Spawning, Veliger Growth and Desiccation of Dreissena bugensis

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SPAWNING, VELIGER GROWTH AND DESICCATION

OF DREISSENA BUGENSI

By

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University of California, Davis
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A thesis submitted in partial fulfillment
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ABSTRACT

Spawning, Veliger Growth and Desiccation of *Dreissena bugensis*

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Lynn Marie Swanson Schaebe

Quagga (*Dreissena bugensis*) mussel veliger growth was compared under two dietary conditions: 1) only seston present in Lake Mead water; and 2) Lake Mead seston plus algae (*Isochrysis galbana*). Shell length, width, and area were compared as a function of time for the two treatments. It was expected that mussels would exhibit faster growth when supplemented with *I. galbana* than with Lake Mead water alone. However, no significant difference was observed between the control and *I. galbana* treatments. This result was unexpected and may have been caused by water quality, the nutritional content of Lake Mead seston, and/or over feeding.

The first experimental investigation of desiccation tolerance for *Dreissena bugensis* (quagga) veligers is presented. Desiccation tolerances of veligers four weeks post capture was examined under low, medium, and high humidity conditions. Veligers were exposed to dry conditions for fixed periods of time to determine the minimum dry time required to achieve 100% mortality. Data collected shows a positive correlation between desiccation tolerance time and humidity level. There is currently no data regarding desiccation tolerances of quagga veligers. Experiment results will be applicable to management strategies regarding control of quagga mussel colonization as well as watercraft and equipment decontamination. Additional research is required to examine the desiccation tolerance of younger veligers.
Spawning of *Dreissena bugensis* under laboratory conditions has been reported to be a challenge. Due to the time and labor-intensive nature of the process, little research has been conducted. A comprehensive understanding of spawning is necessary to better predict the implications that introduction of quagga mussels will have on an ecosystem. We evaluated three methods to induce spawning in quagga mussels: external application of serotonin, temperature shock, and gonad slurry. Mussels were individually exposed to the spawning treatments, and success or failure to produce gametes was observed. We also examined the relationship between shell length, time to spawn, and gamete production. We found that temperature shock produced a maximum of 22% spawning success. The addition of gonad slurry showed a slight increase over temperature shock producing a response of 32%. Serotonin was found to produce the highest percent spawning with a 77% success rate. Our results show that a clear dose response relationship exists between serotonin concentration and spawning response, and that consistent spawning of both males and females is observed following exposure to serotonin at concentrations at or above $5 \times 10^{-4} \text{ M}$. We found no correlation between mussel size and clutch size or time to spawn; however, the time delay between serotonin exposure and spawning onset was found to be greater in females than males.
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CHAPTER 1
INTRODUCTION

*Dreissena bugensis*, commonly known as the quagga mussel, is an invasive species originating in the Dneiper River catchment in the Ukraine (Mills et al., 1996). Quagga mussels were first introduced to the Great Lakes region of the United States through contaminated ballast water release by ships travelling from infested areas. Since the first confirmed identification in 1989, subsequent transport and colonization has occurred through overland transport, as well as downstream flow (USBR 2010).

Quagga mussels are an invasive species, and considered a nuisance in many water bodies. Due to their high fecundity, with females reproductive capacity estimated at up to one million eggs per season based on zebra mussel studies (Borcherding 1991), a free floating larval (veliger) stage, and the ability to attach to both hard and soft substrate (May and Marsden 1992, Dermot and Munawar, 1993) the potential for significant and rapid colonization is very high (Ram et al., 1996). Veliger density in Lake Mead has been reported as >100/L (LaBounty and Roefer 2007), and population estimates from areas in the Great Lakes are reported to be over 700,000 mussels/m² (USACE 1992). The rapid colonization and prolific growth rate carries negative economic impacts, as they clog pipes and interfere with boating operations. The presence of quagga mussels in infested water bodies results in millions of dollars in expenditures in maintenance, upkeep, and recreational losses (Pimental et al., 2005).

At the time of this writing, the majority of existing quagga mussel research has focused on adult populations. Little is known regarding the growth and development of veliger and juvenile quagga mussels. To better manage the threat of invasion, a more
thorough understanding is required. However, further exploratory research cannot be conducted without a foundational knowledge regarding basic spawning, growth and development under laboratory conditions. This research included three main objectives: 1) to better understand the feeding requirements of quagga veligers under laboratory conditions, 2) to contribute to the knowledge base regarding spawning of quagga mussels in laboratory conditions, and 3) to analyze desiccation tolerance in quagga veligers.

My research includes preliminary analysis of the effect of feeding the algae *Isochrysis galbana* on growth of quagga veligers. This algae was previously reported by Wright et al. (1996a), to increase survival of zebra and quagga mussel juveniles under laboratory conditions due to the ideal fatty acid content.

Currently there are very few published papers regarding the quagga mussel veligers and spawning. Wright et al. (1996b), reported that spawning and rearing veligers is a time consuming and difficult task. The purpose of this research is to contribute to the general knowledge base in order to create a platform from which to conduct further research regarding spawning, and growth of veligers under laboratory conditions. Data from this project will broaden the existing knowledge base and reduce the trial and error time demand of future research, thereby allowing future studies to be more easily conducted.

The spread of quagga mussels occurs primarily through overland travel of contaminated boats or equipment, as well as through downstream transport of veligers in river/aqueduct systems. In spite of measures to educate boaters and inspect equipment, quagga mussel contamination continues to spread. The mussels’ ability to withstand desiccation during overland transport is dependant on mussel size, ambient temperature and humidity levels (Ricciardi et al., 1994).
During early life stages, juvenile mussels can cling to equipment or vessels, however due to their small size they are difficult to detect with the naked eye, making identification of contaminated equipment virtually impossible. A thorough understanding of veliger and juvenile desiccation will allow for the establishment of a concrete window of dry time requirements, thereby eliminating guesswork and unnecessary decontamination procedures.

While research has been conducted regarding desiccation tolerances of adult quagga mussels, this thesis includes the first investigation of desiccation tolerances of veliger and juvenile quagga mussels. The results provide a partial baseline of knowledge regarding decontamination and dry time requirements for control of quagga in their early attachment phases.

Each research objective was investigated separately. The following three chapters are organized to separately cover the research into growth, desiccation, and spawning respectively.
CHAPTER 2
GROWTH

Abstract

Quagga mussel veliger growth was compared under two dietary conditions: 1) only seston present in Lake Mead water, and 2) Lake Mead seston plus algae (*Isochrysis galbana*). Shell length, width, and area were compared as a function of time for the two treatments. It was expected that mussels would exhibit faster growth when supplemented with *I. galbana* than with Lake Mead water alone. However, no significant difference was observed between the control and *I. galbana* treatments. This result was unexpected, and may have been caused by water quality, the nutritional content of Lake Mead seston, and/or overfeeding.

Introduction

The mollusk species, *Dreissena bugensis*, commonly known as the quagga mussel, has been spreading across North American waters since 1989. Veligers are the microscopic larvae of quagga mussels, and can be transported overland in ballast tanks, bilge water, and/or any other area containing water, through downstream currents from a contaminated water body (USBR 2010), as well as transported upstream via boats or other watercraft (USFWS 2008). In contrast to adult quagga mussels, factors affecting the growth and survival of veligers are poorly understood.

Few papers exist examining quagga veligers under laboratory conditions. Wright et al. (1996a) found that feeding of the algae, *I. galbana*, to zebra and quagga veligers resulted in increased survival and growth. Wacker and von Elert (2002) examined growth in zebra mussels over 16 day periods and found that poor diet during early veliger stages
of zebra mussels led to a permanent reduction in growth rate. No other studies have been identified regarding growth rates of quagga veligers under laboratory conditions. Nichols (1993) found zebra veliger mortality of over 90% from egg to settlement, and that food and substrate requirements for veliger survival are poorly understood. Wright et al. (1996b) found that salinity and temperature tolerance in zebra and quagga veligers increases with age, as well as a greater salinity tolerance in zebra vs. quagga embryos and larvae. Seltzer-Hamilton et al. (1995) examined the effect of temperature and salinity on development of zebra and quagga veligers. Stoeckel et al. (2004) reported that zebra veliger survival was size dependent, and increased when veligers were reared at lower densities. Overall, high mortality rates have proven *Dreissena* veligers to be difficult subjects for laboratory studies.

