Using Pressurized Hot Water Spray to Kill Quagga Mussels on Watercraft and Equipment: Field Testing on the Effects of Water Temperature and Duration of Exposure

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David Wong has 10 years experience in studying quagga/zebra mussels in the Mid-West and East. He has a Ph.D. in Ecology (Bivalve) and a Master and Bachelor in Fisheries. Principal or Co-Principal investigator (PI or Co-PI) for the following projects: 1) Effectiveness of using redear sunfish to control invasive quagga mussels in Sweetwater Lake, California (Funded by Sweetwater Authority, 2009-2010); 2) Trophic interactions among threadfin shad (*Dorosoma petenense*) and quagga mussels (*Dreissena bugensis*) in Lake Mead (Funded by Great Basin Ecosystem Study Unit, 2008-2009); 3) Selective feeding behavior of zebra mussels (Funded by National Science Foundation and Hudson River Foundation, 2000-2002). He's been an invited editor and reviewer for more than 10 scientific journals and federal agency proposals.

Shawn Gerstenberger has facilitated a quarterly quagga mussel meeting in the Lower Colorado Region since early 2007. There are 29 agencies from Nevada, California, and Arizona attending this meeting to update discoveries and progress about their projects. He has Ph.D. and Master in Aquatic Toxicology and a Bachelor in Reclamation. He is the PI for these projects: 1) Quagga mussel and boat survey in Lake Mead (Funded by 100th Meridian, 2007-2009); 2) Limnological Assistance for the Lake Mead NRA in meeting the challenge of the Water 2025 Initiative (Funded by Nevada Public Lands Management Initiative, 2008-2012).

Wen Baldwin is currently a NPS Volunteer. He is a past President of the Lake Mead Boat Owners Association and ANS Technical Representative for the Lake Mead National Recreation Area and consultant for natural resource agencies throughout the west, is generally considered to be the leading expert on the inspection and decontamination of trailed watercraft in the western US. He has inspected hundreds and decontaminated dozens of quagga mussel infested watercraft in the Colorado Basin and has conducted many trainings on this topic all over the west.

Emily Austin is currently the NPS Lake Mead National Recreation Area Quagga Mussel Coordinator. She is responsible for synchronizing containment efforts for invasive mussels in Lake Mead, and cooperates with other agencies in the southwest US to monitor and educate the public about mussels. She has a Master and Bachelor degree in Science.

This team has been actively involved in the monitoring, control, and prevention of invasive quagga mussels. Representative publications: 1) A standardized design for quagga mussel monitoring in Lake Mead, USA. *Lake and Reservoir Management*, in review; 2) Abundance and size of quagga mussels (*Dreissena bugensis*) veligers in Lake Mead, Nevada-Arizona. *The Veliger*, in review; 3) Assimilation of micro- and mesozooplankton by zebra mussels: a demonstration of the food web link between zooplankton and benthic suspension feeders. *Limnology and Oceanography* 48: 308-312 (2003); 4) Development of a fish contaminant monitoring protocol for Lake Mead, Nevada. *Lake and Reservoir Management* 18: 18-128 (2002).

Proposal Summary: **Herein we propose a scientific investigation of pressurized hot water wash applied to watercraft as a decontamination practice to determine minimal thresholds (temperature and duration) for 100% quagga mussel mortality. This project will provide valid field data on the most efficient use of hot water to decontaminate watercraft and equipment by systematically testing combinations of temperatures and application durations. In this study, the field experiments will: (1) Establish the relationships between quagga mussel mortality, hot-water spray temperatures and exposure times for watercraft areas that can be directly exposed to high-temperature spray water; (2) Determine the minimum amount of time required to reach and sustain the lethal temperature in areas (i.e., gimbal areas) which can only be indirectly exposed to hot-water sprays; 3) Ascertain the time necessary to reach and sustain the lethal temperature in areas (i.e., ballast tanks) with special temperature requirements; and (4) Validate the experimental data by decontaminating actual watercraft and equipment infested with quagga mussels at Lake Mead National Recreation Area with various combinations of spray water temperatures and exposure durations to achieve 100% quagga mussel mortality.**

Introduction - Quagga mussels (*Dreissena bugensis*) were recently found in Lake Mead, NV, and zebra mussels (*Dreissena polymorpha*) in San Justo Reservoir, CA. In the western United States, many federal, state, regional, and local agencies have initiated watercraft interception programs to prevent further infestations by these invasive mussels (Zook and Phillips 2009). These agencies most commonly decontaminate watercraft with a pressurized hot-water spray exceeding 140 ^oF. This temperature is based on acute (short-term) upper-thermal-limit data generated for continuously immersed mussels (Morse 2009). The first data set on use of hot-water spray for mitigation of emersed zebra mussels 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 ≥ 140 ^oF for 10 s or 176 ^oF at ≥ 5 s was 100% lethal to zebra mussels, which indicates that current decontamination recommendations of spray temperature of ≥ 140 ^oF may not kill all the mussels if the exposure duration is < 10 s (Morse 2009).

