Final Project Report
to the Fox River Navigational System Authority
Aquatic Invasive Species Committee

Tests of Lethal Temperature Limits for Invasive Species in the Lower Fox River

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Introduction

The proposed use of a boat lift to transport vessels across the current invasive species barrier at the Rapide Croche dam on the lower Fox River, WI would potentially allow upstream passage of invasive invertebrate species attached to the hull, motor, and other external surfaces of the transported boats. To prevent this occurrence, the Fox River Navigational System Authority (FRNSA) has proposed to immerse all transported boats in a water bath maintained at 110 ^{O}F (43 ^{O}C). Motors and pumps will be run for a minimum of 1 minute after immersion in the water bath. Total immersion time will therefore be closer to a minimum of 3 – 4 minutes. At public forums conducted by FRNSA during January and February of 2008 when the planned procedures were described, some concerns were raised about the effectiveness of this protocol for killing attached invertebrate species that might be invasive. As a result of those meetings and subsequent discussions with representatives of the Wisconsin Department of Natural Resources, it was decided that more information is needed on the effectiveness of this approach.

The information currently available in the literature concerning thermal tolerance limits for potentially invasive invertebrate species of the lower Fox River is limited and variable at best. Three species are of particular importance for the lower Fox River. These are the zebra mussel (*Dreissena polymorpha*), the quagga mussel (*Dreissena bugensis*) and the spiny water flea (*Bythotrephes longimanus*) (Figure 1). Available data

Figure 1. The three species tested in this study for thermal tolerance limits: zebra mussel (left), quagga mussel (center), and spiny water flea (right).

on North American populations of the zebra mussel (*Dreissena polymorpha*) indicate that individuals can survive for extended periods at 86 ^{O}F (30 ^{O}C), but have an upper incipient lethal temperature of 88 ^{O}F (31 ^{O}C), where 50% of the tested individuals can survive for a prolonged period. Animals acclimated to higher temperatures have higher thermal tolerance values, but no zebra mussels survive at temperatures at or above 97 ^{O}F (36 ^{O}C). At or above this temperature death is essentially instantaneous (McMahon & Ussery 1995, McMahon 1996). Comparative studies between populations of zebra mussels in their native range in Europe and the North American populations show that North American populations have higher thermal tolerances than do their ancestors in Europe. This suggests that there may be a greater ability by North American populations to change their thermal tolerances based on local conditions. Generally, the quagga mussel

(*Dreissens bugensis*) is less tolerant of exposure to warm temperatures than the zebra mussel (upper incipient lethal temperatures are approximately 0.5 ^{O}C lower on average; Dom et al. 1993). Studies also have shown that the quagga mussel cannot survive for prolonged periods at temperatures higher than 86 ^{O}F (30 ^{O}C), and that less than 5% of animals survive at 102 ^{O}F (39 ^{O}C) after acclimation to 68 ^{O}F (20 ^{O}C)(Mills et al. 1996). For the spiny water flea (*Bythotrephes longimanus*), Yurista (1999) determined the upper lethal limit to be 74^{o}F (23 ^{O}C), probably due to inactivation of respiratory enzymes. These data are consistent with field and laboratory data for spiny water fleas (Garton et al. 1990).

The fact that North American populations have shown the ability to shift thermal tolerances related to local conditions indicates the need for tests performed on local populations. In addition, none of the datasets available are directly applicable to the situation expected with the Rapide Croche boatlift because of the extremely short, acute immersion of the animals while attached to the external surfaces of the boats. Therefore, in order to provide a robust analysis of the proposed protocol for preventing the upstream transfer of invertebrate invasive species across the Rapide Croche barrier, we conducted a set of experiments to determine the effective combinations of time and temperature of immersion that would meet or exceed lethal temperature limits for the zebra mussel, the quagga mussel, and the spiny water flea.