Because of the difficulty of conducting veliger laboratory studies, the body of research on quagga veligers remains sparse. Field collected veligers do not fare well post capture, thus have proven difficult for use in laboratory studies. Knowledge of optimal veliger dietary information will increase the success of rearing veligers under laboratory conditions, and enhance the ability to study juvenile life states for both ecological and containment purposes. The objective of our research was to investigate if feeding quagga veligers with the algae *I. galbana* would alter the growth rate in a laboratory environment. Our original hypothesis was that supplementing quagga mussel veligers with *I. galbana* would result in an increased growth rate. The efficacy of various feeding techniques is critical for future research, as it will create a better understanding of quagga veliger rearing and provide a foundation to support further veliger studies.
Methods

The study design was to collect quagga veligers from Lake Mead, and collect data regarding growth (length, width, and area) under different food treatments over a one-month period. Veliger selection and culture methods were based on preliminary work conducted late in 2009 and early in 2010. Lake Mead water was selected for use in the trial based on availability, and successful experience in using Lake Mead water during preliminary work. Additionally, we chose not to use Evian water as a non-feeding control due to the absence of nutritional content, and veliger survival time without food has been reported at approximately two weeks (Sprung, 1989). Collection of adequate numbers of viable veligers from shoreline accessible locations of Lake Mead proved to be a difficult task. Plankton tows were regularly conducted leading up to the experiment, and the experiment was run immediately upon completion of a successful tow.

Water

All growth assays were conducted in filtered Lake Mead water (FLMW). Water used in the growth experiments was collected between 4/14/2010 and 5/13/2010 from the Boulder Beach area of Lake Mead in 20 L Nalgene® carboys. Collection took place approximately 10 m from the shoreline. Carboys were triple rinsed with lake water and then submerged such that the mouth of the carboy was a minimum of 10 cm below the surface water to avoid collection of floating surface debris during the filling process. Prior to use in mussel cultures or experiments, water was poured through a 35 μm Nitex mesh filter held between a PVC ring cap and bushing of 2” diameter. Filtered water was held at room temperature in covered five gallon buckets with aeration, and used within seven days of collection.
Algal Culture

The algae *I. galbana* (Carolina Biological Supply Company) was cultured based on the optimal growth conditions described in Kaplan et al. (1986): 26 °C with F/2 media (Guillard and Ryther, 1962) in Instant Ocean® and gentle aeration. The F/2 media was prepared by mixing 0.5 mL Micro Algae Grow™ (Florida Aqua Farms Inc.) with 1 L artificial seawater (26-28 ppt). All media was autoclaved following preparation to reduce bacterial contamination. The initial pH in the algae cultures was 7-7.5, but regularly rose to ~9 within seven days due to utilization of carbon dioxide by the algae. Cultures were gently swirled by hand daily to ensure adequate mixing. Cultures were replenished on a weekly basis by pouring out 40% of the volume and replacing the removed volume with fresh media. The pH of the fresh media was adjusted to 4.5 with HCl before replenishing the culture in order to maintain a culture pH of 7-7.5. The pH of the algae culture was measured (ORION 3 STAR pH Benchtop, Thermo Scientific) before, during, and after replenishment. Prior to use, algae were centrifuged at 4,000 rpm for 10 minutes and re-suspended in FLMW at the desired concentration. Algae were frozen in single portions of known concentration for feeding during the test period. Frozen algae were used for the entirety of the test period. Algae were not defrosted all at once for feeding purposes. Rather, feeding was conducted by placing frozen algae in funnels attached to the side of the test chamber. Frozen algae were then allowed to melt into the veliger culture.

Veliger Collection

Veligers were collected via plankton tow on 4/14/2010 at approximately 5 pm from the east side of the courtesy dock at Lake Mead Marina, in the Boulder Basin area of Lake Mead, (Figure 2-1). A weighted 60 μm net was lowered to a depth of ~10 m and
then dragged the length of the dock, ~40 m. Prior to transport, each sample was filtered through a 250 μm mesh to remove larger zooplankton. Based on microscope examination in the laboratory, predation by larger zooplankton, most notably by copepods, significantly reduced the number of viable veligers. In some instances predation was 100%. This observation is consistent with Karabin (1978), who reported predation by copepods in zebra veligers. The practice of filtering directly following collection removed larger zooplankton, thereby increasing veliger survivorship. After filtering, the sample was re-suspended in a 1 L Nalgene® bottle to reduce density and maintain viability during transport back to the lab. It should be noted that the cohort of veligers captured may not have been representative of the veliger population of Lake Mead in its entirety. Rather, factors including variations in water temperature, the season during which capture took place, the age of veligers upon capture, sampling depth, and the physical and structural description of the sampling location may have skewed our sample towards one population subset.

**Veliger Identification and Culture**

Immediately upon return to the lab, collected veligers were filtered through a 35 μm Nitex filter held between a 2” PVC ring cap and bushing, and re-suspended in 100 mL FMLW in a small beaker. The contents of the beaker were gently swirled. Following swirling, 1 mL aliquots were transferred to a glass watch glass for examination under a dissecting microscope. Veligers were positively identified as quagga under cross-polarized light on a stereomicroscope. As per Johnson (1995), quagga veligers can be identified by the presence of a characteristic iron cross on the shell that appears under polarized light (Figure 2-2). Individual quagga veligers were determined to be active
(viable) through display of filtering, or movement (shell or foot) during microscopic examination. Active veligers were individually removed from the watch glass with a disposable plastic transfer pipette, and placed in one of six glass beakers containing 150 mL FMLW. Identification and selection of veligers continued until six groups of 250 active veligers were obtained.

After selecting six sets of 250 viable individuals, each of these colonies was transferred to a separate 4 L beaker for culturing. Prior to veliger transfer, 3 L of FMLW at room temperature was added to each of the larger beakers. Each larger beaker was placed within a plastic dish tub for secondary containment, and subjected to gentle aeration. The small beakers used for veliger selection were each emptied into one of the large test beakers. To ensure transfer of all veligers, the beakers were rinsed with FMLW three times, with the contents being added to the test beaker. Following transfer of the active veligers to the larger beakers, the volume was brought up to 4 L by the addition of FMLW. Beakers were left uncovered for the duration of the experiment.

**Experimental Procedure**

The growth experiment took place between 4/14/2010 and 5/14/2010. The experimental design was based on comparison of a control group to a treatment group. There were three replicates in each group, and each replicate consisted of a population of 250 veligers. In addition to seston naturally present in FMLW, the treatment group was supplemented with the algae *Isochrysis galbana*, at 1x10⁵ cell/mL every other day for the first week, followed by 1x10⁶ cell/mL every day for the duration of the trial. The feeding concentration and schedule were modified from Wright et al. (1996a), having been reduced to minimize risk from over-feeding due to the reduction in veliger density.
compared to the Wright et al. study. *I. galbana* cell densities of $1.5 \times 10^5$ were also used by Rehmann (2003), with zebra veligers. The control group was not supplemented with *I. galbana* for the duration of the experiment. The test was conducted over a 31-day period, during which the veligers were subject to ambient laboratory light and temperature conditions (23-25 °C, 16-18% humidity, 16 hours of light). Water in the test beakers was changed every three days by replacing approximately 3,500 mL of culture water with fresh FLMW. To avoid losing veligers during the process, culture water was passed through a 35µm Nitex filter. The filter was rinsed with FMLW into a small beaker to collect any veligers captured during the water change process.

**Data Collection**

Measurement of veliger size was performed at the start of the test (day zero) and immediately following each replacement of the culture water. During the water change, five active veligers were collected from either the Nitex filter rinse water, or removed from the walls of the test beaker. The test beaker was swirled before and during removal of water, including before the last ~500 mL removal to ensure adequate mixing of veligers. For selection from the rinse water, a small sample was taken from the rinse beaker with a plastic transfer pipette, and examined under microscope in a glass Petri dish. Following removal and identification of the requisite number of veligers, the contents of the rinse beaker were poured back into the test beaker. The rinse beaker was triple rinsed to avoid veliger loss. When removing veligers from the walls, care was taken to sample from the base as well as varying heights of the beaker wall to ensure a representative sample. Removal from the beaker wall was conducted using a disposable plastic transfer pipette with the narrow tip removed. Beginning with the pipette under a
slight suction, individual veligers were gently scraped from the wall, and then quickly captured with the pipette before they began to sink.

Veligers were not observed to have attached to the beaker walls during the first week, thus all five veligers were taken from the rinse beaker. During the second week, veligers had begun to attach to the walls of the test beaker, and were visible upon close inspection through the glass wall. For this period, two veligers were removed from the wall for measurement, and three taken from the rinse beaker. For the third and fourth weeks, veligers were not readily identified in the rinse water, and all five measured veligers were collected from the beaker walls.