This new information is helpful in making standards for watercraft interception programs (Zook and Phillips 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 1). At the same time, there are also areas on watercraft that hot-water sprays cannot directly reach. We divide decontamination areas into three categories: (1) areas easy to access; (2) areas difficult to access; and (3) special areas (Table 1). These three categories of areas should be treated differently to achieve 100% quagga mussel mortality for legitimate watercraft and equipment decontamination.

Table 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, PFD's, swim platform, wetsuits and dive gear, inflatables, down-riggers and planing boards, water skis, wake boards and tow ropes, 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 have water transfer pumps that require water temperature ≤ 130°F for decontamination	Ballast tanks/bladders, washdown systems, bait and live wells, internal water systems

Morse's (2009) findings are quite useful because his study was the first to test emersed mussels and it provides a solid starting point. However, several unanswered questions remain:

1) Is the quagga mussel more or less susceptible than the zebra mussel to hot water spray? The chosen application method for hot-water spray should be species specific as 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 (McMahon 1996, Spidle et al. 1995). Quagga mussels are also reported to have thinner shells (Zhulicov et al. 2006), less tightly sealing shell valves (Claxton et al. 1997), and lower byssal thread synthesis rate 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 (Morse 2009). On the other hand, quagga mussels are thought to be more competitive since they are displacing zebra mussels in the Great Lakes. They are becoming the dominant dreissenid species due to the following reasons: A) quagga mussels have higher filtration rates and assimilation efficiency than zebra mussels

(Baldwin et al. 2002, Diggins 2001); B) quagga mussels have a lower respiration rate than zebra mussels (Stoeckmann 2003), which saves energy and leaves more available for growth and reproduction; C) quagga mussels have higher growth rates than zebras in lower food conditions; and D) quagga mussels devote a smaller portion of body tissue to reproduction than zebra mussels. In the western United States, the quagga mussel is the most widely spread dreissenid mussel (Benson 2009, Benson 2009). Therefore, to be effective and efficient in preventing further spread of these mussels, mitigation techniques such as hot-water spray need to be developed specifically for quagga mussels.

2) Are the data from studies with tap water-accustomed mussels relevant enough to be used in the field where mussels are living in the natural water? Mussels living in tap water should have very different physiological conditions from mussels living in the field. Their susceptibility to hot water could be very different as well.

3) For those areas that are inaccessible to spray treatment, how long must we apply hot water for decontamination (Morse 2009, Zook and Phillips 2009)? It will take more time to reach the lethal temperature in the hard-to-access areas, such as the gimbal areas, but there are no valid data on exactly how much time is necessary.

4) For areas where spray water cannot be ≥ 130^{o}F (i.e. ballast tanks and bladders), how long do we need to apply this relatively cooler water to kill the mussels (Zook and Phillips 2009)? The system components in these areas are not designed for temperatures greater than 130^{o}F (Zook and Phillips 2009).

In the quagga mussel lifecycle, individuals are most resilient during the adult stage. An effective protocol must effectively eliminate all adult quagga mussels. In other words, if mortality results for all adults, it can safely be said that all juveniles are eliminated as well. Thus, in the present proposal, adult quagga mussels will be used as test targets to establish standards for the conditions unique to the western environment.

The above questions need to be answered with scientifically designed field experiments that provide validated data as the basis for a sound recommendation for standard watercraft and equipment decontamination protocols for the western United States (Zook and Phillips 2009). The highest priority is to obtain valid field data on maintaining lethal temperatures for a minimum but sufficient time to achieve 100% quagga mussel mortality for federal, state, and local watercraft and equipment decontamination programs.

