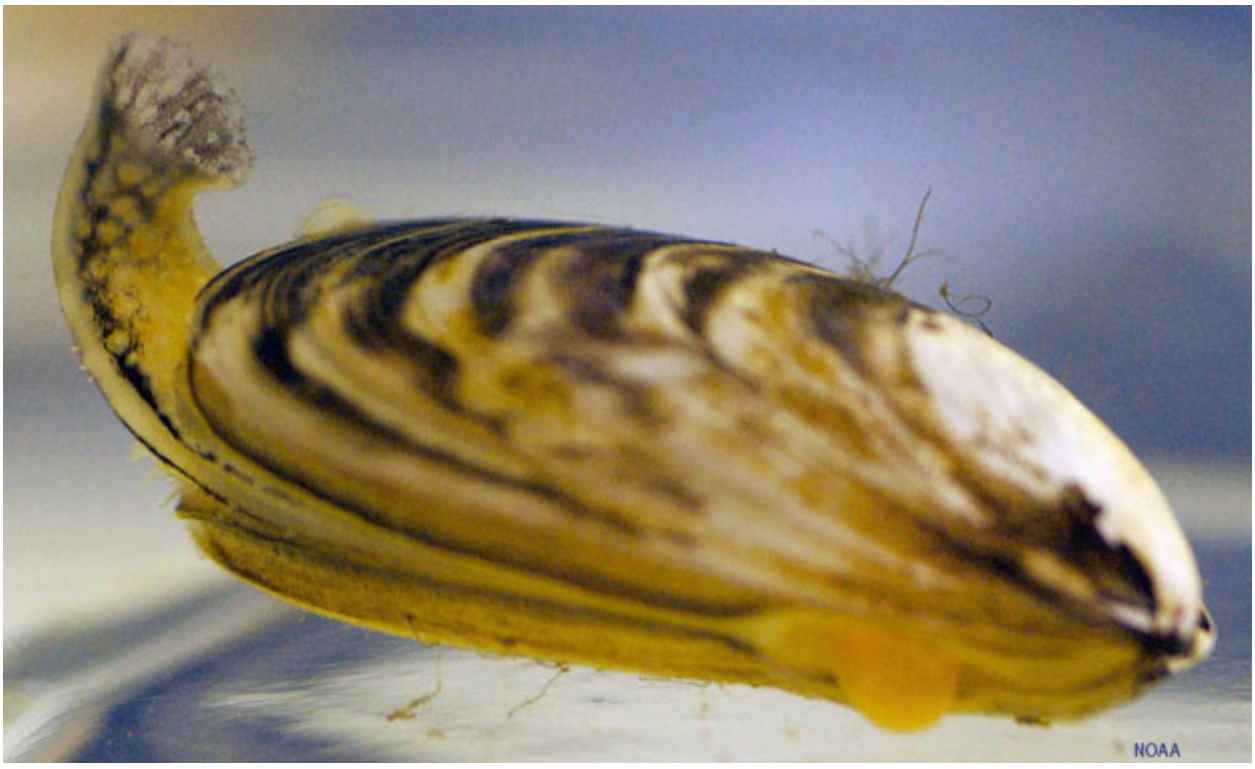
EVALUATION OF QUAGGA MUSSEL VELIGER THERMAL TOLERANCE

FINAL REPORT – JANUARY 2011 RESEARCH SESSION



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Introduction

Quagga mussels *Dreissena rostriformis bugensis* are a mussel species native to the Northern Caspian Sea. Quagga mussels were introduced to Lake Erie via ship ballast water in 1989 (May and Marsden 1992, Orlova et al. 2005). Since their introduction and establishment in North America, quagga mussels have spread to many waters, now occurring in the Laurentian Great Lakes, Mississippi River Basin, Colorado River basin, and other, scattered locations in the Western U.S. (Figure 1, USGS 2011). In Colorado, evidence of quagga mussels has been found in Lake Granby, Grand Lake, Jumbo Reservoir, Lake Pueblo, Shadow Mountain Reservoir, Tarryall Reservoir, and Willow Creek Reservoir. Their presence is suspected but currently unconfirmed in Blue Mesa Reservoir.

