Stephen Phillips
Pacific States Marine Fisheries Commission
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Dear Mr. Phillips,

In the previous three reports (April, June, and September of 2010), we have provided experimental data on Experiments 1, 2, and 3, which were conducted from February to early September, 2010. The current report is about Experiment 4: Field validation of lethal temperatures to kill 100% of quagga mussels in summer (September 2010) and winter (January 2011).

Part I of Experiment 4: Field data validation for killing 100% mussels on surface area

In the previous experiments, it has been found that hot-water spray at 140^{o}F for 5 s or longer can lead to 100% quagga mussel mortality (Wong et al. 2010, Comeau et al. 2011). This is different from the experiment on zebra mussels, which required 10 seconds to reach 100% mortality rate (Morse 2009). At 5 s duration, LT_{50} (time to reach 50% mortality) and LT_{99} (time to reach 99% mortality) for zebra mussels were 54.6^{o}C and 69.1^{o}C, respectively (Morse 2009), while they were 47.2 and 58.8^{o}C for quagga mussels, respectively (Comeau et al. 2011). These results suggest that quagga mussels are more susceptible to hot-water sprays than zebra mussels. This is probably due to the fact that quagga mussels have thinner shells (Zhulidov et al. 2006) and less tightly sealing shell valves (Claxton et al. 1997), which may allow the heating of the soft tissues of the quagga mussel to occur more rapidly than that of the zebra mussel. Another potential reason for this difference is the impact of ambient temperature and seasonal productivity variations on the acute thermal tolerance of dreissenid mussels (Elderkin and Klerks 2005). These factors may account for Morse's (2009) longer application time at 60^{o}C for 100% kill in zebra mussels, as dreissenid mussels tend to have higher acute thermal tolerance temperature if they are acclimated in warmer waters before treatment (McMahon and Ussery 1995). The specimens of zebra mussels from Hedges Lake (New York) used in Morse's study (2009) were acclimated to 20 ± 1^{o}C water for 2 weeks already prior to experimentation. The quagga mussels in our experiment used experienced winter water conditions (i.e., lower temperature) before treatment, may require higher temperature or longer application times to achieve 100% mortality if the mussels were taken from Lake Mead during summer time. When the surface water temperature ranges are higher (ranging from 25^{o}C and 30^{o}C) in summer time, mussels could have had elevated acute thermal tolerances. Although the data from the present experiment is in line with the report that the upper thermal limit of the quagga mussel is lower than that of the zebra mussel (Spidle et al. 1995, McMahon 1996, Mills et al. 1996), studies on quagga mussels acclimated to both summer and winter time temperatures were designed to confirm that 100% morality can be reached in the real field situation (Figure 1).

Figure 1. Field data validation on boat surface area with hot-water spray (140^{o}F/5 seconds duration)

The experiments conducted in summer (September 2010, air temperature 96.5^{o}F) and winter (January 2011, air temperature 40.9^{o}F) demonstrated that all mussels were killed by 140^{o}F hot-water within 5 seconds (Table 1). It is confirmed that **140^{o}F/5 seconds duration or longer can kill 100% quagga mussels on surface area**. It is evident that that quagga mussels are more susceptible to hot-water sprays than zebra mussels is therefore mainly due to their thinner shells (Zhulidov et al. 2006) and less tightly sealing shell valves (Claxton et al. 1997).

Table 1 Data validation on boat surface area with hot-water spray

Season	Area	Number of Mussels	Temperature of Application	Duration	Mortality rate
Summer	Area 1	206	140^{o}F/60°C	5 s	100%
	Area 2	171	140^{o}F/60°C	5 s	100%
	Area 3	293	140^{o}F/60°C	5 s	100%
	Area 4	109	140^{o}F/60°C	5 s	100%
	Area 5	82	140^{o}F/60°C	5 s	100%
	Area 6	102	140^{o}F/60°C	5 s	100%
Winter	Area 1	58	140^{o}F/60°C	5 s	100%
	Area 2	42	140^{o}F/60°C	5 s	100%
	Area 3	48	140^{o}F/60°C	5 s	100%
	Area 4	32	140^{o}F/60°C	5 s	100%
	Area 5	58	140^{o}F/60°C	5 s	100%
	Area 6	31	140^{o}F/60°C	5 s	100%

