Research Article

Comparative efficacy of *Dreissena rostriformis bugensis* (Bivalvia: Dreissenidae) spawning techniques

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Abstract

Spawning of *Dreissena rostriformis bugensis*, commonly known as the quagga mussel, under laboratory conditions has received relatively little attention. 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 (TS), and temperature shock with the addition of gonad slurry (TS+GS). 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 TS produced a maximum of 22 % spawning success. The addition of gonad slurry showed a slight increase over TS at 32 %. Serotonin was found to produce the highest percent spawning at 77.3% success. 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 5×10^{-4} 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.

Key words: quagga mussel; temperature shock; gonad slurry; serotonin

Introduction

Invasion of North American surface waters by *Dreissena rostriformis bugensis* Andrusov, commonly known as the quagga mussel, has had significant environmental and economic effects (e.g., Pimentel et al. 2005; Limburg et al. 2010). The quagga mussel was apparently introduced to the North American Great Lakes region in the 1980s (e.g., Mills et al. 1993), and then spread outwards through the mid-western and eastern portions of the country (e.g., Karatayev et al. 2012). The first appearance of quagga mussels in the western United States was reported in 2007 (Wong and Gerstenberger 2011). Specifically, they were discovered in Lake Mead, a ~640 km² artificial reservoir on the Colorado River near Las Vegas, Nevada. The Colorado River is the most important river in the southwestern United States, with an overall length of ~2300 km and a

drainage basin of ~6.4 ×10⁵ km². Invasion of this watershed has the potential to facilitate the spread of the quagga mussel throughout the western United States and into Mexico. To mitigate continued invasion of the quagga mussel, it is important to conduct experiments on laboratory-raised specimens. Unfortunately, there is a dearth of literature regarding the induction of spawning for *Dreissena rostriformis bugensis*, and none that is directly relevant to conditions in the southwestern United States.

Aquaculture of mollusk species is common in both research and commercial environments. Researchers have successfully used serotonin to induce spawning in bivalves including quagga mussels. McAnlis et al. (2010) were able to induce spawning on three freshwater and marine bivalves including quagga mussels with external application of serotonin. Walker et al. (1996) used serotonin to successfully spawn both quagga and zebra mussels. Similarly, Walz

(1978), Sprung (1992), and Nichols (1993) used serotonin to induce spawning on zebra mussels. Serotonin and other neurotransmitters are known to regulate reproductive processes in bivalves (e.g., Hirai et al. 1988; Ram et al. 1993; Fong et al. 1994; Fong 1998), including oocyte maturation, spawning, and parturition (Osada et al. 1998). Zebra mussels have been observed to spawn shortly after external application of sero-tonin (5-hydroxytryptamine) at concentrations of both 1.0 mM and 0.1 mM (Ram et al. 1993). Freshwater mussels exhibit coordinated spawning (e.g., Ram et al. 1993) triggered by gamete release. Exposure to spermatocytes through introduction of gonad slurry has been shown to induce spawning in zebra mussels (e.g., Sprung 1989). Temperature shock is often used to initiate spawning at commercial aquaculture operations (Carlsbad Aqua Farm, pers. comm., which likely reflects seasonal changes in estua-ries and riverine environments (e.g., Honkoop et al. 1999). Zebra mussels have been reported to spawn in response to temperature shock alone (Tourari et al. 1988), and in combination with the application of gonad slurry (Seltzer-Hamilton et al. 1995; Wright et al. 1996). Wright et al. (1996) also reported that the combined application of temperature shock and gonad slurry induced spawning in quagga mussels.

Here, we compare the efficacy of serotonin, temperature shock (TS), and temperature shock plus gonad slurry (TS+GS) as means to successfully induce quagga mussels to spawn in a laboratory environment. Adult mussels harvested from Lake Mead were cultured in the laboratory under controlled conditions to mini-mize the effects of harvest and transport. Healthy individuals were then subjected to one of the three treatments (serotonin, TS, or TS+GS) before being discarded. Results of this study add to our understanding of quagga mussel spawning processes and have the potential to enhance our ability to study the juvenile life stages for both ecological and containment purposes.