Quagga mussels, and their congener *D. polymorpha*, the zebra mussel, filter feed upon suspended phytoplankton. Adults can achieve very high densities in the habitats they colonize. These mussels often significantly change the aquatic food web through their large-scale removal of suspended organic matter (Kissman et al. 2010). Studies have noted a widespread shift towards oligotrophic conditions in offshore Lake Erie after quagga mussel establishment, similar to conditions found in Lake Superior (Fahnenstiel et al. 2010). The possible impacts of quagga mussels on sport fish populations are not fully understood. However, it is likely that the large-scale removal of suspended phytoplankton could reduce overall food web productivity and drastically alter food web dynamics and structure. Additionally, Dreissenid mussels settle upon solid substrates and attach via byssal filaments, often damaging or fouling machinery (Figure 2). These mussels can negatively affect hydroelectric power plants, water control structures, hatchery water intakes, and boat motors. For these reasons, controlling the spread of Dreissenid mussels is of high priority for many state and federal agencies.

Transportation of Dreissenid mussels via recreational watercraft is a potential vector facilitating their colonization of new bodies of water. During the recreational boating season, the Colorado Division of Wildlife, Colorado State Parks, and various local agencies operate mandatory boat inspection checkpoints at waters known to contain quagga mussels, and at waters deemed to be at high risk of being invaded by the mussels. Boaters are required to clean all plants and sediment from their watercraft and drain all spaces on the boat and trailer that contain water. Boaters are also asked to allow their watercraft to fully dry before launching in another body of water. These requirements are thought to be the best means of preventing the spread of mussels to uninfected waters.

Unfortunately for boaters and boat inspectors, dreissenid mussels are microscopic during their larval, planktonic life stage. Dreissenid mussels at this stage of development are called veligers, and typically range in size from 50 to 400 µm (Ackerman et al. 1994). At this size, they are nearly invisible to the naked eye, and unlikely to be detected during a boat inspection. Very small spaces containing a small amount of water could also contain veligers and potentially facilitate colonization of new waters, if the veligers survive the pre-launch period. Survival will likely depend upon a number of factors, including the availability of dissolved oxygen, possibly the degree of mixing or agitation, and the thermal conditions in those spaces.

Overall, the thermal tolerance of quagga mussel veligers is a key factor influencing their ability to spread to uninfected waters. However, few data on this topic have been collected. The upper thermal limit of the adult life stage of *D. rostriformis* is believed to be approximately 30.5º C (Dyga and Zolotareva 1976, cited by Karatayev et al. 1998). At the time of writing, data regarding the upper thermal limit of the veliger life stage of *D. rostriformis* were lacking. Two studies regarding the veliger life stage of *D. polymorpha* (Lewandowski and Ejsmon-Karabin

1983, cited by Karatayev et al. 1998, Shevtsova 1968a) have recorded upper thermal limits of 29º C and 30º C, respectively. If conditions inside the small volumes of water exceed these temperatures, then the veligers would perish. At lower temperatures, it is likely that veliger survival will be prolonged, possibly as a function of temperature.

During this study, we sought to evaluate the thermal tolerance of the veliger life stage of *D. rostriformis* in conditions similar to those it would encounter on a boat traveling between bodies of water. We hypothesized that veligers contained in interstitial spaces on recreational boats would encounter darkness, limited oxygen availability, and entrainment in a small volume of water. We chose to test veligers at temperatures near the presumed high and low limits of their tolerance, in an attempt to verify these assumptions. We hypothesized that quagga mussel veliger survival would be negatively influenced by both increasing water temperature and increasing time spent in the static water baths.

Materials and Methods

Veliger Collection

Quagga mussel veligers were collected at Willow Beach National Fish Hatchery (WBNFH) in Willow Beach, Arizona, USA from 6 January 2011 to 9 January 2011. A 35 µm nylon mesh plankton net (Aquatic Eco-Systems model PKN1) placed underneath a pipe delivering water from Lake Mojave to the indoor raceways at WBNFH was used to collect veligers. Collecting periods averaged 6 hours in length. After collection, veligers were transferred from the 1-liter cod end of the net into a bucket containing 3 to 3.5 liters of fresh water from the same inflow line (to create six or seven 500 ml samples, respectively). This water was stirred to mix and suspend veligers, detritus, and other collected microorganisms, and a 0.5-liter sub-sample of water was collected from the bucket.

