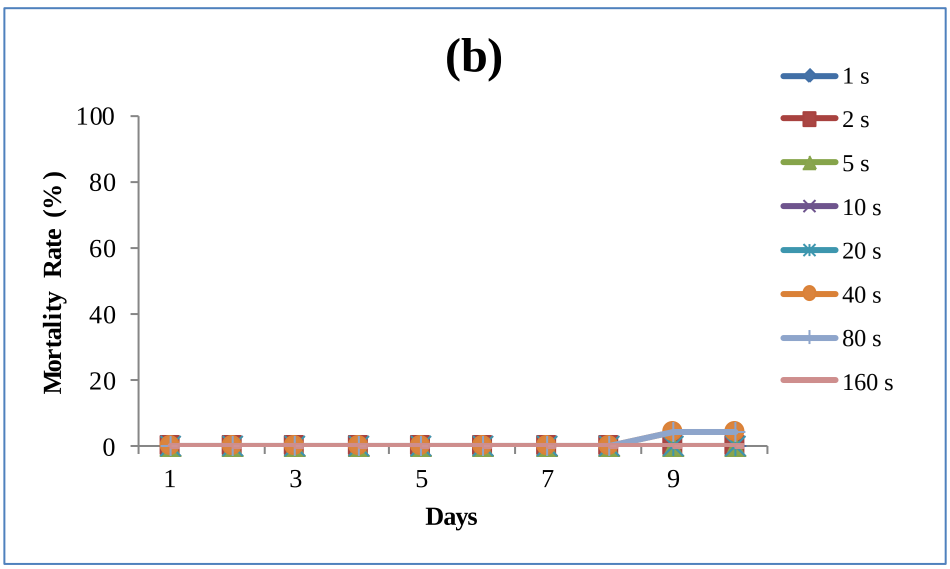

Figure 7b. Mortality rates (%) *of Dreissena polymorpha* in Wilson Lake after hot-water spray treatment: 20°C.

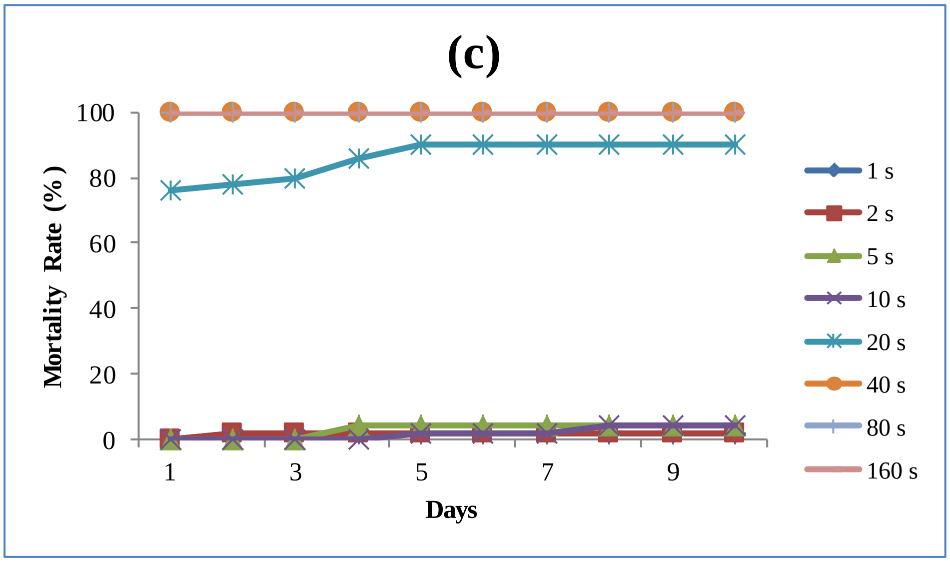

Figure 7c. Mortality rates (%) *of Dreissena polymorpha* in Wilson Lake after hot-water spray treatment: 40°C.