Goal – The overall goal is to provide valid field data on maintaining lethal temperatures for a minimum but sufficient time to achieve 100% quagga mussel mortality for watercraft and equipment decontamination in the western United States. To achieve this goal, the following four objectives must be met:

1. To provide field data on the lethal effect of hot-water spray on emersed quagga mussels in Lake Mead at different combinations of water temperature and duration of exposure. The data from this test can be applied to Category I decontamination areas.
2. To collect field data on the time required to reach and sustain lethal temperatures in the inaccessible areas such as the gimbal unit. The data from this test can be applied to Category II decontamination areas
3. To collect field data on the time required to reach and sustain lethal temperatures in special areas, such as ballast tanks where temperatures should be ≤ 130^{o}F. The data from this test can be applied to Category III decontamination areas
4. To validate the field data (Objectives 1-3) through tests on boats infested with quagga mussels at Lake Mead National Recreation Area to make sure decontamination programs with the identified standards will lead to 100% mortality.

Methods and Experimental Design – To achieve the above goal and objectives, four experiments will be conducted.

Experiment 1: Field tests on the lethal effect of hot-water spray on emersed quagga mussels. Healthy adult quagga mussels (2800 individuals) will be collected from Las Vegas Boat Harbor, Lake Mead and will be subdivided into treatment groups and exposed to air for approximately 10 min prior to treatment. One treatment group will be assigned to each combination of water temperature and duration time. For each treatment, 50 mussels will be placed in a 3 mm mesh bag, suspended over a large tank, and exposed to the pre-determined temperature/time combination (Table 2). The temperature of hot water spray will range from 68 ^{o}F to 176 ^{o}F and the spray times will range from 1 to 160 seconds. The 68 ^{o}F test temperature is used as control. The pressurized spray will be applied to each treatment group from a distance of 15 cm above the mussel-containing mesh bag at a flow rate of 900 mL min^{-1} through a fan-shaped nozzle producing a pressure of 15 psi (Morse 2009). The water temperature, on contact with the treatment group, will be monitored. After treatment, mussel groups will each be transferred to individual 500 ml beakers covered with 3 mm mesh and randomly submerged in the lake. Dead mussels will be identified as described by Morse (2009) immediately after testing and daily thereafter for 10 days (Morse 2009). An additional control group of mussels (50 individuals) is immersed continuously in the lake for 10 days. The mortality rate for different treatment conditions will be calculated (Morse 2009). The results of these calculations will establish the relationship between temperatures lethal to quagga mussels and the minimum time sufficient, at the lethal temperature, to make sure all quagga mussels were killed. The data from this test can be applied to Category I decontamination areas (Table 1) after validation (See Experiment 4 below) as hot water can be directly sprayed onto these areas.

Table 2 Combinations of temperature × duration time during hot water spray treatment to evaluate quagga mussel mortality (N = 50 adult mussels per treatment).

Temperature		1 s	2s	5s	10s	20s	40s	80s	160s
^{o}F	^{o}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

Experiment 2: Evaluation of the time needed to reach and sustain the lethal temperatures in the inaccessible areas. It will take some time for these areas to reach the lethal thermal temperature (See Experiment 1) since these areas are not directly exposed to sprays and some heat loss can occur during the water flow to these areas. The gimbal unit and internal AC cooling system will be tested and the contact temperature (internal temperature) will be monitored until it reaches the lethal temperature. The data from this test can be applied to Category II decontamination areas (Table 1) after validation (See Experiment 4 below). Since weather conditions, especially ambient temperature, could be a confounding factor to affect this, this experiment will be conducted twice, once in winter and again in summer.

Table 3 Time needed to raise contact temperature to the lethal temperature.

Temperature °F	°C	Gimbal	AC Cooling System (Internal)
104	40	x	x
122	50	x	x
130	54	x	x
140	60	x	x
158	70	x	x
176	80	x	x

Experiment 3: Field test on the time required for contact temperature in the special areas to reach and sustain the lethal temperatures. In the areas, the temperature cannot exceed 130°F (Zook and Phillips 2009). The contact temperature (internal temperature) will be monitored until it reaches the lethal temperature. Since weather conditions, especially ambient temperature, could be a confounding factor. Experiment 3 will be conducted twice, once in winter and again in summer. The time data from this test can be applied to Category III decontamination areas after they are validated (See Experiment 4).