After collecting five viable veligers for measurement, each veliger was photographed with a Nikon Stereomicroscope SMZ1000 at 40x magnification. Each photograph was then measured for length, width, and area as described in Figure 2-3. Measurement values were calibrated by measuring a photograph of a micrometer taken under the same conditions. Measurements of shell length, width, and area were taken from the photographs using Infinity Analyze software (Lumenera Corporation). Measurement accuracy reported by Lumenera was 1-2 pixels, corresponding to a length of 0.2-0.4 μm. Growth was calculated based on changes in shell dimensions over time. Following photography and measurement, veligers were discarded and not returned to the test beakers. The test was concluded at the end of the 30-day test period due to lack of sufficient veligers in some test chambers. All remaining veligers were discarded.

**Results**

The progression through three developmental states was seen throughout the study in veliger growth, as well as attachment to the walls of the test chambers. Veligers were
initially free swimming and had an average length of 92.8 μm (SD+/-12.0), fitting the length range of 39-221 μm, and free swimming description of umbonal veligers. Average length was above 150 μm (mean 206.9, SD +/- 36.0) by day nine in both treatment and control groups. This size marks the lower size range for pediveligers (150-228 μm), a transition confirmed by the observation of attachment to beaker walls. By day 19, veliger length in all groups was above 220 μm (mean 287.0 μm, SD +/- 45.9) and veligers were no longer found in the filter rinse water. This coincides with the transition to the plantigrade veliger state, characterized by lengths of 220-410 μm and loss of the ability to swim (USACE, 2010).

Growth results are summarized in Table 2.1, and graphs of length, width, area, and aspect ratio over time are presented as Figures 2.4-2.7. The combined average growth rate in length for the control and treatment groups was 8.38 and 7.07 μm/day respectively. Growth rates for shell width were 5.40 and 4.61 μm/day for control and treatment groups, and growth rates for shell area were 23.71 and 20.20 μm²/day respectively. Pairwise analysis was used to determine the effect of supplementing quagga veligers with Isochrysis galbana algae on veliger growth. For this analysis, all data points for each date were pooled into treatment and control groups, and the two data sets compared. The analyses were carried out using JMP software (SAS Institute, Cary, North Carolina). Pairwise analysis showed no significant difference between treatment and control shell length, width, area, and aspect ratio (width/length) on any date.

The graphs for growth rate (Figures 4-7) show a slight s-curve in the shape, with a period of rapid growth occurring from days 3-19, and a slower growth rate occurring from days 19-31. The control and treatment groups showed growth rates for shell length
of 12.51 and 9.71 μm/day during the rapid period. Growth rates for the same groups were 2.64 and 3.27 μm/day during the slow growth period. Growth rates for shell width in control and treatment groups were 8.20 and 5.98 μm/day during the rapid period. Control and treatment width growth was 1.42 and 2.55 μm/day during the slow period respectively. Rapid shell area growth rates were 36.45 and 28.31 μm²/day, and slow shell area growth rates were 5.99 and 8.27 μm²/day for control and treatment groups.

**Discussion**

The results do not support the original hypothesis that veligers would have a faster growth rate when supplemented with *I. galbana* than when provided with Lake Mead seston alone. For length, width, and shell area, the growth rates calculated were the opposite of our initial hypothesis, with veligers in the control groups showing a very slight however statistically insignificant increase in growth rate over the treatment groups. During the rapid growth period, days 3-19, the control group showed growth rate was slightly higher than the treatment group. However, during the slow period from days 19-31, the treatment group showed a slightly quicker pace.

The rapid growth period during days 3-19 coincides with the end of the umbonal stage, and the pediveliger stage, as identified by veliger length and attachment to the beaker walls. The slow growth period from days 19-31 corresponds with the plantigrade veliger stage, as described by veliger length, morphology, and absence of swimming veligers. The observed range in shell lengths at settlement of 150-220 μm is consistent with the plantigrade size described in USACE (2010), but smaller than the findings of Martel (2001), who reported near shore settlement sizes of 256-284 μm in quagga
veligers, as well an increase in veliger size of up to 322 \( \mu m \) with increased settlement depth.

One hypothesis regarding the differing growth rates is that veligers experience rapid growth in length while still swimming, then following firm attachment and subsequent transition to the plantigrade stage, a period follows during which veligers experience growth in height rather than length and width. Due to the limitations of the measuring technique used, consistent measurement of height was not possible. A longer test period as well as more complex measuring techniques would be necessary to further analyze growth patterns through the varying developmental stages.

The similar growth rates observed in the feeding and control groups suggest three possible hypotheses. One, that over-feeding resulted in poor water quality, potentially impacting veliger growth rate. Previous studies have shown that water and food quality are of great importance to larval growth (Wright et al., 1996a, Wacker and von Elert, 2002), with poor conditions resulting in increased mortality, and stunted or reduced growth. The trial was stocked at a density of 62.5/L, much lower than the 1000/L density described in Wright et al. (1996a). The lower density was chosen based on the theory developed from observations that SNWA plankton tows rarely show veliger densities greater than 40/L, suggesting that Lake Mead water can adequately support veligers up to, but not beyond 40/L (IQMM). To avoid potential dietary deficiencies due to overcrowding in the control group, for this trial a density slightly greater than 40/L was used with expectation of some veliger mortality.

Due to the low densities used, the optimal feeding described in Wright et al. (1996a) potentially over-fed the veligers, and the unfiltered algae in the beakers created sub-
optimal water quality conditions, thus stunting the treatment group early on. Sprung (1984) observed a decrease or stabilization in growth of *Mytilus edulis* larvae when fed with a dense algal culture. The suggested reason being due to larval sensitivities to algal metabolites, and/or the large number of algal cells hampering the larval feeding apparatus.

Second, while previous studies have shown that *I. galbana* provides an optimal fatty acid content for veliger survival and growth (Wright et al., 1996a), it is possible that the natural seston present in Lake Mead may provide a comparable nutritional profile. The algae *I. galbana* has been used in zebra and quagga mussel culture due to the specific fatty acid content (Wright et al., 1996a, Grima et al., 1992). It is the polyunsaturated fatty acid (PUFA) specifically that has been shown to be beneficial to veliger culture. However, Webb and Chu (1983), observed that protein content had greater correspondence with total algal nutritional value than carbohydrate or lipid content. It is possible that the natural seston in Lake Mead water provide nutritional content that is suitable for veliger growth. To further examine this factor, we suggest that future studies include a group held in Lake Mead water filtered at 0.2 $\mu m$ to eliminate any dietary contributions. Also, we suggest examining the fatty acid profile of the natural seston present in Lake Mead water filtered at 35 $\mu m$.

Finally, results suggest that algal supplementation is not required in veliger cultures held in Lake Mead water unless the veligers are stocked at a density high enough to exhaust naturally occurring seston. We hypothesize that the similar growth rates between feeding and control groups was due in part to the different clearance rates of adult versus juvenile mussels. Because adult mussels are capable of filtering out large quantities of
seston, they require additional supplementation in order to thrive. Assuming the hypothesis that Lake Mead seston provides a comparable nutritional profile (percentages of protein, lipids, and carbohydrates) to *I. galbana*, then veligers, with a much smaller filtration capacity than adults, potentially do not require algal supplementation unless stocked at high enough densities as to exhaust the natural seston present in Lake Mead water.