Methods

Test Animal Collection

Zebra mussels (*Dreissena polymorpha*) and quagga mussels (*D. bugensis*) were collected by hand from rocks in approximately 1 m of water from either Lake Winnebago or Green Bay, WI between 25 June and 18 August 2008 (Table 1). Zebra mussels from Lake Winnebago were retrieved from the northwestern end of the lake near Lighthouse Reef (N 44^{O} 11.72', W 88^{O} 25.15'). Quagga mussels were collected from two sites in Green Bay just south of Egg Harbor, WI (Site A: N 44° 59.035', W 87° 21.725'; Site B: N 45° 00.938', W 87° 19.902'). Rocks with mussels attached were transported to the laboratory in ambient lake water. In the laboratory individual mussels were removed from rocks and maintained for at least 24 hours at 68 ^{O}F (20 ^{O}C) before being used in experiments to reduce artifacts due to stress of collection and transfer.

Table 1. Specimen collection information for animals tested during summer 2008 for thermal tolerance limits. See text for latitude and longitude of sampling sites.

Species Collected	Date	Location	Number Collected
Zebra mussel	6/23/08	Lake Winnebago	100
Zebra mussel	6/30/08	Lake Winnebago	400
Zebra mussel	7/9/08	Lake Winnebago	500
Zebra mussel	7/15/08	Lake Winnebago	250
Spiny water flea	7/22/08	Green Bay C	100
Quagga mussel	8/4/08	Green Bay A	130
Quagga mussel	8/4/08	Green Bay B	170
Quagga mussel	8/11/08	Green Bay B	350
Quagga mussel	8/18/08	Green Bay B	100
Spiny water flea	8/18/08	Green Bay C	350
Spiny water flea	8/25/08	Green Bay C	300

Samples of spiny water fleas (*Bythotrephes longimanus*) were obtained from the eastern area of Green Bay, Lake Michigan, northwest of Chaudoir's Dock, Door County, WI (N 44^{O} 45.06', W 87^{O} 42.19') between 22 July and 25 August 2008. Samples were collected with oblique tows from the upper 10 m of the water column using a Wisconsin-type net (opening diameter = 0.52 m, mesh size = 308 um), and diluted into 20 L buckets containing ambient water and covered to exclude light during transport back to the laboratory. Experiments were run either on the same day of collection following a short acclimation period at 68 ^{O}F (20 ^{O}C) or within 48 hours of collection.

Experimental Design

Animals were exposed to combinations of temperature and time of immersion to assess thermal tolerance limits. Replicate sets of 10 individuals were tested in each treatment combination (Table 2). Together all the possible combinations of temperature and immersion time resulted in 25 unique treatments, employing 1250 individual zebra mussels and 750 each of quagga mussels and spiny water fleas. Six separate non-

circulating water baths were filled with approximately two gallons of aged tap water (aerated for a minimum of 2 weeks) treated with AmQuel Plus water conditioner (Kornon, LLC) to remove ammonia, chlorine and chloramines. Before each set of experiments the temperatures and time immersion were randomly assigned to each bath. Only actively siphoning mussels and swimming water fleas were selected for the experiment, ensuring that the animals were alive before exposure to treatment conditions.

Table 2. Number of replicate sets of 10 individual zebra mussels tested in each combination of temperature and time of immersion during summer 2008. The design employed for the quagga mussel and the spiny water flea experiments was the same except that three replicates were run for each treatment combination.

		Temperature				
		90° F	100° F	110° F	120° F	130° F
		32° C	38° C	43° C	49° C	54° C
Time	**1 min**	5 reps	5 reps	5 reps	5 reps	5 reps
	5 min	5 reps	5 reps	5 reps	5 reps	5 reps
	10 min	5 reps	5 reps	5 reps	5 reps	5 reps
	15 min	5 reps	5 reps	5 reps	5 reps	5 reps
	20 min	5 reps	5 reps	5 reps	5 reps	5 reps

Sets of ten individual adult mussels (shell length greater than 5 mm) were placed into the water bath, directly onto the stainless steel plate supported above the heating element on the bottom of the bath, somewhat replicating the situation of individual mussels attached to the surface of a boat. As visual surveys of boats prior to immersion in the hot water bath will exclude boats with attached druses (clumps of mussels), only individual mussels were tested. After the prescribed time of immersion had elapsed, the mussels were removed from the bath with forceps and placed into an ambient recovery bath maintained at 68 ^{O}F (20 ^{O}C). After 20 minutes in the recovery bath, the mussels were removed to separate dishes of aged tap water to determine if they were alive or dead. Mussels were considered dead if the intake siphon was not withdrawn after being poked with a probe (McMahon & Ussery 1995). If mussels were closed they were tested for shell closure ability. Dead mussels opened readily under slight pressure, whereas it was not possible to open the shells of live mussels. The length of each mussel and whether or not it survived were recorded.