Veliger Holding System

Veligers were held in custom-made water baths. To achieve and maintain desired water temperatures, we utilized a system of 94.6 liter Coleman Xtreme coolers connected to Aqualogic Delta Star ¼ hp chillers (model AE3B) 10° and 15° C treatments, a Current USA 1/10 hp Prime Inline Aquarium Chiller for the 30° C treatment, and titanium submersible aquarium heaters (Won Pro Heat) (Figure 4). The 35° C treatment water bath used dual titanium aquarium heaters and no chiller. Water was circulated through high-pressure silicone tubing from the water bath to the chillers by Danner Supreme MD12 model pumps in the 10, 15, and 30° C treatments, while a larger model, the Danner Supreme 36A, was used in the 35° C system (Figure 3). The water bath systems were regularly monitored to ensure proper operation, and temperatures were continuously recorded using temperature loggers (Hobo brand Pendant Temperature Data Logger) to verify that the desired temperatures were achieved (Table 1).

Veliger Thermal Tolerance Testing

The sub-samples from the water inflow pipe were filtered through 35-micron nylon mesh, and the filtered matter was transferred into opaque high-density polyethylene sample bottles (250 ml; Figure 4). These bottles were then filled to capacity with fresh water from the same Lake Mojave water supply line. Slight pressure was applied to the outside of the bottles to compress them and eliminate any dead air space, and the tops were tightly screwed on.

Sample bottles were then tempered to within 2.8° C (5° F) of their static water bath temperature, loaded into the screen used to hold them within the cooler system (Figure 5), and submerged in the recirculating water baths until later observation (due to time constraints related to WBNFH hours of operation, samples in the 30°C water bath were placed in the cooler with both samples and the cooler water temperature near 12°C; warming took place over the next 25 hrs. 45 min. within the cooler). At the time of submersion, for all treatments excluding the 10° C, one bottle was set aside and observed to serve as a "time 0," or initial survival sample. This sample was sorted and the numbers of living and dead veligers were recorded. With these initial observations, we sought to ascertain background levels of veliger mortality, possibly due to collection and handling stress.

Sample bottles were removed and samples examined at regular intervals after immersion (Table 1). As each sample took considerable time to review, the investigators sought to follow this schedule as closely as possible, while still spending ample enough time on each sample to ensure high levels of accuracy. It was expected that samples within the colder temperature treatments (e.g., 10°C and 15°C) would survive for longer periods of time than those in the warmer treatments (e.g., 30°C and 35°C). Therefore, planned intervals between removal events were longer for the colder temperature bottles and shorter for the warmer temperature bottles. Note that the final (120 hrs) sample from the 35°C water bath was omitted no living veligers had been

observed in bottles removed before. All samples were disposed of back into the wastewater effluent at WBNFH

After removal from the cooler, samples were again strained through 35-micron nylon mesh and transferred to a petri dish. Before observation, samples from the 10° and 15° C treatments were allowed to warm for 10 minutes above the microscope lamp to stimulate movement in living veligers. All samples were observed at 30× magnification. Veligers were considered alive if they were seen to move or react to the touch of a dissecting probe, similar to the methodology used by Sykes (2009). Those exhibiting no movement or reaction to touch were considered dead. Data recorded included the time each sample was placed in and removed from the cooler, total time spent within the cooler, and numbers of live and dead veligers. The time and proportion of surviving veligers were recorded for use in the statistical analyses.

Statistical Analyses

All statistical analyses were performed using SAS 9.2. Effects were considered significant at the α=0.05 level. The effects of water temperature, time, and the time × temperature interaction on the proportion of surviving veligers were evaluated using a least squares linear regression analysis with the PROC reg command. Temperature data for all four coolers were also compared via one-way ANOVA using the PROC glm command. Mean temperatures for each cooler were individually compared using Tukey's adjustment for multiple comparisons (SAS 9.2, α=0.05) (Kramer 1956, Tukey 1953).

Results

In total, 25 samples were observed. The 10, 15, and 30°C data were included in the linear regression analysis, but because none of the veligers survived in the 35°C treatment, their data were excluded. As mentioned above, we considered time, temperature, and time × temperature interaction variables in our regression model. The time and temperature × time interaction variables both significantly affected veliger survival (P=0.0170 and 0.0002, respectively) with increased holding time and increased holding time and increasing temperature leading to lower survival rates. Surprisingly, the temperature variable was not found to be significant (P=0.5030). Mean temperatures for all four coolers were found to be significantly different (P<0.0001), and the difference in mean temperature for each cooler when compared to all other coolers individually was found to be significantly different (P<0.0001 for all comparisons).