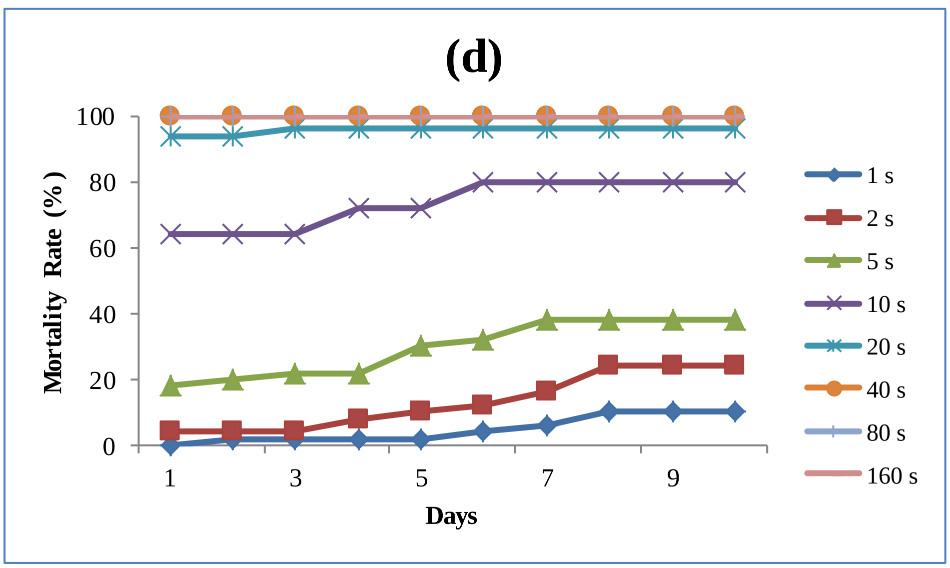

Figure 7d. Mortality rates (%) *of Dreissena polymorpha* in Wilson Lake after hot-water spray treatment: 50°C.

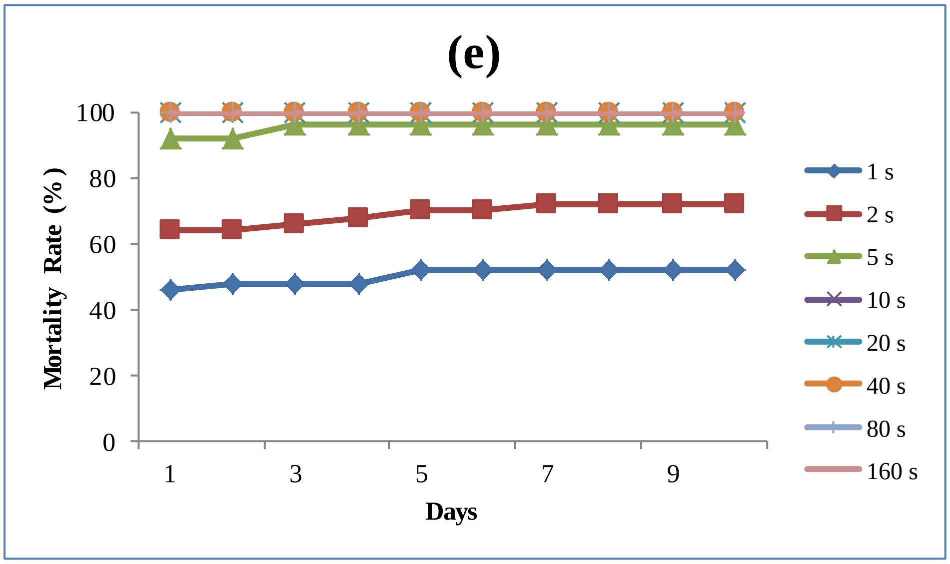

Figure 7e. Mortality rates (%) *of Dreissena polymorpha* in Wilson Lake after hot-water spray treatment: 54°C.