Experiment 4: Validation on the data generated from the above three experiments. After the minimum time to kill 100% quagga mussels is identified from the above three experiments, all the data (Table 4) will be tested using 6-10 boats encrusted with quagga mussels. For each treatment, at least 5 samples from each category will be tested. As described in Experiment 1, after each treatment for each category at different conditions (Table 4), 5 mussel samples at each condition will be transferred to individual 500 ml beakers covered with 3 mm mesh and randomly submerged in the lake. Dead mussels will be identified immediately after testing and daily thereafter for 10 days (Morse 2009). An additional control group of mussels (same number of mussel individuals as each testing mussel sample) is immersed continuously in the lake for 10 days. The mortality rate for different treatment conditions will be calculated. For testing the viability of mussels living in the inaccessible areas (Experiment 2), portions of the lower unit/cooling system from 7-12 boats encrusted with mussels will be torn down. If all the data are confirmed to be valid, then all the minimum times at different lethal temperatures will be recommended as the minimum standards for watercraft and equipment decontamination programs in the western United States. Otherwise, more data need to be generated and validated in the field.

Table 4 Data generated from Experiments 1-3 need to be validated.

Temperature °F	Temperature °C	Category I areas	Category II * areas	Category III * areas
68	20	x (6 samples)		
104	40	x (6 samples)	x (6 samples)	x (6 samples)
122	50	x (6 samples)	x (6 samples)	x (6 samples)
130	54	x (6 samples)	x (6 samples)	x (6 samples)
140	60	x (6 samples)	x (6 samples)	
158	70	x (6 samples)	x (6 samples)	
176	80	x (6 samples)	x (6 samples)	

*** These two categories will be validated in winter and summer**

Statistical Analysis - For Experiment 1, the data set will include 50 mussels treated at every temperature × duration exposure combination. Under each temperature (except the control at 68°F), all the data will be modeled and analyzed together to find the minimum duration time to result in a 100% quagga mussel mortality. This minimum duration time will be used as the reference value to test, in Experiment 4, for100% quagga mussel mortality during *in situ* watercraft decontamination.

Deliverable Products - Valid field data will be provided on maintaining lethal temperatures for a minimum but sufficient time to achieve 100% quagga mussel mortality for watercraft and equipment decontamination in the western United States. These data can be used to set up standards for easily accessible areas, inaccessible areas, and areas with special temperature requirements. These field data will help policy makers to set minimal thresholds for associated decontamination and inspection parameters. A report will be submitted to the Pacific States Marine Fisheries Commission. A scientific manuscript will be submitted to the journal, *Biofouling*, for publication.

Timeline

TIMELINE
[JANUARY 2, 2010] — [SEPTEMBER 31, 2010]

PROJECT PROGRESS	START DATE	END DATE
Order Equipment	**January 2**	**January 15**
Experiment 1: Hot-water spray treatment	**January 16**	**February 15**
Experiments 2 and 3: Time to reach lethal threshold in winter	**February 16**	**February 25**
Experiment 4: Validation on data generated from Experiments 1, 2, and 3 by decontaminating 6-10 encrusted boats	**February 26**	**April 15**
Data interpretation and statistical analysis	**April 16**	**June 15**
Experiments 2 and 3: Time to reach lethal threshold in summer	**July 16**	**July 25**
Experiment 4: Validation on data generated from Experiments 1, 2, and 3 by decontaminating 6-10 encrusted boats	**July 26**	**August 15**
Statistical analysis and report	**August 16**	**September 10**
Final report	**September 11**	**September 31**
Publication*	**October 1**	**December 31**

* DELIVERABLE PRODUCT BEYOND THE PROJECT TIME FRAME

Budget

Budget Category		Cost
Personnel*	**David Wong**	**$0**
	Shawn Gerstenberger	**$0**
	Emily Austin	**$0**
	Wen Baldwin	**$21,052**
	Graduate Student (6 months)	**$9,000**
	Total Salary	**$30,052**
	Graduate Fringe (16%)	**$1,440**
	Total Salary & Fringe	**$31,492**
Equipments/Operations/Other	**General equipments (i.e. spray pumps, heater, tanks, thermometers, plastic nets, and electricity) and mechanics**	**$13,000**
Travel	**Mileage costs for local trips to all sites for supervision of project; training (about 40 trips to Lake Mead)**	**$1,600**
Total Direct Costs (TDC)		**$46,092**
Indirect Costs (44% of TDC)		**$20,280**
Total project costs		**$66,372**

*Emily Austin, Shawn Gerstenberger, and David Wong will contribute 80, 50, and 180 hours in-kind services on this project. David Wong is going to be in charge of the entire project from start to finish and will supervise the experiment (i.e., design, mussel viability test, data collection, report draft and submittal, and so on). Shawn Gerstenberger will be in charge of the operation of the entire project, such as communicating with UNLV Budget Office and submitting reports on time. Emily Austin will provide experimental locations, boats encrusted with quagga mussels, and technical assistance as needed.

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