More work is necessary to determine the feeding requirements for optimal growth of quagga veligers under laboratory conditions. Future trials should examine the effects of water quality, veliger density and nutritional content on veliger growth rates. In order to understand the specific nutritional content required for optimal veliger growth, analysis of nutritional content of natural Lake Mead seston, for lipid, protein, and carbohydrate, should be undertaken and compared to levels found in *I. galbana*. 
Table 2.1: Growth Assay Results

Average Growth Rates

<table>
<thead>
<tr>
<th>Length (μm/day)</th>
<th>Width (μm/day)</th>
<th>Area (μm$^2$/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Days 3-19</td>
<td>Days 19-31 Overall Days 3-19</td>
<td>Days 19-31 Overall Days 3-19</td>
</tr>
<tr>
<td>8.23</td>
<td>10.63</td>
<td>5.24</td>
</tr>
<tr>
<td>8.55</td>
<td>12.91</td>
<td>2.67</td>
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<td>8.35</td>
<td>14.00</td>
<td>0.00</td>
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<tr>
<td>6.79</td>
<td>9.53</td>
<td>3.19</td>
</tr>
<tr>
<td>8.31</td>
<td>9.24</td>
<td>6.32</td>
</tr>
<tr>
<td>6.12</td>
<td>10.35</td>
<td>0.29</td>
</tr>
</tbody>
</table>

Combined Average Growth Rates

<table>
<thead>
<tr>
<th>Length (μm/day)</th>
<th>Width (μm/day)</th>
<th>Area (μm$^2$/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Overall Days 3-19</td>
<td>Days 19-31 Overall Days 3-19</td>
<td>Days 19-31 Overall Days 3-19</td>
</tr>
<tr>
<td>8.38</td>
<td>12.51</td>
<td>2.64</td>
</tr>
<tr>
<td>7.07</td>
<td>9.71</td>
<td>3.27</td>
</tr>
</tbody>
</table>
Figure 2.1: Satellite photo of veliger collection site at Lake Mead Marina, on the Nevada side of Lake Mead at 36.024979°, -114.771747°.
Figure 2.2: Image of *Dreissena bugensis* veliger under cross polarizing light showing the characteristic “Iron Cross.”
Figure 2.3: Diagram outlining shell measurement points for *Dreissena bugensis* veligers.
Figure 2.4a: Combined average shell length of Lake Mead *Dreissena bugensis* veligers under laboratory conditions with and without feeding of *Isochrysis galbana*.

Figure 2.4b: Average shell length of Lake Mead *Dreissena bugensis* veligers under laboratory conditions with and without feeding of *Isochrysis galbana*. 
Figure 2.5a: Combined average shell width of Lake Mead *Dreissena bugensis* veligers under laboratory conditions with and without feeding of *Isochrysis galbana*. Error bars represent standard errors.

Figure 2.5b: Average shell width of Lake Mead *Dreissena bugensis* veligers under laboratory conditions with and without feeding of *Isochrysis galbana*. 
Figure 2.6a: Combined average shell area of Lake Mead *Dreissena bugensis* veligers under laboratory conditions with and without feeding of *Isochrysis galbana*. Error bars represent standard errors.

Figure 2.6b: Average shell area of Lake Mead *Dreissena bugensis* veligers under laboratory conditions with and without feeding of *Isochrysis galbana.*
Figure 2.7a: Combined average aspect ratio of Lake Mead *Dreissena bugensis* veligers under laboratory conditions with and without feeding of *Isochrysis galbana*. Error bars represent standard errors.

Figure 2.7b: Average aspect ratio of Lake Mead *Dreissena bugensis* veligers under laboratory conditions with and without feeding of *Isochrysis galbana*. 
References


Martel AL (2001) Species and epilimnion/hypolimnion-related differences in size at larval settlement and metamorphosis in Dreissena (bivalvia). Limnology and Oceanography 46: (3)707-713


CHAPTER 3
DESICCATION

Abstract

The first experimental investigation of desiccation tolerance for *Dreissena bugensis* (quagga) veligers is presented. Desiccation tolerances of veligers four weeks post capture was examined under low, medium, and high humidity conditions. Veligers were exposed to dry conditions for fixed periods of time to determine the desiccation tolerance, which is defined as the minimum dry time required to achieve 100% mortality. Data collected shows a positive correlation between desiccation tolerance time and humidity level. Experiment results will be applicable to management strategies regarding control of quagga mussel colonization as well as watercraft and equipment decontamination. Additional research is required to examine the desiccation tolerance of younger veligers.

Introduction

Quagga mussels have colonized waters across the North American continent since they were first discovered in the Great Lakes in 1989. Existing colonization models are: 1) overland transport via boats, trailers, or other equipment; 2) downstream current transport from a contaminated water body (USBR 2010); and 3) upstream transport from contaminated water bodies via boats or other watercraft (USFWS 2008). Veligers are the microscopic larvae of quagga mussels, and can be transported in ballast tanks, bilge water, and/or any other area containing water. Adult and juvenile quagga mussels can be transported overland once attached to boat, trailer, or gear surfaces. In spite of measures to educate boaters and inspect equipment, quagga mussel populations continue to spread. National quagga mussel distributions from 2000-2011 are shown in Figures 3.1a-c.
Current measures in place to contain further contamination focus on reducing the risk of overland transport. To achieve this outcome, public outreach and education campaigns have encouraged boaters to drain, clean and dry equipment with the expectation that the mussels will not survive desiccation. Examples of public education signage are shown in Figure 3.2. In addition to mandatory inspections at many water bodies, decontamination and inspection protocols for gear and equipment also exist. An example of cleaning and inspection guidelines designed to prevent the spread of invasive species can be seen in USBR (2010). During inspection procedures, juveniles are too small to detect by visual identification alone. USBR in-house recommendations call for a thorough tactile inspection of all surfaces, with a sandpaper texture indicating possible juvenile contamination. Finally, quarantine periods, as well as physical removal, chemical treatment, and hot pressure washes are common decontamination methods (USBR 2010).

Previous studies have proved desiccation to cause mortality in quagga mussels. USACE (1993) showed a positive correlation between mussel size and desiccation tolerance, and Ricciardi et al., (1994) found that increasing humidity increases survival time in adult quagga mussels. Current estimates for adult survival after being removed from water range from three days in freezing temperatures to greater than 40 days in cool and humid environments (USBR 2010).

This thesis includes the first investigation of the desiccation tolerance of quagga veligers. We will first discuss veliger collection, handling, and culture, followed by presentation of results showing a positive correlation between desiccation tolerance of quagga veligers in the sedentary plantigrade veliger life stage and relative humidity.
Finally we will discuss the implications of these results on decontamination procedures and quarantine recommendations.

**Methods**

The basic research plan was to collect quagga veligers from Lake Mead via plankton tow, and then assess desiccation tolerance after allowing them to thrive for four weeks post-capture. At the end of four weeks of culturing, replicate groups of 30 each were evaluated at three humidity levels (low, medium, and high).

**Collection and Transport**

Veligers were collected via plankton tow on 4/14/2010 at approximately 5 pm from the east side of the courtesy dock at Lake Mead Marina, in the Boulder Basin area of Lake Mead (Figure 3-3). A weighted plankton net with 63 μm mesh was lowered to a depth of ~10 m and then dragged the length of the dock, for a distance of ~40 m. Prior to transport, each sample was filtered through a 250 μm mesh to remove larger zooplankton. After filtering, the sample was re-suspended in a 1 L Nalgene® bottle to reduce density and maintain viability during transport back to the lab.

The practice of filtering directly following collection removed larger zooplankton, thereby increasing veliger survivorship. Based on microscopic observation, predation by larger zooplankton, most notably by copepods, significantly reduced the number of viable veligers. In some instances predation was 100%. This observation is consistent with Karabin (1978), who reported predation by copepods in zebra veligers.

It should be noted that the cohort of veligers captured may not have been representative of the veliger population of Lake Mead in its entirety. Various environmental factors, including water temperature, season, veliger age, sampling depth,
and the physical and structural description of the sampling location may have skewed our sample towards one population subset.

**Water**

Veligers were raised in Filtered Lake Mead Water (FLMW). Water used in the growth experiments was collected between 4/14/2010 and 5/13/2010 from the Boulder Beach area of Lake Mead in 20 L Nalgene® carboys. Collection took place approximately 10 m from the shoreline. Carboys were triple rinsed with lake water and then submerged such that the mouth of the carboy was a minimum of 10 cm below the surface to avoid collection of floating debris. Following collection, carboys were transported by car to the laboratory. Prior to use in mussel cultures or experiments, the collected water was poured through a 35 µm Nitex mesh filter to remove zooplankton, plant matter, and any other debris. Filtered water was held at room temperature in covered five gallon buckets with aeration and used within seven days of collection.

**Veliger Culture**

Veligers were cultured for 4 weeks post-capture in 1 L glass beakers with 800 mL FLMW and gentle aeration for four weeks following collection. The water was changed every three days by replacing 70% of the culture water with FLMW. Culture water was poured through a filter, which was rinsed back into the culture chamber to avoid veliger loss. Cultures were supplemented with an overall concentration of 1X10^5 cells/mL of frozen Isochrysis galbana every other day for the first week and every day thereafter. This feeding schedule was modified from the schedule described by Wright et al. (1996a). Algae were frozen in single 15 mL portions at 5.33X10^6 cells/mL for feeding. Frozen algae aliquots were used for the entirety of the veliger culture period. Algae were
not defrosted for feeding purposes. Rather, feeding was conducted by placing frozen algae in funnels attached to the side of the test chamber. Frozen algae were then allowed to melt into the veliger culture.