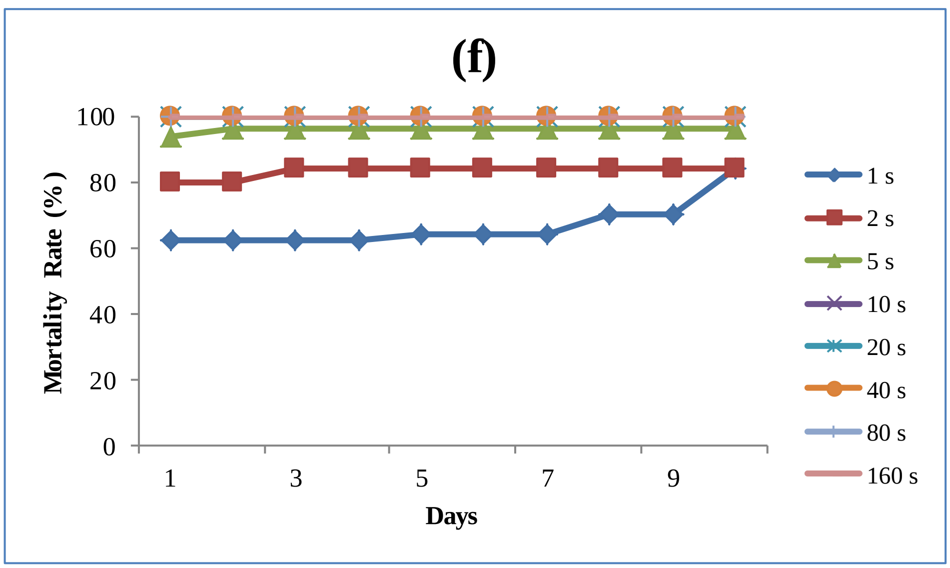

Figure 7f. Mortality rates (%) *of Dreissena polymorpha* in Wilson Lake after hot-water spray treatment: 60°C.

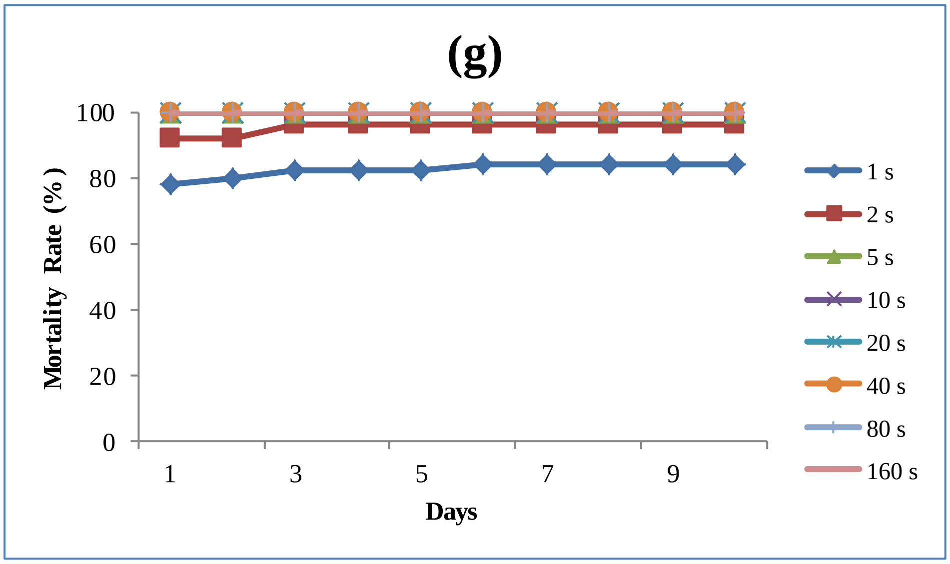

Figure 7g. Mortality rates (%) *of Dreissena polymorpha* in Wilson Lake after hot-water spray treatment: 70°C.

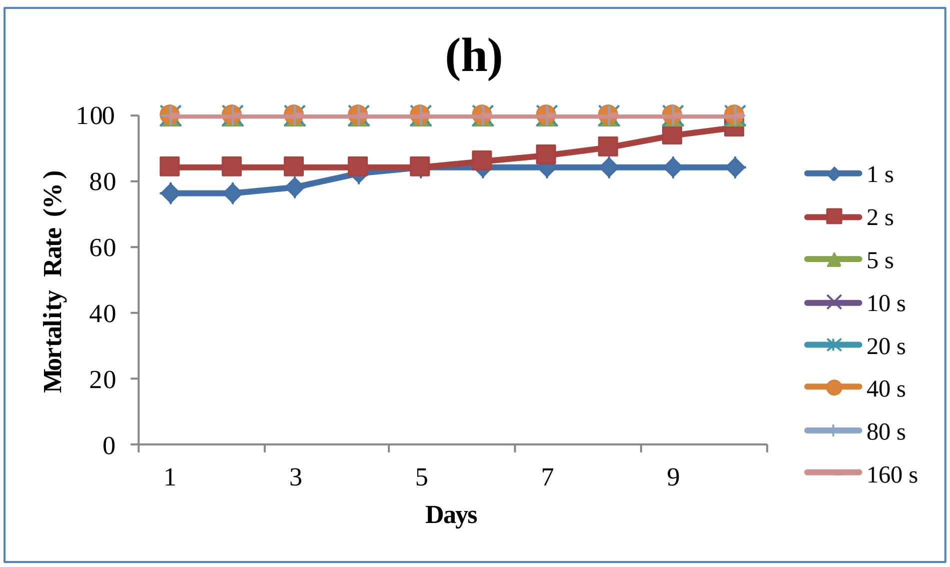

Figure 7h. Mortality rates (%) *of Dreissena polymorpha* in Wilson Lake after hot-water spray treatment: 80°C.

