

**U.S. Fish and Wildlife Service
Columbia River Fisheries Program Office**

Western Pearlshell Mussel Life History in Merrill Creek, Oregon: Reproductive Timing, Growth, and Movement

2010 - 2014 Project Completion Report



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On the cover: Two western pearlshell mussels sit in the streambed of Merrill Creek, OR. Photo by Marci Koski, USFWS.

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WESTERN PEARLSHELL MUSSEL LIFE HISTORY IN MERRILL CREEK, OREGON: REPRODUCTIVE TIMING, GROWTH, AND MOVEMENT 2010 - 2014 PROJECT COMPLETION REPORT

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Abstract

Most freshwater mussel species in North America are imperiled. Life history traits of many freshwater mussels have been documented but information regarding western pearlshell mussels (*Margaritifera falcata* Gould) is scarce. The goal of this study was to document, thus improve our understanding of, western pearlshell mussel reproduction, growth, and movement. The study area was a 250 m stream reach in Merrill Creek, Oregon. We examined 1,389 mussels for signs of gravidity and examined water samples for presence of glochidia during presumed spawning times over a 4-year period. We tagged 416 mussels to conduct mark and recapture observations for growth and movement analyses. No mussels sampled within our study transects showed any visible signs of gravidity. However, four western pearlshell mussels outside of the study transects (but within the study area) were observed releasing conglutinates. Glochidia were present from approximately April to mid-June. In each year, glochidia were not detected until maximum daily water temperature had reached 10°C, and were no longer detected once minimum daily water temperature remained above 9°C. The overall growth rate of the mussels we evaluated was imperceptible. However, there was a negative relation between growth and mussel size. For large mussels, growth rate was not significantly different than 0 mm/d; however, the growth rate for small mussels was 0.0011 mm d⁻¹. Relative to where they were originally marked, 60% (n=15) of these mussels were recaptured in the same transect, 32% (n=8) were recaptured 3.7-115.6 m downstream and 8% (n=2) were recaptured 12 m upstream. This basic life history information is essential to consider when developing management plans associated with the conservation of western pearlshell mussels and their habitat, particularly as our results indicate that western pearlshell mussels are slow-growing, slow-moving, long-lived, and thus likely slow to adapt to environmental change and respond to habitat perturbations.

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Introduction

Nearly 300 species of freshwater mussels are native to North America (Toy 1998; Nedeau et al. 2009), and three-quarters of these species are imperiled as a result of degraded water quality and invasive species (Williams et al. 1993; Nedeau et al. 2009). Within the Pacific Northwest, freshwater mussels once abundant in the Columbia River Basin are becoming increasingly scarce (Helmstetler 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). Many 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). One of the goals of the national strategy for the conservation of native freshwater mussels 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 (National Native Mussel Conservation Committee 1998). 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 may direct the successful conservation of freshwater mussels.

The general reproductive strategy of freshwater mussels has been well documented (Murphy 1942, Young and Williams 1984; Toy 1998; Hastie et al. 2003). Briefly, during breeding, females filter sperm from the water, embryos develop internally in the gills which then swell, and glochidia are released into the stream. Some mussels, such as pearlshell species (*Margaritifera* spp.), can also release aggregates of glochidia (i.e., conglomerates; Barnhart et al. 2008). Both eastern pearlshell mussels (*Margaritifera margaritifera* Linnaeus) and western pearlshell mussels (*M. falcata* Gould) generally appear to spawn in the spring and summer (Young and Williams 1984; Hastie and Young 2003); however, while numerous studies have investigated eastern pearlshell mussel reproduction, relatively few studies have investigated that of western pearlshell mussels. In particular, the specific timing of and cues driving reproduction in western pearlshell mussels are unclear. In *Margaritifera* spp., the timing of reproduction and glochidial release may be influenced by temperature. Hastie and Young (2003) found that eastern pearlshell mussels in warmer rivers tended to release glochidia earlier than those in cooler rivers. Studies have shown periods of western pearlshell mussel glochidial release during late spring to early summer (Murphy 1942; Toy 1998). Murphy (1942) also found that one population of western pearlshell mussels was entirely rid of their glochidia three weeks earlier than another population of mussels 16 km downstream where the temperature of the river averaged 5°C cooler. Western pearlshell mussels spawning in relatively warm western Washington streams appeared to spawn earlier than those in relatively cool streams (Toy 1998). However, a specific relationship between water temperature and reproductive timing in western pearlshell mussels has not been described and, in general, information regarding the reproductive timing and triggers of western pearlshell mussel populations is scarce.

Understanding growth is also an important consideration in mussel conservation. When considered as a whole, mussels are relatively slow growing and long-lived organisms. Despite growing slowly, there can be substantial differences between the growth rates of various mussel species (Haag and Rypel 2011). Freshwater mussel growth may be influenced by water

temperature, discharge, food availability, and stream habitat conditions such as substrate and water depth (Hruska 1992; Hastie et al. 2000). These factors can result in variable growth rates in freshwater mussels. *Margaritifera* spp. may be some of the slowest growing and longest lived of the freshwater mussels (Bauer 1992). However, much of what is known about growth in *Margaritifera* comes from studies on the eastern pearlshell mussels (e.g., Hastie et al. 2000), whereas little information exists regarding western pearlshell mussel growth rates (e.g., Toy 1998; Fernandez 2013).

Freshwater mussels are regarded as sessile organisms. However, understanding if and how much they move is also an important consideration for conservation because it may be difficult for mussels to avoid detrimental changes in their environment and move to more suitable habitat. Mussels have been shown to move both upstream and downstream (Balfour and Smock 1995; Amyot and Downing 1998; Schwalb and Pusch 2007). Passive movements, usually downstream, may be the result of disturbance to the stream substrate from humans, animals, or high flow events (see Balfour and Smock 1995). Freshwater mussels may also move actively, for example, to avoid adverse conditions such as dewatering or to bring mussels closer together during times of reproduction (Amyot and Downing 1998). While movement has been documented in some freshwater mussels, movement of western pearlshell mussels has not been well characterized. The goal of this study was to document and improve our understanding of western pearlshell mussel life history in the Pacific Northwest to inform future aquatic conservation activities and environmental management decisions. The specific objectives were to: 1) determine western pearlshell mussel reproductive timing and its relationship to water temperature; 2) describe western pearlshell mussel growth rate; and 3) describe western pearlshell mussel movement. Addressing these objectives is intended to provide fundamental information on basic biology of western pearlshell mussels, which is supportive of the national conservation strategy for native freshwater mussels (National Native Mussel Conservation Committee 1998).

Study Area

Merrill Creek (Columbia County, Oregon, USA) is a 12.7 km tributary of Tide Creek, which drains into the lower Columbia River through the sloughs on Deer Island (Figure 1). The reach we studied is located 3.3 km upstream from the mouth of the creek, 250 m in length, and approximately 5.0 m in width. We chose this reach for our study because western pearlshell mussels are located throughout the reach, both loosely scattered and in dense patches. The study reach is low gradient (<1.0%) with a substrate of approximately 90% gravel and sand. The remaining substrate consists of cobble and bedrock.