Temperature logger data showed that all water baths, aside from the 35° treatment, stayed within 2°C of the nominal temperature during the study period (Table 2). The difference between the lower temperature water baths and the 35°C water bath likely stems from the different system design wherein there was not a chiller counter-balancing the output of the heaters. Temperature data from the 35° cooler shows a higher range of variability, as well as one event during which the temperature dropped significantly due to heater failure. Furthermore, maximum temperature in this cooler approached 40° C, instead of the 35° C that was desired. Possible solutions to this wider variability in temperature control are discussed below.

Discussion

Our results suggest a direct relationship between quagga mussel veliger mortality and increasing temperature and time spent in the static water baths (Figure 6). As expected, increasing the water temperature resulted in a decreased survival rate for the quagga mussel veligers; the predicted survival times ranged from less than a day at 35°C to at least 24 days at 10°C. Interestingly, while the temperature variable itself was not found to be significant at the $\alpha=0.05$ level, this could result from the small sample size (n=25) used in this iteration of the study. Overall, these findings support our hypotheses that increasing temperature and time within the water baths will increase mortality levels of *D. rostriformis bugensis*. Subsequently, understanding the relationship between time and temperature will prove useful for managing the spread of the mussels in residual water in boats and other water craft.

One hundred percent mortality was noted in all samples from the 35°C treatment. This supports the findings described by Karatayev et al. (1998), and suggests that pressure-washing boats at a high temperature, a disinfection method practiced by multiple agencies, is an effective and rapid decontamination method. It should be noted, however, that our level of precision was inadequate to determine the exact upper thermal maxima for quagga mussel veligers collected from Lake Mojave during the winter; such a study would be beneficial from both a mussel management and ecology standpoint.

It is noteworthy that we initially had difficulty determining whether a veliger was alive and dormant, or dead, in the 10°C treatment, due to a lack of movement in all veligers observed. Future investigators should note that allowing these samples to warm before observation stimulated movement in the cilia, velum, and other internal organs. Typically, 10 to 15 minutes of warming was sufficient to increase activity levels in living veligers to where they could be detected.

The methodology used in our study does not allow us to rule out anoxia as a factor that influenced mussel survival in our sample bottles. All bottles that contained veligers were designed to be airtight, so it is possible that as the metabolic activity of veligers and other microorganisms consumed the oxygen trapped within each bottle, hypoxia influenced the mortality of veligers. However, veligers being transported between bodies of water within the interstitial spaces of a recreational boat would likely encounter similar conditions. So, while our data may not give exact thermal tolerances of these veligers, it should serve as a general prediction of mortality rates in veligers transported by recreational watercraft. Nevertheless, future studies (see below) should compare mortality of veligers in open (but not actively aerated) and closed containers to see how dissolved oxygen levels are related to veliger mortality.

Proposed Future Work

The work complete in January 2011 did not completely exhaust the funds that the Colorado Division of Wildlife provided to Colorado State University for this project. Therefore we would like to request permission to conduct a set of follow-up studies to clarify some of the results we observed during the January trip to the Lake Mojave area. The goals of this follow-up trip would be:

1. To replicate the study conducted in January 2011, but with greater precision (more replicates per time interval) and resolution (more time intervals) to allow better prediction of the effects of time and temperature on veliger mortality.
2. To conduct a parallel study using *open* holding containers to allow comparison of mussel veligers in open, unstirred containers with veligers in sealed containers.
3. If time and facilities are available, conduct a short study on the upper temperature tolerance of the veligers.

This work would be conducted either at the Willow Beach National Fish Hatchery or at the Nevada Department of Wildlife Lake Mead Hatchery by CSU personnel. Unlike the January 2011 trip, a group of 3 – 4 individuals will conduct this work, which will facilitate the processing of larger numbers of water samples.

Future studies, with greater sample sizes and shorter times between sampling intervals would be helpful in determining survival times at temperature of *D. rostriformis bugensis* with greater confidence.

