CHARACTERISTICS OF THREE WESTERN PEARLSHELL (*MARGARITIFERA FALCATA*) POPULATIONS IN THE CHEHALIS RIVER BASIN, WASHINGTON STATE

by

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ABSTRACT

CHARACTERISTICS OF THREE WESTERN PEARLSHELL (*MARGARITIFERA FALCATA*) POPULATIONS IN THE CHEHALIS RIVER BASIN, WASHINGTON STATE

Freshwater unionoid mussels are the most imperiled family of freshwater organisms in North America. In Washington State, documentation of mussel populations, abundances, and investigations of environmental conditions influencing their morphology are limited to a few studies. Here, I describe three populations of the western pearlshell (*Margaritifera falcata*) in the lower Chehalis River basin occurring along an ecological and physical gradient from a headwater stream to a major regional river. Quantitative analysis revealed a trend of increasing shell size as well as shell weight to length ratio along this gradient, but I found no difference in external shell measurement ratios as watershed area increased. Environmental conditions that coincide with an increase in western shell size and proportional shell weight are discussed within. Additionally, information regarding mussel distributions was gleaned from opportunistic interviews with individuals encountered during this research as well as from field notes during surveys for native fish populations. These were then compared to existing records of mussel distributions in Washington. This information led to the reporting of 15 specific mussel localities in this thesis not yet documented in existing databases. The number of previously undocumented populations and the existence of local ecological knowledge about mussel populations make the recognized need for a regionally specific central database for mussel records more important. Information found in this document will increase our understanding of the variation among populations of western pearlshell in Washington and provide support for documenting existing and perhaps historic populations while the mussels, and knowledge of them, is extant.

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Chapter 1: Introduction

Freshwater ecosystems are some of the most critically imperiled systems in the world (Richter et al. 1997, Dudgeon et al. 2006, Strayer and Dudgeon 2010). The obvious and obligate human, biological, cultural, and public health needs for fresh water, along with declines in water quality and species richness, have led to contemporary protection, conservation, and restoration actions in North America. Recently, these efforts have included the management and restoration of freshwater mussel populations throughout North America. However, the status of freshwater mussels in the northwestern United States including Washington State is not well known.

Freshwater mussels are recognized as the most imperiled group of organisms in North America (Williams et al. 1993, Stokstad 2012). Despite this knowledge, only recently have natural resource managers focused their attention on this diverse and abundant group and its ecological role. In North America, 35 species are already extinct and 70 more are considered threatened with extinction (Stokstad 2012). In Washington State there are a number of reports of declining and disappearing populations of freshwater mussels (Hovingh 2004, Krueger et al. 2007, Hastie and Toy 2008, Helmstetler and Cowles 2008, Cowles 2012, Jespen 2012). Washington State is home to three genera of freshwater mussels, yet in many of these rivers, little is known about them and their presence is often overlooked. Washington State’s effort to document distributions of freshwater mussels, describe the variation among populations in their habitats, or monitor their populations has been left to a small number of impassioned individuals and workgroups.

In this study, I focus on the lower Chehalis River basin in southwestern Washington because of prior documentation of mussel populations in the area, as well as its proximity to active agricultural, industrial, and forest land use practices. In this thesis, I describe the abundance, demographics, and physical habitats of three populations of western pearlshell (*Margaritifera falcata)* within this watershed, and investigate anecdotal knowledge about freshwater mussels using information from informal interviews.

Western pearlshells are one of the more common and widespread mussel species in running waters in Washington State and inhabit a wide variety of habitats from the Columbia River (one of the North America's largest rivers), to small, roadside ditches with permanent water. It stands to reason that the physiology and life history of a sessile organism living in such a large spectrum of environments would also have many variations. This study will provide a general synopsis of western pearlshell populations in the East Fork Satsop River and the Chehalis River, as well as specific information that can be used for more expansive detection and monitoring of the western pearlshell of the Lower Chehalis watershed. I compared western pearlshell size and growth at three distinct sites to determine if a difference in western pearlshell morphology exists and if so, what environmental conditions are driving those morphological variations.

The importance of understanding how mussels grow and respond to different environmental conditions within their known habitat is of importance for future monitoring, restoration of habitat, and reintroduction should western pearlshell abundance decline further within this, or other, watersheds. Monitoring is especially important for mussels because a population of long-lived individuals can appear stable for decades but actually be composed mainly of older mussels, lacking the younger cohorts that are vital in replenishing the population (Jespen et al. 2010b). Studies in Northern Europe have found that physical and chemical differences in instream conditions can cause measurable differences in shell morphology

(Preston et al. 2010). The differences between populations of pearlshell can even cause reductions in survivorship when individuals are translocated between streams, perhaps influencing the success of restoring or supplementing populations (Valovirta 1990, Preston et al. 2010).

I completed a pilot study evaluating the cost-effectiveness of different visual survey methods for freshwater mussels and verifying historic and new populations of native freshwater mussel fauna in 2011. Many of the surveys were carried out in the East Fork of the Satsop River from Satsop Springs Hatchery (47° 6.726' N, 123° 26.393' W) to the confluence of the mainstem Satsop River and the Chehalis River (46° 58.609' N, 123° 29.145' W). This effort covered approximately 31.2 km and roughly 100 person hours (F. Waterstrat, unpublished data). These surveys resulted in locating two previously undocumented populations of freshwater mussels in the lower reaches of the Satsop River, one of western pearlshell, another of *Anodonta* Clade II (historically *A. oregenensis*), and a failure to relocate a historic western pearlshell locality near Schafer State Park (47° 5.916'N, 123°27.951'W) on the East Fork of the Satsop River. Additional surveys were conducted in January 2012 in Stillwater and Phillips creeks which are both in the headwaters of the East Fork Satsop River after a review of Green Diamond survey databases and communication with former biologists indicated the presence of freshwater mussels in that system. One population of western pearlshell was identified in Stillwater Creek upstream of its confluence with Phillips Creek and an additional population was found in early August 2012 at roughly 4 river km upstream of the convergence of Stillwater and Bingham Creeks. Other water bodies in southwest Washington were systematically or opportunistically surveyed during 2011 – 2013 for native and non-native freshwater mussels and all confirmed observations were reported to Washington Department of Fish & Wildlife. These surveys provided the initial observations that led to the completion of this thesis.

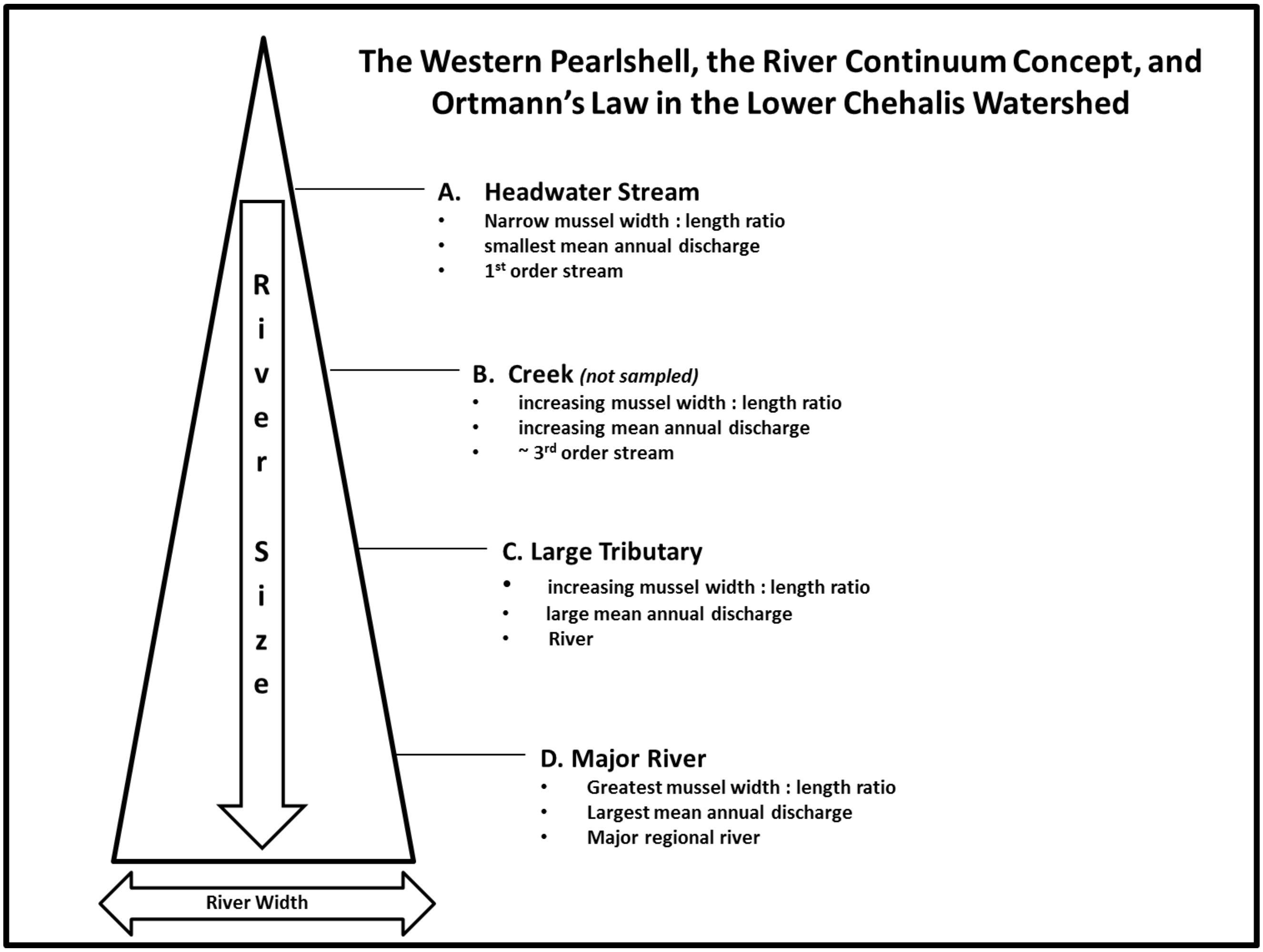


Figure1: Conceptual diagram relating Ortmann's "Law" (1920) and the River Continuum (1980) for predictions of western pearlshell morphology in the lower Chehalis watershed.

Selected survey sites, which ranged from the headwaters to the mainstem of a major regional river, framed this investigation with the theories outlined in the River Continuum Concept (Vannote et al 1980) and Ortmann's Law of Stream Position (Ortmann 1920). Visual inspection of western pearlshell at each site led to the hypothesis that size would increase and shell morphology would change in a predictable manner as watershed area and water discharge increased (Fig 1).

Ortmann's Law describes a broad trend of increasing shell width to length ratio (valve inflation) for lotic (riverine) mussels increasing in a downstream fashion in the Mississippi River drainage (Ortmann 1920). This relationship was not found to hold true for eastern species of Margaritiferidae and to my best knowledge, remains untested in western populations. It has been hypothesized that the variation in shell morphology, as described in Ortmann's Law, is a strategy to either stay anchored in place or allow for quick reburial during a disturbance event such as swift flows (Stanley 1981, Watters 1994). Shells with increased sculpture or ornamentation have been proven to help retain sediment and substrate in high flows and shells where ornamentation is lacking are able to rebury themselves more quickly after or during disturbances (Stanley 1981). In this study I hypothesized that although mussels in the Pacific Northwest lack ornamentation and that eastern North American Margaritiferidae do not follow Ortmann's law, there may be other undescribed phenotypic variation in freshwater mussels related to environmental conditions (Preston et. al 2010). As previously mentioned, mussel populations in the Pacific Northwest and Washington State (Jepsen 2010) are declining and many eastern freshwater mussel species are listed as threatened or endangered according to the US Endangered Species Act (Stokstad 2012). These designations mandate population recovery and may lead to the reintroduction of mussels to areas in which they have been extirpated. There is sparse documentation about the variation among populations of mussels in Washington and there is a body of evidence that not all populations of mussels are equally successful under various conditions. A study in Great Britain found differences in shell morphologies and lower mortality rates of pearlshell within similar environmental conditions than those from outside watersheds (Preston et. al 2010). This suggests that there is some degree of specialization within species to differing environmental conditions.

Chapter 2: Literature Review:

An alarming decline in freshwater mussel populations and a continuing number of mussel species extinctions is currently underway in North America. The disappearance of mussels will be summarized along with an explanation of a number of the possible causes and why immediate conservation efforts are needed. As freshwater mussels are not commonly to the public, a general understanding of their biological classification and basic life histories is needed in order to frame this thesis. A summary of freshwater mussels in the Pacific Northwest, with an emphasis on ecological trends and environmental conditions affecting western pearlshell, will describe the research and general knowledge about mussels in Washington State that provide the foundation for this study.

Freshwater Mussels

Freshwater mussels, hereafter mussels, are molluscs (Linnaeus, 1758) of the class Bivalvia (Linnaeus, 1758) and the order Unionoida (Fleming, 1828). Literature prior to the 1970's may refer to mussels as naiads, but most contemporary literature uses the term "mussel" and they should be considered the same organisms. Globally there are approximately 840 species of freshwater mussels (Graf and Cummings 2007). North America contains the greatest diversity of species with between 297 and 302 species represented by the: 1) *Margaritiferidae* (5 species) and 2) *Unionidae* (~295 species) families (Williams et. al. 1993, Graf and Cummings 2007). The exact number of species is currently in question as genetic tools are being employed to reevaluate species designations and taxonomic investigations are ongoing (*see* Mock et al. 2004, Campbell and Lydeard 2012*).*

Mussels are primarily aquatic infaunal filter-feeding organisms found in perennial freshwater systems. Although mussels are capable of short vertical migrations in benthic substrates and short horizontal movements across the substrate, they are typically regarded as mostly sessile organisms once larvae have settled in the substrate.

