2010

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Hydrodynamic forces affect larval zebra mussel (*Dreissena polymorpha*) mortality in a laboratory setting

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Received: 18 May 2010 / Accepted: 30 August 2010 / Published online: 7 September 2010

**Abstract**

Mortality of zebra mussel, *Dreissena polymorpha*, larvae was quantified in a laboratory experiment that was designed to assess the role played by both intensity and duration of the exposure to hydrodynamic forces. Larvae were collected in a plankton net and distributed in 100-ml aliquots to 125-ml Erlenmeyer flasks. The flasks were spun on an orbital shaker at different speeds, 100 rpm and 400 rpm, to change the intensity of the hydrodynamic forces experienced by larvae inside the flasks. Actual shear forces were not quantified. A parallel set of control flasks were not spun. Flasks were spun for 1, 24 and 48 hours. Mortality was highest in the 400 rpm, 48-h trial. Both intensity and duration were highly significant variables in the ANOVA model (p<0.001). However, the interaction term was also highly significant (p<0.001). Larval mortality was significantly higher in the treatments than in the non-spun flasks in only the 400 rpm, 24-h and 400 rpm, 48-h trials. Thus, longer duration of exposure to high intensity hydrodynamic forces increases larval mortality. These results help explain natural recruitment patterns of zebra mussels in natural streams and may be of interest to management and conservation efforts.

**Key words:** veliger, turbulence, dispersal, invasive species, streams

**Introduction**

The zebra mussel, *Dreissena polymorpha* (Pallas, 1771), is a highly adaptive freshwater mollusk. Life cycle and life history characteristics, such as byssal attachment to various hard and soft substrates, high filtering activities, high fecundity, and dispersible planktonic larvae, has allowed the species to colonize many inland lakes and rivers in the Northeastern and Midwestern United States since 1990, with expansions into the western United States in 2009. Native to the Black and Caspian seas region and present in European waters for most of the last century (Kinzelbach 1992), and it will likely continue to colonize new waters in both Europe and North America (Kraft et al. 2002).

Human behaviors have contributed greatly to the successful range expansion of this species. Canal systems created across Europe have given the mussel new avenues for dispersal either associated with barge traffic, or via passive downstream dispersal of its planktonic larvae. Similarly in North America canals gave access to the Mississippi drainage system from the Great Lakes. Dispersal as adults is human-mediated, and initial colonization events within watersheds is often due to recreational boating activities (Johnson and Carlton 1996; Buchan and Padilla 1999; Kraft et al. 2002), although other mechanisms are possible (see Horvath and Lamberti 1997). Since lakes are often used by recreational boaters, lakes are often the point of first introduction within watersheds. Passive dispersal of larvae can then distribute the mussel throughout all connected water bodies downstream (Horvath et al. 1996; Lucy et al. 2008).

Environmental characteristics of habitats have long guided the probable distribution of zebra mussels, especially in North America (e.g., Neary and Leach 1992; Strayer 1991). For example, suitable environments are those with a pH between 7.4 and 9.4, temperatures ranging from 12°C to 24°C for optimal reproduction, and with a minimum calcium ion concentration of 20 mg l$^{-1}$ present (Sprung 1993; Hincks and Mackie 1997). Similar
environmental variables have also generated sophisticated predictive models of probable distributions (Bossenbroek et al. 2001; Leung et al. 2004; Bossenbroek et al. 2007). Although these processes have been instructive in terms of large scale distribution patterns, smaller scale phenomenon have received less attention (Kraft et al. 2002). Of particular interest may be the fate of potential colonizing individuals (i.e., larval propagules), which ultimately determine small-scale distributions such as settlement patterns in a single stream.