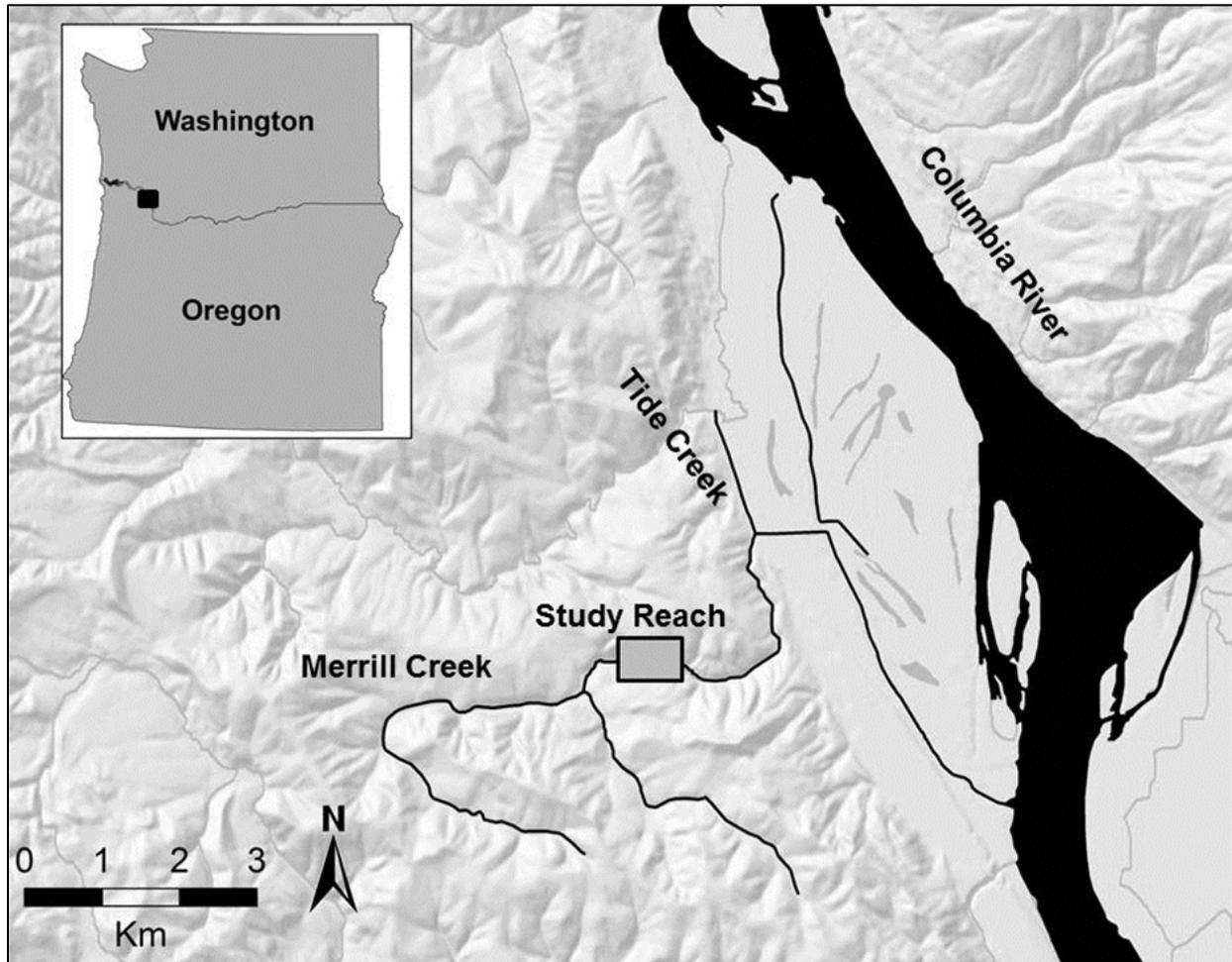


Figure 1. Map of the study area located in Merrill Creek, Oregon.

Methods

To characterize the abundance of potentially reproductive mussels in the area, density was estimated using 1 m² quadrats. Throughout the study area, 40 quadrat locations were randomly selected. Biologists used an underwater viewer (Aqua Scope II™, Water Monitoring Equipment and Supply, Seal Harbor, Maine) to inspect quadrats and enumerate all visible mussels. Given that western pearlshell mussels must reach approximately 40-50 mm in length before they can begin reproducing (Toy 1998), we also determined the proportion of visible mussels that were ≥ 40 mm in length (P_{40}). Up to four mussels from each quadrat were randomly selected, their length was evaluated, and P_{40} was calculated for all mussels that were evaluated. The density of visible western pearlshell mussels (D_{VM}) was calculated as the mean (\pm SE) number of visible mussels per m² (quadrat). The density of potentially reproductive western pearlshell mussels (D_m) was estimated as $P_{40} \times D_{VM}$.

To survey mussels for gravidity, growth, and movement, 10 permanent bank-to-bank transects perpendicular to stream flow, each 2 m wide and an average of 5 m long, were established within the reach and sampled during each field visit (Figure 2). Three high-density groups (HDG) of mussels were identified within the study area in 2010 and remained for the duration of surveys conducted in 2011, 2012, and 2014. Each HDG was roughly 20 m² in size and exhibited an approximate density more than 3X that of the overall study area. Two transects were placed haphazardly in each of the three HDGs for a total of six transects. An additional transect was placed upstream and downstream of the HDGs, and between each HDG for a total of four additional transects (Figure 2).