Mussels have a unique and complex reproductive life history that includes an obligate parasitic stage. Mussels use a sperm-cast mating pathway for fertilization, in which sperm released by males is captured by females during filter-feeding and fertilizes eggs in suprabranchial gills, outside the reproductive organs of the female, in contrast to true internal fertilization (Bishop and Pemberton 2006). Once fertilized, embryonic mussels develop into glochidia, or larval mussels, and hosts (often fish) are attracted to the gravid female in what can be a stunning variety of behavioral and morphological adaptations. Glochidia are released singularly or in a discrete mass called a conglutinate and infect an intermediate host organism by attaching to the gills, scales, fins, and other tissues. Host organisms are almost exclusively bony fish (*Osteichthyes* Huxley, 1880) or very infrequently salamanders (*Caudata* Scopoli, 1777) (Thomas Watters and O'Dee 1998). The glochidia clamp to the host's tissue and become encysted for days to months, sometimes absorbing nutrients from the host, before metamorphosing into juveniles, releasing from the host organism, and settling in the aquatic substrate. After settlement, mussels grow into maturity, filter-feed, and for the most part stay *in situ* for the remainder of their lives.

Much progress has been made in the past twenty-five years to understand the role of mussels in freshwater systems. As long-lived and stationary organisms, they are long-term indicators of ecosystem health within aquatic systems composed primarily of organisms with relatively short lives and motile life histories. They provide records of environmental conditions and sequester the chemical composition of water in their tissue and deposit visible annual rings in their shells (Cope et al. 2008, Black et al. 2010, Farris and Hassel 2010). Mussels remove particulate matter from the water column, which improves water quality, cycling nutrients, and concentrating dilute nutrients to the benthos subsequently providing food for other organisms.

One of the many ecological processes performed by native freshwater mussels is suspension-feeding which removes particulate matter from the water column, therefore improving water quality and concentrating nutrients otherwise unavailable to benthic organisms (Vaughn et al. 2009). Mussels create a vacuum through the movements of cilia on the gill mantle, which suctions water, and with it particulate matter, through the incurrent aperture located posteriorly and ventral to the excurrent aperture. Particulate matter is sorted coarsely by papillae and then further sorted into food and non-food items by internal labial palps. Non-food items are encased in a mucus coating and expelled as pseudofeces. Accepted food is digested and fecal waste is expelled through the excurrent valve. Suspension feeding rates can be more than 1 Liter per hour per mussel and in dense populations mussels are capable of filtering water volumes in excess of a river’s daily discharge (Vaughn et al. 2008, Haag 2012). Without the presence of healthy freshwater mussel populations, stream conditions for native organisms can deteriorate.

As epi-benthic organisms, partially in the substrate and partially in the water column, mussels transfer energy from the water to the substrate and are described as couplers between benthic and water column nutrient sources (Vaughn et al. 2008). Mussels also create important micro- and meso-habitats within and around their aggregations, or beds. Within the bed they provide stable micro-habitats for macroinvertebrates and microorganisms during high flow events (Vaughn et al. 2008). They also provide a reliable source of nutrients which can lead to higher densities of macroinvertebrates found within mussel beds than outside of them (Spooner and Vaughn 2006). For example, the growth and health of Pacific lamprey (*Lampetra tridentate)*are greater for those raised within a mussel bed than those excluded from mussels (Limm and Power 2011). The shells of living or dead mussels provide structures for periphyton and macroflora to establish and grow upon (Vaughn et al. 2008).

In addition to their unique life histories and important role in water quality and nutrient cycling, mussels are consumed as food by many organisms such as: muskrats, raccoons, birds, turtles, sunfish, white sturgeon, and invertebrates (Bauer and Wächtler 2001, Wydoski and Whitney 2003, Nedeau et al. 2009, Haag 2012). Mussels often occur in large stationary aggregations, making them both a reliable and abundant food source year round. In North America muskrats (*Ondatra zibethicus*) are a primary predator and their consumption of mussels can influence mussel abundance and species distribution (Neves and Odom 1989).

To humans, mussels have historically and contemporarily provided food sources, material resources, decorative and functional ornamentation, and sources of income. In the Pacific Northwest, Native Americans used mussels as a source of food from at least the central Puget Sound to the Tri-cities vicinity (Wong 1993, O'Brien et al. 2013), and also for tools and ornamentation (O'Brien et al. 2013). Contemporarily freshwater mussels were of major economic importance in eastern North America as the raw material for button manufacturing until post World War II, for the pearl harvest into the 1950's, and as material to seed the foreign pearl production industry into the 1990's (Haag 2012).

Declines of Freshwater Mussels

Freshwater mussels are in steep decline globally (Strayer 2008), with sources stating global declines of 72%, 69%, and 72% (Williams et al 1993, Stein et al. 2000, Turvey 2009 respectively) for the families imperiled in North America. Although estimates of decline within the Unionid family vary slightly, it is consistently considered the most imperiled family of organisms in North America. North America had lost 21 species of mussels by the early 1990's (Williams et al. 1993) and twenty years later that number increased to 35 extinct species (Stokstad 2012).

From the 1800's to post World War II mussel declines in North America were primarily a result of over harvesting of mussels for the pearl and button trade in the eastern and southeastern regions. The creation of buttons from shells of freshwater mussels ended after the advent of modern plastics created a cheap and reliable substitute. This historic commercial use of mussels created dramatic local declines in mussel populations, but remarkably did not push any species into extinction (Haag 2012). The harvest of mussels for seeding pearl growth in the Asian pearl production market continued into the late 1990's in the southeastern US until the simultaneous collapse of pearl oyster stocks, the declining Japanese economy, and new pearl seeding methods by the Chinese and Japanese pearl producers reduced the demand for American mussel shells (Haag 2012).

Modern extinctions and declines in freshwater fauna including mussels are attributed to four anthropogenic disturbances: habitat alteration (often because of hydraulic impoundments; Bogan 1993), declining water quality (Williams et al. 1993, Richter et al. 1997, Dudgeon et al. 2006), invasive species (Williams et. al. 1993), and global climate change (Hastie et al. 2003, Pandolfo et al. 2010).

Impoundments

The alteration of many of North America's waterways by impoundment, diversions, channelization, and other means has changed the hydrology and instream water conditions from their historic natural states. These shifts from lotic to lentic (lake-like) systems have contributed to lowered numbers and species of freshwater mussels, alteration of temperature regimes and sediment transport, elimination of host fish to upstream reaches, and deviation from the historic high and low water discharge periods (Williams et al. 1993, Bogan 1993, 2008, Vaughn and Taylor 1999, Stokstad 2012). Water pooling behind dams and impoundments increases sedimentation, smothering riverine species adapted to coarser substrates, increases temperatures to levels detrimental or fatal to species adapted to cooler temperatures, eliminates the habitat used by host fish, and isolates mussel populations from each other (Bogan 1993, Vaughn and Taylor 1999). Riverine mussels, typically thicker shelled than still water mussels, evolved to withstand the erosional force of moving water and sediments. However, heavy shelled riverine species are unable to dig out of fine sediments which accumulate behind impoundments and sink into or are buried by the substrates where they suffocate (Vannote and Minshall 1982). Dramatic changes in water depth due to draw downs for spring melts or to electric power demands can lead to mussels becoming stranded out of the water and perishing. Native host fish required for reproduction of freshwater mussels often share habitat preferences with their freshwater mussel parasites at some point in their life history. Impoundments can, in some situations, physically block fish access to upstream habitats eliminating the possibility for reproduction or altering the habitat to a degree that the lack of fish hosts becomes an ecological barrier to reproduction (Bogan 1993). Interestingly, impacts of dams and impoundments in many cases also create habitats that are beneficial to non-native species or to native mussel species not typically found in lotic habitats. Indeed often native lentic-adapted mussel species may replace their lotic counterparts behind impoundments and a shift from lotic-loving to still water species may occur (Watters 1999). Mussels require a specific set of physical and biological attributes for growth and reproduction and the construction of impoundments can alter water bodies and create unsuitable conditions for historic native populations to exist.

Water Quality and Pollution

Declining water quality from pollution, land use practices, and watershed alterations have been identified in the decline of freshwater life worldwide. In North America, pollution was implicated in the destruction of freshwater mussel populations as early as 1909 (Ortmann 1909). As stationary creatures, mussels have little ability beyond “clamming up” and burrowing into the substrate to avoid unfavorable water conditions. While this strategy may be useful for short term avoidance it is of little benefit when encountering chronic conditions of poor water quality. Pacific drainage mussels in the genera *Margaritifera* and *Anodonta* can be found living in highly urbanized creeks and ponds as well as more pristine environments (Nedaeu et al 2010). Reports of mussels in urban areas document declining populations and evidence of decreasing recruitment possibly from the effects of urbanization on habitat (Hastie and Toy 2008). In general, mussels need waters rich in calcium carbonate to grow and maintain their shells and are absent in highly acidic conditions which can lead to shell deterioration. The exception to this common theme is *M. margaritifera*, a congener of western pearlshell, which is often the only mussel found in soft, calcium-poor waters. Water quality thresholds for *Margaritifera* reported in Great Britain state that levels greater than 1.0 mg/L^{-1} for nitrate and 0.03 mg/L^{-1} for phosphate are detrimental especially to the larval forms (Young et al. 2003). *Margaritifera* prefer oligotrophic (nutrient-poor) conditions with a neutral to basic pH and conductivity less than 100 μS^{-1} (Young et al 2003).

As previously stated, mussels have complex life cycles and various life stages. Each life stage is exposed to the contaminants in the water through a different vector, of varying durations, at different locations in the watershed, and with differing levels of tolerance to pollutants (Cope et al. 2008). It is likely that the behavioral and physiological responses of mussels vary with life stage and species. Once the mussel has settled in the sediment and begun its life as a filter feeder it is exposed to every particle in the water for the duration of its life and readily accumulates metals and other pollutants (Naimo 1995). Currently, mussels are being extensively tested for a host of chemical compounds to understand their effect on mussel physiology. Due to the high number of species in North America, and the cocktail of pollutants and their unknown interactions, the full suite of pollutants and their effects on mussels will likely never be fully documented.

Aquatic Invasive Species

Aquatic invasive species (AIS) are one of the great challenges for native organisms worldwide including North America's native freshwater mussel assemblages. Invasive species can impact mussel populations in several ways. Invasive *Dreissena* mussels directly compete with native freshwater mussels for food resources In addition, these invasive mussels attach to native mussel shells as substrate which impairs their ability to function physiologically, restricts their ability to move, and exhausts them to death (Haag et al. 1993). The Asian clams in the *Corbicula* genus have been in North America since the early 1900's and also compete with native mussels for food and habitat (Counts III 1986). However, the impacts of Asian clams on native mussels varies in the literature from detrimental to neutral (Leff et al. 1990). Some invasive fish may consume native mussels (Poos et al. 2010) and non-native plants can alter the substrate, planktonic abundance and velocity of streams, limiting habitat and food resources throughout aquatic ecosystems (Strayer 2010). On the other side of the coin, freshwater mussels are also among the most successful AIS themselves, including the zebra (*Dreissena polymorpha*) and quagga (*Dreissena bugensis*) mussels, Asian clams (*Corbicula* spp) (non-unionid mussels), and the Chinese mussel (*Anodonta woodiana*) (Douda et al. 2012).

Climate Change

Climate change is a global phenomenon that will affect most organisms on this planet to some degree. Freshwater mussels are particularly susceptible to shifts in climate because of their complex and host-obligate life histories and the inability of individuals to migrate in adverse conditions (Hastie et al. 2003). Mussels have thermal tolerances that may be exceeded as the seasonal maximum and minimum temperatures expand in range and shift in timing. In fact, some species may already exist at the edge of their thermal tolerances (Pandolfo et al. 2010). Climate change has brought measurable changes in the magnitude and timing of precipitation and snowmelt events that control in-stream water volume. These changes can negatively affect mussels in a number of ways. An increase in major flood events from increased precipitation could cause mussels to be scoured from the substrate, and alter host fish assemblages and movement patterns, in turn decoupling the mussel’s reproductive life history. Rising sea levels could impact low-lying coastal populations exposed to tidal fluctuations and incursions of salt water into freshwater mussel habitats (Hastie et al 2003). In the Pacific Northwest climate change models have predicted wetter, warmer winters and drier, hotter summers (Mote and

Salathe 2010). Predicted hotter and drier summers may reduce water volume and discharge. This can potentially expose shallow mussel beds, cause rivers to deposit sediments and organic materials, and increase algal growth on the stream bed making it less suitable for mussels (Hastie et al. 2003).

Mussels in Washington State

Currently, North American freshwater mussels are divided into four broad geographic regions and 17 faunal provinces (Haag, 2010). The Pacific Region, which includes Washington State, contains a singular Pacific province which encompasses all waters flowing into the Pacific Ocean from North America, including the Gulf of California and the Bering Sea. Within this region, there are at least five species of Pacific freshwater mussels represented by 3 genera: *Margaritifera* (1 sp.), *Gonidea* (1 sp.) and *Anodonta* (unknown number of spp.) (Nedeau et. al. 2009, Haag 2010), which is the lowest species diversity for any region (Table 1). Nevertheless, the Pacific Region is unique in that all species are endemic to the region. Washington State is inhabited by all but one of the five species with western pearlshell being the most widespread and common (Jepsen et. al. 2010) and all species but the Yukon floater (*A.bergingiana*) present (Nedeau et. al. 2009). Three of the five species of freshwater mussels have been recorded in the Chehalis watershed with the *Anodonta Clade I* complex not represented (Washington Department of Fish and Wildlife 2012).