Hot-water Spray Watercraft Validation

The hot-water spray field test showed that 100% mortality of *D. polymorpha* was reached using 54ºC for 10 s of exposure time (Figure 7e). These results were validated on a zebra mussel encrusted Crestliner pontoon vessel (Figure 6). Immediate mortality was observed in the six treatment groups (N = 72). No mortality was observed in the six control groups three days post-experimentation (N = 46). The average shell length of the treatment and control group mussels was 8.16 mm (range = 4.84—14.26 mm) and 8.15 mm (range = 6.17—14.15 mm), respectively.

Hot-water spray is sustainable, effective, and economical compared to chemical applications which could lead to further financial and ecological issues (Piola 2009). By using tap-water cultured zebra mussels, Morse (2009) found that water sprayed at ≥ 60°C for 10 s or 80°C at ≥ 5 s was 100% lethal to *D. polymorpha*, which indicates that current decontamination recommendations of spray temperature of ≥ 60°C may not kill all the mussels if the exposure duration is < 10 s. The current study found the same results as Morse (2009) in regards to 10 s application using 60°C will result in 100% *D. polymorpha* mortality. The results also showed that with a 5 s application using 60°C resulted in a 98% mortality rate, as opposed to Morse's study that concluded 5 s application resulted in an 87% mortality rate. However, at a cooler temperature of 54°C, 100% mortality of *D. polymorpha* was also attained by 10 s in this study. The 54ºC/130ºF was not tested in Morse's study, but the model result shows that the LT99 is 53.9ºC (with variations from 52 to 60.2ºC) which is very close to the present study (100% mortality at 54ºC). In Morse's (2009) study, the LT50 and LT99 at 1 s duration were both >80°C while they were 60.3°C and >102.8°C, respectively, for *D. polymorpha* in the present study (Table 8). At 5 s duration, LT50 and LT99 for *D. polymorpha* in the first study were 54.6°C and 69.1°C while they were 51.2°C and > 55.0°C in the current study. With 10 s exposure, the LT50 and LT99 for *D. polymorpha* in the first study were 46.9°C and 53.9°C, and in the current study, they were 49.8°C and 51.1°C. Clearly, relatively lower temperatures with the same exposure time, or relatively less time under the same treatment temperature, is needed to reach the same lethal rate in the present study than the study by Morse (2009). The only difference is that *D. polymorpha* in Morse's study have been acclimated in laboratory conditions while the present study used mussels fresh from the native reservoir. Therefore, the physiology of *D. polymorpha* tested in these two studies and their responses may differ (Costa et al. 2008). The effort of adaptation of physiological responses of *D. polymorpha* to hot-water treatment needs to be studied in the future. Morse's experimental design was set up to examine the 60ºC/140ºF lethal temperatures at 5 and 10 s, whereas 6, 7, 8, and 9 s were not tested. Through the results of this study, it is suggested that *D. polymorpha* could reach 100% mortality between 6-9 s when exposed to 60°C. In that case, boat inspectors can save time in decontaminating a mussel fouled watercraft. Field tests would be needed to validate this assumption.

Another study examined the susceptibility of quagga mussels to hot-water sprays. The researchers found that at hot-water temperatures ≥60°C, with contact duration of only 5 s was sufficient to induce 100% mortality in *D. rostriformis bugensis* (Comeau et al. 2011). These results indicate that *D. rostriformis bugensis* are more susceptible to hot-water sprays than *D. polymorpha* when comparing to the results from the current study and Morse's study. *Dreissena rostriformis bugensis* have thinner shells and less tightly sealing shell valves compared to *D. polymorpha* (Claxton et al. 1997). Because the shell valves may not close as tightly in *D. rostriformis bugensis*, heating of the soft tissues may occur more rapidly than that of *D.*