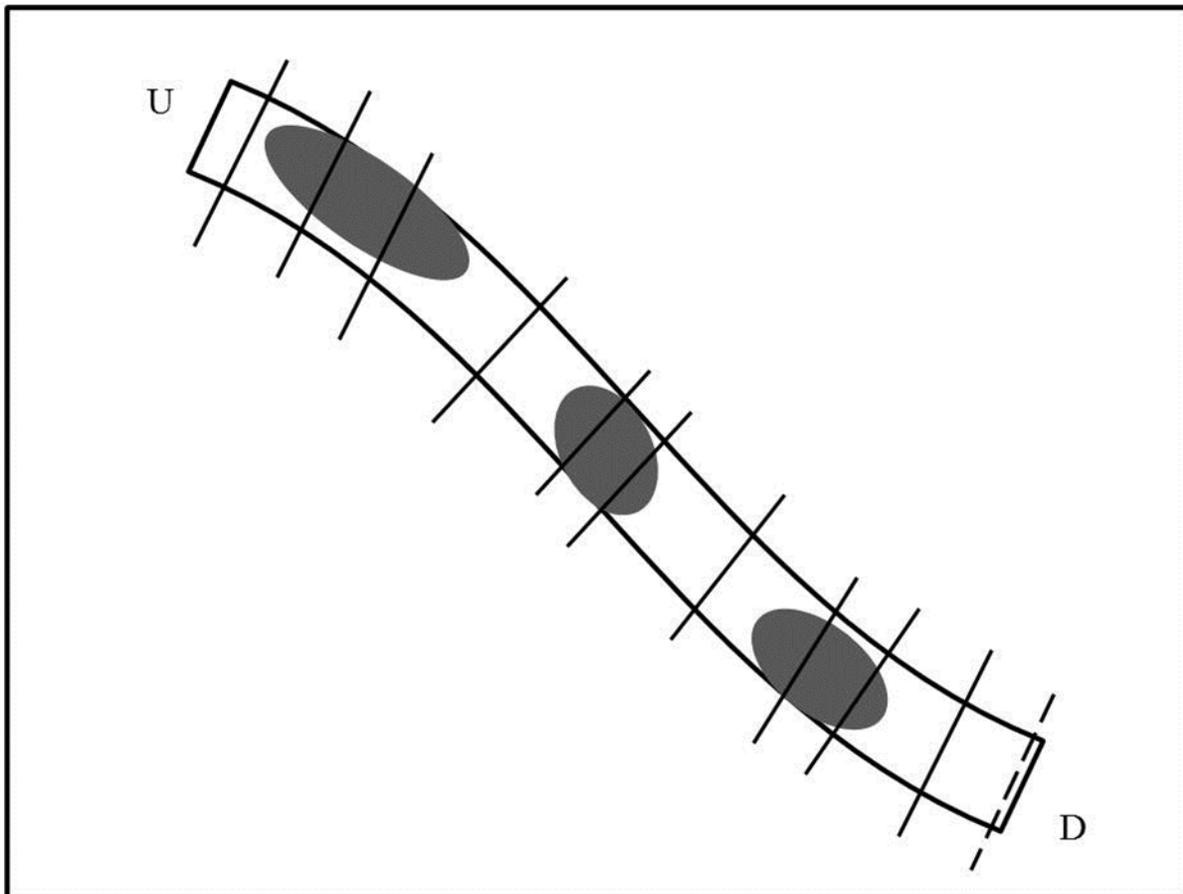


Figure 2. Schematic of the study area (not to scale) in Merrill Creek. High density groups (HDG) of western pearlshell mussels (approximately 20 m² areas in which mussel density was approximately 28.9 per m²) are represented by shaded ovals. Solid lines illustrate the placement of 10 transects relative to HDG of mussels. The dashed line illustrates an additional transect where plankton net-sets were conducted. Direction of flow is indicated by U (upstream) to D (downstream).

Reproductive Timing

Reproductive timing was assessed using two indicators of reproduction, direct examination of individual mussels for signs of gravidity and water sampling for larvae. During 2010-2012, three mussels were haphazardly selected and examined for signs of gravidity at each transect, for a total of 30 mussels examined during each survey. In an attempt to increase the chance of detecting gravid mussels, in 2014, we increased our sample effort so that six mussels were examined at each transect for a total of 60 mussels during each survey. After selection of a mussel, the mussel was gently removed from the sediment, where a marker was placed at its location. To detect signs of gravidity, a mussel was pried open approximately 1 cm using modified snap ring pliers so that the marsupial gills could be examined. A gravid mussel is distinguished by inflated, opaque gills containing visible patches or striations of eggs or embryos (Spring Rivers 2007). During each survey, the substrate 0.5 m above and below each transect was examined for the presence of conglomerates using an Aqua Scope. During 2010-2012, mussels were surveyed beginning in early spring to summer. Since the onset of mussel spawning appeared to be occurring prior to spring, in 2014, mussels were surveyed beginning in January through early summer.

In addition to inspecting transects for conglomerates as evidence of larval mussels, the water column was sampled during each survey and inspected for glochidia. A plankton net (50- μ m mesh) was deployed along one additional transect that was located 25 m downstream of transect 1, perpendicular to stream flow (i.e., from bank-to-bank; Figure 2). Five water samples were typically collected during each survey, with the plankton net set at equidistant locations along the transect as flow and water depth allowed (i.e., locations with water velocity >0.01 m s⁻¹ and depth >0.10 m). Fewer than five water samples were collected when water velocity and depth were not conducive to sampling. The plankton net was left in place for 5-26 minutes (depending on flow and debris accumulation) to collect each water sample. Water depth and velocity were recorded either at the approximate midpoint of a net-set (i.e., during 2010-2012) or at the start and end of each plankton net-set (i.e., 2014) using a top-setting rod and flow meter (Marsh-McBirney, Inc., Frederick, Maryland). Stream discharge was measured at the plankton net transect. The volume of water sampled by each net-set was calculated and expressed as the proportion of discharge sampled during each sample period. Each water sample was preserved in 100% ethanol or 95% isopropyl alcohol and taken back to the laboratory for examination under a dissecting scope (40X microscope) to determine presence of glochidia. The proportion of water samples containing glochidia (\pm 95% CI) during each survey was calculated.

To assess the relation of reproductive timing to water temperature, one temperature logger (Onset Computer Corporation, Bourne, Massachusetts) was deployed in Merrill Creek within the study reach and another was installed at the mouth of Merrill Creek. The loggers recorded temperatures every hour. Neither logger was operated continuously throughout the study. However, both loggers were often operated simultaneously. Given that the water temperatures at each site were linearly related ($P < 0.01$, $df = 368$, $R^2 = 0.99$), when the study reach logger was not operational, we used the relationship between water temperatures at the two loggers to estimate water temperatures in the study reach. Actual and estimated water temperatures were used to generate daily mean, minimum, and maximum water temperature in the study reach for each year. This data was used to describe prevailing thermal conditions during mussel reproduction. To try and identify possible temperature thresholds associated with reproduction,

we described characteristics of the minimum and maximum water temperatures being experienced by the mussels near the onset and conclusion of reproduction.

In addition, we used degree-days (the accumulated product of time and temperature between the developmental thresholds, see UC 2014) to assess thermal conditions mussels likely experienced between reproductive cycles. To determine annual degree-days (DD_{annual}), calculations began on June 17 and went through June 16, the latest date we observed evidence of reproduction. This was done for 2011-12 and 2013-14. To determine degree-days from the end of a given reproductive period to the beginning of the next reproductive period (DD_{partial}), calculations began on June 17 and went through March 28, the earliest date we observed evidence of reproduction. This was done for 2013-2014. We used 0°C as the lower threshold for degree-day calculations. A single sine method (UC 2014) was used to calculate the number of degree-days. We then described the number of degree-days that were associated with the onset of reproduction.

Growth

To assess growth, we measured the length (L) of all collected mussels to the nearest millimeter (mm) using dial calipers (Allard et al. 2013). From 2010-2012 a subset of the mussels being handled for the first time was haphazardly selected and the shell of each mussel marked with an individually numbered oval tag (Floy Tag and Manufacturing, Inc., Seattle, Washington) attached using cyanoacrylate (Krazy Glue, Westerville, Ohio) (LeMarié et al. 2000). After marking, each mussel was gently returned to its original location and orientation in the substrate. Tag number, date, and transect number were recorded. If a tagged mussel was recaptured, the tag number, recapture date, and length were measured and recorded. Growth rate (change in length expressed as mm d^{-1}) was determined for all recaptured individuals. All of the following growth analyses were considered significant at the $\alpha = 0.05$ threshold.

Preliminary inspection of the data showed that some of the mussels, specifically those recaptured relatively soon after tagging, appeared to exhibit negative growth. This observation prompted an evaluation of measurement error (precision; see Gutreuter and Krzoska 1994). In April 2014, six mussels were haphazardly selected from the study area. The length of each mussel was measured 10 times in a blind manner (60 total measurements). To accomplish this, one biologist collected mussels, presented a mussel (one at a time) to a second biologist, and recorded all the data. The second biologist made all measurements, without knowledge of which mussel was being selected or in what order, and then returned the mussel to the first biologist. The mean length for a given mussel (based on the 10 measurements) was considered the Empirically Derived, True (EDT) length (see Gutreuter and Krzoska 1994). The Absolute Error (AE) in length measurement was calculated as the difference between a measured length and the EDT length while the relative error (RE) in length was calculated as AE/EDT (Harvey et al. 2001). To evaluate any size bias associated with measurement error, RE was regressed on EDT length. To determine whether measurement error was unbiased and approximated 0, the distribution of the error around the EDT length was evaluated for normality (D'Agostino and Pearson 1973).

