Western Pearlshell Mussel Reproduction Timing: Merrill Creek, Oregon

FY 2011 Annual Report

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On the cover: A group of Western Pearlshell mussels in Merrill Creek, Oregon. Photograph by Donna Allard, CRFPO.

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Abstract

Three-quarters of the nearly 300 native species of freshwater mussels in North America are imperiled as a result of degraded water quality and invasive species. Although the reproductive life cycle of most freshwater mussels has been fairly well documented, information regarding the reproductive timing and triggers of western pearlshell mussel (WPM) populations is scarce. The goal of this ongoing study was to improve our understanding of WPM reproductive timing and to determine if it is correlated with water temperature.

The study area was a 250 m reach in Merrill Creek, Oregon with an estimated population of approximately 2400 WPM. Beginning in late-April and through mid-August 2011, every two weeks, 30 WPM were examined for signs of gravidity. Thirty two drift net samples were collected and examined for glochidia. A total of 267 mussels were examined in 2011. No mussels showed visible signs of gravidity. Glochidia were present in drift net samples from April 21 to June 14, 2011. The average daily temperature during this time was 10.6 °C. These results are very similar to 2010 results when glochidia were present from May 5th (1st sample date) to June 16th with an average daily temperature of 10.7 °C.
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Introduction

Nearly 300 species of freshwater mussels are native to North America (Toy 1998, Nedeau et al. 2009). Nearly three-quarters of these are imperiled as a result of degraded water quality and invasive species (Nedeau et al. 2009, Williams et al. 1993). Within the Pacific Northwest, freshwater mussels were once abundant in the Columbia River Basin but are becoming increasingly scarce (Helmstedtler and Cowles 2008). Causes for this decline include dams, water quality impairment, changes in fish populations, introduction of non-native species, and siltation (King County 2005, Nedeau et al. 2009). Freshwater mussels can be an indicator species of ecosystem health because they require clear, clean water in which to live and reproduce (Nedeau et al. 2009). As outlined in the National Strategy for the Conservation of Native Freshwater Mussels (1998), one of the goals of the strategy is to increase fundamental knowledge of basic biology and habitat requirements of mussels so that managers can more effectively conserve and manage our mussel fauna. By learning more about the life history of freshwater mussels, conservation and management plans can be developed to assist resource managers in minimizing or eliminating threats and protecting mussel habitat. Implementation of these plans will direct the successful conservation of freshwater mussels.

The general reproductive strategy of freshwater mussels has been well documented (Hastie 2003, Murphy 1942, Toy 1998, Young and Williams 1984). During breeding, males release sperm into the water. For fertilization to occur within the female’s marsupium, females must filter sperm from the water. In the marsupium, the embryos develop into larvae called glochidia. This incubation period, before glochidial release can take between two and four weeks (Murphy 1942, Toy 1998). Some species such as the western pearlshell mussel (Margaritifera falcata) (WPM) release conglutinates, aggregates of glochidia loosely bound by mucus (Barnhart et al. 2008). These conglutinates mimic worms or insect larvae (Figure 1), and rupture if a fish attacks, giving the fish a mouthful of glochidia. Otherwise, the conglutinates disintegrate rapidly, leaving the free glochidia to find a host on their own. The glochidia complete their development as parasites on the gills and/or fins of suitable host fish such as cutthroat trout (Karna and Millemann 1978, Nedeau et al. 2009). To survive, glochidia must attach to a suitable host within days to a couple of weeks after being released into the water (Murphy 1942, Jansen et al. 2001). After attaching to a host, they form a cyst around themselves and remain attached for several days or months depending upon the species and water temperature (Barnhart et al. 2008). When the glochidia metamorphose into juvenile mussels, they release from the fish, burrow into the sediment, and begin their existence as free-living mussels (Hastie et al. 2003).

Although reproduction timing has been documented for eastern pearl mussels (Margaritifera margaritifera), few studies have been done for the western species (Hastie and Young 2003, Young and Williams 1984. Murphy (1942) observed that WPM had spawned a full three weeks earlier in warmer water temperatures than mussels located 16 km downstream in cooler waters. The reproductive cycle of WPM spawning in two western Washington streams appeared to be related to water temperature as well (Toy 1998). A study of eastern pearl mussels also indicated differences in the timing of spawning between rivers was related to water temperature (Hastie and Young 2003). Mussels spawned earlier in warmer rivers in all three studies. In addition, eastern pearl mussel spats (the release of glochidia from the marsupium) are likely triggered by an environmental cue such as water temperature or changes in discharge or dissolved oxygen
levels (Hastie and Young 2003, Nedeau et al. 2009). The gills of female gravid mussels are filled with spawn thereby making them sensitive to decreased levels of dissolved oxygen. Gravid mussels were shown to shed their glochidia soon after being held in non-aerated buckets of river water (Young and Williams 1984). However, in general, information regarding the reproductive timing and triggers of western pearlshell mussel populations is scarce.