Methods

Mussel collection and culture

Mussels were collected in August 2010 from an undisclosed location on the Colorado River that is ~18 km downstream from Lake Mead. The collection method was to scrape live mussels from a concrete surface at water depths of 30-100 cm, capture the free-floating mussels in a net, and then transfer them to a 20 L (five-gallon) plastic bucket. Following collection, mussels were separated from one another using a razor blade to sever byssal thread attachments. Each mussel was then carefully inspected for damage, and those with crushed or damaged shells were removed from the harvest. Shells of the intact specimens were scrubbed with a nylon brush to remove debris and fouling organisms. After cleaning, the mussels were rinsed with fresh water from the collection site and placed in sealed plastic containers for transport to the laboratory.

The collected mussel specimens were acclimated to laboratory conditions for one week prior to the start of spawning trials. During this time, the specimens were cultured in aerated 4 L plastic beakers at a density not exceeding 10 mussels per L; ambient conditions were held at $15^{\circ}C$ and 24 hr darkness using a laboratory incubator (VWR International LLC. Model 2015). Feedstock consisted of the natural seston (<35 µm) present in Lake Mead water and was replenished by replacing 80% of the water four to five times per week. Water used in mussel cultures and experiments was collected ~10 m from shore at the Boulder Beach area of Lake Mead in 20 L Nalgene® carboys, then passed through a 35-µm Nitex mesh filter. This filtered Lake Mead water (FLMW) was held in covered 20 L buckets with aeration and used within seven days of collection.

Spawning

All spawning trials were conducted on individual specimens that had been identified as actively filtering. Prior to use in the serotonin assays, mussels were placed in a 5.7 L (six-quart) plastic vessel filled to a depth of ~5 cm with FLMW at $15^{\circ}C$, and then allowed to equilibrate slowly to room temperature. Mussels for the TS and TS+GS assays were held in the incubator at a temperature of $15^{\circ}C$ to prevent any warming on the laboratory bench prior to the abrupt tempe-rature increase during the spawning trial. After observation, actively filtering mussels were transferred a second time to a separate 5.7 L plastic storage container filled to an approximate depth of 10 cm of FLMW for holding.

Active mussels were identified and randomly selected, being placed into individual beakers (one mussel per beaker) for use in spawning trials. Beaker specifics for each trial are further

described below. In each trial, mussels were observed for a four hour period following stimulation by TS, TS+GS, or serotonin. Mussels that were not observed spawning within the four-hour period were designated as non-spawning. The four hour window was based on a number of preliminary trials in which spawning was observed to occur within 4 hours or not at all.

The serotonin assay was conducted over six separate sessions; TS and TS+GS were tested over four sessions. For each session, a control group of 10 mussels was subjected to the same handling procedures as the test groups (except for the artificial stimulation), and then examined for spawning. At the end of each session, all mussels (test and control groups) were discarded to preclude reuse.

Gamete identification

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 was detected under microscopic analysis of all beakers testing positive for odor presence (n=48), including beakers showing no increase in turbidity. Therefore, a positive odor test was deemed an effective rapid assessment for male spawning activity. During data collection for this paper, all beakers testing positive for presence of odor and showing no visible turbidity were analyzed under microscope to verify sperm presence; odor was a positive indicator for sperm presence in 100% of the non-turbid cases.

Temperature shock

Temperature shock (TS) was induced by transferring mussels from a holding temperature of 15°C to test temperatures of 22, 24, or 26°C. Individual 200-mL glass 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 8 cm of water with a submerged heater (EHEIM GmbH and Co. KG, JAGER 365090 Aquarium 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 observed for spawning activity over a four-hour period. Time to spawn, sex, and shell length was recorded for all mussels.