Larval zebra mussels remain planktonic for 2-4 weeks (Sprung 1989). During this stage, they move passively with water currents. The larval cycle is completed when they reach the pediveliger stage (220-320µm). Pediveligers use byssal threads to settle and attach to hard substrates before metamorphosing into the adult form. In riverine systems larvae produced by a population are transported far from the parent population before they reach settlement stages. Populations of zebra mussels in such lotic systems remain dependent on new propagules from upstream sources, as these populations are rarely if ever self-sustaining (Horvath et al 1996; Stoeckel et al. 2004). However, even when seemingly abundant numbers of larvae make it to reaches of a small stream, the successful recruitment of these propagules are consistently limited to reaches near the source population (Bobeldyk et al 2005; Horvath et al. 1996). Horvath and Lamberti (1999a) looked for biological factors that may have limited recruitment in a small North American stream, but found none conclusive. Data on larval mortality during downstream transport revealed that patterns in mortality matched well the recruitment pattern in that stream (Horvath and Lamberti 1999b). They suggested exposure to turbulence as a possible mortality-causing factor, and Rehmann et al. (2003) showed convincing data that this is indeed possible. Although they showed that higher levels of exposure to turbulence increased larval mortality, their experimental trials lasted only 24 h at each turbulence intensity level. We believe that an additional variable of time is important because larvae may spend variable lengths of time being subjected to turbulence during downstream transport.

Our experiment was designed to test the hypothesis that exposure of larvae to low levels of hydrodynamic forces (i.e., turbulence) over longer time periods can cause mortality. Knowing what factors contribute to zebra mussel mortality at this highly vulnerable stage may give insight into recruitment patterns in natural systems. This may then be of interest to natural resource managers and conservation efforts.

**Methods**

Canadarago Lake (N42°48.9’, W75°00.4’), in Otsego County, NY, was chosen as our collection site because it is a good source of zebra mussel larvae and it is close to the laboratory. Zebra mussels were first reported in Canadarago Lake in July of 2002, but it was estimated that they have been there since 2000 (TH Personal Observation). Larvae were collected from Canadarago Lake on 19, 21, and 25 June, as well as 2, 9 and 12 July. We used a 63-µm mesh plankton net towed behind a boat driven by an electric motor. Multiple tows were made each sampling day. The average length of time per tow for collection was approximately 3 minutes. After each tow, the plankton bucket was carefully washed into a 30-liter cooler that had a few liters of raw lake water and ice. Collection sessions never lasted more than 1 h, and larvae were transported to the laboratory within a travel time of 25 minutes.

A new trial was set up immediately upon arriving at the laboratory on each collection date. The collected lake water was homogenized by gently stirring the contents of the cooler, and aliquots of 100-ml were put into 24 pre-washed 125-ml Erlenmeyer flasks. The homogenization assured that each flask had similar starting conditions of veliger density and composition of other organisms (predators included). Half of these flasks were randomly picked to be placed onto an orbital shaker and subjected to shaking (i.e., treatments); the other 12 flasks were placed next to the shaker at 0 rpm (i.e., no shaking). The experimental design was fully factorial with all possible combinations of spin intensity (100 rpm and 400 rpm) and duration of spin (1, 24, 48 h) being run on separate dates - the June 19 collection was 400 rpm for 1 h; the 21 June collection was 400 rpm 24 h; the 25 June was 400 rpm 48 h; the 2 July collection was 100 rpm 24 h; the 9 July collection was 100 rpm 48h; the 12 July collection was 100 rpm 1 h. We expected some differences in the physiological conditions of the veligers collected on the different days, therefore the flasks that were not on the orbital spinner allowed us to correct for mortality due to the
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handling of the veligers. These 0 rpm flask were not used as a level of treatment because of the differences expected among the sampling days. Temperature of water in all flasks was recorded at the end of each trial.

At the end of each trial, samples were counted immediately by both authors simultaneously in order to assure similarity of identifying live and dead larvae (Figure 1). A dissecting microscope (200x; Zeiss Stemi 11) and a compound microscope (200-400x; Zeiss Axioskop) each fitted with filters enabling cross-polarizing light to easily identify the zebra mussel larvae (Johnson 1995) were used. Sedwick-Rafter Counting Cells (1-ml cells) were used on the compound microscope, while a petri dish lid with a thin layer of sample water was used on the dissecting microscope. We wanted at least 100 larvae counted per flask. On average, 3 Petri dishes were counted per 125-ml flask of sample using the dissecting scope, and 2 Sedwick-Rafter slides on the compound microscope. Under the microscopes the internal organ movement, blood circulation and respiration can easily be seen to determine whether or not the larvae are alive. Noticeably damaged and empty shells were counted as dead. Counts were calculated as proportional mortality per treatment.