Using a large bore bulb pipette (1 cm inner diameter), spiny water fleas were transferred from the storage bucket to a PVC cup with a mesh bottom (308 μm-mesh). The mesh bottom allowed immersion of individuals in the water bath but retained them in the cup for easy removal and counting. Care was taken to ensure that water fleas were continuously immersed in water during the entire procedure. After the prescribed

immersion period the cup containing water fleas was placed in the recovery bath for 20 minutes. The cup was removed to a dissecting microscope and the individuals were examined to determine if they were alive (observable movement) or dead (no movement; as used by Garton et al. 1990). The eggs of gravid females were examined for viability. Opaque eggs were considered nonviable. For spiny water fleas used in the experiments the number of opaque versus translucent brood pouches was determined to provide information on the thermal tolerance of eggs carried by females.

Results

Zebra mussel thermal tolerance

We tested 1249 adult zebra mussels for thermal tolerance to immersion in heated water for various amounts of time. Zebra mussels showed a clear response to increasing temperature of immersion. Very few individuals were killed at 90 ^{O}F whereas all individuals were killed at temperatures of 120 ^{O}F or greater, no matter how long they were immersed (Table 3 and Figure 1). In 100 ^{O}F water, time of immersion was critical and mortality increased with time. Percent killed jumped from 6% after 1 min of immersion to between 30 – 40% for 5 min to 15 min of immersion. Mortality again increased to over 80% when animals were immersed for 20 min. At 110 ^{O}F about 25% of the animals were killed after 1 min of immersion. All individuals were killed when immersed at 110 ^{O}F for 5 min or longer.

Table 3. Mean percent of zebra mussels that were dead at the end of the recovery period in the thermal tolerance experiments during summer 2008. Percent values are based on five replicate trials of 10 individuals immersed for the indicated period of time.

		Temperature				
		90° F	100° F	110° F	120° F	130° F
		32° C	38° C	43° C	49° C	54° C
Time	**1 min**	2.0	6.0	24.0	100.0	100.0
	5 min	0.0	38.0	100.0	100.0	100.0
	10 min	0.0	34.0	100.0	100.0	100.0
	15 min	4.0	30.0	100.0	100.0	100.0
	20 min	0.0	84.0	100.0	100.0	100.0