Four weeks following collection, all surviving veligers were observed to have anchored to the walls of the holding beakers. At this age, veligers were visible upon close inspection through the glass. Removal of veligers was required for use in the desiccation assay.

**Algae Culture**

The process used to culture *I. galbana* (Carolina Biological Supply Company) was based on the optimal growth conditions described in Kaplan et al. (1986): water at 26 °C with F/2 media (Guillard and Ryther, 1962) in Instant Ocean® and gentle aeration. The F/2 media was prepared by mixing 0.5 mL Micro Algae Grow™ (Florida Aqua Farms Inc.) with 1 L artificial seawater. All media was autoclaved following preparation to reduce bacterial contamination. The initial pH in the algae cultures was 7-7.5, but regularly rose to ~9 within seven days due to utilization of carbon dioxide by the algae. Algae cultures were gently swirled by hand daily to ensure adequate mixing. Cultures were replenished on a weekly basis by pouring out 40% of the volume and replacing the removed volume with fresh media. The pH of the fresh media was adjusted to 4.5 with HCl before replenishing the culture in order to maintain a pH of 7-7.5. The pH of the algae culture was measured (ORION 3 STAR pH Benchtop, Thermo Scientific) before, during, and after replenishment. Prior to use, algae were centrifuged at 4,000 rpm for 10 minutes and re-suspended in FLMW at 5.33X10⁶ cells/mL, and frozen in 15 mL aliquots for feeding.
**Desiccation Experiments**

After considerable experimentation, it was determined that veligers could be safely removed from the beaker walls using a disposable plastic transfer pipette with the narrow tip removed. With the pipette under a slight suction, the tip can be used to gently scrape a single veliger from the wall. The veliger is then quickly captured with the pipette before it can sink.

Veligers selected for the desiccation experiments were first examined to assure viability. Specimens were placed on a slide in a small pool of water in groups of ten and examined under a Nikon Stereomicroscope SMZ1000 at 40x magnification. Due to the agitation of removal and transfer to a microscope slide, a period during which veligers remained inactive with shell closed was consistently observed. A ten-minute recovery period on a microscope slide with the microscope light off was found to reduce the amount of observation time required to identify active veligers. Only veligers determined to be active through observation of foot or shell movement and/or filtering activity, were selected for the trial. Active veligers were transferred to a small plastic Petri dish with care to ensure that a small amount of water remained around each veliger. Prior to commencement of the desiccation period, water was removed from around the veligers with a disposable plastic transfer pipette and a tightly rolled up Kimwipe™ while under the microscope. During this process, the microscope light was dimmed to minimize external heating. Care was taken to remove water quickly so that all veligers were “dry” within a two-minute period.

The desiccation test period began when all veligers were observed to be dry, and lasted over defined time periods, ranging from five minutes to 24 hours. During each test,
veligers were held in a fixed location, lab bench for low humidity (16.6-17.1%), and humidity chamber for both medium (54.3-76%), and high humidity (81-82%). The humidity chamber consisted of a clear plastic tub overturned on the counter top enclosing a humidity meter, one to four containers of water, and test desiccation dishes. The chamber was set up one to two days in advance of the desiccation trials to allow humidity levels to equilibrate. At the end of each trial, veligers were re-hydrated through addition of FLMW to the test dish and stored covered on the lab bench at room temperature. The mussels were examined under a microscope 48 hrs post re-hydration and determined viable if foot or shell movement, filtration, or internal activity was observed.

Ambient temperature, humidity, and evaporative water loss (mg/min) were recorded for all test periods. All tests took place at room temperature, ranging from 20.6 to 24.7 °C. Humidity levels were 16.6-17.1%, 54.3-76%, and 81-82% for the low, medium, and high ranges respectively. Evaporative water loss was determined as follows: 100 mL water was placed in a 150 mL plastic beaker on a balance directly adjacent to the desiccation area, either on the laboratory counter or within the humidity chamber. Water mass (mg) was recorded at the start and end points for each trial; microenvironment trial length ranged from five minutes to 24 hours.

Results

Initial experimental design called for examining the desiccation tolerance of veligers immediately post capture, as well as 1, 2, 3 and 4 weeks post capture to provide a spectrum of data describing changes in desiccation tolerance through developmental stages. Results collected thus far are only for plantigrade veligers 4 weeks post-capture.
Results are summarized in Table 3-1. All tests took place at ambient room temperature, ranging from 20.6 to 24.7 °C. Desiccation tolerance at low humidity (16.6-17.1%) was found to be 50 minutes. Desiccation tolerance at high humidity (81-82%) was found to be 120 minutes. Desiccation tolerance at medium humidity (54.3-76%) was found to be at most 150 minutes. The results for medium humidity are preliminary as best, as only two time durations were examined, 65 minutes and 150 minutes. Following the desiccation period, many of the veligers shells remained open, indicating mortality. An image of a plantigrade veliger with open shell is shown in Figure 3-4 below.

Results show a positive correlation between relative humidity and desiccation tolerance in quagga veligers, which is consistent with the results from Ricciardi et al. (1994) for adult quagga mussels. Evaporative water loss was recorded to provide a means to extrapolate desiccation time for temperature/humidity combinations not examined in this study.

**Discussion**

Veligers transition through several developmental stages: straight hinged or D-shaped, umbonal, pediveliger, and plantigrade veliger. Straight hinged, or D-shaped veligers range in size from 39 to 71 μm. This early stage is characterized by swimming in a circular motion, and a physical shape that is mostly rounded with one flat side, giving the name D-shaped. Umbonal veligers are primarily round in appearance, but unlike the straight-hinged veligers they show the development of an umbo, giving a slight v-shape to the hinge. Umbonal veligers swim using a velum, and range in size from 39 to 221 μm. Pediveligers have a clam shaped shell ranging from 150-228 μm, and utilize their foot to both swim and maneuver. The shell of plantigrade veligers is elongated in a manner
similar to the adult form, and has a length of 220-410 μm. Like pediveligers, plantigrade veligers show presence of a foot, however plantigrade veligers do not swim (USACE 2010). Based on size, attachment, and inability to swim, the veligers used in this study fall under the classification of plantigrade veligers. Images of pediveligers and plantigrade veligers are shown in Figures 3-5 & 3-6.

The developmental transition removing the ability to swim is a critical factor in considering decontamination procedures for equipment or watercraft following short periods of deployment into contaminated waters. In considering equipment or boats deployed for periods ranging from several hours to a few days, we can surmise that with the exception of objects placed in such a manner that would allow direct contact for sedentary plantigrade veligers to crawl onto an attachment point, that gear used in open water would carry the potential contamination risk from veligers up to, but not beyond the still swimming pediveliger developmental stage.

It should be noted that while the high humidity tests were conducted at 82%, further testing is recommended to examine desiccation tolerance at 99% humidity in order to present a recommendation that would be protective under extreme humidity conditions. Assuming the hypothesis that with increasing age comes an increase in size, and a corresponding increase in desiccation tolerance, we can presume that older non-swimming plantigrade veligers would have a higher desiccation tolerance than younger, still swimming pediveligers.

The results for mid-range humidity (54.3-76%) require further tests. Results for this range were reported as a desiccation tolerance of at most 150 minutes. The description, at most, is used as due to the fact that examination of only two time periods were
completed, 65 minutes and 150 minutes rather than the original experimental design that called for analysis of incrementally increasing time periods, to identify more closely the desiccation period required to achieve 100% mortality. A review of the low and high humidity results shows that for both groups, 100% mortality was achieved within 30 minutes following an observation of 30% mortality. Based on this trend, we can expect that 100% mortality would be achieved at around 95 minutes for mid-range humidity conditions; further testing is required to validate this hypothesis.

These findings have implications for current decontamination procedures calling for physical removal, chemical treatment, or hot pressure washing (USBR 2010). Given that our results show 100% mortality after a reasonable drying period, the extra time, expense, and wear on material under certain conditions (i.e., bleaching of rubber products, expense of hot pressure washing equipment) may not be necessary. Rather, decontamination of goods deployed for short periods of time, and showing no adult mussels, may be achieved following a desiccation period of at least two hours under 82% humidity and 23 °C or equivalent drying conditions.