polymorpha. Another potential reason for this increased vulnerability may have to do with the impact of ambient temperature conditions and seasonal productivity variations on the acute thermal tolerance of dreissenid mussels (Elderkin and Klerks 2005). The upper thermal limit of *D. rostriformis bugensis* is lower than that of *D. polymorpha* (McMahon 1996). This suggests that *D. rostriformis bugensis* are more susceptible to death by hot-water sprays at a lower temperature and less exposure time than *D. polymorpha*. However, the difference thermal tolerance between the two species is not great. For example, it is also found 54°C/10 s will result in 100% mortality for quagga mussels (Table 9, Comeau et al. 2011). Compared to 60°C (140°F), 54°C (130°F) is more appropriate because it is the highest temperature at which the system components of heat sensitive areas in watercraft (i.e. ballast tanks and bladder) are designed to withstand (Zook and Phillips 2009). In addition, lower temperature will be safer for boat inspectors (Morse 2009) and it is relatively cheaper and environment friendly (i.e. less energy will be consumed). Based on results from Morse (2009), Comeau et al. (2011), and the present study, it is recommended that 54°C (130°F)/10s is to be used for boat decontamination.

Table 9 Quagga mussel mortality (%) under different treatments at day 10

Temp °C/°F	**Exposure Duration (s)**							
	1	**2**	**5**	**10**	**20**	**40**	**80**	**160**
20/68	4	4	6	0	0	2	2	0
40/104	2	2	8	12	94	100	100	100
50/122	10	22	36	82	100	100	100	100
54/130	54	72	98	100	100	100	100	100
60/140	72	92	100	100	100	100	100	100
70/158	88	98	100	100	100	100	100	100
80/176	86	98	100	100	100	100	100	100

There are a couple of other methods for watercraft decontamination that have been explored, such as dry time acceleration and dry ice blasting (Zook and Phillips 2012). The rate of desiccation for dreissenid mussels is a function of temperature, humidity, and mussel size (Morse 2009). Increasing ambient temperatures and lower humidity decrease the time needed for desiccation, while larger mussels require more time to dry-out than smaller mussels. To assist lake managers in knowing how long a watercraft needs to remain out of the water to ensure the mussels are dead through desiccation, a dry time estimator was developed (Mangin 2011). The estimator is a tool that can be used to estimate the minimum time a vessel should remain out of the water before launching into an uninfested water body. Accelerated dry times should be used after a fouled vessel has gone through a high pressure, hot-water spray service. The use of dry ice (CO_2) pellets for cleaning and removing attached dreissenids is an alternative decontamination method. Dry ice blasting uses compressed air to propel tiny dry ice pellets onto fouled watercraft. The dry ice freezes the mussels with the intent of killing them. The pellets quickly dissipate into the air so there's no wastewater or other media to dispose (Zook and Phillips 2012). This will only remove the mussels, not kill them. For high density colonization, 100% mortality rate could not be reached for removed mussels (WH Wong, personal observation). The effectiveness of dry ice blasting has not been reviewed in the literature and it should be systematically investigated prior to implementation. Combining the methods of pressurized (3000 psi) and hot-water spray (60°C for 10 s) to the surface of the fouled watercraft is the best way to decontaminate a vessel to prevent the further spread of dreissenids. For the inaccessible areas, such as the gimbal area, inside the engine, generator and AC cooling systems, treatments of 60°C for 10 s will not suffice. According to Comeau et al. (2011), the amount of time needed to achieve the target lethal temperature is 43 s for the summer time, and 2 minutes and 7 s for the winter time. The time variations are because of the different surface area temperatures present between the two seasons. The hot water needs to warm up these internal compartments. In addition, most watercraft have special areas that have water transfer pumps that require water temperature ≤ 54°C for decontamination, such as ballast tanks/bladders, wash-down systems, bait and live wells, and internal water systems. For these sensitive areas, 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 to ensure 100% mussel mortality (Comeau et al. 2011).