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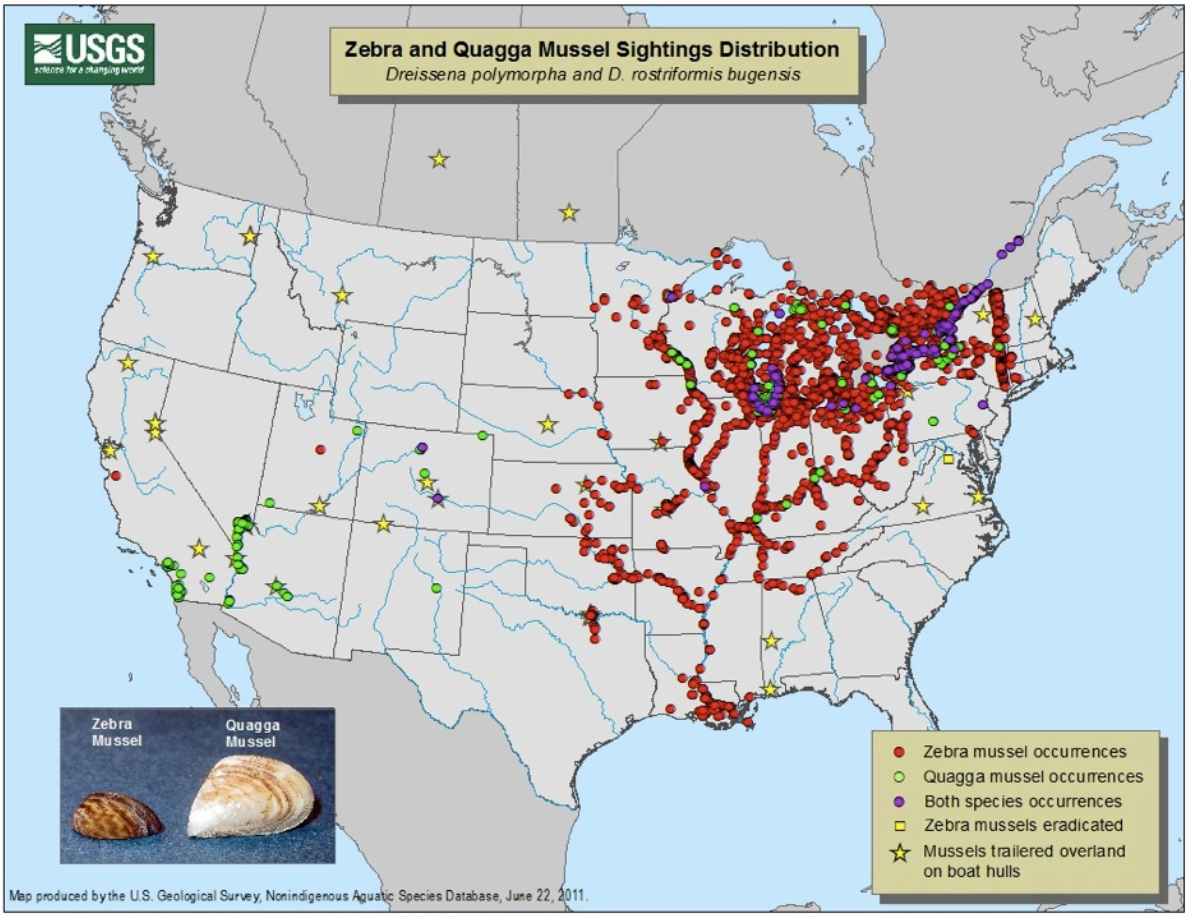

Figure 1. Map showing the reported distribution of zebra and quagga mussels in the Continental United States as of 22 June 2011 (USGS 2011).

Figure 2. Photograph of quagga mussels clogging a pipe. Note the dime in the foreground of the photo for scale. Photo found at http://www.pe.com/imagesdaily/2009/05-21/quagga21_400.jpg (June 2011).

Figure 3. Photograph of the cooler, chiller, and heater system used for each temperature treatment.

Figure 4. Photograph showing one of the opaque 250-ml bottles used to hold veligers within the water baths.

Figure 5. Photograph of the screen apparatus used to hold bottles within each cooler.