Table 1: Taxonomy of freshwater mussels in Washington State is represented by 3 genera of mussels in the Pacific region as defined by Haag 2012. * Clade I was formerly two species the California floater (*A. californiensis*) and the winged floater (*A. nuttalliana*.). ** Formerly two species the western floater (*Anodonta k.)* and the Oregon floater (*A. oregonensis*)

Native Freshwater Mussel Species of Washington State			
Common name	**Scientific name**	**Family**	**Tribe**
"winged" floaters	*Anodonta Clade I**	Unionidae	Anodontini
"western" floater	*Anodonta Clade II***	Unionidae	Anodontini
western ridged mussel	*Gonidea angulata*	Unionidae	-
western pearlshell	*Margaritifera falcata*	Margaritiferidae	-

No formal survey of the distribution of freshwater mussels in Washington State has been undertaken, but regional efforts have been made to document the presence of native mussels. The state of Montana recently completed a state-wide inventory and outreach program to assess the status of their freshwater mussel populations. Montana's inventory could act as both a precedent and guide for other states in the region as concern and documentation about freshwater mussel declines in the western states becomes a management concern for wildlife and conservation entities (Stagliano 2010). Historic and contemporary mussel locality records have been exhaustively researched and compiled by the Xerces Society at a Pacific Northwest regional scale. The Washington Department of Fish and Wildlife maintains verified state locality observation data within the Priority Habitat-Species Database (Washington Department of Fish and Wildlife 2012) (Fig 2) (http://wdfw.wa.gov/conservation/phs/ 2012). However, these databases are not the result of a concentrated survey effort for mussels and access and additions to these databases is limited.

The main body of freshwater mussel research in Washington State investigates population abundance and habitat associations of the western pearlshell , though several researchers have also investigated its reproductive traits at different sites (Toy 1998, Adair et al.

2009, Allard et al. 2012) and the effects of anthropogenic change on population structure (Hastie and Toy 2008, Helmstetler and Cowles 2008, Krueger et. al. 2007, Cowles et. al 2012). The theme for many of these studies is the need for long-term monitoring of population trends and the quality of their habitats as summarized in a USFWS report (Lohr and Glasgow 2005), yet this review found only two studies revisiting populations for monitoring in Washington State. Toy revisited her 1998 thesis study in 2006 and found significant population declines at both sites (Hastie and Toy 2008). An additional survey found total extirpation of western pearlshell from the mid-Columbia River (Helmstetler and Cowles 2008).

In addition to the locality information collected and housed by management and conservation entities there may be information about the distribution of freshwater mussels known by fishermen, property owners, and other river users in the local communities. The knowledge of individuals and communities that live in or frequent areas is referred to as local ecological knowledge (LEK). The declines, disappearances, or new occurrences of mussel populations can be documented through incorporation of local ecological knowledge (Azzurro et al. 2011). LEK is often overlooked during scientific studies and can provide a rich spatially and temporally long-term record of information if it is both accurate and reliable (Brook and McLachlan 2008).

While LEK can be a valuable resource for documentation and therefore conservation of a species, it is also the responsibility of the conversation community to educate and excite the local populous about the organisms that surround them. Outreach to the community about freshwater mussels is not only an effective method in engaging individuals in their environments, it also aids conservation efforts by creating invested and concerned stewards of the biological community (Mazzacano 2012)

Western pearlshell in Washington State

This review will focus primarily on the pearlshells, but information from other studies and mussel species will be used to inform and contrast findings and will be included if applicable.

By far the most abundant and widespread mussel in the Pacific Northwest is the western pearlshell (Toy 1998, Jespen et al. 2010b), and for that reason this and the majority of studies within Washington State have focused on this species. The western pearlshell typically inhabits cool, clean fast-flowing streams. It possesses a thick shell with a brown to black outer coloration often with erosion on the umbo region and a white, purple, or salmon colored nacre on the shell interior (Toy 1998, Nedeau et al. 2009). In western North America, the western pearlshell often inhabit low gradient lotic systems with stable substrates and low shear stress (Stock 1996, Howard and Cuffey 2003, Nedeau et al. 2009, Jespen et al. 2010b), where they can occur in great numbers and densities (Murphy 1942, Nedeau et al. 2009). Western pearlshell become sexually mature between 9 – 12 years and differentiation of sex has been observed in western Washington (Toy 1998). Fishes in the family Salmonidae, especially those within the genera *Oncorhynchus*, *Salmo*, and *Salvelinus*, have been identified as key hosts for the western pearlshell glochidia in our region. Their interactions are summarized in Jepsen et al. (2010).

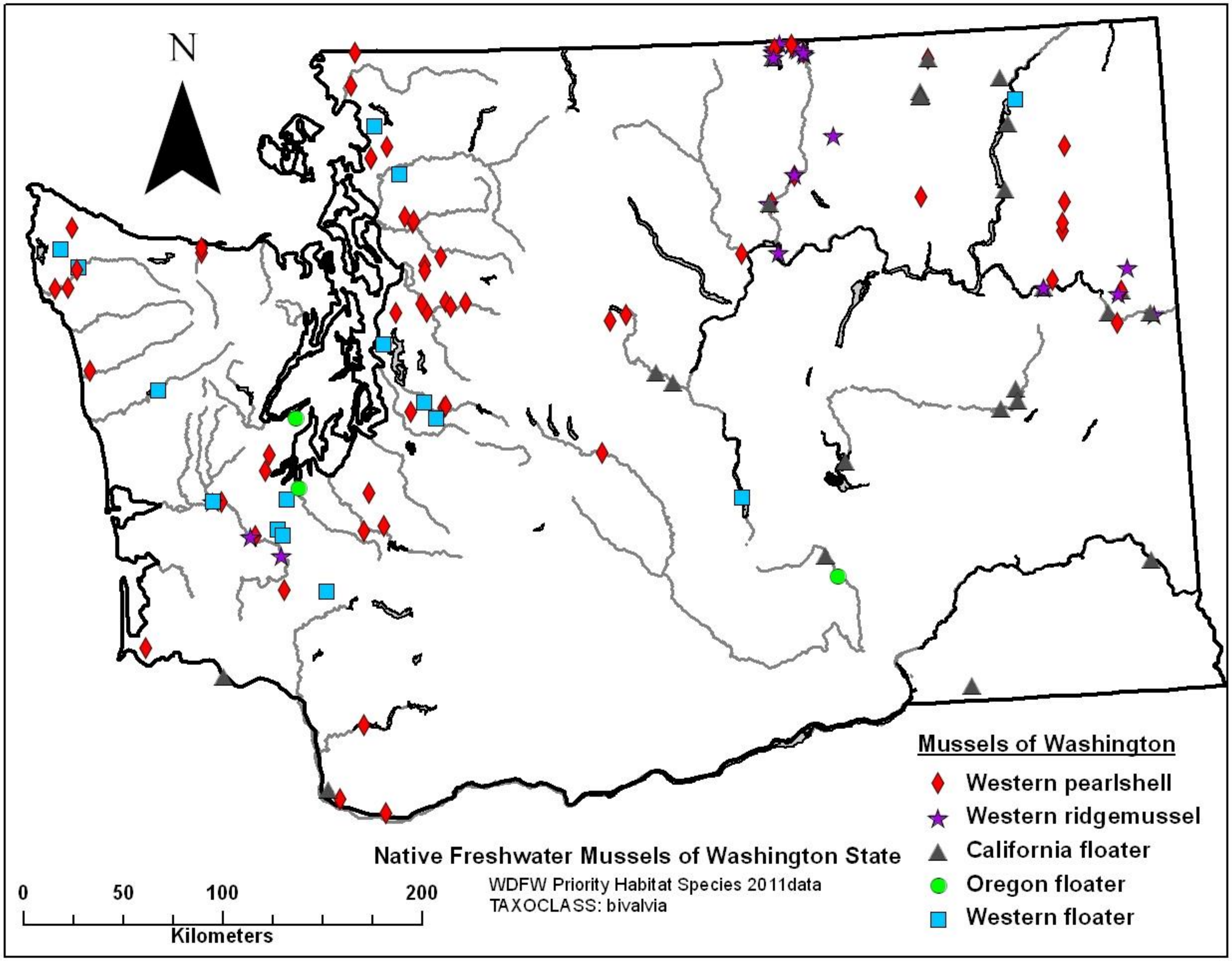


Figure 2: Freshwater mussel distribution in Washington State created with WDFW Priority Habitat Species data (2011). Note that all species reported to exist in Washington are represented on this map.

As a testament to a sedentary organism's plasticity and to the difficulty in defining exact pearlshell habitat associations, western pearlshell exist and reproduce in highly variable areas wherever suitable habitat and host fish co-occur. Western pearlshells can occur in low gradient sections of headwater streams at elevations exceeding 1,500 m (Jespen et al. 2010). They have been observed living in roadside ditches, and have even crossed the Rocky Mountains into western Montana on their trout hosts. Mussels in the genus *Margaritifera* can live upwards of a century (Ziuganov et al. 2000), making them among the longest lived animals on the planet and the focus of several studies reconstructing the environmental history of lotic habitats (Howard and Cuffey 2005, Black et al. 2010). However, more typical life spans of mussels in Washington State reach 60 to 80 years old (Bauer 1992, Ziuganov et al. 2000, Black et al. 2010).

Ecology: Biotic and abiotic controls of western pearlshell

The physical environment, including temperature, water discharge, shear stress, water chemistry, and nutrient levels, has been documented to have effects on the longevity and growth rates of freshwater mussels (Bauer 1992). These environmental parameters have been investigated in Great Britain extensively and throughout the range of the critically endangered pearlshell (*Margaritifera margaritifera*) (Bauer 1992, Skinner et al. 2003, Young et al. 2003). These studies found that human alteration of hydrology and subsequent substrate composition change as well as the decline of native host fish has led to the near elimination of pearlshells in Great Britain.

Temperature

Temperature is a major driver of mussel growth, size, and age. Cold conditions in higher latitudes create slower growth, larger sizes, and longer lifespans in the pearlshell than in populations at lower latitudes and warmer waters in Europe (Bauer 1992). Bauer (1992) hypothesized that metabolic rates are influenced by water temperature. Colder waters reduce annual growth rates and slow metabolic processes, but cooler habitats allow for longer lifespans and ultimately larger mussels. Toy (1998) found that pearlshell mussels in east-central Puget Sound rapidly increased their seasonal growth rates during the summer months of the year and that winter conditions showed reduced growth rates. She states that this increase in growth occurs after the reproductive cycle is complete, indicating that energy may be shifted from reproduction to growth. Warmer temperatures also tend to increase productivity in aquatic systems unless dissolved oxygen level or reach a critical low threshold for the organism.

Discharge and velocity:

Discussion of water discharge and velocity excludes lentic habitats, which by definition are still waters where velocity remains very slow or is nonexistent. Water velocity is determined by the steepness, or gradient, of the river channel and the roughness of the channel bed which is determined largely by instream substrate and large woody debris, as well as the sinuosity of the channel itself. Pearlshell mussels are typically found in streams and rivers that have suitable habitat for their host fish species, allow for the successful settlement of larval mussels, and have suitable flow rates, low shear stress, or refugia within the channel (Vannote & Minshall 1982, Strayer 1999, Howard and Cuffey 2003, 2005, Stone *et al.* 2004). In most rivers, mussels exist in areas where velocity maintains a stable non-aggrading substrate or where there are sufficient refugia, such as boulders or large woody debris, for mussels to remain in place during high flows (Vannote & Minshall 1982).

Pearlshell mussel habitat preferences can be identified at different resolutions within aquatic systems. Most studies have examined abundance and habitat associations within a single stream reach or described a single population, but have not examined their distribution from the headwaters to the mainstem of a watershed (Hastie & Toy, 2008; Lohr & Glasgow, 2005; Stock, 1996; Toy, 1998). Only one investigation in western Washington by Jennifer Stone (2004) examined freshwater mussels over a significant distance. While it is possible to accurately predict mussel presence in a lotic system based on physical characteristics defining a stream reach, their distribution within the reach tends to be highly patchy (Stone et al. 2004).

Theses on western pearlshell in Washington

Two of the earliest studies to address freshwater mussels in Washington State are theses, one from The Evergreen State College in 1996 by Amy Stock followed by one by Kelly Toy in 1998 at the University of Washington. Stock (1996) focused her research on habitat associations in a tributary of the Wenatchee River and found that mussel populations existing in poor salmonid habitat had no juvenile recruitment in over 45 years. Toy contrasted 2 populations of western pearlshells in eastern Puget Sound drainages to determine age, growth, reproductive timing, habitat size, and included detailed histology of the reproductive organs. Both studies found similar substrate and habitat conditions typical of the western pearlshell throughout its range. They both assessed age and growth by measuring and counting bands on the hinge ligament. Stock (1996) censused populations based on visual counts and Toy (1998) estimated populations from transect subsampling within sites.

Toy (1998) investigated mussel abundance at Battle and Bear Creeks and found average densities of 80 mussels/m^2 and 55 mussel/ m^2 respectively. She found that these mussels became sexually differentiated, and later sexually mature, at the same size. Individuals in Battle Creek mature 2 years later than mussels in Bear Creek. She attributed this difference in age at maturation to the difference in temperatures between the two creeks and proposed that warmer waters can cause the mussel to mature and grow more quickly. Toy also recognized the impending peril mussels in urbanizing Seattle might face. Earlier investigations targeting freshwater mussels in Washington State have set the stage for the synthesis and expansion of information to create a comprehensive assessment of mussels within Washington State. Interest and knowledge about freshwater mussels in Washington State is increasing, yet much work remains to identify mussel localities and explain variation observed among populations. Documentation of extant mussel population abundance and demographics is crucial to the monitoring of population stability, and can provide evidence for species protection before populations are lost (Nedeau et al. 2009, Jespen et al. 2010a, 2010b, 2010c). Understanding the range of environmental and biological parameters in which mussels exist explains the plasticity within a species and the limitations of their distribution. More efforts to document mussel localities, and investigations to scope ecological variation within species and populations, are needed before a complete assessment of freshwater mussels in Washington State is possible.