In summary, the results described above show potential to influence recommendations regarding veliger transport and decontamination. However, further research is required including additional trials of plantigrade veligers at mid-range humidity levels, as well as trials on umbonal and pediveligers at low, medium, and high humidity in order to provide a comprehensive analysis of veliger desiccation tolerance.
Table 3.1: Desiccation Results

<table>
<thead>
<tr>
<th>Dry Time (minutes)</th>
<th>Temp</th>
<th>Humidity</th>
<th>Evaporative loss (mg/min)</th>
<th>Number Sampled</th>
<th>48 hr Viable</th>
<th>Survival %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Low Humidity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>5</td>
<td>24.7</td>
<td>16.6</td>
<td>6.84</td>
<td>30</td>
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</tr>
<tr>
<td>30</td>
<td>24.7</td>
<td>16.6</td>
<td></td>
<td>30</td>
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<td>40</td>
<td>24.1</td>
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<td>17%</td>
</tr>
<tr>
<td>50</td>
<td>24.1</td>
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<td></td>
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</tr>
<tr>
<td>24hrs</td>
<td>24</td>
<td>17.1</td>
<td></td>
<td>30</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>High Humidity</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>75</td>
<td>23.5</td>
<td>82</td>
<td>0.53</td>
<td>30</td>
<td>10</td>
<td>33%</td>
</tr>
<tr>
<td>90</td>
<td>23.5</td>
<td>82</td>
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<td>6</td>
<td>20%</td>
</tr>
<tr>
<td>105</td>
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<td>Medium Humidity</td>
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<tr>
<td>65</td>
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<tr>
<td>150</td>
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<td>54.3</td>
<td>1.28</td>
<td>30</td>
<td>0</td>
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Figure 3.1a: National Zebra and Quagga Mussel Distribution, 2010

Figure 3.1b: National Zebra and Quagga Mussel Distribution, 2006

Figure 3.1c: National Zebra and Quagga Mussel Distribution, 1990
Figure 3.2: Quagga Mussel Public Outreach Signage
Figure 3.3: Satellite image of veliger collection site at Lake Mead Marina, on the Nevada side of Lake Mead at 36.024979°, -114.771747°.
Figure 3.4: *Dreissena bugensis* Plantigrade Veliger Post Desiccation
Figure 3.5: *Dreissena bugensis* Pediveliger
Figure 3.6: *Dreissena bugensis* Plantigrade Veliger
References


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CHAPTER 4

SPAWNING

Abstract

Spawning of *Dreissena bugensis*, commonly known as the quagga mussel, under laboratory conditions has been reported to be a challenge. Due to the time and labor-intensive nature of the process, little research has been conducted. A comprehensive understanding of spawning is necessary to better predict the implications that introduction of quagga mussels will have on an ecosystem. We evaluated three methods to induce spawning in quagga mussels: external application of serotonin, temperature shock, and gonad slurry. Mussels were individually exposed to the spawning treatments, and success or failure to produce gametes was observed. We also examined the relationship between shell length, time to spawn, and gamete production. We found that temperature shock produced a maximum of 22% overall spawning success. The addition of gonad slurry slightly showed an increase over temperature shock at 32%. Serotonin was found to produce the highest percent spawning at 77.3% overall success. Our results show that a clear dose response relationship exists between serotonin concentrations and spawning response, and that consistent spawning of both males and females is observed following exposure to serotonin at concentrations at or above $5 \times 10^{-4}$ *Mol*. We found no correlation between mussel size and clutch size or time to spawn, however the time delay between serotonin exposure and spawning onset was found to be greater in females than males.
Introduction

Invasion of North American surface waters by *Dreissena bugensis*, commonly known as the quagga mussel, has had significant environmental and economic effects in the Midwestern United States. Prior to the discovery of quagga mussels in the western United States, estimates of costs due to the presence of zebra and quagga mussels were estimated at up to $1 billion per year (Pimentel et al., 2005). Rapid colonization by quagga mussels is enhanced by the species’ high fecundity, with estimates of females reproductive capacity based on zebra mussel studies at up to one million eggs in a single spawning season (Borcherding 1991). In spite of their high fecundity in natural environments, research efforts directed at slowing the spread of this invasive species have been hampered by the difficulty of breeding quagga mussels in the laboratory. Current reproductive understanding of the quagga mussel has been applied from studies on its nearest congener the zebra mussel (Nichols 1993, Ram et al., 1993, Ram et al. 1996, Sprung 1992, Walz 1978, and Wright 1996a).

Aquaculture of mollusk species is common in both research and commercial environments. However, at the time of this writing, few papers have been published regarding the spawning of quagga mussels. Three techniques have been described in inducing laboratory spawning in zebra and quagga mussels: application of serotonin, temperature shock, and gonad slurry. Serotonin is a hormone/neurotransmitter that is known to stimulate spawning behavior and regulate gamete maturation in zebra mussels (Ram et al., 1993, Wright et al., 1996a). In the case of gonad slurry, coordinated spawning efforts are critical for successful external fertilization. Application of gonad slurry exposes mussels to chemicals released from the gonads along with eggs and sperm.
during spawning processes, inducing spawning through activation of a coordinated response mechanism. The exact identity of the stimulatory chemicals is unknown. Temperature shock is often used to initiate spawning at commercial aquatic farms, as species tend to propagate when exposed to rapid environmental changes in a breed or die effort to prevent extinction (Carlsbad Aqua Farm, personal communication).

Ram et al. (1993) was the first to report on spawning induction in zebra mussels via external application of serotonin (5-hydroxytryptamine). In their experiments, zebra mussels were observed to spawn shortly after external application of serotonin (5-hydroxytryptamine), at concentrations of both 1 mM and 0.1 mM. The neurotransmitter serotonin and serotonin-like compounds are known to regulate reproductive processes in bivalves (Ram et al., 1993, Hirai et al., 1988, Fong et al., 1994, and Fong 1998), including oocyte maturation, spawning, and parturition.

Sprung (1989) used gonad slurry to induce spawning in zebra mussels. Temperature shock alone was used by Tourari (1988) to induce spawning in zebra mussels. Setzler–Hamilton et al. (1995) and Wright et al. (1996b) reported the use of temperature shock with gonad slurry to induce spawning in quagga and zebra mussels. This is the first description of a dose response curve describing spawning response to serotonin in quagga mussels. Spawning response to temperature shock alone has not been studied in quagga mussels. In addition, the comparative efficacy of each of the three techniques in inducing a spawning response in quagga mussels has not been critically analyzed. A better understanding of the aforementioned spawning techniques will support method selection for future spawning studies.
The objective of this research was to investigate if quagga mussels could be induced to successfully spawn in a laboratory environment using serotonin, temperature shock or gonad slurry. The comparative efficacy of the three techniques will create a better understanding of quagga mussel spawning processes and provide a foundation to support future studies. This information is critical to understanding quagga mussel population dynamics. Additionally, a myriad of studies regarding zebra mussel physiology and life history have been conducted on adult mussels, but the body of research on quagga mussel adults and veligers remains sparse. Field collected veligers do not fare well post capture, thus successful use in laboratory studies is a reputedly difficult task. The ability to spawn veligers for laboratory purposes has the potential to enhance our ability to study juvenile life stages for both ecological and containment purposes.

Methods

Water Collection

Water used in mussel cultures and experiments was collected from the Boulder Beach area of Lake Mead in 20 L Nalgene® carboys. Collection took place approximately 10 m from the shoreline. Carboys were triple rinsed with lake water and then submerged such that the mouth of the carboy was a minimum of 10 cm below the surface of the water to avoid collection of surface debris during the filling process. Prior to use in mussel cultures or experiments, water was poured through a 35 µm Nitex mesh filter held between a PVC ring cap and bushing of 2” diameter. This filtered Lake Mead water (FLMW) was held in covered five gallon buckets with aeration and used within seven days of collection. All mussel cultures and tests were conducted in Lake Mead water filtered as described above.
Mussel Collection and Culture

Mussels were collected in August 2010, 11 miles downstream from Lake Mead. Collection depth was approximately one to three feet below the surface and was conducted from dry land. Mussels were removed from a concrete wall using a scraper attached to the end of a pole. Free-floating mussels were then collected with a net and transferred to a five-gallon bucket. Following collection, mussels were separated from one another using a razor blade to sever byssal thread attachments. Mussels were inspected for damage, and those with crushed or damaged shells were removed from the harvest. The remaining mussels were scrubbed with a nylon brush to remove debris, algae and other organisms attached to the shells. Following cleaning, the mussels were rinsed and placed in sealed plastic containers within secondary containment for transport to the laboratory.