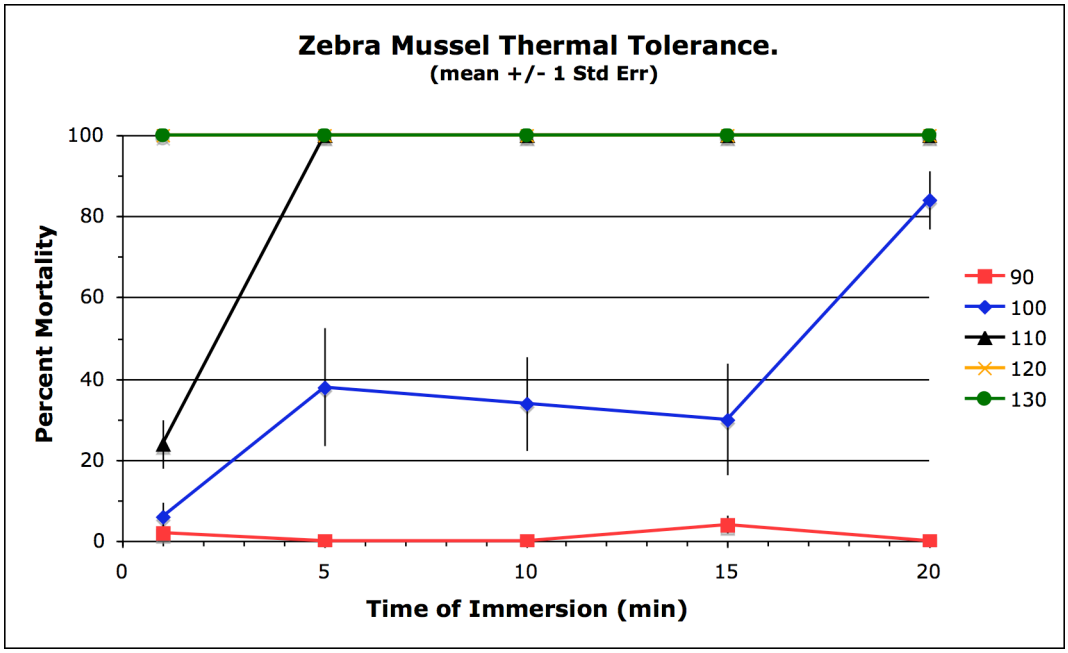

Figure 2. Mean percent mortality (+/- 1 standard error) of zebra mussels versus time of immersion at five water temperatures (degrees Fahrenheit) during summer 2008.

Quagga mussel thermal tolerance

Quagga mussels demonstrated thermal tolerances very similar to those observed for zebra mussels, but had slightly greater mortality at a given temperature (Table 4). Based on our results for the 750 adult quagga mussels tested, immersion in 90 ^{O}F did not kill any individuals but exposure to 100 ^{O}F killed an increasing percentage as time of immersion increased (Figure 3). Exposure to 110 ^{O}F for 1 min killed approximately 60% of individuals, and immersion for 5 min or longer killed 100% of the specimens tested. No animals survived any length of exposure at 120 or 130 ^{O}F.

Table 4. Mean percent of quagga mussels that were dead at the end of the recovery period in the thermal tolerance experiments during summer 2008. Percent values are based on three replicate trials of 10 individuals immersed for the indicated period of time.

		Temperature				
		90° F	100° F	110° F	120° F	130° F
		32° C	38° C	43° C	49° C	54° C
Time	**1 min**	0.0	3.3	60.0	100.0	100.0
	5 min	0.0	16.7	100.0	100.0	100.0
	10 min	0.0	80.0	100.0	100.0	100.0
	15 min	0.0	90.0	100.0	100.0	100.0
	20 min	0.0	100.0	100.0	100.0	100.0