To evaluate whether growth was related to size, we regressed growth rate on length at tagging. Because the growth over relatively short periods of time was difficult to determine precisely, only mussels recaptured at least two years after marking were used for this analysis. It became

apparent that growth rate was related to size, thus, we also evaluated the growth rate of three length classes of mussels, small (47.0-64.9 mm), medium (65.0-82.9 mm), and large (83.0-100.9 mm). Since we determined that measurement error was unbiased and approximated 0, to determine growth rate for each size class, change in length was regressed on time between captures over the course of the study.

Finally, to begin to understand age at length relationships, we developed an inverted von Bertalanffy equation (Anthony et al. 2001). A von Bertalanffy relationship is considered the best model to fit pearl mussel length at age data (Hastie et al. 2000). Growth rates for various size classes were used to estimate Brody's growth constant (K) (see Richardson et al. 1998). The longest mussel we observed was used to estimate asymptotic length (L_{inf}) (see Reátegui-Zirena et al. 2013). These parameters allowed age to be expressed as a function of length.

Movement

We used information on the date and location of recaptures to evaluate mussel movement. This information was generated from surveys of predetermined transects in a specific study area. The study area was not closed, mussels could immigrate to or emigrate from the area. Thus, to assess whether tagged mussels were likely to stay within the study area during the course of the study, we inspected the pattern of initial and final transects of capture. Mussels marked from transects 5-10 appeared likely to remain in the study area and were used for subsequent analysis. To evaluate the rate of movement, the distance between the marking and recapture site was regressed on time between marking and recapture over the course of the study. All analyses related to movement were considered significant at the $\alpha = 0.05$ threshold.

Results

The 40 quadrats that were sampled to characterize abundance contained a total of 379 western pearlshell mussels (see Appendix A for a complete summary of data collected between 2010 and 2014). P_{40} was 0.93 ($n=87$). D_{VM} in the study area ranged from 0-55 per m^2 , with a mean density of 9.5 (± 2.30). The mean density of potentially reproductive mussels (those ≥ 40 mm in length) throughout the study area was estimated to be 8.8 (± 2.14) per m^2 , which expanded to an estimated 11,027 ($\pm 2,675$) in the entire study area. The mean density of potentially reproductive mussels in areas of high density groups was estimated to be 28.9 (± 4.14) per m^2 .

Reproductive Timing

A total of 222, 267, 180, and 720 mussels (≥ 40 mm in length) were examined for signs of gravidity in 2010, 2011, 2012, and 2014 respectively. No mussels exhibiting characteristics of gravidity were observed during any transect survey. However, on May 5, 2010, four western pearlshell mussels were observed releasing conglutinates in the upper portion of the study reach. Because these western pearlshell mussels were within the study reach but not on a transect, they were not physically examined (pried open). Conglutinates also were observed on the substrate downstream of these western pearlshell mussels. In 2010, glochidia were found in water samples collected between May 18 (first water sample collected) and June 16; in 2011, glochidia were detected between April 21 (first water sample collected) and June 14; in 2012, glochidia were

detected between April 17 (first water sample collected) and June 6; and in 2014, between March 28 and June 13 (last sampling date).

Between the beginning of the year (January 1) and the time we first detected glochidia (approximate onset of reproduction), minimum and maximum water temperatures had reached a peak of at least 8.3°C and 10.0°C, respectively (Figure 3). Glochidia were not detected until minimum and maximum water temperatures remained warmer than (i.e. reached thresholds of) 5.5°C and 8.0°C, respectively. Glochidia were no longer detected (approximate end of reproduction) once minimum and maximum water temperatures had reached a peak of at least 13.8°C and 18.3°C, respectively. Furthermore, glochidia were not detected after minimum and maximum water temperatures remained warmer than 9.0°C and 13.3°C, respectively. For 2011-2012 and 2013-2014, DD_{annual} was 3,670 DD and 3,793 DD, respectively. For 2013-2014, DD_{partial} was 2,890.

Growth

For mussels used to assess measurement error, EDT length ranged from 45.1 to 69.1 mm. The AE associated with these measurements ranged from -0.77-0.41 mm. The RE had a mean of 0.000 (± 0.000 SE), ranged from -0.003 to 0.007 and was unrelated to length ($P = 0.88$, $df = 9$). The error associated with measuring length was normally distributed around the EDT length ($P = 0.08$, $df = 59$, Figure 4).

During 2010-2014, the length of 1,482 total mussels was measured and 44 recaptured mussels provided information for analysis of growth rate. Mussel recaptures occurred from 13-1,492 days after marking. Range in length of these mussels was 47.0-97.0 mm. Mean growth rate was -0.0005 mm d⁻¹ (range -0.0430-0.0570) and not different than 0 mm/d ($P=0.85$, $df = 43$). Growth rate was negatively related to size ($R^2 = 0.43$, $df = 10$, $P = 0.03$, Figure 5). For small mussels (47.0-64.9 mm), change in length had a positive, linear relation to time ($R^2 = 0.45$, Figure 6). The growth rate (slope) was 0.0011 mm d⁻¹ and significantly different than 0 ($P=0.02$, $df = 11$) while the amount of growth after 0 days (intercept) was 0.01 mm and not significantly different than 0 ($P=0.98$, $df = 11$). For medium mussels (65.0-82.9 mm), change in length also had a positive, linear relation to time ($R^2=0.27$). The growth rate was 0.0008 mm d⁻¹ and significantly different than 0 ($P=0.01$, $df = 24$) while the amount of growth after 0 days was -0.03 mm and not significantly different than 0 ($P=0.85$, $df = 24$). For large mussels (83.0-100.9 mm), change in length was not related to time ($R^2=0.08$). The growth rate was -0.0003 mm d⁻¹ while the amount of growth after 0 days was 0.12 mm, neither value significantly different than 0 ($P = 0.55$ and 0.71, $df = 6$, respectively). For the inverted von Bertalanffy growth equation, L_{inf} was estimated to be 97.3 mm (longest mussel we observed), K was estimated to be 0.012, and the majority of the western pearlshell mussels we observed (those ≥ 40 mm in length) were estimated to be over 45 years old (Figure 7).