Following collection, mussels were held in aerated four liter plastic beakers at a density not exceeding 10 mussels per liter, within secondary containment in a laboratory incubator at 15 °C in 24 hr darkness. Mussels were not supplemented with algae in the incubator, the only food present being natural Lake Mead seston smaller than 35 µm that were not removed during the filtration process. One week of acclimation time was allowed between collection and spawning trials. Mussel cultures were maintained with 80% water changes four to five times per week.

Spawning

All mussels included in the spawning trials were identified as actively filtering to ensure selection of healthy/active individuals. Prior to spawning assays, mussels were removed from the incubator and placed in six-quart clear plastic storage containers
holding approximately two inches of 15 °C water. Mussels for serotonin assays were held on the laboratory bench to allow a gentle warming to room temperature prior to serotonin exposure. Mussels for the temperature shock and gonad slurry assays were held in the incubator at a temperature of 15 °C to prevent any warming on the laboratory bench prior to the abrupt temperature increase during the spawning trial. Containers were observed and filtering mussels were individually removed by hand, then immediately transferred to a separate six-quart plastic storage container filled to an approximate depth of four inches of FLMW for holding prior to the identification of an adequate number of specimens for each trail.

All trials conducted for each of the three techniques: temperature shock, gonad slurry, and serotonin, were allowed to run for a four-hour period. Mussels that were not observed spawning within the four-hour period were designated as non-spawning. Following identification of active individuals, mussels were randomly selected from the filtering group and placed into individual beakers (one mussel per beaker) for use in spawning trials. Following each trial, all mussels were discarded, and no mussel was used in more than one spawning trial. In addition, controls were run with each spawning trial. The serotonin assay was conducted over six separate sessions. Temperature shock, with and without slurry, was tested over four sessions. One control group of ten mussels was run in conjunction with each spawning session for a total of 100 control mussels. Control mussels were randomly selected, handled, and observed for spawning behavior following the same processes as the test procedures described below, excluding treatments with temperature shock, gonad slurry, and serotonin.

Gamete Identification
Release of gametes was rarely directly observed; therefore onset of spawning was determined by the presence of eggs for females and characteristic odor and/or cloudy water for males. Eggs were visually identified as small whitish spheres present at the base of the beaker. High output male spawning was visually identified by cloudy water. It was observed that low output male spawning did not consistently produce a cloudy effect in the water, and thus could easily be overlooked. Through significant experimentation, it was discovered that a characteristic pungent odor was emitted during male spawning. During preliminary trials, sperm presence in odor positive beakers was consistently identified when viewed under a microscope, so olfactory analysis was deemed an effective rapid assessment for male spawning activity.

**Temperature Shock**

Temperature shock was induced by transferring mussels from a holding temperature of 15 °C to test temperatures of 22, 24, or 26 °C. Individual beakers were filled with 50 mL of FLMW and placed in a water bath at 22, 24, or 26 °C. The water bath consisted of a plastic dish tub holding approximately three inches of water with a submerged fish tank heater to maintain the specified test temperature. All beakers were allowed to equilibrate with the constant temperature bath before a single active mussel was added to each beaker. Mussels were held at test temperature and observed for spawning activity over a four-hour period. Time to spawn, sex, and shell length was recorded for all mussels.

**Gonad Slurry + Temperature Shock**

The combination of gonad slurry plus temperature shock was tested by adding a twenty-minute exposure to a homogenate of mussel gonad tissue and water at the beginning of the temperature shock period. To prepare the gonad slurry, mussels were
sliced along the shell junction with a razor blade, and gently pried apart with gloved fingernails to open. Gonad tissue was removed using a razorblade and tweezers. Following collection, tissues from multiple individuals were homogenized with 10 mL culture water per mussel using a mortar and pestle.

Individual beakers were prepared with 50 mL of FLMW as described above plus 5 mL gonad slurry and placed in a water bath at 22, 24, or 26 °C. Mussels were selected and transferred as described in the temperature shock method above. Following 20 minutes of exposure to the slurry homogenate, mussels were triple rinsed in FLMW at test temperature, and returned to clean beakers holding 50 mL FLMW at test temperature within the water bath. Mussels were left undisturbed at test temperature for the remainder of the four-hour spawning period. Time to spawn, sex, and shell length was recorded for all mussels.

**Serotonin**

A 1 mMol stock solution of serotonin was prepared using serotonin creatinine sulfate and FLMW. Test concentrations of 0.01, 0.08, 0.1, 0.2, 0.3, 0.4, and 0.5 mMol were prepared using serial dilutions of the 1 mM stock solution. Active mussels were identified as described above. Mussels were randomly selected from the active group and transferred by hand to individual 25 mL plastic beakers containing 10 mL of the desired serotonin concentration and left undisturbed for 20 minutes. Following the treatment period, mussels were removed from the serotonin solution using tweezers, triple rinsed in FLMW, and placed in glass beakers containing 25 mL of FLMW at room temperature. The glass beakers were placed on a black laboratory bench in puddle de-ionized water such that the area under the beaker contained no air, to better allow for identification of
gametes. Following exposure to serotonin, mussels were observed on the laboratory bench for a four-hour period. Time to spawn, sex, and shell length was recorded for all mussels. Gender was not determined for non-spawning mussels.

**Gamete Production**

Mussels were spawned using exposure to 0.5 mmol serotonin and time to spawn as described above, was recorded for all mussels. Mussels not spawning within four hours following serotonin exposure were noted as non-spawning. One hour following onset of spawning, mussels were removed from the spawning chamber with tweezers, measured for shell length, and discarded. Contents of the spawning chamber were gently stirred with a disposable transfer pipette prior to gamete counting. For males, a disposable plastic transfer pipette was used to fill a hemocytometer, from which sperm were counted under a Nikon ECLIPSE TS100 inverted microscope at 200X. For females, 100 µL aliquots of water from the test beaker were transferred with an adjustable pipette to a microscope slide and the total number of eggs present was counted under a Nikon Stereomicroscope SMZ1000 at 40X magnification. The process was repeated until a minimum of 100 eggs had been counted. Total gamete production for eggs and sperm was calculated as: gametes/µL x 25 mL. Gamete production was reported as the average of three counts for each spawning chamber.

**Data Analysis**

One way Analysis of Variance (ANOVA) was used to determine effect of serotonin, temperature, and temperature and gonad slurry treatments on spawning, as well as gamete production. Separate analyses were run for each treatment and for each sex (male and
female) in the case of serotonin. The analyses were carried out using JMP software (SAS Institute, Cary, North Carolina).

**Results**

Serotonin significantly affected both male \((p<0.001)\) and female \((p<0.01)\) spawning. A clear dose response curve was found between serotonin concentration and spawning success (Figure 4-1). Consistent spawning (\(>50\%\) success) was observed at concentrations at or above \(0.5\) \textit{mMol}, \((p<0.001)\). There appears to be significant individual differences in sensitivity in both males and females to serotonin treatments, females doing better than males at lower concentrations and males doing better at higher concentrations. Females showed a slightly lower threshold for serotonin sensitivity, responding to concentrations as low as \(0.08\) \textit{mMol}, while males initially responded at a concentration of \(0.1\) \textit{mMol} (Figure 4-1). Males showed a distinct threshold between \(0.3\) and \(0.4\) \textit{mMol}, with spawning success rising from \(16\%\) to \(64\%\) \((p<0.738, p<0.001)\). The gradual slope of the dose response curve in females vs. the steep slope in males indicates greater individual variation in spawning stimuli sensitivity in females than in males.

Temperature shock, and gonad slurry with temperature shock (Figure 4-2) were shown to be less effective in inducing spawning than serotonin, with overall response of \(22\%\) and \(32\%\) spawning respectively \((p<0.051, p<0.003)\), compared to an overall spawning response of \(77\%\) in serotonin\((p<0.001)\). There appears to be a difference between male and female sensitivity to temperature and slurry treatments, with male response ranging from \(0\%\) to \(16\%\), and female response ranging from \(0\%\) to \(48\%\). Treatment with a \(22 ^\circ C\) shock did not induce spawning in male or female quagga mussels; however the addition of slurry to the \(22 ^\circ C\) shock increased spawning response.
to 8% in both male and females. Temperature shock with gonad slurry showed the highest response, with 48% spawning in females and 16% in males. The addition of gonad slurry had a greater positive effect on spawning response in females than in males, with an increase of 16% response over temperature shock alone in females compared to a maximum increase of 8% response in males.