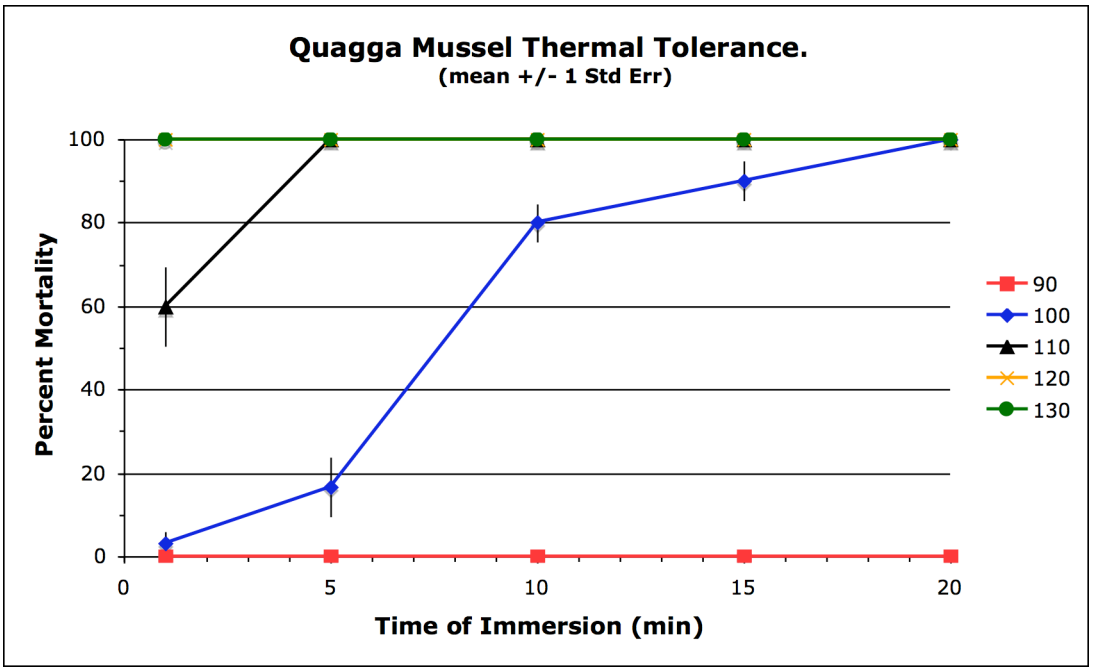

Figure 2. Mean percent mortality (+/- 1 standard error) of quagga mussels versus time of immersion at five water temperatures (degrees Fahrenheit) during summer 2008.

Spiny water flea thermal tolerance

Results obtained from the 750 spiny water fleas tested showed a similar pattern as for the mussels, but with more variability. Essentially no individuals were killed at 90 ^{O}F unless they were immersed for 20 min (Table 5). With increasing time of exposure an increasing percentage was killed at 100 ^{O}F (Figure 4). Thirty percent of individuals were killed after 1 min immersion at 110 ^{O}F, while essentially all individuals were killed with 5 min or longer immersion. The mortality level observed after 5 min immersion at 110 ^{O}F was not significantly different than 100%, as shown by the 95% confidence interval including 100% (P>0.05, df=2). No individuals survived immersion at 120 or 130 ^{O}F.

Table 5. Mean percent of spiny water fleas that were dead at the end of the recovery period in the thermal tolerance experiments during summer 2008. Percent values are based on three replicate trials of 10 individuals immersed for the indicated period of time.

		Temperature				
		90° F	100° F	110° F	120° F	130° F
		32° C	38° C	43° C	49° C	54° C
Time	**1 min**	0.0	13.3	30.0	100.0	100.0
	5 min	0.0	63.3	96.7	100.0	100.0
	10 min	5.0	50.0	100.0	100.0	100.0
	15 min	0.0	75.0	100.0	100.0	100.0
	20 min	26.7	83.3	100.0	100.0	100.0

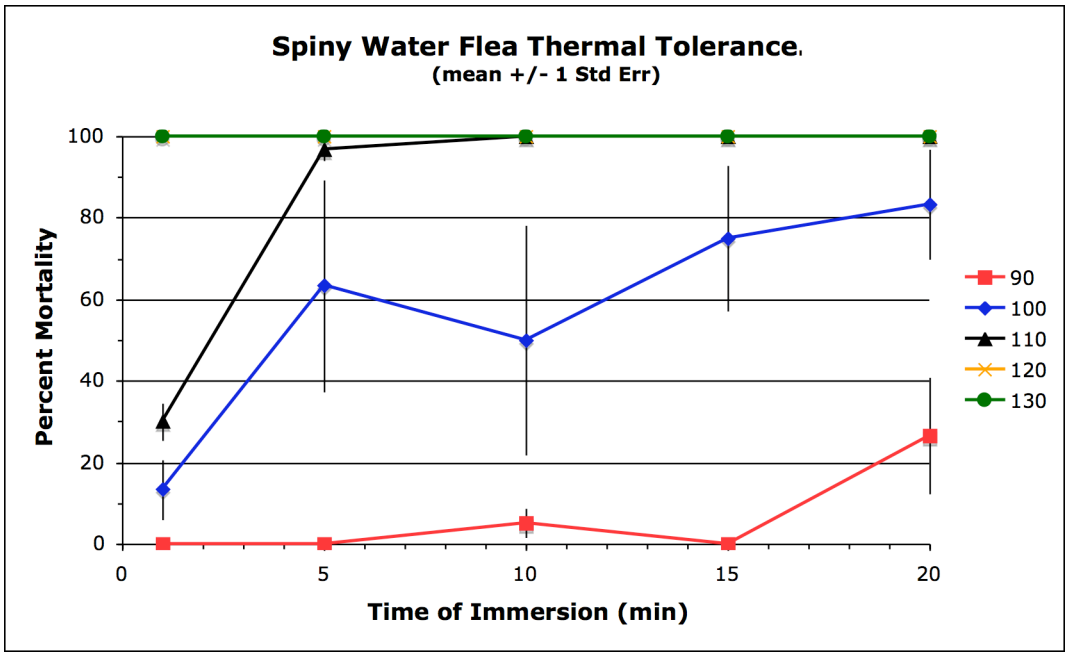

Figure 3. Mean percent mortality (+/- 1 standard error) of spiny water fleas versus time of immersion at five water temperatures (degrees Fahrenheit) during summer 2008.