Chapter 3: Methods

Site Descriptions

This study was conducted within the Lower Chehalis watershed as defined by Water Resource Inventory Area (WRIA) 22 (Washington Administrative Code 173-500-040), located in southwestern Washington State (Figure 3- inset). This is the first investigation of mussels in the Chehalis River beyond opportunistic locality reporting. The Chehalis River runs 125 miles from its headwaters to Grays Harbor and its watershed is the second largest watershed in Washington State draining ~7000 km² (USGS Washington Water Science Center, 2013). It was never glaciated and its mainstem is free of dams. The Chehalis watershed can also be considered biologically rich as its waters are home to nearly half of all species of freshwater fish in Washington, including: Chinook salmon (*Oncorhynchus tshawytscha),* chum salmon (*O. keta*), coho salmon (*O. kisutch*), steelhead (*O. mykiss*), and Olympic mudminnow (*Novumbra hubbsi*) (Wydoski and Whitney 2003) and is home to all three mussel genera occurring within the state (http://wdfw.wa.gov/conservation/phs/, 2012).

Following initial mussel surveys conducted in WRIA 22, three sites were selected with easily accessible populations of pearlshell mussels, in close spatial proximity to each other, and in increasing watershed area. The sites are referred to as Stillwater Creek (headwaters), Lower Satsop (tributary), and South Elma (mainstem; Fig 3). Two sites, South Elma and Lower Satsop, are located in Grays Harbor County and Stillwater Creek is located in Mason County. The Stillwater and Lower Satsop sites are within the Satsop River drainage and South Elma is located on the Chehalis River 6.5 river km upstream of the Satsop River's confluence with the Chehalis River.

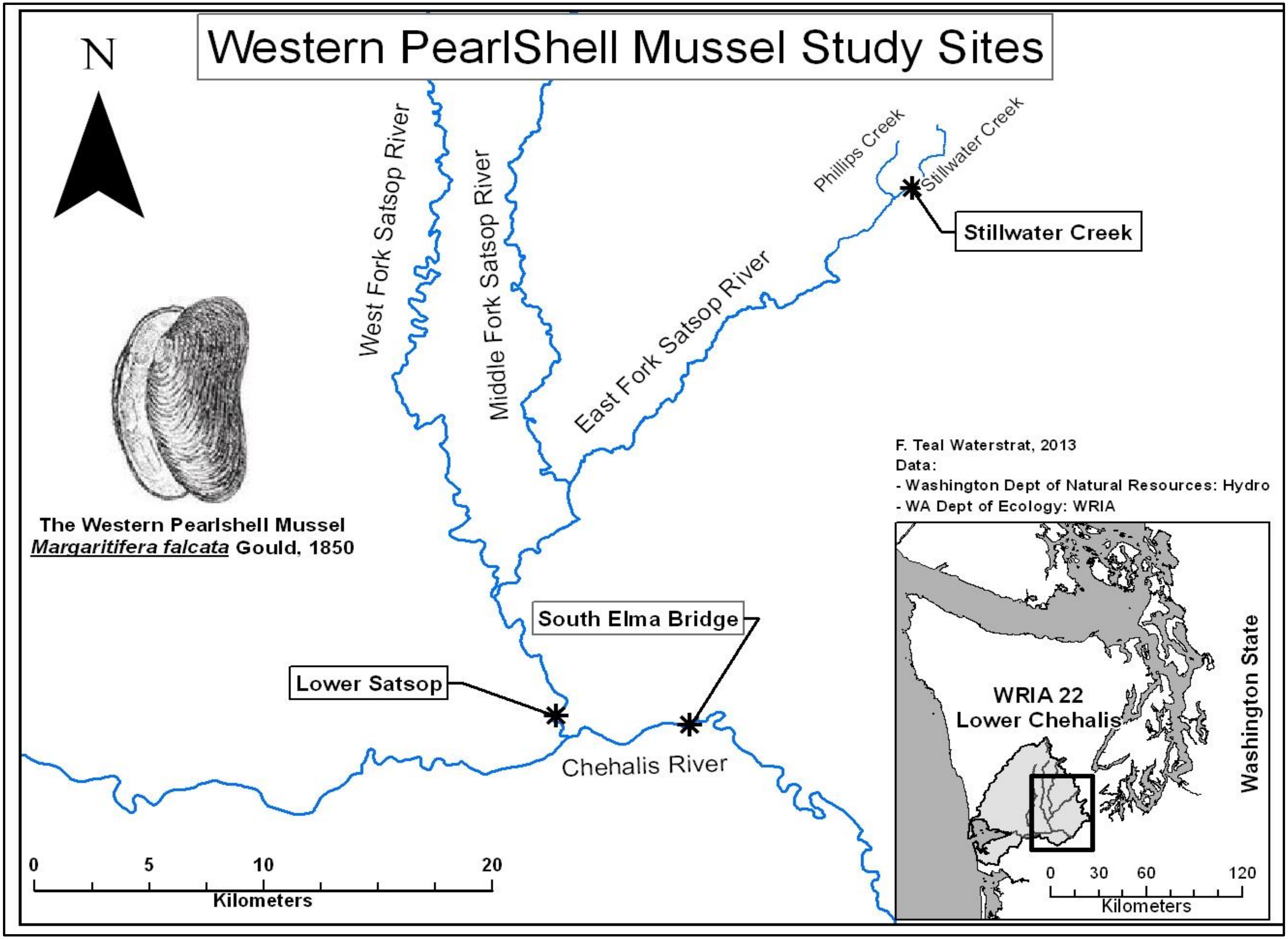


Figure 3: The location of the three study sites in the Lower Chehalis Watershed (WRIA 22)

The Stillwater Creek site is located on a first-order segment of Stillwater Creek, 3.7 km below its initiation point at an unnamed, spring-fed permanent wetland (~ 47° 13.225’N, 123° 15.729” W) south of the Shelton-Matlock Road and immediately upstream of the convergence of Stillwater and Phillips Creeks (47° 12.257’N, 123° 12.764’W) (Fig 3). The area is a low gradient anastomosed reach with pool-riffle stream habitats and enough flow to maintain clean substrate. Pearlshell mussels appear in patchy but continuous numbers for nearly 0.5 km upstream of the convergence of Stillwater and Phillips Creeks. The site is surrounded by managed forest land owned and operated by Green Diamond Resource Company with an extensive graded gravel road system and riparian buffer.

The surrounding forest is primarily a Douglas-fir (*Psuedotsuga menziesii* Franco) plantation, but the immediate riparian forest (33 m buffered area) is a much older heterogeneous evergreen forest with species typical to this region. Between multiple channels within the overall stream channel are vegetated areas composed of a mixture of sedges (*Cyperaceae spp.*), alder (*Alnus rubra* Bong), willows (*Salix spp*.), Pacific nine bark (*Physocarpus capitatus* Kuntze), devil’s club (*Oplopanax horridus* Miquel), and other wetland-associated plant species. This reach is used by coho and trout as a spawning area and it is likely other salmonids use this reach for reproduction as well. Reaches up- and downstream had extensive hydrologic alteration by beaver (i.e. dams) leading to the slowing and widening of channels with extensive beds of hornworts (*Ceratophyllaceae* sp) and the buildup of greater than 1 m depth of fine silts and sediments that may exclude pearlshell mussels.

The Lower Satsop site (46°59.261’ N, 123°29.454’W) is located 2.5 km upstream of the mouth of the Satsop River where it converges with the Chehalis River and 2 km downstream of the Highway 12 Bridge crossing the Satsop River. The site is immediately upstream of a deep side channel pool armored by extensive rip-rap in a long riffle-dominated reach. The Satsop watershed drains 593.1 km^2 at the site and the twenty year average discharge for the Lower Satsop River is 60.77 m^3/s at the Highway 12 Bridge and was recorded at USGS gaging station 12035000. The entire site consists of several hundred individual pearlshell mussels and is centered around a large fallen tree on the west bank of the river. The Lower Satsop site is surrounded by agricultural land, primarily producing dairy and vegetable crops, with Keyes Road to the east. The immediate east bank of the river is owned by an unmaintained Washington State Department of Fish and Wildlife parcel that is used mainly by fishermen to access salmon and steelhead fishing sites and is primarily composed of a mixed black cottonwood (*Poplus trichocarpa* Brayshaw) and willow riparian floodplain forest. The WDFW parcel was formerly the location of a gravel extraction site presumably for the construction of the Satsop Nuclear Power Plant, now the Satsop Industrial Park, and extraction of the gravel has left three deep ponds in the floodplain.

The South Elma site is located 2.5 km south of Elma on Wakefield Road immediately downstream of the Wakefield Bridge at the head of the southern of two channels in the mainstem Chehalis River. The two channels are divided by an established mid-channel island (46° 58.915'N, 123° 24.845'W) covered in woody vegetation and roughly 300 m in length. Upstream of the study site, the river makes an abrupt 90° turn and changes direction from SW to NW. At this bend there is a deep pool that extends from the corner to the edge of the site where the river then enters a shallow, swift riffle that continues downstream for at least 200 m. The watershed drains 3768.4 km^2 and the twenty year average discharge for the Chehalis River is 117 m^3/s at the

Porter Bridge and was recorded at USGS gaging station 12031000. Delzene, Mox Chehalis, and Eaton Creeks enter the Chehalis River between this USGS gage and the South Elma study site. The pearlshell population extends in either direction upstream of the bridge with the density decreasing to an individual or small pockets of mussels found occasionally. The surrounding area is agricultural to the north, east, and west, but immediately to the south of the site the landscape rises into the northern edge of the Willapa Hills. Both sides of the river have high bluffs composed of loose fine sediments. Above the bluffs is a mixed stand of black cottonwood, willow, and mixed shrubs. See Appendix A for a list of observed flora and fauna at all three sites.

Sampling design

A simple random sampling design was selected for this study after a review of methods outlined in Strayer and Smith (2003). Initially a systematic sampling design was developed, but due to the high level of channel heterogeneity at the Stillwater Creek site, the systematic design was abandoned for logistical simplicity and a random design was adapted.

The methods used to calculate sample reach area included measuring reach length by average width and dividing the resulting area into a 1-m^2 grid of the reach. This created a standard x, y map of the site (x: channel width from river right to river left, y: stream longitudinal distance from upstream to downstream) that was used to divide the reach into regular sampling plots (Fig 4).

	Lower Satsop River Random Sampling locations																
meter **dist.**	**X, stream width**																
Y, Stream Length	**0**	**1**	**2**	**3**	**4**	**5**	**6**	**7**	**8**	**9**	**10**	**11**	**12**	**13**	**14**	**15**	**total at x meters**
0				**1**					**1**				**1**	**1**			4
1	**1**		**1**		**1**												3
2			**1**			**1**					**1**				**1**	**1**	5
3			**1**			**1**	**1**					**1**	**1**				5
5		**1**								**1**							2
6								**1**									1
7						**1**		**1**						**1**			3
8	**1**			**1**				**1**						**1**	**1**		5
9	**1**	**1**						**1**									3
10	**1**	**1**				**1**											3

Figure 4: Depiction of random sampling map used to identify sample plot locations in the field. Cells marked “1” in this example were sampled for mussels and blank cells were not sampled.

The number of plots sampled was determined by multiplying the average segment width by the segment length. During surveys, I attempted to randomly sample twenty percent of the plots by calculating sample area and dividing by five (# plots = {L x $W_{average}$)/5}. Sample plot locations were determined using a random number table composed of the longitudinal and latitudinal axis positions of each segment.

Establishment of a survey segment at each site required that it meet three criteria: 1) it included a population of pearlshell mussels, 2) its depth and flow velocity during the summer low flow period was such that an individual snorkel sampler could both stay in place and sample the benthic environment, and 3) channel structure was simple enough to allow for reach division into a grid of sampling plots. Once a segment from each site met the criteria, a continuous 100 m longitudinal distance was measured with an LTI® Impulse hypsometer along an average channel azimuth as determined by compass on-site. Upstream and downstream extents of the sampling reach were delineated by flagging and GPS waypoints were taken on a Garmin® Etrex HCx for reference. The segments spanned the wetted stream width of the channel and were measured to the nearest tenth of a meter. At South Elma only the left channel was used as a segment, at Lower Satsop the channel was restricted to the specific habitats where mussels existed and at the Stillwater site the sum of the braided channel widths was used.

All sampling at plots occurred within a 0.25 m^2 (0.5 x 0.5 m) sampling quadrant placed in the upstream right hand corner of selected plots. The quadrants were made of 0.75 inch diameter PVC piping and 90° PVC joints. The quadrants were drilled with regular holes to allow water to enter causing them to sink. In plots with water flow strong enough to move quadrant squares they were anchored by rebar pounded into the substrate inside the upstream corner.

Mussel data collection

All freshwater mussel handling and collections were performed under the terms of Washington Department of Fish and Wildlife Scientific Collection Permit #11-400. No mussels were sacrificed for this study. Enumeration and measurement of mussels, with the exception of collecting discarded shells (see Aging mussel shells) occurred within the delineated survey reach. After the sampling quadrant was placed in the selected plot, a snorkeler sampled the quadrant for live mussels with a visual and tactile search of the quadrant. The sampler removed all mussels encountered into a mesh bag for identification, census, and valve measurement. The top layer of substrates, cobble and smaller, was also removed, but the quadrant was not excavated. If any portion of a mussel shell was inside the border of the quadrant it was included in the total quadrant. All mussels in the quadrant were identified, counted, and measured for valve length, width, and height to the nearest tenth of a millimeter using a caliper (Pittsburgh® venier scale 6” utility caliper #7914, CA, USA) (Fig 5). If more than 30 mussels were encountered in a single plot, measurements were taken from every fourth mussel picked randomly from the sample bag.