Proportional data were generated, and all were transformed via an Arc-sin square-root to correct for normality (Zar 2009). General linear models were used to detect differences within and between treatments, whereas Student t-tests were used to test for mean differences between controls and treatments in each time-spin trial. To assure that assumptions of these parametric tests were met, equality of variance was checked via Levene’s Test and normality of residuals was checked with a Kolmogorov-Smirnov one sample test. All data were analyzed using Minitab15®.

Results

With exception of the first round of sampling (19 June), where we averaged 106 (± 19 SD) larvae counted per flask, we could not get much more than 30-50 individuals counted per flask. All flasks from a given trial (12 treatments and 12 controls) were always quantified within an 8-h period. One trial (400 rpm, 48 h control) had a standard deviation of 28% of the mean, but most were less than 5% of the mean. Thus we didn’t feel the need to further analyze any differences in interpretation of live versus dead larvae among the two authors. Environmental conditions in the laboratory, including light and temperature, remained fairly consistent across all the days when trials were run. Temperatures in the flasks did not exceed 22.5° C during any trial.

We did not detect significant differences between means of treatments and controls at 100 rpm for any of the trials (Table 1). We only detected a significant difference between control and treatment groups at 400rpm for the 24 and 48h trials (Table 1). At 100 rpm, both the 1 h and 24 h treatment and control flasks had only 4% of the larvae counted as dead. In the 48 h trial, control (18% dead) and treatment (19% dead) were also very similar. Only 2% of the larvae were dead in the 1-h trials at 400 rpm in both control and treatment. The small difference in mortality in the 24-h trial, 13% in the control versus 21 % in the treatment, was significant. The greatest mortality was found in the treatment spun for 48 h at 400 rpm, with 95% of the larvae encountered being dead and 39% being dead in the control.

Because proportional mortality in controls differed among the trials (mean = 0.13, SD = 0.14), we used the control data not as a level of treatment, but as an internal control. In fact, when we ran a factorial ANOVA using mortality from the control data only, with duration of spin and intensity of spin as independent and crossed factors, we found all factors in the model to be significant at the p<0.001 level. So to adjust treatment data in each trial, we took the average mortality from the set of control flasks and subtracted it from the mortality of each treatment replicate. This was then considered our adjusted mortality data.

Figure 1. Damaged and intact veligers at 200× magnification. The intact veliger is 296 microns at its widest axis. Photo by L. Crane.
Table 1. T-test results comparing means from spin trials at variable durations and spin intensities (rpm) compared to controls (not spun) run separately for each duration-intensity combination. P-values are two-tailed with equal variances assumed and confirmed with a Levene’s Test.

<table>
<thead>
<tr>
<th>Duration (h)</th>
<th>rpm</th>
<th>t</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>100</td>
<td>-0.10</td>
<td>0.92</td>
</tr>
<tr>
<td>24</td>
<td>100</td>
<td>0.97</td>
<td>0.34</td>
</tr>
<tr>
<td>48</td>
<td>100</td>
<td>-0.31</td>
<td>0.76</td>
</tr>
<tr>
<td>1</td>
<td>400</td>
<td>-0.98</td>
<td>0.34</td>
</tr>
<tr>
<td>24</td>
<td>400</td>
<td>-2.66</td>
<td>0.01</td>
</tr>
<tr>
<td>48</td>
<td>400</td>
<td>-12.29</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

Table 2. ANOVA table from the factorial analysis using adjusted mortality data.

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>Time</td>
<td>2</td>
<td>7.78</td>
<td>3.89</td>
<td>254.92</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>RPM</td>
<td>1</td>
<td>3.23</td>
<td>3.23</td>
<td>211.46</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Time*RPM</td>
<td>2</td>
<td>3.02</td>
<td>1.51</td>
<td>98.90</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>Error</td>
<td>66</td>
<td>1.01</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>71</td>
<td>15.03</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 3. ANOVA table for comparison of treatments of trials spun at 100 rpm (source A) and 400 rpm (source B).