Western Pearlshell Mussel Reproduction, Growth, Movement

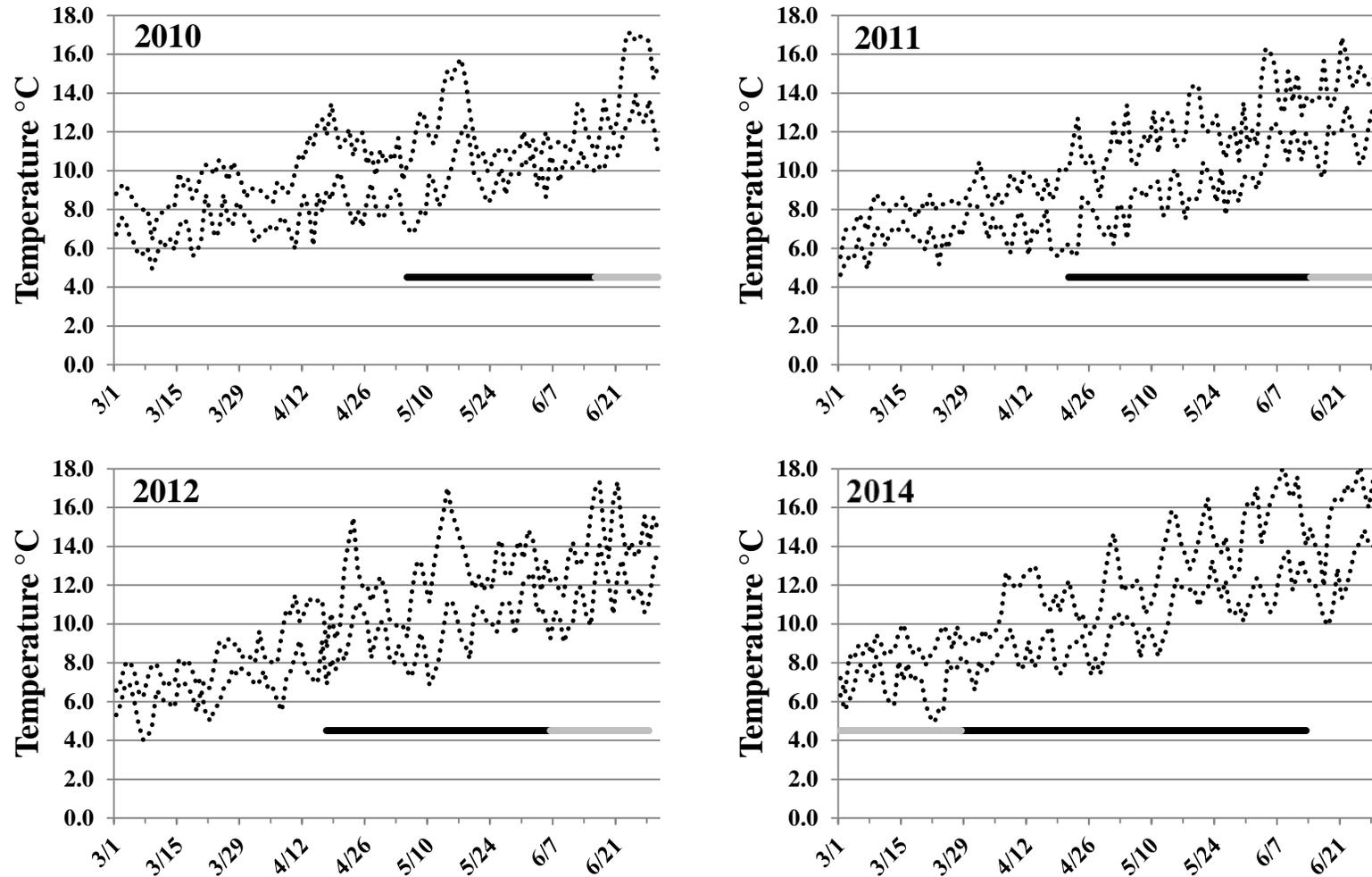


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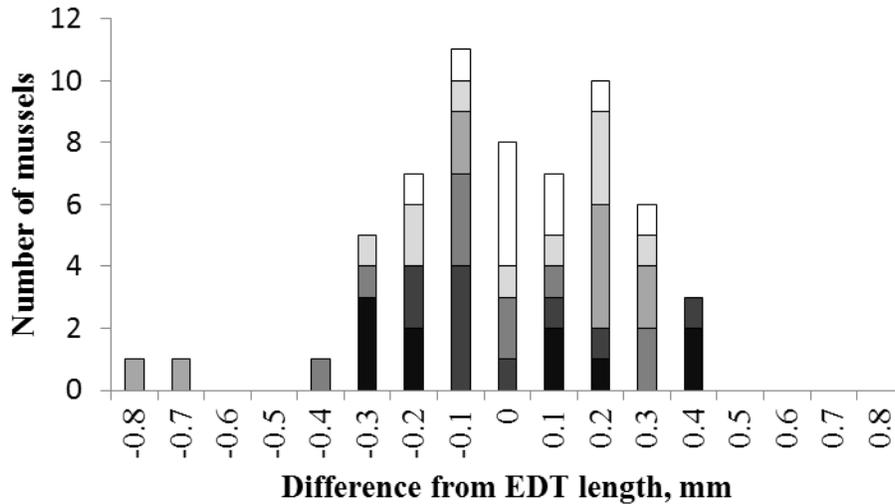


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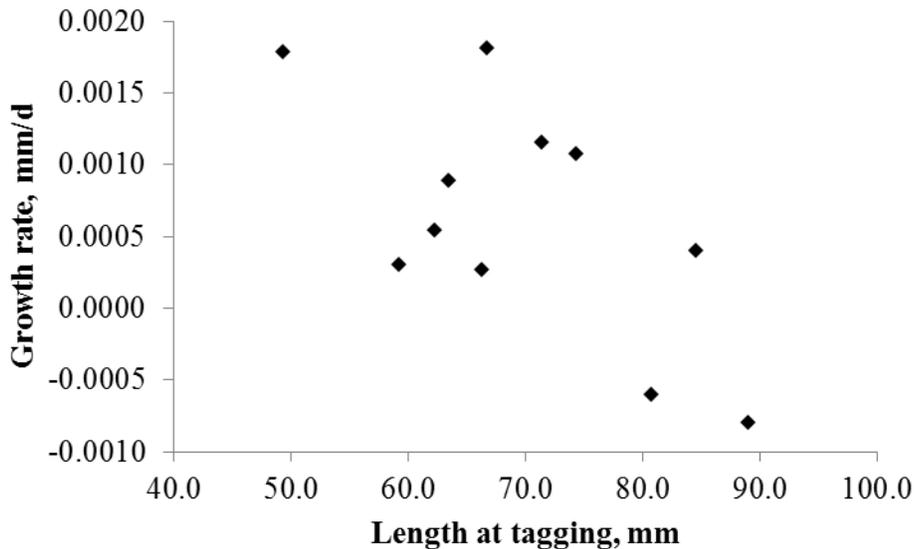


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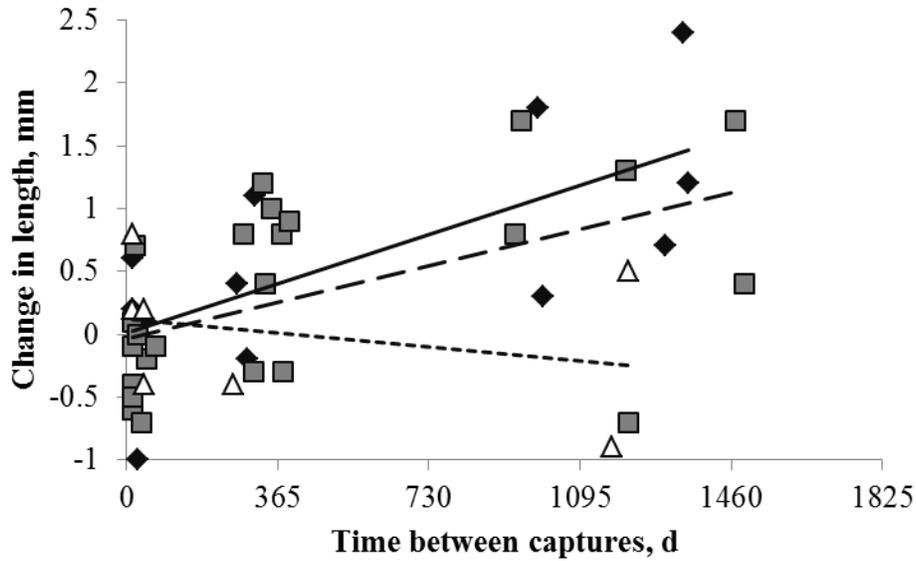