Complete valves in good condition were opportunistically collected from all sites. However, because only one valve was found at the Lower Satsop (tributary) site, assessment of age, growth, and length to mass ratios were completed only for the Stillwater (headwaters) and South Elma (mainstem) sites.

Complete right valves were collected from the Stillwater and South Elma sites and were retained to calculate length to mass ratios. The valve was weighed to the nearest hundredth of a gram using a balance (Denver Instruments Model 220 Balance, Bohemia, NY, USA) and its length measured with a caliper (Pittsburgh® 6” digital caliper #68304, CA, USA).

Aging mussel shells

The left valves of complete mussel shells collected at the Stillwater and South Elma sites were retained for aging and growth analysis. Only complete valves, without cracks or excessive erosion were considered for this analysis. At the South Elma sites all valves had a large degree of erosion in the umbo (or dorsal area) and the ones in the best condition were selected for analysis. Following methods described for aging bivalves (Schöne 2005) and specifically *Margaritifera falcata* (Howard and Cuffey 2006, Black et al. 2010), acceptable valves were measured for length, height, and width and then incased in epoxy (JB Kwik Weld® Sulphur Springs, TX, USA). A dime sized area of the valve was covered in white fingernail polish and the sample number was written on the polish. At the Washington Department of Natural Resources (DNR) Geology Division Laboratory the valves were cut transversely from the umbo to the edge of the shell perpendicular to external growth lines with a heavy liquid rock-saw and mounted to a 46 mm x 26.75 mm (1.05" -1.81") glass slide with epoxy (Loctite® translucent yellow Westlake OH, USA).The mounted section of the mussel was then cut to roughly 0.5 mm with an Ingram thin-section cut-off saw (Ingram model 135) and ground to ~0.25 mm with a thin section grinder (Ingram model 400). The mounted thin sections were then polished with very fine grit sand paper and aluminum powder until imperfections from cutting and grinding were removed. The finished thin section mounts were stained with a modified Mutvei's staining solution for four hours at 37°C (Schöne et. al 2005). In this stain Alcian blue was replaced by Coomassie Blue® (Brilliant Blue) for cost and safety reasons. Finally the mounted specimen had a small amount of mineral oil applied to clarify annuli by coating small scratches and imperfections not removed by polishing (MacLellan 1976).

Once prepared and stained, the sections were observed under a compound microscope (Nikon SMZ-2T) at 50x magnification and each annuli was counted from beak to edge. Average growth was calculated by dividing the length of the shell by the number of years observed. For individual valves with erosion at or around the area of the umbo which obscured early annuli, a minimum age was established from readable annuli.

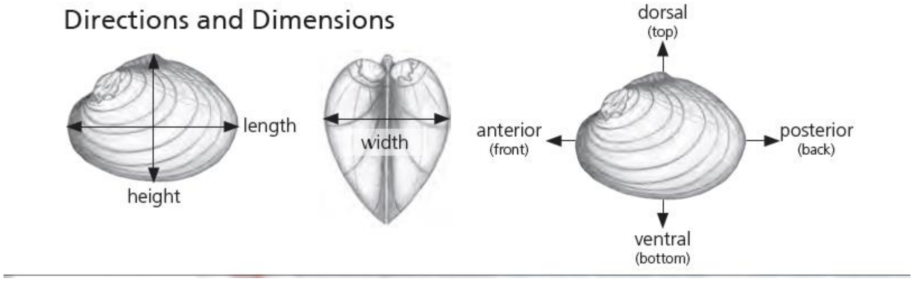


Figure 5: Basic mussel valve orientation and measurements. Image created by Ethan Nedeau, 2009: Freshwater Mussels of the Pacific Northwest. 2nd edition p.16

Stream habitat assessment

To examine the differences in mussel habitat preferences among sites, physical characteristics of each site were recorded. Stream habitat assessment followed the Timber-Fish-Wildlife Monitoring Program's *Method Manual for the Habitat Unit Survey* (*AM9-99-003)* which was designed to quantify major physical characteristics of wadeable streams commonly encountered in Washington State (Pleus et al. 1999). This survey was designed to provide guidance in quantifying standardized data about habitat units, stream morphology, and habitat characteristics. The inorganic dominant and sub-dominant surface substrates were assessed at the habitat unit level as well as within the sampling quadrant. Substrate categories included: mud/silt, sand, gravel, cobble, boulder, bedrock, and compacted clay (Appendix II). Gradient was measured at the segment start to end points while standing on the bed of the river or creek. Measurements were taken to the nearest tenth of a degree (0.0°) with a range finder (Impluse 200, Laser Technology Inc., Centennial, CO, USA). See Appendix II to reference the field sampling protocol.

Water temperature, quality, and discharge field measurements

Water temperature at each study site was recorded at hourly intervals by temperature data loggers (Onset® HOBO® Tidbit v2 Temperature Data Logger, Bourne, MA, USA) which were fully submerged and placed on the river substrate within the mussel bed at each site. Temperature was also recorded opportunistically at site visits with a hand held thermometer or with a multimeter probe (YSI® Model 85 Multiparameter Meter, Carlsbad CA, USA).

Dissolved oxygen levels, conductivity, and salinity were measured at each site with the same YSI multimeter probe. The YSI multimeter was calibrated between site visits. In late summer 2012 and early spring 2013, water samples were collected for analysis of nitrate, total phosphorus, pH, alkalinity, and hardness levels. The water samples were analyzed by Dragon Analytical Laboratories (530 Ronlee Lane NW, Olympia, WA 98502).

Discharge and velocity were measured at each site on September 6, 2012 using a velocity meter (Marsh-McBirney Flo-Mate Model 2000, Loveland, Colorado, USA). Measurements were taken following USGS standard methods and additional measurements were taken at the substrate level to evaluate flows as experienced by mussels. On two occasions velocity and discharge were measured at the Stillwater site using a neutrally buoyant object and a stopwatch. The Lower Satsop and South Elma sites both have USGS gaging stations upstream of the study sites which were used to determine estimates of discharge year-round. High and fast waters made discharge measurements using the tools available logistically difficult and unsafe at times other than the low-flow period. At the Stillwater site the velocity and discharge were measured multiple times throughout the year to create a partial hydrograph.

Informal Interviews

Serendipitous interviews were conducted with individuals encountered when surveying for mussels at or near the study sites to gather information about potentially unknown mussel localities and to deduce the local level of knowledge about freshwater mussels. The discussions were commonly initiated by an individual or individuals curious about study activities or by myself to ease tensions about having an individual working in the area. All information pertaining to freshwater mussels was recorded in a field notebook without names or other identifying details and later transcribed.

Statistical Analysis

Mussel measurements

Data collected from living mussels were used to calculate a population estimate of mussels at each site by multiplying the average number of mussels found in all plots sampled at each site by the wetted area at each site. At the Lower Satsop site the area of mussel occupation was small enough that a complete visual census was conducted. The population census at the Satsop site was contrasted with estimated population size from subsampling to determine an estimate of sampling accuracy at this site

Site level differences in the mean size of measured mussel valves metrics (length, width, and height) were determined among sites using an ANVOA. Additionally, Ortmann's Law (Ortmann 1920) for increasing valve inflation as river volume increases was tested among the three study sites as defined by a ratio of length to width. The ratios were calculated by dividing the mussel width by length (w/l) for each individual measured. Raw and log-transformed data of shell metrics and Ortmann's ratio failed to meet the assumptions of normality for parametric tests so resampling ANOVA and regression statistics (Resampling Add-in for Excel 2007) were used to assure the validity of each test.

Distribution of age and age at size as determined from measurements taken from complete left valves at study sites in Stillwater Creek and South Elma were compared using a t-test to verify if differences in average population age between these two sites. Regressions for size at age and growth rate were created and contrasted in ANCOVA analysis at each site.

The resulting length to mass slopes for the two sites were analyzed in an ANCOVA to describe the different rates of mass at size as a surrogate for valve density.

Habitat measurements

Potential preferential use of substrate habitats by mussels at each site and collectively among sites were evaluated by analyzing the presence or non-detection of pearlshell mussels in dominant substrates recorded within each sampled plot. A Chi-squared analysis compared the expected distribution of mussels, based on the available types of habitat, against where they were observed. Average and maximum discharge in 2012 for the Satsop and South Elma sites, as recorded by the Satsop and Porter USGS gage flow stations, respectively were used for analysis. An average stream discharge rate from Stillwater Creek was calculated from readings taken in the field using the above described methods. The highest recorded flow was used as a surrogate for maximum flow at the Stillwater site. Discharge rates from single occurrence where discharge was measured at all sites in September of 2012 were contrasted. Flow velocities taken at the substrate level at each site in September 2012 were compared using an ANOVA.

Temperatures recorded in-stream were graphed to depict seasonal variation within and among sites. Temperatures recorded during the July to September period were compared using

ANOVA to contrast temperatures at the warmest and perhaps most productive time of year. Mean, maximum, and minimum temperatures during the low flow period were also compared. Water quality measured in field and analyzed in the laboratory was contrasted to existing literature about Pearlshell tolerances to determine is any locations exceeded critical levels.

Local Ecological Knowledge

Information garnered from informal interviews was compiled and location notes were taken. These localities were added to known mussel localities as compiled by the WDNR in the database. Additional useful information was annotated on the map created showing existing mussel populations in 2013.

Chapter 4: Results

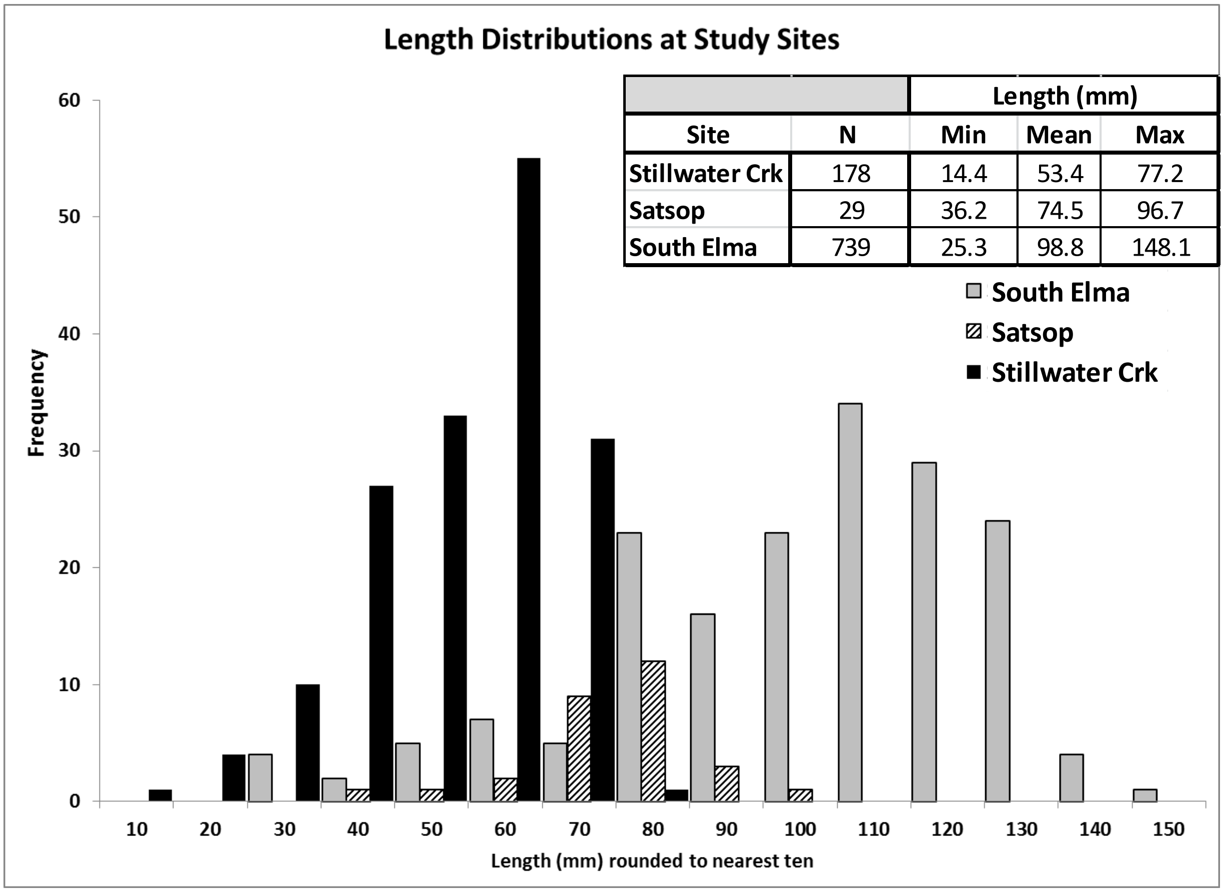
Western pearlshell abundance estimates

Nine hundred forty-six total western pearlshells were counted within the quadrant squares at all sites (Table 2). Western pearlshell abundance estimates were extrapolated from the average of all quadrant densities at each site and showed an order of magnitude difference between each population (Table 2). Satsop had the lowest density with an estimated 2.3 pearlshells/m, with Stillwater at 14.2 pearlshells/m and South Elma at 89.6 pearlshells/m. The highest recorded density at any site was 99 western pearlshells in one 0.25 m^2 plot at the South Elma site.