<table>
<thead>
<tr>
<th>Source</th>
<th>DF</th>
<th>SS</th>
<th>MS</th>
<th>F</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>A-time</td>
<td>2</td>
<td>0.62</td>
<td>0.31</td>
<td>15.98</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>A-error</td>
<td>33</td>
<td>0.65</td>
<td>0.02</td>
<td></td>
<td></td>
</tr>
<tr>
<td>A-total</td>
<td>35</td>
<td>1.27</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-time</td>
<td>2</td>
<td>10.1</td>
<td>5.09</td>
<td>463.76</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>B-error</td>
<td>33</td>
<td>0.36</td>
<td>0.01</td>
<td></td>
<td></td>
</tr>
<tr>
<td>B-total</td>
<td>35</td>
<td>10.5</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Both duration (time) and intensity of spin (rpm) proved to be highly significant in the model (Table 2). However, the interaction term was also significant, which makes a clear interpretation of duration and intensity effects more difficult.

We also analyzed the time component separately for each spin intensity. Duration of spin was significant in the ANOVA model (Table 3). However, the model was largely influenced by the increase in mortality after 48 h of spinning. The post-hoc pairwise comparison was insignificant (p = 0.90) between 1 and 24 hours. However, when larvae were spun for 48 hours, a significant increase (p<0.001) in mortality was observed (Figure 2). When the spin intensity was raised to 400 rpm, time is again significant (Table 3). Each pairwise comparison becomes significant (each at p<0.001 level), with mortality again increasing with increased duration of spin (Figure 3).
**Discussion**

Our initial goal of counting at least 100 larvae in each flask was only met on the first sampling day. Larval mortality in the flasks increases with time. Thus, we needed to get all 24 flasks counted at the end of a trial within at most an 8 h period because the condition of larvae seemed to deteriorate rapidly as time went on. Counting enough larvae often required close to an hour of microscope time, due largely to the handling time needed to load slides onto the microscopes, locate and identify larvae, and then closely examine each to see if it was alive or dead. Larval condition often required observing an individual for a few minutes or even touching it with a microprobe (insect pinning needle) to see if it would react or not. As the sampling went on into July, larval densities in Canadarago Lake also declined from the early June maximum, making it even more difficult to encounter enough individuals in the sample. However, we did manage to count over 30 individuals in each flask and we were confident that we correctly identified live and dead individuals.

We made note of the physical condition of larvae. For example, some individuals had shells that were broken, some severely, and others had the two valves twisted and separated as if they were wrenched apart. Most of these were counted as dead, but some obviously alive larvae also showed signs of physical damage. We counted these as live, but clearly they would have died in the near future. The amount of damaged alive larvae was the highest in the 400 rpm, 48 h trials averaging 1 damaged shell per replicate. We did on occasion also see damaged larvae in the control flasks (22 of the 3355 larva observed), which may indicate that the collection method itself was somewhat stressful. We occasionally also observed zebra mussel larvae ingested by rotifers. We did not include these individuals in our counts. Images of damaged larvae are available upon request.

Our experiment had some obvious shortcomings. We were not able to quantify the hydrodynamic forces in our experiments as had others (Rehmann et al. 2003). However, these types of spinning experiments have been done before (Thomas and Gibson 1990; Mead and Denny 1995), and forces were quantified. In those experiments, greater speeds of rotation increased shear forces inside the flasks. We do not extrapolate their values to our trials, nor can we say that increasing the spin from 100 rpm to 400 rpm is a four-fold increase in force experienced by larvae in the flasks. We do feel confident, however, that by increasing the rpm of the orbital shaker table, that we are increasing the shear forces encountered by the zebra mussel larvae in the flasks.