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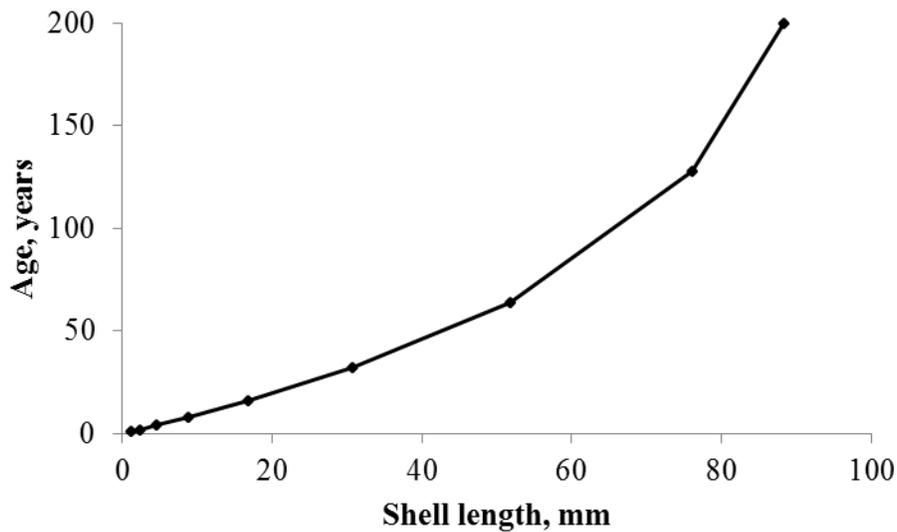


Figure 7. Age at length for western pearlshell mussels from Merrill Creek (OR), estimated from an inverted von Bertalanffy growth equation. This relationship was derived from estimates that $L_{inf} = 97.3 \text{ mm}$ and $K = 0.012$.

Movement

Since we assessed movement using transect surveys, it was important to consider whether tagged mussels may have moved out of the study area. In terms of downstream movement, only mussels marked from transects 1 and 3 were recaptured as far downstream as transect 1 (the transect that was furthest downstream) and none of the mussels marked in transect 5-10 were recovered any further downstream than transect 4 (Figure 8). In terms of upstream movement, none of the mussels marked from any transect were recaptured in transect 10 (the transect that was furthest upstream) and only mussels marked in transect 9 or 10 were recovered as far upstream as transect 9. These results suggest that mussels marked in transects 5-10 remained in the study area and were useful for further evaluation of movement. During 2010-2014, 25 mussels were marked in transects 5-10 and recaptured (13-1,492 days after marking) during this study. Relative to where they were originally marked, 60% ($n=15$) of these mussels were recaptured in the same transect, 32% ($n=8$) were recaptured 3.7-115.6 m downstream and 8% ($n=2$) were recaptured 12 m upstream. The mean rate of movement was 0.11 m d^{-1} (downstream) (range 0.025 to -1.821 m d^{-1}). The relationship between movement and time was insignificant ($R^2 < 0.01$, Figure 9), with an intercept (-13.8 m) and slope (0.0004) not significantly different that 0 ($P=0.09$ and 0.97 , respectively, $df = 24$).

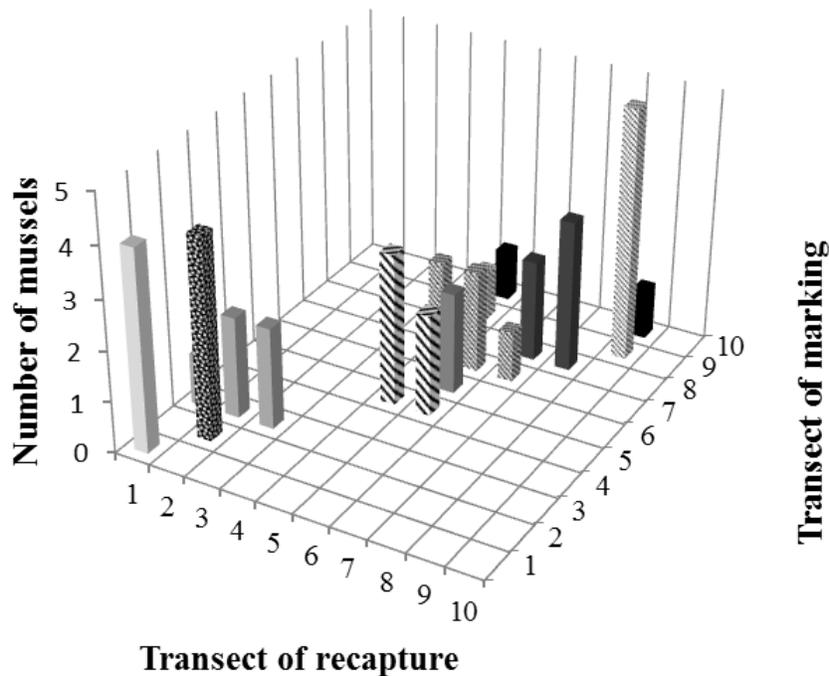


Figure 8. Relationship between the transect where a mussel was originally marked and the transect where a marked mussel was recaptured. All mussels that were marked in and returned to a given transect are represented by bars that have a distinct shade or pattern. Since mussels were marked in and returned to all transects except transect 4, there are nine different patterns or shades or bars.

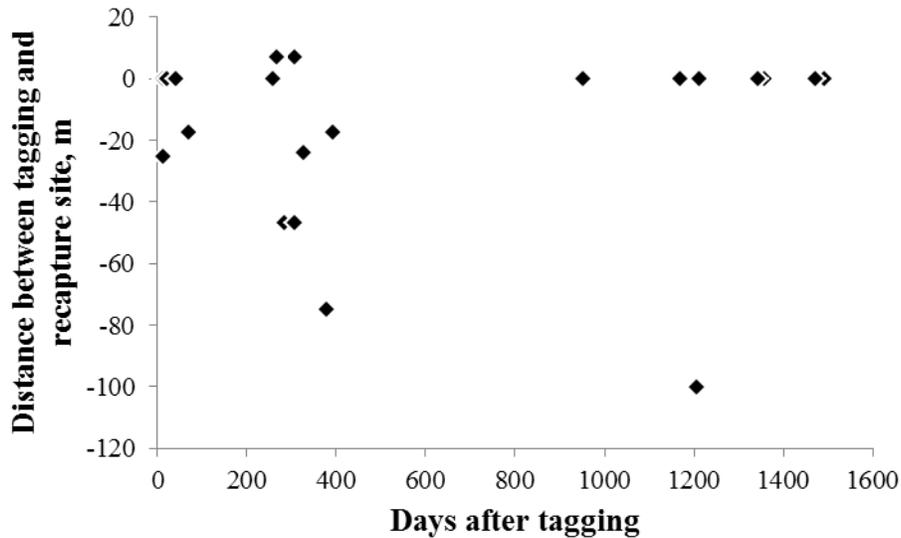


Figure 9. The relationship between time and distance moved. Distance moved was not associated with time ($R^2 < 0.01$) and neither the slope (rate of movement) nor intercept were different than 0 ($P = 0.97$ and 0.09 , respectively, $df = 24$). Negative distances represent downstream movements. Positive distances represent upstream movements.