Table 2: Summary table displaying sampling effort and abundance of western pearlshell at each site. There was a large variation in western pearlshell density in the areas sampled with an extremely high mean density found at the South Elma site within the area sampled despite not detecting mussels in 15 of the 33 plots sampled.

Site	# Plots sampled (0.25 $m^{2)}$	Total Mussels Found	Site Area (m^2)	Mean Density mussels/ m^2	Site Abundance Estimate
Stillwater Crk	50	178	616.9	14.2	8784
Satsop	50	29	210	2.3	487
South Elma	33	739	715.6	89.6	64104

Shell Morphology



		Length (mm)		
Site	N	Min	Mean	Max
Stillwater Crk	178	14.4	53.4	77.2
Satsop	29	36.2	74.5	96.7
South Elma	739	25.3	98.8	148.1

Figure 6: Length distribution of western pearlshell valve length at three study sites in the lower Chehalis Basin showing shorter individuals at Stillwater, an intermediate size at Satsop, and both the largest range and overall longest mussels at South Elma.

Overall, western pearlshell showed a wide range of size from 8 to 142 mm. The difference between the shortest and longest western pearlshell was 60.5 mm at Satsop, 62.8 mm at Stillwater, and 127.8 mm at South Elma. The restricted range of mussel sizes at the Satsop and Stillwater sites may be related to the relatively smaller populations indicating that recruitment is infrequent and may have occurred when environmental conditions were suitable for larval recruitment. Stillwater had a similarly small size range of valve sizes, but the smallest measured mussel was observed at this site (Figure 6) and gravid individuals with viable glochidia were observed in early spring 2012. The large number of individuals at this site, the anecdotal reports that indicate long term presence of mussels in the area, and evidence suggest contemporary spawning events point toward restrictions of maximum size of mussels. South Elma had the largest range of sizes with more individuals above the mean number than below, typical of species with a high fecundity – low survivorship strategy.

Western pearlshell were found to significantly increase in length (ANOVA, $P = 0.38$), width (ANOVA, $P = 0.38$), and height (ANOVA, $P = 0.04$) from the first order headwater stream site (Stillwater) to the Chehalis River. This confirms our observations that within this study, western pearlshell tends to increase in size and volume as the distance from stream initiation point increases. The abundance of western pearlshell is not a good indicator of the maximum length of mussel (i.e., it is not true that more mussels mean bigger mussels) and likely there is an overlying biological control restricting the growth of western pearlshell at the Stillwater site.

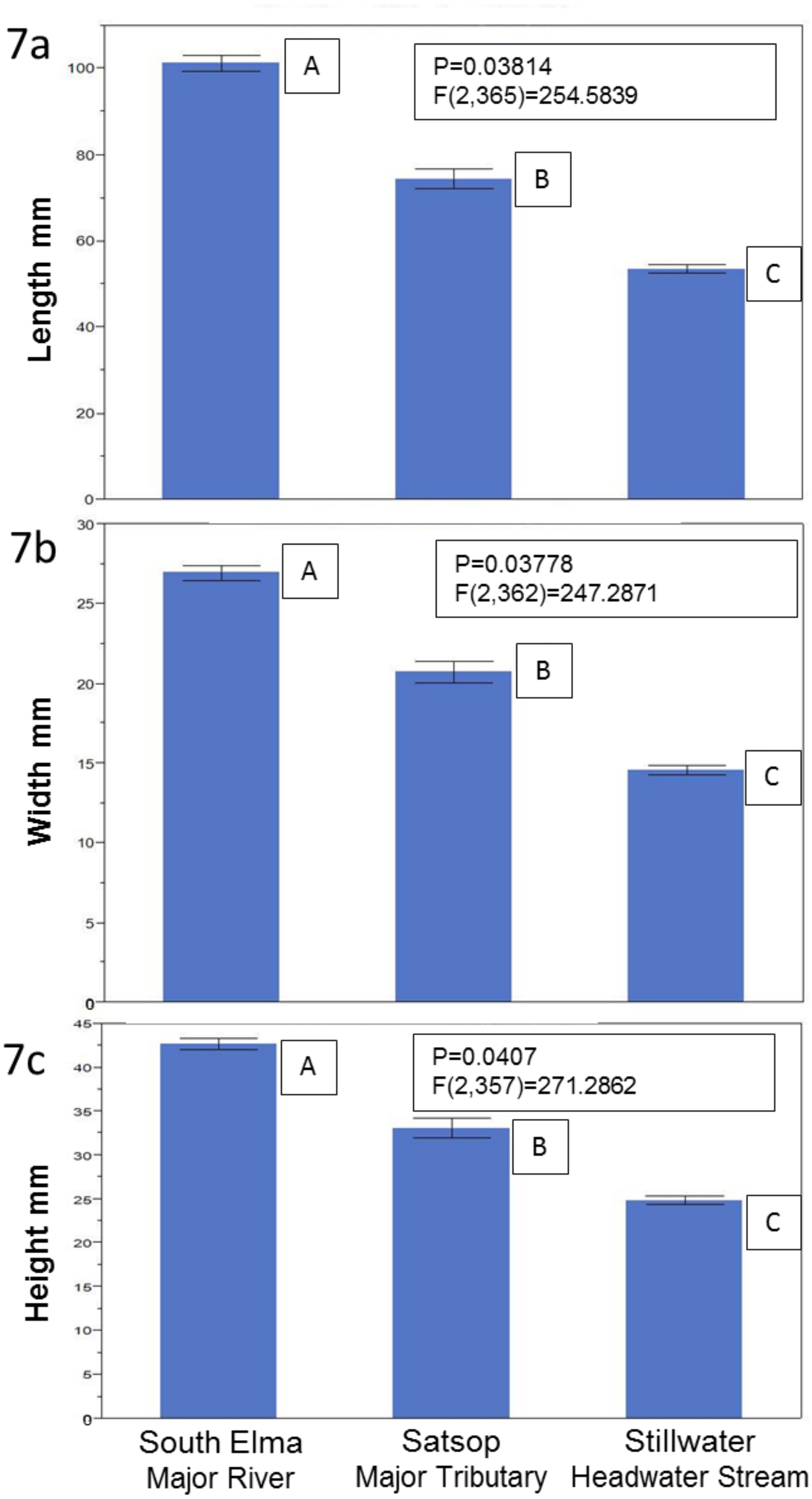


Figure 7: Mean ($^{+}/_{-}$ 1 SE) length, width, and height of western pearlshell in three sites of the lower Chehalis Watershed in 2012. Significant differences (ANOVA test) are indicated by unique letter characters next to each bar.

In contrast to increasing mussel sizes, the ratio of increasing length to width remained consistent at all three study sites (Fig 8). This supports previous reports stating *Margaritiferidae* do not conform to Ortmann's Law which predicts increasing mussel valve inflation, or width to height rations, in a downstream direction. These results further support findings that mussels are volumetrically larger as river size increases and are not changing the proportional shapes of their shells for differing environmental conditions.

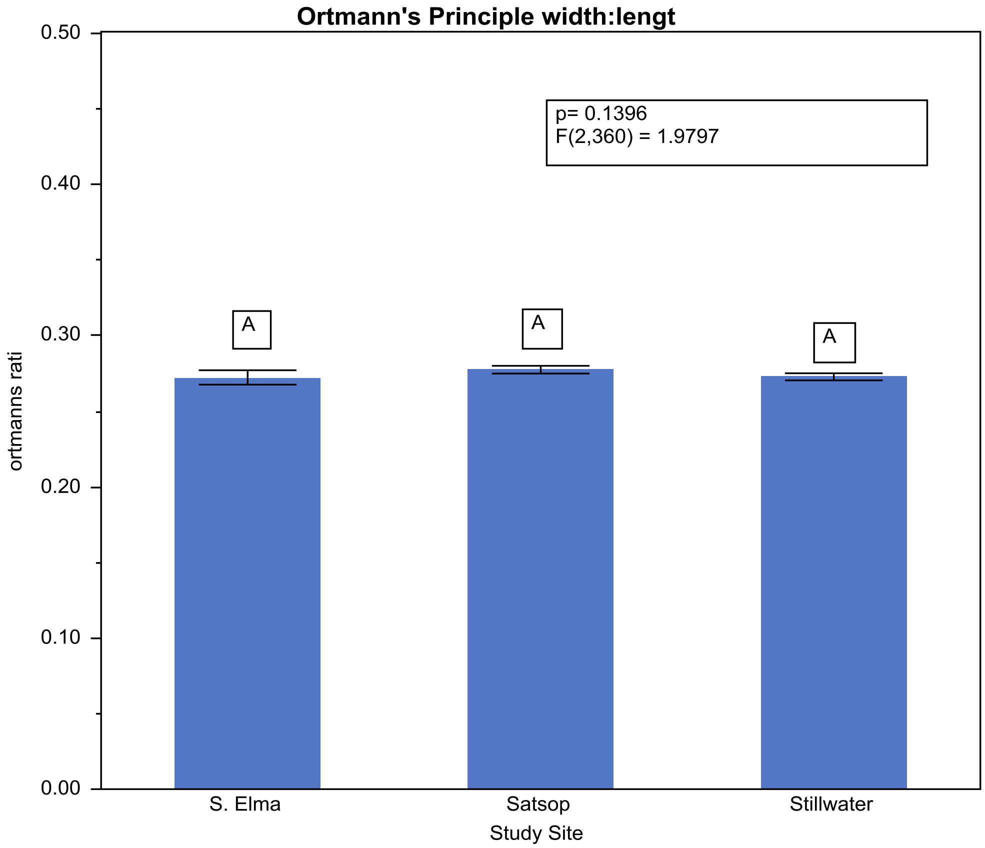


Figure 8: Mean ($^{+}/_{-}$ 1SE) width to length ratio (Ortmann's ratio) of western pearlshell in three sites of the Lower Chehalis River basin, 2012. Significant differences (ANOVA test) are indicated by unique letter characters above each bar.

An interesting differentiation of weight per unit valve length was apparent when the right valves of mussels were compared between South Elma and Stillwater Creek sites (Fig. 9). The weight: length ratio at the South Elma site ($y = -57.13 + 0.793*x$) was found to be significantly greater than that of Stillwater Creek ($y = -7.128 + 0.188*x$), indicating either a more dense shell or a thicker shell at South Elma. The lack of overlapping valve lengths at the sites leaves the possibility that a transition from proportionally lighter valves to heavier ones occurs at a length of 70 – 80 mm, but no mussels >77 mm were found at Stillwater Creek to extend the dataset and intact smaller valves were not found at South Elma.

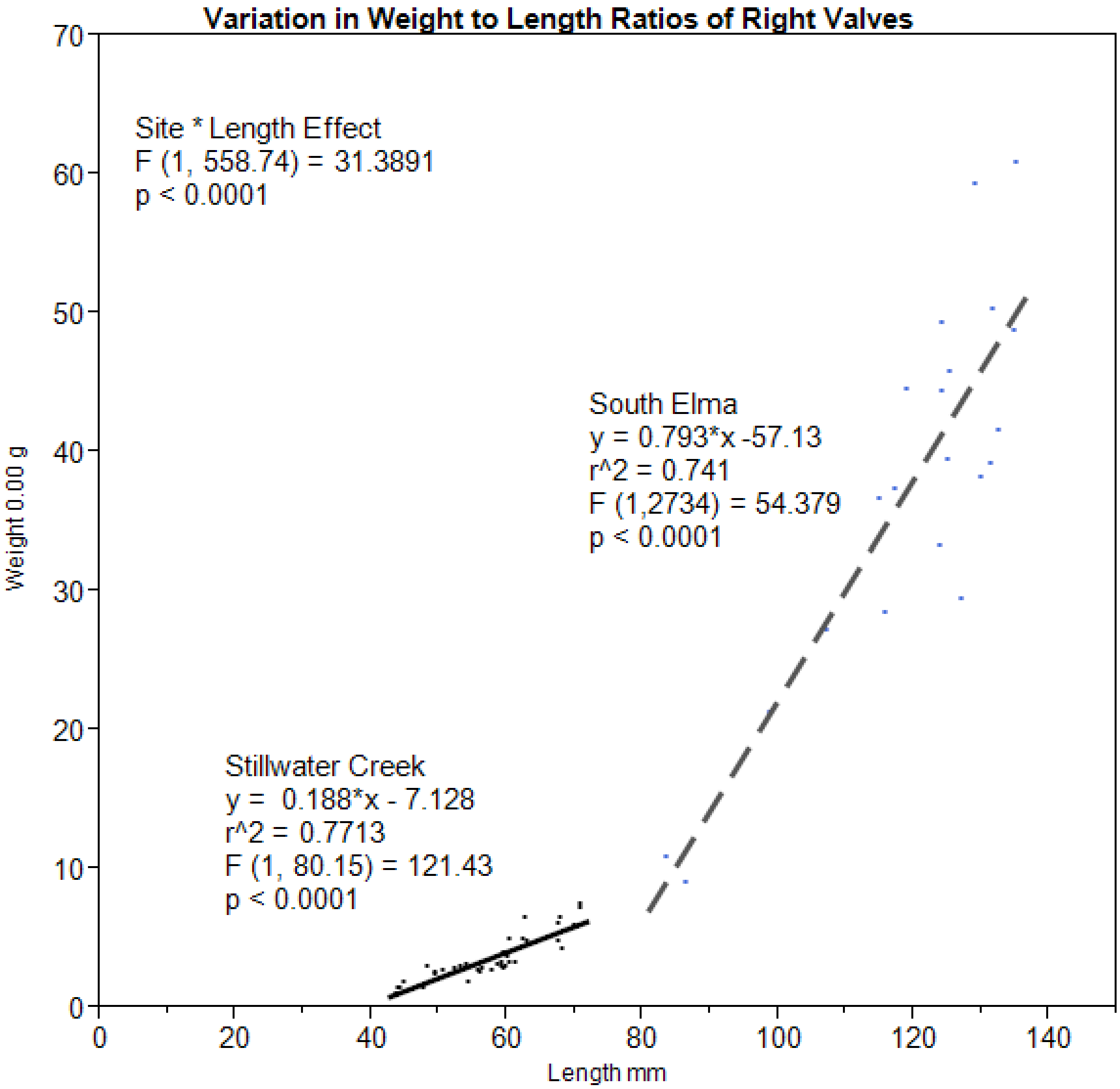


Figure 9: Variation in the dry weight to length ratios of right valves of western pearlshell in Stillwater Creek (headwater stream) and South Elma site (Chehalis River), 2012. ANCOVA analysis was used to compare the difference in slope (ratio) at each site. Linear regression results are also given for each site.