Temperature shock plus gonad slurry

A 20-minute exposure to a homogenate of mussel gonad tissue and water at the beginning of the temperature shock period was imposed to considered TS+GS. 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 200-mL glass 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

Serotonin was tested as spawning stimulant at concentrations of 0.01, 0.08, 0.1, 0.2, 0.3, 0.4, 0.5, and 1.0 mM in FLMW. A 1 mM stock solution was prepared by adding serotonin creatinine sulfate, monohydrate (5-hydroxytryptamine) (TCI America) to FLMW, and the remaining test concentrations were produced through serial dilution. 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 then 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 200-mL glass beakers containing 25 mL of

FLMW at room temperature. 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 the non-spawning mussels.

Gamete production

Mussels were induced to spawn using exposure to 0.5 mM serotonin for the purpose of measuring gamete production. Time to spawn was recorded over the first four hours following serotonin exposure, the remaining test specimens were noted as non-spawning. One hour following the 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 × 25,000. 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 the effects of serotonin, TS, and TS+GS treatments on spawning, as well as gamete production after testing for normality and homogeneity of variances using Shapiro-Wilk and Levene's tests. 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

The experimental results showed that serotonin clearly affected the spawning of quagga mussels. Temperature shock, and TS+GS shock (Figures 1 and 3) were shown to be less effective in inducing spawning than serotonin.

Serotonin significantly affected both male ($p<0.001$, $F_{8,21}=52.17$) and female ($p<0.001$, $F_{8,21}=51.83$) spawning. The results of ANOVA indicated that there were differences among the means of spawning responses of eight serotonin concentrations in the confidence level of 99.9% for male and 99% for female. A clear dose response curve was found between serotonin concentration and spawning success (Figure 2). Consistent spawning (>50% success) was observed at concentrations at or above 0.5 mM. There were statistical differences between a group of at or above 0.5 mM and a group of lower concentration treatments ($p<0.001$, $F_{8,21}=192.94$). The percent spawning was compared between male and female at each serotonin dosage. The spawning differences between genders varied except for serotonin concentrations of 0.4 and 0.5 mM. Females showed a slightly lower threshold for serotonin sensitivity, responding to concentrations as low as 0.08 mM, while males initially responded at a concentration of 0.1 mM (Figure 2). Males showed a distinct threshold between 0.3 and 0.4 mM, with spawning success rising from 16% to 64%. 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 (Figure 2).

The overall maximum spawning responses produced by TS only and TS+GS treatments were 22% ($p<0.05$, $F_{6,7}=6.22$) and 32% ($p<0.003$, $F_{6,7}=6.22$) respectively, compared to an overall response of 77% in serotonin ($p<0.001$, $F_{8,21}=192.94$). There appears to be a difference between male and female sensitivity to temperature and slurry treatments at 24 and 26°C ($p<0.05$, $F_{6,7}=6.22$) when male and female were pooled from both treatments: with and without gonad slurry), with male response ranging from 8% to 16%, and female response ranging from 12% to 48%. Treatment with a 22°C shock alone did not induce spawning in either male or female quagga mussel; however, the addition of gonad slurry to the 22°C shock increased spawning response to 8% in both male and females. Temperature shock to 26°C with gonad slurry showed the highest response, producing 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 a 50% increase in response seen with the addition of gonad slurry over 26°C TS alone in females, compared to a 30% increase in spawning response in males at the same temperature.
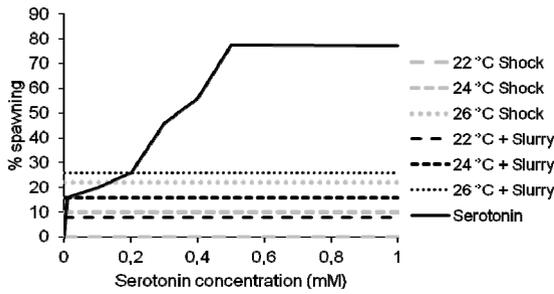
Figure 1. Spawning percentages of Lake Mead *Dreissena rostriformis bugensis* under laboratory conditions under serotonin, temperature shock and gonad slurry treatments.
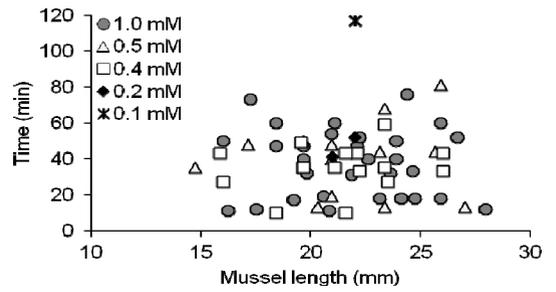