Part II of Experiment 4: Field data validation for killing 100% mussels inside gimbal units

For areas where the mussels would not receive a direct thermal spray and/or may come in contact with sprayed water as runoff from other surfaces where it may have cooled to an ineffective temperature, the application times needed to reach lethal temperature are provided in our previous reports (Wong et al. 2010). However, field validation to confirm 100% mortality is still needed. Here we reported the summer (September 2010, air temperature 96.5^{o}F) and winter (January 2011, air temperature 40.9^{o}F) validation experiment for mussels living inside the gimbal units (Figure 2).

Figure 2. Field data validation on gimbal unit area with hot-water spray (140^{o}F/5 seconds duration)

The two experiments conducted in summer confirmed that all mussels were killed while only 97% of mussels were killed in winter (Table 2). Several mussels from the 2nd sample of winter experiment were collected from the gimbal unit. The gimbal unit was flushed from the top and may not have adequately heated the mussels on the side of the gimbal unit to the appropriate lethal temperature. **In addition to the top flush at the predetermined time, in order to ensure 100% mortality, there should be a side flush on the gimbal unit for the predetermined time**. This will adequately ensure that the entire gimbal unit will be heated to the predetermined lethal temperature.

Table 2 Data validation on gimbal area with hot-water spray

Season	Area	Number of Mussels	Temperature	Time to Reach 140^{o}C	Killing Duration	Mortality rate
Summer	Gimbal 1	150	140^{o}F/60°C	42.9 s*	5 s	100%
	Gimbal 2	94	140^{o}F/60°C	42.9 s*	5 s	100%
Winter	Gimbal 1	48	140^{o}F/60°C	122 s**	5 s	100%
	Gimbal 2	70	140^{o}F/60°C	122 s**	5 s	97%

* N = 3, air temperature = 96.5^{o}F; ** N = 3, air temperature = 40.9 ^{o}F

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n the previous report, we measured the time to reach lethal temperature (130^{o}F) in the live well by filling the entire tank. In that way, the amount of time necessary to fill the entire live well is completely dependent upon the flow rate of the hose which can vary depending on the type and power of the decontamination device. In this winter, we tried a new way to test the time to reach lethal temperature (130^{o}F) in the live wells: the flush technique. In this way, we just placed the hot water down the drain directly and measured how long it reached the appropriate temperature. For the new data on live wells, the time to reach 130^{o}F was about 1'.05'' (N = 3, Air Temperature =38^{o}F). While in previous experiments, times to reach 130^{o}F, 122^{o}F, and 104^{o}F were 3'15'', 3'12'', and 3'09'' (Air Temperature= 65^{o}F), respectively. It took much longer in the previous tests. The reason is that we used an entire fill technique: Filling the entire live well with hot water and waiting for it to all drain at the predetermined lethal temperature (130^{o}F).

Recommendations

For mussels in Category I area (Table 3), hot-water sprays at 140^{o}F/60ºC for a minimal duration of 5 s should be utilized to ensure 100% quagga mussel mortality.

For mussels in the gimbal area of Category II area (Table 3), hot-water sprays at 140^{o}F/60ºC for a minimal duration of 127 s (winter validation data) should be used for boat decontamination; more importantly, both a top flush and a side flush (at the predetermined time of 127 s) are needed to ensure 100% mortality.

For mussels in live and bait wells of Category III area (Table 3), data generated from winter flushing experiments are recommended, which is hot-water sprays at 54ºC/130^{o}F for a minimal of 68.2 s for live wells and 69.3 s for bait wells.

Table 3 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

Finally, attached please find manuscript based on our previous experiment. It is accepted for publication by the journal, *Biofouling*.

Thanks again for your continuous support and we look forward to working more with you in the future.

If you have any questions, please let us know.

Sincerely,

David Wong
Shawn Gerstenberger
Wen Baldwin
Emily Austin

References

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