Figure 1. Conglutinates from the western pearlshell mussel, *Margaritifera falcata*.

This was the second year in an ongoing study whose goal is to improve our understanding of WPM reproduction timing. For the purpose of this report, reproduction is defined as when any mussels show signs of gravidity, glochidia are present in drift net samples, or conglutinates are found. The specific objectives of this study were to: 1) determine when WPM reproduce, and 2) determine whether WPM reproduction is correlated with water temperature.

**Study Area**

Merrill Creek (Oregon, USA) is a 12.7 km long tributary of Tide Creek. It enters Tide Creek approximately 1.8 km upstream from its confluence with South Deer Island Slough (Figure 2). The stream reach we studied is located approximately 3.3 km upstream from the mouth of the creek. The reach is approximately 250 meters in length and on average approximately 5.0 m wide. Mussels are located throughout the reach, both loosely scattered and in patches. Three areas within the study reach with relatively dense populations were identified. Dense populations of mussels were defined as being an area containing more than 50 mussels embedded in the substrate, within centimeters of one another. The creek in this vicinity is low gradient with a substrate mostly of gravel and sand. A few areas throughout the reach are comprised of cobble.
Figure 2. Merrill Creek study area (indicated by green square).
Methods

Objective 1: Determine when western pearlshell mussels are reproducing

We generally followed the methods of Allard et al. (2012). Ten permanent transects, each 2 m wide, were established within the reach. The transects were surveyed throughout the study (i.e., the same transects were surveyed during each field visit) (Figure 3). Three dense areas of mussels were identified in the reach in 2010 and remained in 2011. These dense areas of mussels were approximately 20 m² and had patches of 50 mussels or more within centimeters of one another throughout the area. Two transects were placed haphazardly in each of the three densely populated (DP) areas for a total of six transects. One transect was placed above, below, and in between each of the DP areas for a total of four more transects (Figure 3). At each of those transects, a total of three mussels were haphazardly chosen and examined for signs of gravidity. Thirty mussels were sampled during each sampling event. An additional transect (T-11) that spanned the width of the stream was established at a fixed location 25 m downstream of the study area.

Beginning on April 21, 2011, through August 11, 2011, 30 haphazardly-selected mussels were examined once every two weeks. After selection of a mussel, the mussel was gently removed from the sediment, where a marker was placed at its location in the substrate. The length, height, and width of the mussel were measured to the nearest millimeter with dial calipers (Figure 4). To determine gravidity, the mussel was pried open approximately 2-3 mm using modified snap ring pliers (Figure 5). The marsupial gills were then examined. A gravid mussel was distinguished by inflated, opaque gills containing visible patches or striations of eggs or embryos (Spring Rivers 2007, Figure 6). If the mussel was not previously marked (from the 2010 study or previous 2011 sampling events), the shell of the mussel was marked with an individually numbered floy tag (Lemarie et al. 2000) and returned to its original location and orientation in the substrate. Marking the shell allowed us to make secondary observations of survival in the
A plankton net (50 µm mesh) was deployed at T-11 at the beginning of each sampling event to survey for the presence of glochidia in the creek water. Five plankton net samples set equidistant along the transect were collected during each sampling event, or as flow allowed. As velocities along the transect neared 0.01 m/sec the plankton net was not effective, in which case we collected as many samples as possible. The plankton net was left in place for ten minutes for each sample. Each sample was preserved in ethanol and taken back to the lab for examination to determine the presence of glochidia.

The proportion (+/- 95% CI) of plankton net samples containing glochidia was determined. The length frequency of the mussels was plotted to provide an indication of the age distribution of the
mussels sampled during the year. Length measurements were plotted against height measurements.

Figure 6. An example of a gravid mussel’s gills.