Age and population structure

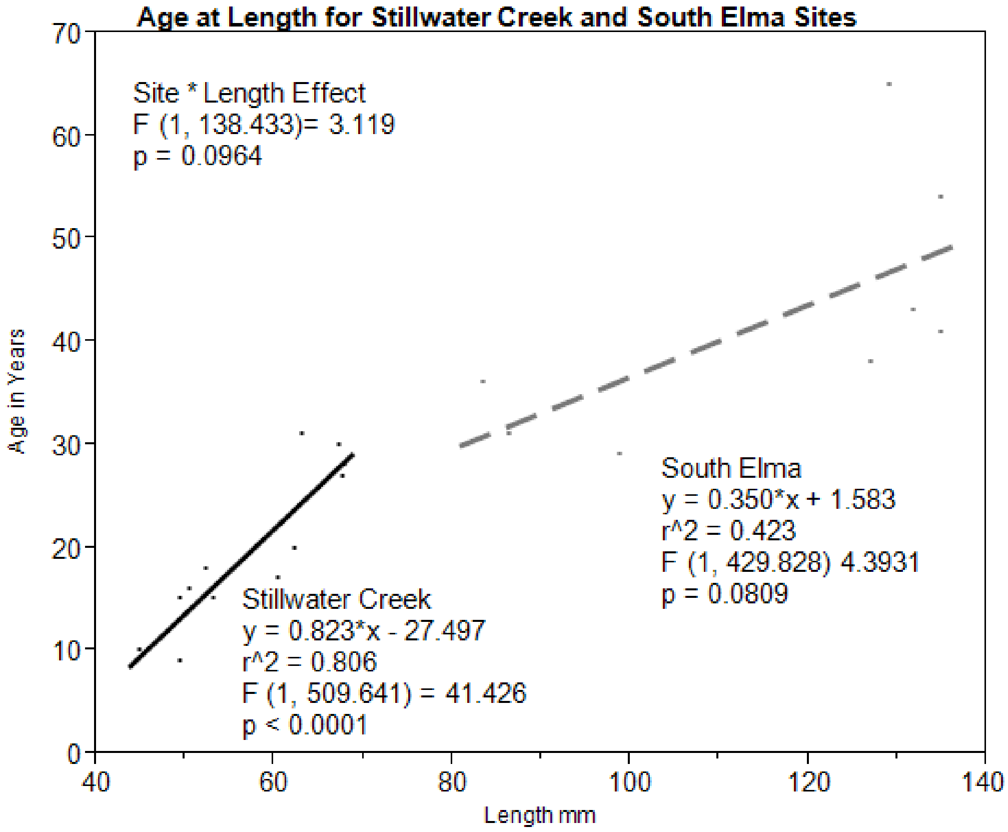


Figure 10: Two growth trajectories calculated from left mussel valves at the Stillwater Creek and South Elma study sites. Valves sizes <40 mm were absent from this analysis as only live mussels of this size were encountered in 2012. ANCOVA analysis was used to determine if site affect the slope of the growth rate equation.

Counts of internal annuli from collected shells at the Stillwater and South Elma sites were plotted separately and resulting slopes were contrasted in an ANCOVA analysis. The analysis revealed that mussel growth rates were similar at both sites (p = 0.0964) (Fig 10). Only one shell was found at the Satsop site. Because of the small population at Satsop, the possibility of declining populations, and a non-lethal sampling permit, no mussels were retained for aging and that site is excluded from these results.

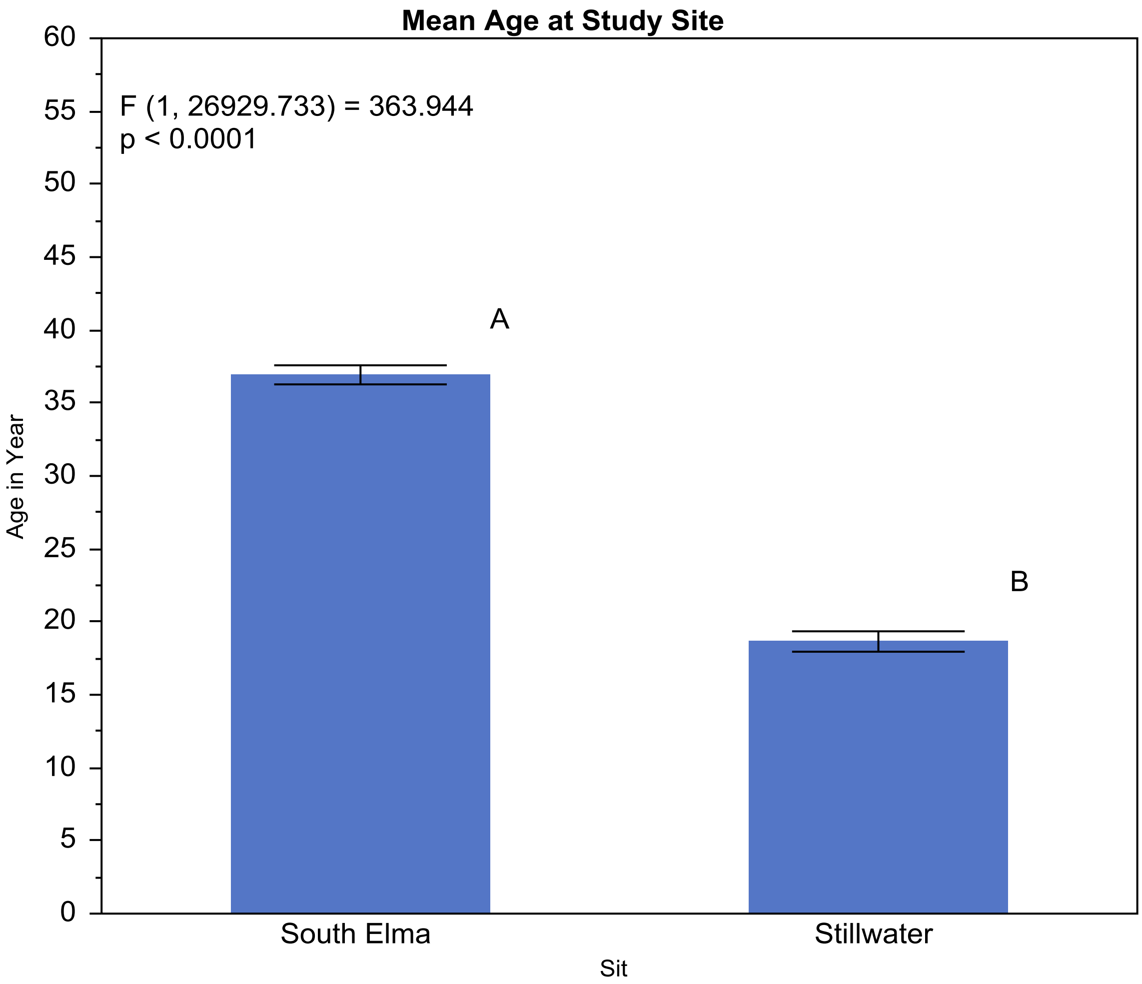


Figure 11: Mean age ($^{+}/_{-}$ 1 SE) of western pearlshell sampled in 2012 at the Stillwater Creek and South Elma sites were compared using an ANOVA. Mussel age of the sample population was derived from age at length estimates for these sites as shown in Figure 9.

Although the overall growth rate in mussels appears similar between the sites, the South Elma site was found to have a significantly ($p < 0.0001$) older population than that of Stillwater Creek (Fig. 11). A rough estimation of population age structure was generated from the slopes found in the aging process (Fig. 12). At the Stillwater Creek the site age was calculated using the equation for the growth line, $y = 0.82x – 27.5$, and at South Elma site the line equation was $y = 0.35x + 1.58$ where y is length and x is age. Because these lines cross the age axis before the length is zero, an age category of less than 10 years old was created for those individuals and is displayed.

Figure 12: Percent of western pearlshell from South Elma and Stillwater sites in seven age categories, 2012. Ages were determined from counting annuli in mussel shells at various sizes and lengths to create an age at length calculator for each site.

Habitat Preferences

Habitat conditions and preferences were investigated at each site to examine differences in habitat selection within the sites by mussels and describe the physical conditions at each site. Habitat units were identified at each study site, but were fairly homogenous at every site except the Stillwater site which had a large number of riffle (33) and pool (16) habitat units and four main braided channels. Habitat at the Satsop site consisted of a glide with a mean sampled depth of 88.5 cm (max 164 cm) that terminated in a deep pool, and the South Elma site consisted of parallel continuous riffle (mean depth 19.2 cm) and glide (mean depth 54 cm) habitats. Among the plots sampled, a Chi-squared test found mussels were not found to preferentially occupy one substrate type over another at Stillwater (p= 0.756), Satsop (p= 0.054), South Elma (p= 0.142), or overall (p= 0.0627). Although there was no statistically preferred selection of one habitat substrate, gravel substrates represented the most common substrate occupied by mussels at each site and overall suggest that gravel substrate is important to pearlshell mussels for occupancy.

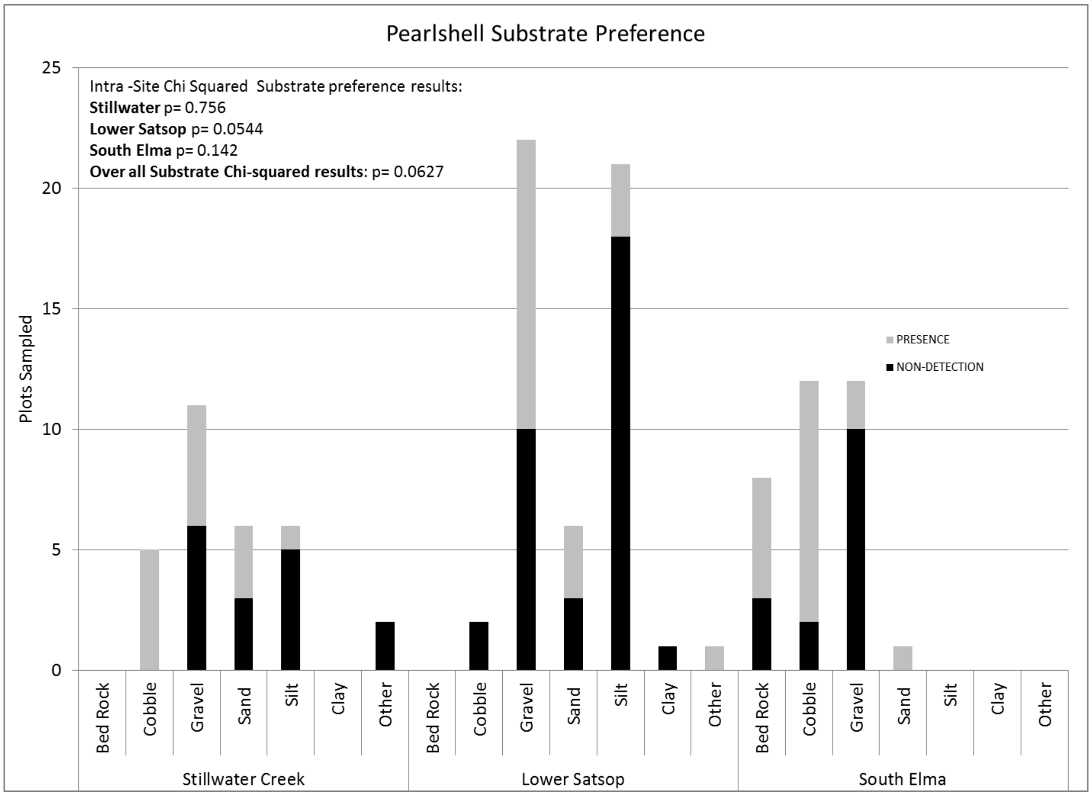


Figure 13: Habitat substrate preference does not vary within study sites selected for the presence of mussel. Gravel and cobble substrate always contained mussels and composed a substantial proportion of the substrate matrix.