Discussion

Timing of reproductive events in western pearlshell mussels is variable. In Merrill Creek, the initiation of reproduction began in late March (the earliest we observed glochidia), which is similar to the timing (15 March) of when Meyers and Millemann (1977) observed gravid western pearlshell mussels in the Willamette River, Oregon. In contrast, western pearlshell mussels initiated reproduction later in other areas; i.e., early May in the John Day River, Oregon (O'Brien et al. 2013), and mid-May in the Siletz River, Oregon (Karna and Millemann 1978), and the Truckee River, California (Murphy 1942). Signaling the end of reproduction, we stopped seeing glochidia in Merrill Creek after mid-June, which is consistent with the timing reported by others (Karna and Millemann 1978; O'Brien et al. 2013). Relative to these dates, it appears that the duration of mussel reproduction is greater in Merrill Creek than elsewhere (i.e., ~3 months versus ~1 month).

Water temperature may be a cue associated with the timing and duration of reproduction in western pearlshell mussels. Water temperature could affect the speed at which mussels develop gametes, and/or serve as a trigger for releasing glochidia. According to Jungbluth and Lehmann (1976), the thermal influence on the timing of *M. margaritifera* reproduction could be both/either 1) a summation effect (e.g., a minimum number of cumulative degree-days), or 2) a critical minimum water temperature required for the onset of reproduction. For example, Hastie and Young (2003) determined that 3,000 degree-days were necessary between releases of glochidia in eastern pearlshell mussels, which is similar to our estimation of 3,700 degree-days for western pearlshell mussels in Merrill Creek. In Merrill Creek, while we did not directly observe

gravid females over the three month spawning period, we might hypothesize that the number of gravid females increased gradually in response to a summation of degree days, causing a prolonged release of glochidia from the population as a whole. On the other hand, others (e.g., Wellman 1943; Young and Williams 1984) observed that a rapid increase in the minimum water temperature caused gravid *M. margaritifera* to release glochidia, indicating that a critical minimum temperature threshold may be required for some aspects of the reproductive process. Because we observed glochidia for a relatively long period of time (3 months, compared to 1 month of other observations), it is difficult to identify whether a degree-day summation and/or minimum temperature threshold triggered the onset of reproduction.

Once they settle and become established, western pearlshell mussels appear to exhibit little to no movement over relatively long periods of time. This was evidenced, in part, by an average rate of movement of only 0.11 m d^{-1} in Merrill Creek. Although it has been reported that average mussel movement can be as much as two body lengths per week (Schwalb and Pusch 2007), the rate we observed is within the rates typically described for various mussel species (see Balfour and Smock 1995). The net, average movement of western pearlshell mussels in Merrill Creek was driven by relatively few individuals. However, when individual western pearlshell mussels were evaluated over time, most did not exhibit perceptible movement during this study. This corroborates reports that mussels are a sessile taxa for which individuals exhibit little to no movement over long periods (Sheehan et al. 1989; Amyot and Dowling 1998; Villella et al. 2004; Schwalb and Pusch 2007). For individuals that moved in Merrill Creek, the predominant direction was downstream. This is also similar to most reports that the net movement of mussels in streams is in the direction of the flow (Schwalb and Pusch 2007) and may be an active or passive process (Balfour and Smock 1995). However, we also detected upstream movement of western pearlshell mussels in Merrill Creek. Upstream movement has been reported previously in mussels (Balfour and Smock 1995; Villella et al. 2004; Schwalb and Pusch 2007) and would require movement against the current. It has been suggested that movement can be triggered by environmental conditions (Schwalb and Pusch 2007) such as dewatering, or biological circumstances such as reproduction (Amyot and Dowling 1998). Reports on movement of *Margaritifera* in general, and western pearlshell mussels in particular, are rare or non-existent (Schwalb and Pusch 2007). While our findings generally correspond with those for other species, there may be species-dependent differences. Once larval western pearlshell mussels settle it appears they are not likely to move great distances and easily colonize far-off areas.

The growth rate of western pearlshell mussels appears to be exceptionally slow. This was evidenced by an average growth rate that was not different than 0 mm d^{-1} for all western pearlshell mussels examined in this study. Reports of imperceptible or negative growth rates are not uncommon for mussels (Haag and Commens-Carson 2008; Reátegui-Zirena et al. 2013). Slow growing species includes western pearlshell mussels (Fernandez 2013) and it appears that *Margaritifera* species (in general) are some of the slowest growing of all mussel species (Haag and Rypel 2011). Even after considering previous reports (Johnson and Brown 1998; San Miguel et al. 2004; Fernandez 2013), however, the rates we observed are among the slowest reported in *Margaritifera*. The specific conditions in Merrill Creek may result in particularly slow growth of western pearlshell mussels. Although they tend to be relatively synchronous among individuals within a population (Rypel et al. 2008), specific growth rates can vary between populations, streams or years (Johnson and Brown 1998; Haag and Rypel 2011) and be influenced by

numerous environmental conditions (Hastie et al. 2000; Black et al. 2010). The inclusion of relatively large mussels in our study may also have been partly responsible for no average growth being detected over periods as long as four years. The growth rate of the western pearlshell mussels we examined was negatively related to mussel size (and presumably age); relatively large western pearlshell mussels did not exhibit any detectable growth whereas relatively small western pearlshell mussels exhibited positive growth rates. Several studies have described a similar, negative, linear relationship between mussel size and growth rate (see Anthony et al. 2001; San Miguel et al. 2004; Haag and Commens-Carson 2008; Reátegui-Zirena et al. 2013). The maximum average growth rate we observed for any size class was 0.0011 mm d^{-1} . This is similar to the average rate Fernandez (2013) observed for western pearlshell mussels in a Washington stream (Headquarters Creek) and Reátegui-Zirena et al. (2013) observed for Fuzzy Pigtoe (*Pleurobema strodeanum*) in Florida streams, but slower than most average growth rates that have been reported (see Haag and Rypel 2011; Fernandez 2013). This finding further emphasizes that the growth we observed for western pearlshell mussels in Merrill Creek is some of the slowest ever reported for mussels. This finding also supports the claim that mussels may grow slower than suggested by previously used techniques (Anthony et al. 2001).

In addition to growing extremely slowly, western pearlshell mussels in Merrill Creek appeared to be long-lived. This was evident when we explored size at age curves from von Bertalanffy growth equations (see Anthony et al. 2001; Haag and Rypel 2011), and estimated maximum ages exceeding 100 years. Our findings are consistent with other reports that western pearlshell mussels grow slowly (Haag and Rypel 2011), reach maximum sizes from 77-158 mm (Hastie et al. 2000), may live well over 100 years (Bauer 1992) and be older than previously thought (Anthony et al. 2001). At the growth rates observed in our study, it would likely take western pearlshell mussels in Merrill Creek more than 45 years to reach the length of 40-50 mm at which they are considered to start becoming mature (Toy 1998).

The presence of glochidia is a useful tool for examining reproductive timing in western pearlshell mussels. During the four years of our study, glochidia were detectable from water samples each year even though gravid mussels were never observed. Other researchers have also used the presence of glochidia in the water column to successfully evaluate reproductive timing (Watters and O'Dee 2000) or complement information on gravidity (Hastie and Young 2003). Alternatively, some have focused on the presence of gravid mussels to assess reproductive timing (see O'Brien et al. 2013). While the presence of gravid mussels does provide information related to when fertilization and brooding occur, gravidity may be more difficult to detect than glochidial presence. In *Margaritifera* spp., it may be common for fertility rates to be lower than 50% (Young and Williams 1984) and fewer than 67% of the females to reproduce (Bauer 1987) whereas reproductive females may release 1-4 million glochidia (Hastie and Young 2003). Thus, the sample effort necessary to detect gravid females and prevent inaccurate claims of their absence may be substantially higher than the effort necessary to detect glochidia in the water column. Using either approach, when trying to determine the timing of reproduction, it is necessary to define events clearly and recognize there is likely a 2-4 week lag between spawning and glochidial release (Hastie and Young 2003) in freshwater pearlshell mussels. The use of multiple metrics may be the best approach to clearly describe reproductive timing.