Average shell length for male and female specimens used in this study were 21.6 and 21.4 mm, respectively. Male and female shell length ranged from 15 to 28mm and 15 to 27 mm, with standard deviations of 3.2 and 3.0 respectively. This shell length range indicates mussels used in this study were of reproductive age, based on Claudie and Mackie (1994), who reported reproductive viability in zebra mussels at a shell length of 8-10 mm. No correlation was found between mussel length and time to spawn in male or female mussels (p>0.38, Figures 4.3-4.4). Males and females differed in time needed to spawn after they were exposed to serotonin. Males spawned within 10 to 117 minutes, with an average time to spawn of 39 minutes, (SD 19 min), whereas spawned within 32 to 194 minutes, with an average of 109 minutes (SD 29 min) (Figure 4.5). Additionally, no correlation was found between shell length and gamete production in male or female mussels (p>0.78, p>0.73). Female egg production ranged from 147 to 13,500 per mussel. Male sperm production ranged from 125,000 to over 35,000,000 per individual in one spawning session. Spawning output was characterized by the majority of specimens showing low gamete counts, with few specimens producing high outputs. As sperm are free floating in the water column, and mussels were left in the spawning chamber for one hour following spawning onset, it should be noted that sperm counts represent minimum
estimates of actual gamete production due to potential removal of sperm from the water column by mussel filtration.

**Discussion**

Quagga mussels were successfully spawned through application of temperature shock, gonad slurry with temperature shock, and serotonin. This is the first study directly comparing the efficacy of the aforementioned techniques to induce spawning in quagga mussels. We found that exposure to serotonin produced the greatest spawning response, with spawning at 77%, followed by gonad slurry with temperature shock, at 32%. Temperature shock alone was found to be the least consistent method to induce spawning, with a maximum response of 22%.

There have been several reports describing temperature shock to induce spawning in *Dreissena* species. Wright et al. (1996a) used temperature shock and gonad slurry to induce spawning in quagga and zebra mussels. Seltzer and Hamilton (1995) used a combination of temperature shock and gonad slurry to induce spawning in zebra mussels. Tourari et al. (1988) reported a 50% spawning rate in zebra mussels following temperature shock treatment. Our results for temperature shock alone showing 22% response in quagga mussels were much lower than the 50% response in zebra mussels reported by Tourari (1988).

Serotonin has been used to induce spawning in both zebra and quagga mussels. Ram et al. (1993) described spawning in zebra mussels after injection or external application of serotonin. Similarly, serotonin has been successfully applied to spawning of other bivalves. Fong (1998) and Honkoop et al. (1999) used serotonin reuptake inhibitors to induce spawning in both marine and freshwater bivalves. Ram et al. (1993) and Stoeckel
and Garton (1993) found concentrations of 0.1 mMol and above to induce spawning in zebra mussels. We observed that while some quagga mussels did start spawning following exposure to 0.1 mMol, consistent spawning in both male and female mussels occurs at concentrations of 0.5 mMol and above.

Our data suggest a range of individual sensitivity to spawning stimuli in quagga mussels. We think it is important to note the possibility that individual differences in sensitivity present in the original colonizing mussels of a given population could lead to a founder effect resulting in significant variation in spawning response between separate populations. Further research is necessary to analyze the potential variations in spawning response between populations collected from geographically isolated locations.

Results show that males begin spawning quicker than females following exposure to serotonin. This is consistent with Sprung (1987) and Stoeckel et al. (2004), who observed a quicker time to spawn in male vs. female zebra mussels. Similarly, Ram et al. (1993), reported that time to spawn began within 15 min for males and 90 min for females. In our experiments, we found no relation between shell length and time to spawn. Also, no correlation was observed between mussel length and gamete production. This result differs from the findings of Stoeckel et al. (2004), who reported a linear relationship between egg production and shell length in zebra mussels, and Walz (1978), who reported an exponential relationship between the total egg production observed over multiple spawning events and shell length in zebra mussels. Further observation of spawning output over a complete spawning season would be necessary to assess a relationship between mussel size and annual spawning capacity.
Finally, we observed that males emit a characteristic pungent odor when spawning. Through significant experimentation, it was discovered that detection of this odor provides a rapid assessment for the onset of male spawning. Successful identification of the odor was also found to reduce false negatives in detecting low volume releases as water may not become cloudy and low sperm counts may require careful scrutiny under microscope.

**A User’s Guide to Spawning Quagga Mussels**

Throughout the spawning assays, several procedural factors were identified that assisted in identification of spawning events.

- Recommended egg viewing minimum magnification 40x, dark field provides good contrast for easy identification
- Recommended sperm viewing minimum magnification 200x, precise focus and lighting is necessary to view.
- Males spawning gives off a distinct characteristic pungent smell. Females do not.
- This smell is a quick way to determine when spawning begins, as it appears before water turns cloudy and does not require the time of microscope viewing, just a quick sniff. Additionally, when small quantities of sperm are released it is possible to observe a false negative under microscopic analysis.
- Too large of a spawning chamber volume will make it difficult to view male spawning unless it is a particularly prolific specimen.
- Glass chambers are strongly recommended over plastic, which obscures identification of eggs/cloudy water.
• Cloudy water from male spawning is more easily identified when viewed from the side.

• Eggs will be visible at the bottom of the beaker upon careful inspection when viewed against a dark background. If viewed against a light background, they will be difficult or impossible to identify and you can miss a spawning event.

• For best viewing, wet a black laboratory table and place beakers in the puddled water with care to avoid air under the beaker. The gloss provides a contrast allowing for easier identification of eggs.

• To ensure spawning of a maximum number of individuals, expose mussels to serotonin in individual beakers. We hypothesize that due to some mussels filtering later than others, those who start filtering towards the end of the 20 min exposure period take in water that has a reduced serotonin concentration due to filtration by other mussels, thus show lower spawning rates.
Figure 4.1: Spawning percentages of Lake Mead _Dreissena bugensis_ under various serotonin concentrations in the laboratory conditions. Each pair represents a single concentration. Error bars represent standard errors.
Figure 4.2: Spawning percentages of Lake Mead *Dreissena bugensis* under temperature shock and gonad slurry treatments in the laboratory conditions. Error bars represent standard errors.
Figure 4.3: Time to spawn following external application of various serotonin concentrations in male Lake Mead *Dreissena bugensis*. 
Figure 4.4: Time to spawn following external application of various serotonin concentrations in female Lake Mead *Dreissena bugensis*.

Figure 4.5: Comparison of male and females time to spawn following external application of various serotonin concentrations in Lake Mead *Dreissena bugensis*
References


Spawning of *Dreissena bugensis* and working with veligers of the species have been reported to be difficult to achieve under laboratory conditions, and as such little data currently exists on the topics. The work presented in this thesis describes successful attempts at both working with veligers and spawning adults in the laboratory. Additionally, the data contributes to the knowledge base regarding quagga veligers, and spawning of adult populations, and presents a valuable starting point for future spawning studies.

The results from the growth study showing no significant difference in veliger growth between Lake Mead seston and *I. galbana* supplemented groups were unexpected, and have left the door open for further studies regarding the relationship between veliger growth and nutritional content. Future studies are recommended to analyze and compare the nutritional content of *I. galbana* and natural Lake Mead seston, as well as the effects of water quality on veliger growth.

Results from the desiccation assay showed a positive correlation between relative humidity and desiccation tolerance in plantigrade quagga veligers. This result was consistent with our original hypothesis, however further research is required to analyze the desiccation tolerance of younger veligers, as well as the analysis of tolerance under extreme humidity conditions such as 99%. Completion of a comprehensive scope of study is recommended to more fully support veliger decontamination and transport requirements.
Great success was observed with the spawning assay, with the study marking the first assessment directly comparing the efficacy of temperature shock, gonad slurry, and serotonin in inducing spawning in quagga mussels. We found that a clear dose-response relationship exists between spawning response and serotonin concentration, with consistent spawning following exposure to serotonin at concentrations at or above $5 \times 10^4$ M in both males and females. Our results found serotonin to be the most effective of the three methods examined, followed by temperature shock with gonad slurry, and temperature shock alone. Maximum spawning response of the three methods was 77.3%, 32%, and 22% respectively. Additionally, we found no correlation between mussel shell length and clutch size, and that the spawning delay between serotonin exposure and spawning to be greater in females than males.

The users guide to spawning quagga mussels presented in this thesis represents the result of a significant investment of time during preliminary stages of the investigation, and will provide a valuable starting point for future spawning studies. Spawning of quagga mussels under laboratory conditions has been reported to be a challenge. The ability to consistently spawn quagga mussels under laboratory conditions provides both a baseline for future spawning studies, as well as a source of veligers for study of juvenile life stages.
References


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