Objective 2: Determine if WPM reproduction events are correlated with water temperature

On March 23, 2011, one Onset Hobo thermograph was deployed in Merrill Creek in the lower end of the stream study reach, approximately at river kilometer (RKm) 3.5 (Figure 2). A temperature and depth probe was installed at the mouth of Merrill Creek in October of 2009 by the Columbia Soil and Water Conservation District. The probes recorded temperatures once every hour. Neither probe operated continuously throughout the investigation. However, at any point in time, one of the probes was generally in operation and both were often operated simultaneously. We evaluated the relationship between water temperatures at the two probe sites and developed equations to estimate water temperatures at a given site from temperature data at the other site. Actual and estimated water temperatures were used to generate daily water temperature data (average, maximum, and minimum) experienced by the mussels in the study reach for the entire year.
Several other stream and water measurements were taken just below the stream reach during each sampling event. A discharge measurement was taken using a Marsh McBirney Flo-Mate. Water quality measurements were collected with a YSI 85 meter; these measurements included water temperature, dissolved oxygen, and conductivity. On May 19, 2011, we started collecting pH measurements with a YSI 556. Turbidity measurements were taken with a Hach 2100p turbidimeter.

**Results**

**Objective 1: Determine when western pearlshell mussels are reproducing**

Transects established to detect mussel reproduction were sampled on nine different events. Thirty mussels were examined on all sample dates except for August 11, 2011, when only 27 mussels were examined (only 9 transects sampled) (Table 1). A total of 267 mussels (approximately 11% of the estimated population) were examined for gravidity. Gravidity was not observed in any of the mussels examined during the transect sampling events.

The length of the western pearlshell mussels examined in Merrill Creek approximated a normal distribution, with the majority of the mussels sampled falling within the 55 to 80 mm range (Figure 7). Shell length of sampled mussels ranged from 42.9 mm to 93.2 mm. Mussels at sizes less than 25 mm were difficult to discern within the substrate and were therefore not sampled. The relationship between length and height was linear (Figure 8).

Stream drift samples were collected during eight sampling events. During the 9th sample date, August 11, 2011, the discharge of 0.02 cubic meters per second (cms) was not sufficient to deploy the net. Glochidia were found in the stream drift samples (Figure 9) collected during five of the sampling events. The latest observation of glochidia in the samples occurred on June 14, 2011 (Table 2).

<table>
<thead>
<tr>
<th>Date</th>
<th>Number Examined</th>
<th>Number Gravid</th>
<th>Conglutinates Present</th>
<th>% Gravid (95% CI)</th>
</tr>
</thead>
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<tr>
<td>4/21/2011</td>
<td>30</td>
<td>0</td>
<td>No</td>
<td>0 (0-11)</td>
</tr>
<tr>
<td>5/4/2011</td>
<td>30</td>
<td>0</td>
<td>No</td>
<td>0 (0-11)</td>
</tr>
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<td>5/19/2011</td>
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<td>30</td>
<td>0</td>
<td>No</td>
<td>0 (0-11)</td>
</tr>
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<td>No</td>
<td>0 (0-11)</td>
</tr>
<tr>
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</tr>
<tr>
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<td>27</td>
<td>0</td>
<td>No</td>
<td>0 (0-12)</td>
</tr>
</tbody>
</table>
Figure 7. Length frequency of WPM mussels sampled in Merrill Creek in 2011.

Figure 8. Length vs. height of WPM mussels sampled in Merrill Creek in 2011.
Table 2. Occurrence of glochidia in stream drift samples.

<table>
<thead>
<tr>
<th>Date</th>
<th>Volume of Water Sampled (m³)</th>
<th>Plankton Net Samples</th>
<th>Glochidia Present</th>
<th>Discharge (cms)</th>
<th>Proportion of Samples Present (95% CI)</th>
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<td>4/21/2011</td>
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<td>5</td>
<td>Yes</td>
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<td>.60 (.19 -.92)</td>
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<td>5/19/2011</td>
<td>5.4</td>
<td>5</td>
<td>Yes</td>
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<td>.80 (.34 -.99)</td>
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<td>4.6</td>
<td>5</td>
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<td>.20 (.01 -.66)</td>
</tr>
<tr>
<td>6/14/2011</td>
<td>3.8</td>
<td>5</td>
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<td>.20 (.01 -.66)</td>
</tr>
<tr>
<td>6/30/2011</td>
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<td>3</td>
<td>No</td>
<td>0.05</td>
<td>0 (0 -.63)</td>
</tr>
<tr>
<td>7/13/2011</td>
<td>1.8</td>
<td>3</td>
<td>No</td>
<td>0.05</td>
<td>0 (0 -.63)</td>
</tr>
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<td>7/28/2011</td>
<td>0.3</td>
<td>1</td>
<td>No</td>
<td>0.03</td>
<td>0 (0 -.95)</td>
</tr>
</tbody>
</table>

Objective 2: Determine if WPM reproduction events are correlated with water temperature

The mean daily temperature in Merrill Creek from March 24, 2011 to August 11, 2011 ranged from a low of 7.1°C on March 23rd to a high of 17.4°C on August 4th (Figure 10). The mean daily temperature during the period when glochidia were observed ranged from a low of 8.1°C on April 21st to a high of 13.8°C on June 6th. Glochidia of WPM range in size from 0.05 to 0.06 mm (Murphy 1942, O’Brien et al. 2013). The glochidia found in our plankton net samples fell in this size range, confirming their identification as WPM larvae. The mean daily temperature from April 21st when glochidia were first observed to June 14th was 10.5°C. Other stream and water measurements collected are included in Appendix A.