Figure 4. Time to spawn following external application of various serotonin concentrations in male Lake Mead *Dreissena rostriformis bugensis*.
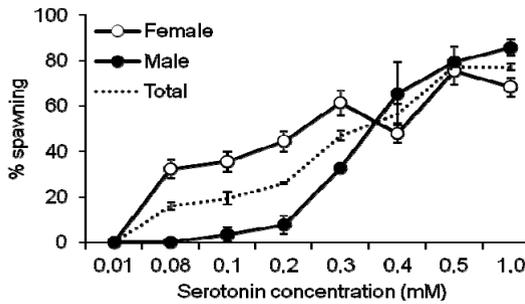

Figure 2. Spawning percentages of Lake Mead *Dreissena rostriformis bugensis* under laboratory conditions for various serotonin concentrations. Vertical bars represent standard error of the mean. n= 365.
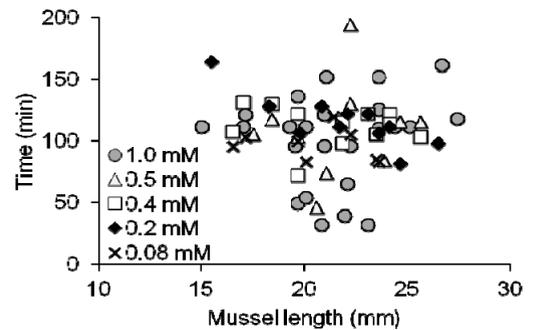

Figure 5. Time to spawn following external application of various serotonin concentrations in female Lake Mead *Dreissena rostriformis bugensis*.
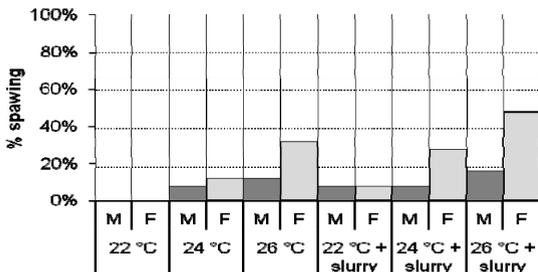

Figure 3. Spawning percentages of Lake Mead *Dreissena rostriformis bugensis* under laboratory conditions for temperature shock and gonad slurry treatments. Each treatment had 25 male and 25 female quagga mussels. M and F represent male and female *Dreissena bugensis*. n=300.
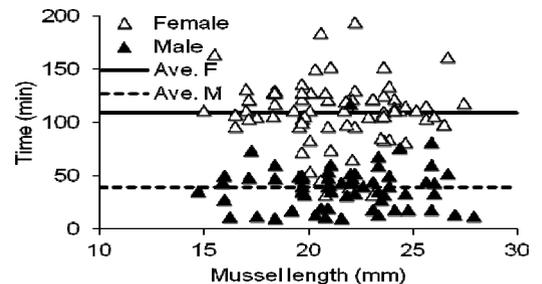