Mussel size and mortality

We did not specifically test whether size of adult zebra mussel affected survivorship in our experiments, but we did measure the size of all mussels that survived and all individuals that died following immersion. Based on these measurements there do not appear to be any differences related to shell length in ability to survive immersion in hot water (Figure 4). The size distribution of animals that died at each temperature essentially mimicked those that survived. Demonstrating that size does not determine ability to survive immersion would require tests that employ larger numbers of some size classes. However, our data do suggest strongly that mortality following immersion in hot water should not depend on size for zebra mussels larger than 1 cm shell length.

Spiny water flea egg viability

Similar to our assessment of size and mortality of zebra mussels, we did not specifically test spiny water flea egg mortality following immersion in hot water. Out of a total of 77 brood pouches observed on females, only 11(14.3%) appeared viable following immersion (Table 5). Viability may have been higher at lower temperatures and longer times of immersion, but overall numbers are low and make such conclusions tentative at best. Some females on 22 July 2008 and some during the August sampling dates were carrying resting eggs instead of immediately hatching eggs. There was no way to determine viability of resting eggs during the experiments.

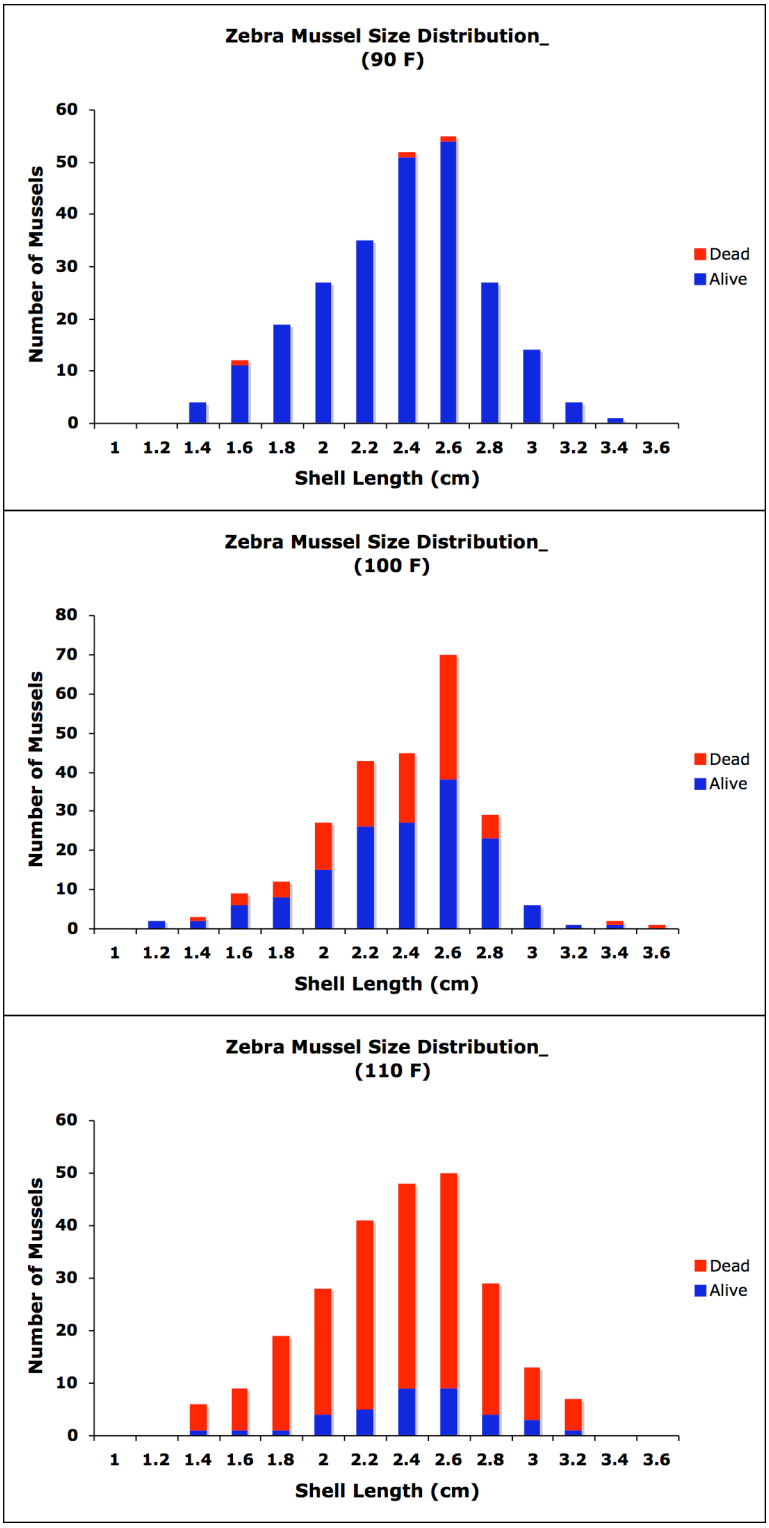

Figure 4. Size distributions of zebra mussels that survived and died after immersion at 90 ^{O}F (top), 100 ^{O}F (middle), and 110 ^{O}F (bottom) during experiments in summer 2008. Data are not shown for experiments at 120 ^{O}F and 130 ^{O}F since all animals died at those temperatures.

Table 6. Number of spiny water flea brood pouches observed in the experiments and categorization of viable brood pouches according to temperature and time of immersion.

					Viable Pouches			
	Total	Total						
Temp	Pouches	Viable	Percent	1 min	5 min	10 min	15 min	20 min
90	1	1	100.0					1
100	10	8	80.0		1	1	4	2
110	26	2	7.7		1			1
120	25	0	0					
130	15	0	0					
Totals	77	11	14.3	0	2	1	4	4

Summary