Figure 9. Glochidia collected in plankton net during 2011.
Figure 10. Reproductive activity and maximum, minimum, and mean daily water temperatures in Merrill Creek from April 21st to August 11th, 2011.

Temperature data collected at the mouth of Merrill Creek was compared to that collected at the study site. Water temperature at the study site was positively and linearly related to water temperature at the mouth (Fig 11). Average water temperature (for example) at the mouth of Merrill Creek was an excellent predictor of water temperature at the study site (P<0.01, R²=0.99). The predictive equation was:

\[ WT_{ss} = WT_{m} \times 0.9244 + 0.4484; \]

where \( WT_{ss} \) = water temperature at the study site and \( WT_{m} \) = water temperature at the mouth. Actual and estimated data on \( WT_{ss} \) was used to determine the water temperature from January 1-December 31 (Figure 12).
Figure 11. Correlation between Temperatures collected at the mouth of Merrill Creek and the Study Site

Figure 12. Average Daily Temperature at Merrill Creek Study Site

Conclusions

Findings

1. The estimated population size in the study reach was approximately 2,390 WPM (Allard et al. 2010). Given that freshwater pearl mussels generally do not reach sexual maturity until
an age of approximately 15 years (Bauer 1987, Skinner et al. 2003) at which time they generally reach a shell length of approximately 50 mm (Toy 1998), we estimate that 99% of the mussels sampled could have been reproductive (255 of 261 unique mussels sampled). If we assume a 1:1 sex ratio (Young and Williams 1984, Toy 1998, Spring Rivers 2007, King County 2005), and that we were equally likely to sample males and females, we would have sampled approximately 127 potentially reproductive females. If we assume that as many as 35% of the potentially reproductive females are not reproducing in a given year (Bauer 1987, Spring Rivers 2007), we would estimate that a maximum of 83 of the mussels we sampled could have been reproductive this year. No WPM examined (pried open) were suspected of being gravid.

2. Evidence of WPM reproduction was found beginning April 21, 2011 (1st sample date) by the presence of glochidia in drift samples. On April 21, 2011, the mean daily temperature was 8.1°C (range 6.8-9.9°C).

3. Glochidia was present in drift samples until June 14, 2011. On June 14, 2011, the mean daily temperature was 12.4°C (range 11.4-13.5°C).

4. No signs of WPM reproduction was found on June 30, 2011 to August 11, 2011 (last sample date).

5. WPM reproduction lasted a minimum of 41 days.

Acknowledgements

We would like to thank Jennifer Poirier, Jeff Jolley, Steve Lazzini, Amy Horstman, Christina Luzier, and STEP students (Jason, Mike, Doug, and Rachael) for their assistance with field sampling. A special thanks to Armin Halston for graciously allowing us to conduct this study on his property.
Literature Cited


Appendix A: Water quality and stream discharge measurements

<table>
<thead>
<tr>
<th>Date</th>
<th>Time</th>
<th>Temp (°C)</th>
<th>DO (mg/l)</th>
<th>Conductivity (μS)</th>
<th>Turbidity (NTU)</th>
<th>Flow (cms)</th>
</tr>
</thead>
<tbody>
<tr>
<td>4/21/2011</td>
<td>10:23</td>
<td>7.2</td>
<td>12.08</td>
<td>29.7</td>
<td>5.53</td>
<td>0.33</td>
</tr>
<tr>
<td>5/4/2011</td>
<td>9:07</td>
<td>6.8</td>
<td>12.05</td>
<td>31.4</td>
<td>6.82</td>
<td>0.23</td>
</tr>
<tr>
<td>5/19/2011</td>
<td>8:56</td>
<td>8.7</td>
<td>12.81</td>
<td>34.3</td>
<td>6.45</td>
<td>0.21</td>
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<tr>
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<td>9.8</td>
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<td>41.0</td>
<td>6.68</td>
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<td>47.0</td>
<td>5.70</td>
<td>0.07</td>
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<td>0.02</td>
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