Summary

Margaritifera species are thought to be longest lived of all freshwater invertebrates (Hutchinson 1979), commonly exceeding 100 years of age (Vannote and Minshall 1982). However, relatively little detailed information exists about much of the basic biology of freshwater mussels (Schwalb and Pusch 2007; Haag and Rypel 2011). For western pearlshell mussels in Merrill Creek, and perhaps western pearlshell mussels in general, conservation efforts will benefit by a keen awareness that the population response to adapt to or recover from disturbances may be very protracted. In addition to growing extremely slowly and moving very little, the western pearlshell mussels in Merrill Creek appeared to be long-lived. Once they mature, western pearlshell mussels appear to be reproductively active, possibly triggered by changes in water temperature, during most of the spring. This suggests that spring may be a particularly critical period to western pearlshell mussel populations. In addition, at the movement rates we observed, adult western pearlshell mussels are unlikely to move into areas very rapidly (if at all) and timely (re)colonization (especially in areas upstream of any known populations) would likely be dependent on glochidia being transported by host fish or water currents (Balfour and Smock 1995). Understanding specific life history characteristics is essential for proper conservation strategies to be developed. Conservation efforts should consider that once western pearlshell mussels are established in an area, they are not necessarily able to leave rapidly from an area when there is a problem or disturbance. This emphasizes the importance of protecting areas where mussels have become established.

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Appendix A: Summary of Survey Data Collected 2010 - 2014

Summary of surveys noting number of adult mussels examined for gravidity, stream discharge, mean (range) volume of water sampled by plankton net-sets, mean (range) proportion of stream discharge sampled by plankton net-sets, detection of glochidia, and proportion (95% CI) of net-set samples for which glochidia were detected in Merrill Creek by survey date during 2010, 2011, 2012, and 2014. (Volume was based on five net-sets for 10 minutes each unless otherwise noted.)

Survey date (month/day)	Mussels examined (n)	Discharge (m ³ s ⁻¹)	Volume water sampled (range; m ³)	Proportion discharge sampled (range)	Glochidia detected (Y/N)	Proportion samples detected (95% CI)
2010						
5/05 ^a	12	0.20	---	---	---	---
5/18	30	0.09	3.4 ^c (0.1-7.6)	0.06 (<0.01-0.14)	Y	0.60 (0.15-0.92)
6/03	30	0.58	12.5 ^c (6.3-20.6)	0.04 (0.02-0.06)	Y	0.80 (0.28-0.99)
6/16	30	0.23	7.0 (0.8-12.6)	0.05 (0.01-0.09)	Y	0.20 (0.01-0.72)
6/30	30	0.06	4.3 ^{bc} (1.7-7.2)	0.11 (0.05-0.19)	N	0.00 (0.00-0.60)
7/14	30	0.04	9.7 ^{bc} (7.7-11.7)	0.21 (0.20-0.21)	N	0.00 (0.00-0.84)
7/28	30	0.02	2.4 ^b (1.9-2.9)	0.19 (0.15-0.23)	N	0.00 (0.00-0.84)
8/18	30	0.01	---	---	---	---
2011						
4/21	30	0.33	15.6 (6.3-18.9)	0.08 (0.03-0.09)	Y	0.40 (0.05-0.85)
5/04	30	0.23	11.8 (6.9-15.6)	0.09 (0.05-0.12)	Y	0.60 (0.15-0.95)
5/19	30	0.21	11.1 (4.8-17.3)	0.09 (0.04-0.14)	Y	0.80 (0.28-0.99)
6/01	30	0.12	9.1 (5.4-13.4)	0.12 (0.07-0.18)	Y	0.20 (0.01-0.72)
6/14	30	0.07	6.1 (4.6-7.0)	0.14 (0.11-0.16)	Y	0.20 (0.01-0.72)
6/30	30	0.05	5.8 ^b (4.8-6.7)	0.20 (0.16-0.23)	N	0.00 (0.00-0.71)
7/13	30	0.05	4.7 ^b (4.2-5.0)	0.16 (0.14-0.17)	N	0.00 (0.00-0.71)
7/28	30	0.03	3.4 ^b	0.22	N	0.00 (0.00-0.98)
8/11	27	0.02	---	---	---	---

Survey date (month/day)	Mussels examined (n)	Discharge (m ³ s ⁻¹)	Volume water sampled (range; m ³)	Proportion discharge sampled (range)	Glochidia detected (Y/N)	Proportion samples detected (95% CI)
2012						
4/17	30	0.27	14.7 (11.8-18.7)	0.09 (0.07-0.11)	Y	--- ^d
5/02	30	0.38	17.7 (10.6-21.2)	0.08 (0.05-0.10)	Y	1.00 (0.48-1.00)
5/08	30	0.40	18.1 (13.0-22.1)	0.08 (0.06-0.09)	Y	1.00 (0.48-1.00)
6/06	30	0.11	7.2 (4.9-8.6)	0.11 (0.07-0.13)	Y	0.80 (0.28-0.99)
6/20	30	0.06	4.3 (3.1-5.4)	0.12 (0.08-0.15)	N	0.00 (0.00-0.52)
6/28	30	0.09	6.1 (4.8-7.7)	0.12 (0.09-0.15)	N	0.00 (0.00-0.52)
2014						
1/08	60	0.12	3.1 (2.5-4.1)	0.04 (0.04-0.06)	N	0.00 (0.00-0.52)
1/29	60	0.18	3.7 (2.3-5.3)	0.03 (0.02-0.05)	N	0.00 (0.00-0.52)
2/12	---	1.06	---	---	---	---
2/26	60	0.25	3.6 ^b (2.5-4.7)	0.02 (0.02-0.03)	N	0.00 (0.00-0.60)
3/12	60	---	---	---	---	---
3/28	60	0.30	10.9 (6.4-13.9)	0.06 (0.04-0.08)	Y	--- ^d
4/02	66	0.85	10.3 (8.7-12.7)	0.02 (0.02-0.02)	Y	0.60 (0.15-0.95)
4/11	60	0.15	2.8 (0.8-5.4)	0.03 (0.01-0.06)	---	--- ^e
4/16	60	0.15	4.4 (1.7-6.5)	0.05 (0.02-0.07)	Y	0.60 (0.15-0.95)
4/30	60	0.30	9.8 (4.0-15.1)	0.06 (0.02-0.09)	Y	1.00 (0.48-1.00)
5/09	60	0.22	9.3 (6.4-16.7)	0.07 (0.05-0.13)	Y	1.00 (0.48-1.00)
5/21	60	0.12	6.7 (2.9-11.1)	0.09 (0.04-0.15)	Y	0.80 (0.28-0.99)
6/13	60	0.08	4.0 (3.2-4.7)	0.09 (0.07-0.10)	Y	0.40 (0.05-0.85)

^a Conglutinates were observed in the stream.

^b Fewer than five net-sets used.

^c Time for at least one net-set \neq 10 minutes.

^d One net-set examined.

^e No net-sets examined.

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