Figure 6. Comparison of male and female time to spawn following external application of various serotonin concentrations in Lake Mead *Dreissena rostriformis bugensis*. The lines in the figure represent average time for female (solid line) and male (dotted line).
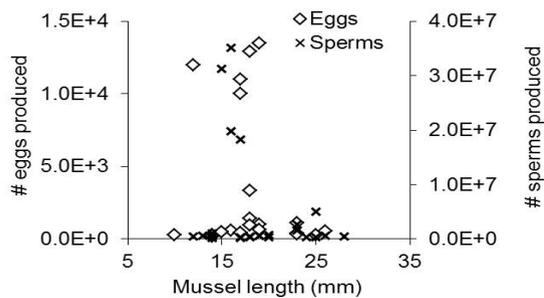
Figure 7. Spawning output of male and female *Dreissena rostriformis bugensis* compared to shell length (mm).

Mean shell length for male and female specimens used in this study were 21.6 ± 3.2 mm and 21.4 ±3.0 mm, respectively. Male and female shell length ranged from 15 to 28 mm and 15 to 27 mm. This shell length range indicates mussels used in this study were of reproductive age, based on Claudi 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 for either male or female mussels (Figures 4 and 5). Males and females differed in time needed to spawn after they were exposed to serotonin. Males spawned within 10 to 117 minutes, with a mean time for spawning of 39 ± 19 minutes, whereas females spawned within 32 to 194 minutes, with a mean time for spawning of 109 ± 29 minutes (Figure 6). Additionally, no correlation was found between shell length and gamete production in either male or female mussels. 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. Quagga mussels of approximately 15-20 mm length group produced the highest number of sperm or eggs (Figure 7).

Discussion

Quagga mussels were successfully spawned through application of TS, TS+GS, and sero-tonin, albeit there was a significant variation in effectiveness of each method. 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 (77%), followed by TS+GS (32%). Temperature shock alone was found to be the least consistent method to induce spawning, with a maximum response of 22%. Our results for TS alone, showing 22% response in quagga mussels were much lower than the 50% response in zebra mussels reported by Tourari et al. (1988).

Serotonin has been used to induce spawning in wide variety of bivalves including zebra mussels. Ram et al. (1993) described spawning in zebra mussels after injection or external application of serotonin. Fong (1998) and Honkoop et al. (1999) used serotonin reuptake inhibitors to induce spawning in both marine and freshwater bivalves. Gibbons and Castagna (1984) success-fully induced spawning in six bivalve species with the dosage of 0.4 ml of 2 mM serotonin solution. Ram et al. (1993) and Stoeckel and Garton (1993) found concentrations of 0.1 mM and above to induce spawning in zebra mussels. Velasco et al. (2007) successfully used serotonin via intragonadal injection on scallops for spawning. We observed that while some quagga mussels did start spawning following exposure to 0.1 mM, consistent spawning in both male and female mussels occurs at concentrations of 0.5 mM and above. The females were more responsive than males at lower concentrations, while the reverse is observed at higher concentrations.

Our data suggest a range of individual sensiti-vity 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, thus resulting in significant variation in spawning response between separate populations. Phillimore et al. (2010) suggest that environ-mental differences can arise when phenotypes are influenced by environmental factors that vary across populations. It is also important to note the effect of environmental conditions on reproductive readiness. This becomes important in evaluating spawning output across different population groups, as environmental differences (temperature, light, nutrition etc.) between the southwest and Great Lakes regions may support varying levels of internal gamete maturation, making accurate comparison of spawning responses between cohorts of diverse geographic origin difficult. Also of importance is spawning sensitivity with seasons and gonadal develop-

ment. Gerstenberger et al. (2011) found that there was a spike in veliger larvae in Sept-Oct in Lake Mead, therefore August was not a bad time for spawning experiment. However, more 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), Stoeckel et al. (2004) and McAnlis et al. (2010), 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. Detection of odor was something that we observed during the experiment; it was not intended and therefore no data has been formally recorded. Additional research will be needed in the future to verify this discovery.

Acknowledgements

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