Results from our study provide a rigorous determination of the combinations of immersion time and temperature required to achieve 100% mortality of three major invertebrate invasive species of concern in the lower Fox River. A 5 min immersion at 110 ^{O}F will ensure 100% mortality of all three species tested (zebra mussel, quagga mussel, and spiny water flea). Immersion for 1 min at 100 ^{O}F will only ensure approximately 25% mortality for zebra mussels, 60% for quagga mussels and 30% for spiny water fleas.

Our findings are consistent overall with the data presented in the literature, except that the populations of zebra and quagga mussels tested appear to have somewhat higher thermal tolerances than those previously studied. While neither species could tolerate long-term immersion at temperature higher than 90 ^{O}F, many did survive exposure to 110 and 110 ^{O}F. Published reports indicated that zebra mussels would be killed essentially instantaneously at any temperature above 97 ^{O}F (McMahon 1996). Also, our results are consistent with previous reports showing that quagga mussels are less tolerant than zebra mussels of exposure to high temperatures. Published relationships describing survivorship using acclimation temperature, exposure temperature and shell length suggest that larger zebra mussels are less tolerant of heat exposure than smaller individuals (McMahon et al. 1994, McMahon 1996). Our data do not support this finding, but the lack of specific manipulation of test specimen size in our experiments prevent us from drawing firm conclusions from these data.

Finally, the results for spiny water fleas indicate that essentially all adult water fleas will be killed after a 5 min exposure to 110 ^{O}F, and that only 2.3% of the egg pouches observed appeared to survive that treatment or anything longer or warmer. We did not directly test viability of spiny water flea eggs, so this conclusion is also speculative at best. The observation that female spiny water fleas were carrying resting eggs as early as July in Green Bay is of concern since those resting eggs are likely to survive better than the immediately hatching type. Production of resting eggs in the middle of summer is unexpected based on previously published information on the life history of spiny water fleas, where resting egg production typically has been seen in fall (e.g. Brown and Branstrator 2005).

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