The interpretation of our data was also complicated by the mortality quantified in the control flasks. Although control mortality was generally low, the longer the larvae remained in the laboratory, the greater their mortality. Thus, the controls for the 1-h trials had the lowest mortality (<5%), whereas the mortality in the 48-h trials were 18 and 39%. We did not observe a pattern in the control mortalities related to the date the larvae were collected, which was a bit surprising to us. Mortality in the 1-h trials when larvae were collected on 19 June was 2% and only 4% when the larvae were collected on 12 July. When larvae remained longest in the lab, those collected on 25 June ended with 39% mortality, whereas those collected on 9 July ended with 18% mortality. We originally considered that larvae collected later in the summer would be significantly harder because there would tend to be a higher proportion at later development stages. Schneider et al. (2003) reported highest larval mortality in the transition between D-stage and umboal-stage individuals. Thus any of the weakened individuals would have already died and settled out of the water column before collection. We did not record size or development stage data while determining mortality; however, we did not notice great differences among the samples in terms of proportions of any given development stage. Thus, we feel that our adjustment of the treatment mortalities was the best way to handle any differences we observed in mortality not related to the actual treatment condition.

Microscopic larvae are known to be susceptible to increased mortality in the presence of hydrodynamic forces. Mead and Denny (1995) simulated shear stress in the laboratory and observed physiological damage to sea urchin, *Strongylocentrotus purpuratus* (Stimpson, 1857), gametes and larvae. Similarly, the dinoflagellate *Gonyaulax polyedra* Stein, 1883 showed lower production values when exposed to shear forces associated with turbulence (Thomas and Gibson 1990). In a lake-outlet stream, phytoplankton showed signs of physiological damage caused by exposure to physical stresses associated with turbulence (Uehlinger 1993).
Zebra mussels have also been shown to be sensitive to turbulent forces. In an elegant set of experiments, Rehmann et al. (2003) quantified the turbulence and found that even small scale forces were enough to induce mortality when larvae were as large or larger than the smallest eddy associated with turbulent flow. They showed that increasing exposure to turbulent forces increased larval mortality. However, they only ran a test trial for 24 hours. In our experimental trials, duration of the exposure along with increasing intensity play a role in causing larval mortality. Increasing the intensity over a short period of time, in this case 1 hour, did not produce a meaningful increase in mortality. Even at a 24 hour exposure period, the adjusted larval mortalities were similar in the 100 rpm and 400 rpm trials, being only about 7% greater at the higher intensity. But when the exposure is lengthened to 48 h, the adjusted mortality is about 50% higher in the 400 rpm trials. At low intensity, when the exposure to the stress was lengthened to 48 hours, we detected a significant increase in mortality. Given that the interaction term in our factorial model was highly significant, we concluded that it is really a product of exposure time to elevated hydrodynamic forces that may be most relevant to determining larval mortality in zebra mussels.

Zebra mussel larvae are susceptible to harsh environmental conditions, including the hydrodynamic forces acting on them during downstream transport. Larval mortality is certainly a factor contributing to the distribution of zebra mussels in streams. Mortality during downstream transport ultimately reduces the propagule pressure, which is critical in starting and maintaining populations (Drake and Lodge 2006). It is still not clear if zebra mussels are able to successfully reproduce in highly turbulent waters such as small streams and contribute additional propagules to downstream populations. Thus populations in streams may not reach the high densities seen in lakes and larger more lotic-like rivers. This has conservation consequences because zebra mussel impacts are often related to density (Ricciardi 2003). This is especially true for direct impacts on freshwater clams (Unionidae) many of which are threatened or endangered (Ricciardi et al. 1996; Martel et al. 2001). Larval development can last weeks (Sprung 1993), during which the likelihood of turbulence-induced mortality increases according to our laboratory results. Small, unimpounded streams may then serve as refugia from colonization for these species similar to other localities in lakes where currents are not delivering high abundances of zebra mussel larvae (e.g., McGoldrick et al. 2009). Structures that reduce turbulence in streams, such as impoundments and channelization, may exacerbate the negative effects that these invasive mussels have on native, endangered bivalves. Vigilance and continued monitoring remain our best management strategies (Lucy 2006) especially given the additional threat of the congener Dreissena bugensis as it turns up in lotic ecosystems.

Acknowledgements

This project was partially supported with a grant from the National Science Foundation to TH (DBI-0420880). We thank the College Foundation (SUNY Oneonta) for providing student support to LC, the Research Foundation (SUNY Oneonta) for a stipend support to TH, and the DeWaals for use of their boat. Two anonymous reviewers improved the content of the manuscript.

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