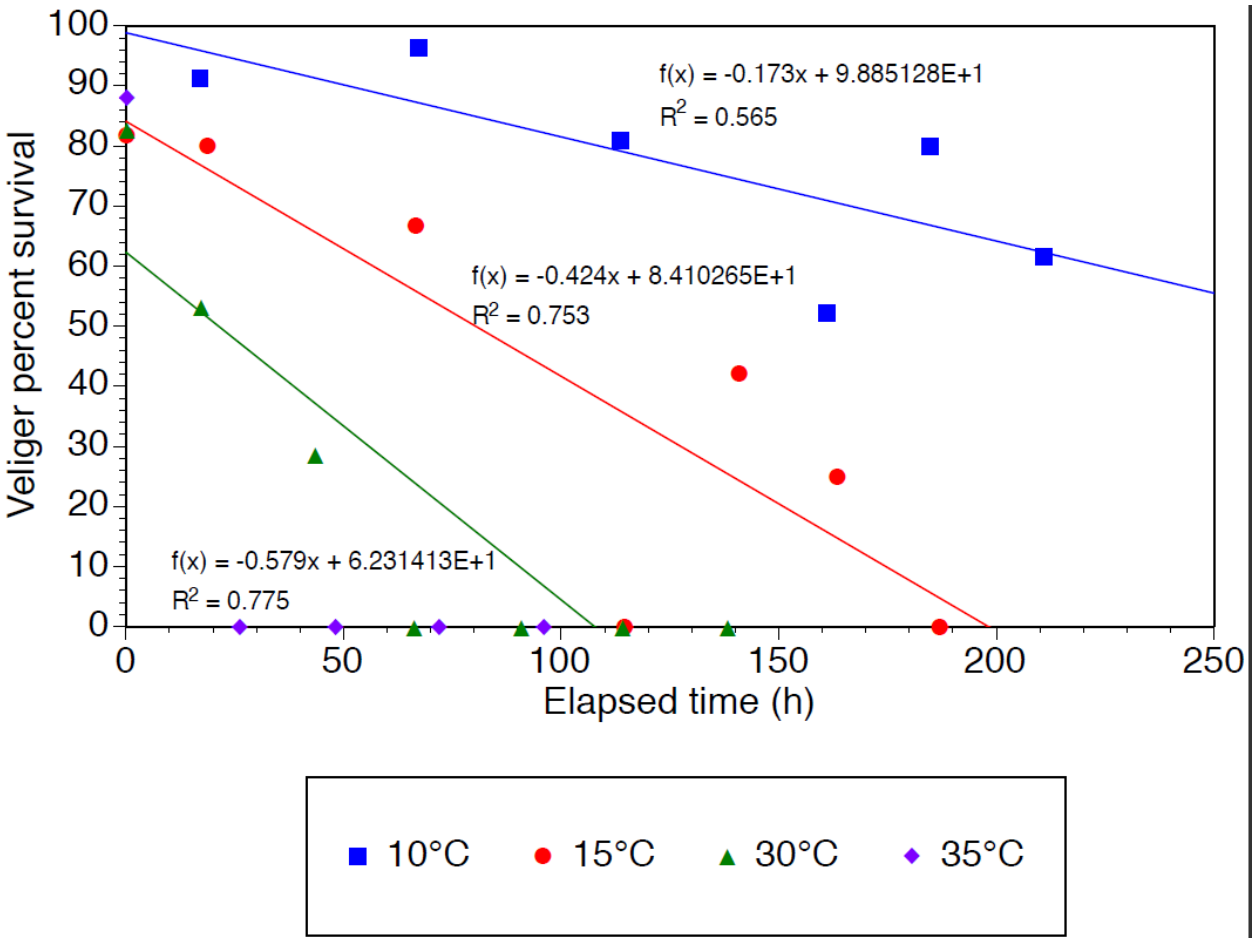

Figure 6. The combined effects of time and temperature on the percentage survival of quagga mussel veligers held at 10, 15, 30, and 35°C. No regression line was plotted for veligers in the 35°C treatment due to zero survival at that temperature. The estimated times for 0% survival for veligers in the 10, 15, and 30°C treatments were 571 h (24 days), 198 h (8.25 days), and 108 h (4.5 days), respectively.

Table 1. Planned and actual bottle removal times for the static water baths at all temperatures. Note that no bottle was observed at 120 hrs for the 35°C cooler as no living veligers had been observed in the previous 4 removal intervals.

	Time Interval (hrs)	
Temperature (°C)	**Planned**	**Actual**
10	24	17
10	72	67.2
10	120	113.7
10	168	161
10	192	184.8
10	216	210.9
15	24	18.6
15	72	66.5
15	120	114.5
15	144	140.8
15	168	163.3
15	192	192
30	24	17.1
30	48	43.3
30	72	66.1
30	96	90.7
30	120	113.9
35	24	26
35	48	48
35	72	71.8
35	96	95.9
35	120	x

Table 2. Mean, minimum, and maximum temperatures for each of the treatments, with calculated standard deviations (SAS 9.2).

Nominal Temperature	Mean (°C)	Minimum (°C)	Maximum (°C)	Standard Deviation
10	9.8	9.5	10.1	0.2
15	14.5	14.2	14.8	0.2
30	31.3	30.2	31.7	0.2
35	38.1	27.1	40.1	3.0