CONCLUSION AND RECOMMENDATION

Hot-water Spray Watercraft Validation

The results of the study showed that 3000 psi is superior to 1500 psi, as it is strong enough to remove the mussels without damaging the vessel. However, when decontaminating certain areas on the vessel, such as the gimbal unit, it is recommended to avoid using pressurized spray as it can damage some of the seals and parts in that area (Zook and Phillips 2012). If the equipment is capable, 3000 psi should be used when removing dreissenids when the watercraft is just out of water. While 3000 psi is the recommended protocol for removing dreissenids from watercraft, 1500 psi may be useful when the mussels are dead and the byssal threads have dried out. To save time and to be less of an inconvenience to boaters, if hot water is unavailable for AIS removal, it is suggested that the vessel remain out of the water for at least one week in the summer and two weeks in the winter before the high pressure spray decontamination method is used. However, this may be difficult to achieve at some water-bodies, as there may not be places to store the vessel prior to decontamination. However, using 3000 psi on a watercraft fresh out of the water will suffice for removing attached mussels.

Susceptibility of Zebra Mussels to Hot-water Spray

When decontaminating watercraft, it is important not only to remove the mussels, but also ensure they are dead. When removing the mussels with high pressure spray, it is difficult to remove every mussel as some cannot be reached or they can be dislodged, landing on a different spot or on the trailer; however, using hot-water spray will certify that the mussels are at least dead. The results from hot-water spray watercraft validation test were not surprising. Once the laboratory test was completed and the temperature and time needed to reach 100% mortality in *D. polymorpha* was determined, the field validation was conducted. The field tests exposing *D. polymorpha* to 54°C for 10 s, verified the laboratory tests and 100% mortality was observed immediately after treatment.

It is extremely difficult to remove every single mussel from an infested watercraft. Combining the methods above ensures that the decontamination process is removing mussels and killing them. To fully kill and remove dreissenids from watercraft, reducing the biological risk, a combination of high pressure and hot-water spray should be used.

REFERENCES

Claxton, W.T., A.B.Wilson, G. L. Mackie and E. G. Boulding. 1998. A genetic and morphological comparison of shallow- and deep-water populations of the introduced dreissenid bivalve *Dreissena bugensis.* Canadian Journal of Zoology, 1998, 76(7): 1269-1276, 10.1139/z98-064.

Comeau, S., S. Rainville, B. Baldwin, E. Austin, S. Gerstenberger, C. Cross, and W.H. Wong. 2011. Susceptibility of quagga mussels (*Dreissena rostriformis bugensis* Andrusov) to hot-water sprays as a means to mitigate biofouling. Biofouling *27:* 267-274.

Elderkin, C.L. and P.L. Klerks. 2005. Variation in thermal tolerance among three Mississippi River populations of the zebra mussel, *Dreissena polymorpha.* Journal of Shellfish Research 24(1):221-226. 2005.

Harrington, D.K., J.E Van Benschoten, J.N. Jensen, D.P. Lewis, and E.F. Neuhauser. 1997. Combined use of heat and oxidants for controlling adult zebra mussels. Water Research *31:* 2783-2791.

Kappel, M. 2012. *Dreissena rostiformis bugensis*: desiccation of adult quagga mussels found in Lake Mead as a preventive measure against overland dispersal in the western United States. UNLV Theses/Dissertations/Professional Papers/Capstones. Paper 1744.

Mangin, S. 2011. The 100th Meridian Initiative: a strategic approach to prevent the westward spread of zebra mussels and other aquatic nuisance species. US Fish and Wildlife Service.

McMahon, R.F. and T.A. Ussery. 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, E.L., G. Rosenberg, A.P. Spindle, M.L. Ludyanskit, Y. Pligin and B. May. 1996. A review of the biology and ecology of the quagga mussel (*Dreissena bugensis*, a second species of freshewater dreissenid introduced to North America. Amer. Zool. (1996) 36: (3) 271-286.

Morse, J.T. 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.

Piola, R.F., K.A.Dafforn and E.L. Johnston. 2009. The influence of antifouling practices on marine invasions. Biofouling *25*(7): 633-644.

Spidle, A.P., B. May and E.L. Mills. 1995. Limits to tolerance of temperature and salinity in the quagga mussel (*Dreissena bugensis*) and the zebra mussel (*D. polymorpha*). Can. J. of Fish. and Aquat. Sci. 1995, 52 (10):2108-2119, 10. 1139/F95-804.

Zook, B. and S. Phillips. 2012. 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. http://www.aquaticnuisance.org/wordpress/wp-content/uploads/2010/01/UMPS_II_doc2_APRIL_5_2012_FINAL_final_edits.pdf. (Retrieved 05 April 2014).