Table 3: Water quality thresholds found by Stock and Toy for western pearlshell and by Bauer and Oliver for *M. Margaritifera* fall within most measured parameters at the study site. * *Margaritifera margaritifera*

Water Quality		Alkalinity mg/L	Hardness mg/L	Nitrate mg/L	pH	Total Phos mg/l	D.O. mg/L-1	Conductivity µs/cm
Stillwater	***Fall***	no result	39.00	nd	7.36	nd	10.10	75.6
	Spring	33.00	34.00	0.12	7.29	nd	10.56	73.3
Satsop	***Fall***	28.00	27.40	0.12	7.32	0.14	10.23	70.6
	Spring	22.20	28.40	0.49	7.29	0.13	10.86	50.8
South Elma	***Fall***	35.60	39.50	0.39	7.29	nd	9.92	74.3
	Spring	20.60	19.10	0.24	7.21	nd	11.07	76.2
Stock 1996	*1996*	NA	NA	NA	6.96 - 7.36	NA	9.21- 10.14	NA
Toy 1998	*1998*	NA	NA	NA	6.5 -7.4	NA		57 - 106
Bauer*	1988	Ca CO3 2 mg/l		1	NA	<0.03	NA	<70
Oliver*	2000	<10 mg/l Ca CO3		0.5	6.5-7.2	<0.03		<100

Water quality measurements taken during the fall of 2012 and spring of 2013 are within the acceptable limits found in other theses in Washington and slightly higher compared to conditions reported for its congener *Margaritifera margaritifera* in Great Britain (Table 3). The reported alkalinity levels which greatly exceeded most commonly reported levels in Great Britain (Bauer 1988, Oliver 2000, Sime 2005) may be explained results from previous studies by the loss of mussels from much of their former range with more variable alkalinity levels (Morreken 1992, Lucey 2006). There was little variation in water quality variables tested at the sites at the time of the sampling, suggesting that these variables are not likely to negatively affect mussel populations.

Water temperatures collected from in-stream temperature sensors show a trend of increasing temperature as watershed size increased with the warmest temperatures in July – August and coldest temperatures in January (Fig. 14). During the warmest period of the year, Stillwater Creek had the coolest mean and recorded temperature, South Elma the warmest mean and recorded temperature, and Satsop the greatest range of temperatures (Table 4). Annual water temperature recorded at the USGS station immediately upstream of the South Elma site in 1975 is used here for a reference of mean annual water temperature. The complete dataset at the South Elma site could not be recovered but likely followed a similar seasonal pattern as Satsop and Stillwater.

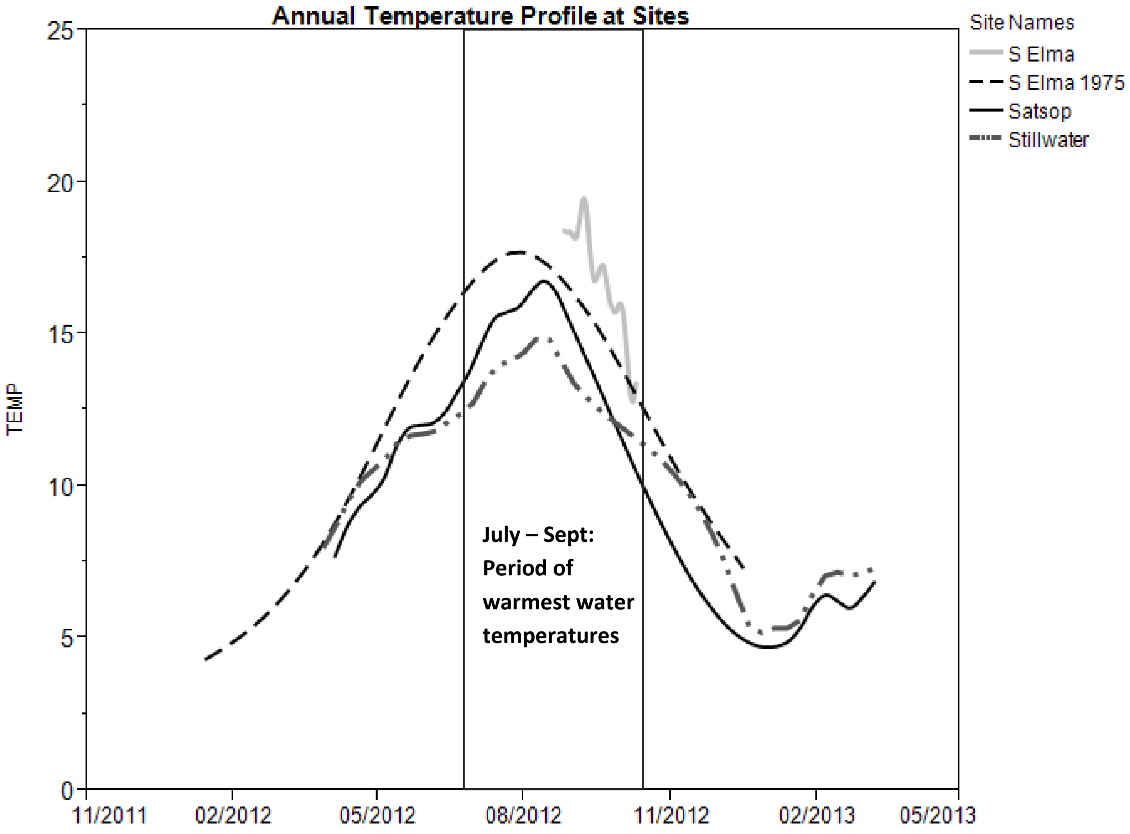


Figure 14: Temperature measurements taken in the Lower Chehalis watershed in 2012 and 2013 using Onset Tidbit monitors recording each hour. Historic temperature data records are from Washington Department of Ecology. Note that the larger the body of water the larger range of temperature fluctuation.

During the warm summer period when concurrent temperatures were being recorded, the South Elma site averaged 17.5 °C, 3.4 degrees warmer than the Stillwater site and was never cooler than Stillwater Creek. Summer water temperatures were found to be significantly warmer at South Elma than Stillwater Creek ($p < 0.0001$) (Fig. 15). However this difference may not be biologically significant to the mussels. The Stillwater site had the smallest range of temperature. This may be attributed to the close proximity of Stillwater Creek to a cold groundwater spring and its narrow channel and dense riparian forest creating a high degree of shading.

Table 4: Summary statistics for water temperatures taken at the substrate level during the warmest part of the year. Bold font indicates the highest value within each category.

	Temperature °C (July - Sept 2012)			
Site	**Average**	**Max**	**Min**	**Range**
S Elma	**17.5**	**20.8**	14.6	6.3
Satsop	15.9	19.6	11.9	**7.6**
Stillwater	14.1	16.7	**11.6**	5.0

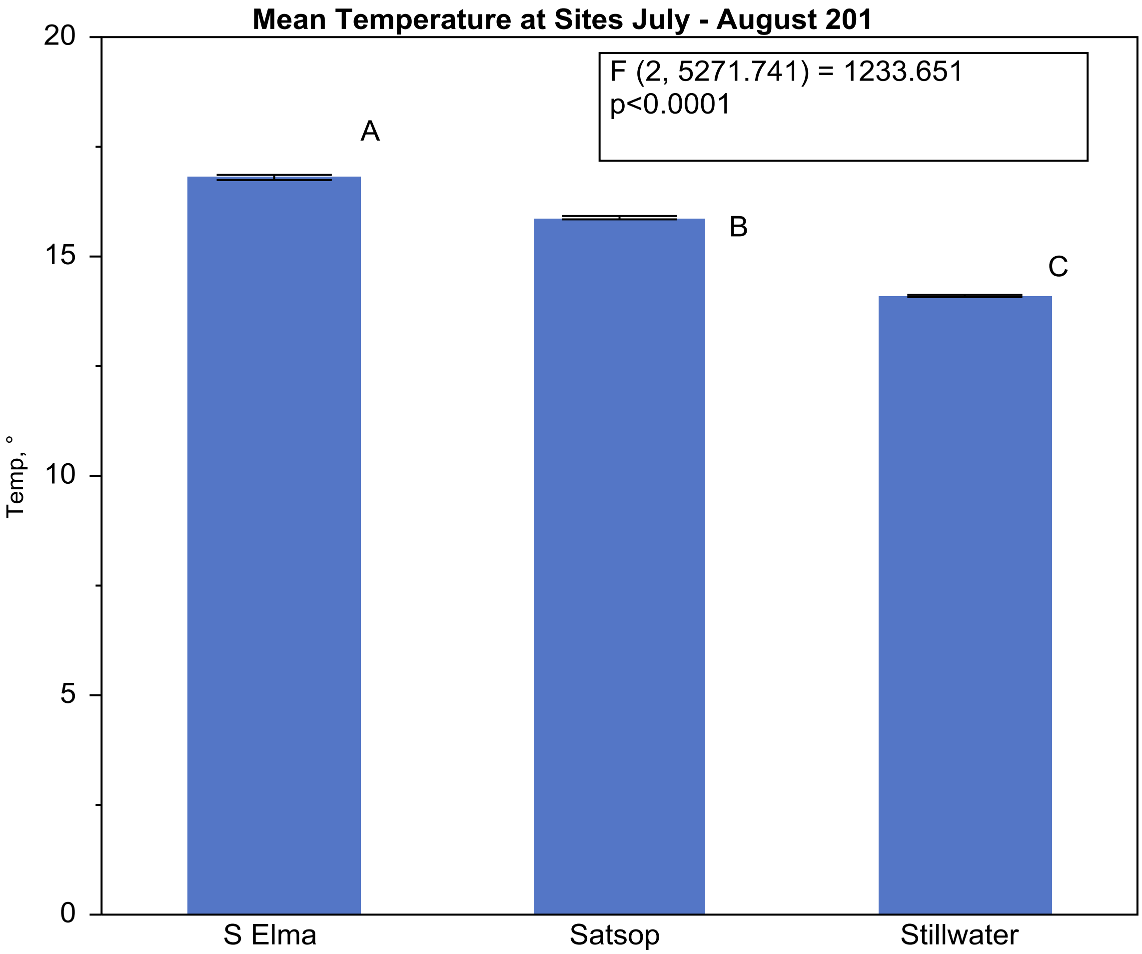


Figure 15: Mean daily water temperature (+/- range) during July-September 2012 at each study site. The two larger sites have larger ranges of temperature values and there is more stable temperature at the smaller spring-fed site.

Water Discharge and Velocity

Annual discharge was measured once at each site on September 6th, 2012. Although discharge is only roughly ten times greater at the Satsop and South Elma sites during low flow periods Stillwater Creek does not display the same magnitude of discharge from <300 cfs to 30,000 cfs (Fig. 16). The less variable discharge rates and lack of channel disturbance or flooding observed at Stillwater Creek were markedly different than the large increases in water discharge and velocity observed at the Chehalis and Satsop River sites in 2012 and 2013 which resulted in flood and near flood conditions.

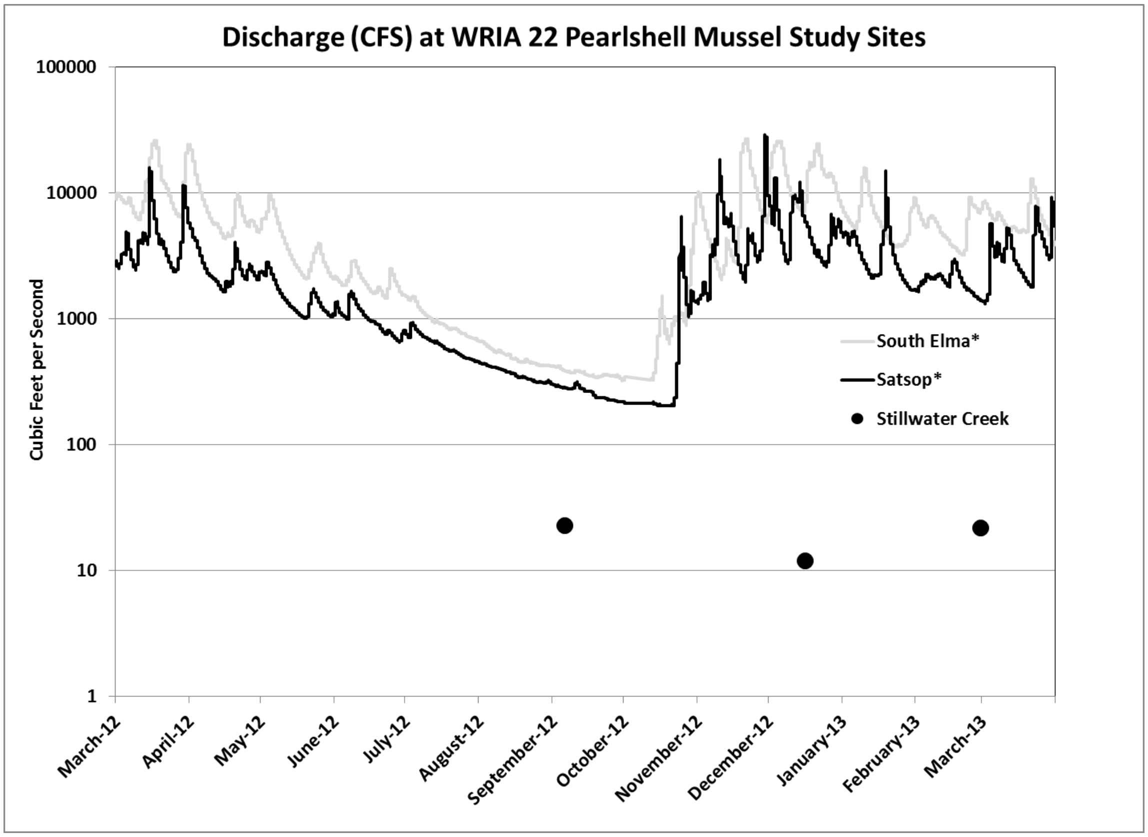


Figure 16: Discharge measurements at Stillwater Creek were made on Sept 6th and Dec 6th, 2012 with a final measurement on Feb 27th 2013. Scale of the graph is large to replicate USGS generated graphs for comparison across sites. Discharge measurements made at Satsop and South Elma sites on September 6th 2012 are noted for comparison.* indicates measurements by USGS river discharge stations in 2013-2013. Both stations are upstream of the sample sites

All substrate velocity measurements were taken on the same date, September 6th 2012, as concurrent discharge measurements in riffle or riffle/glide habitats that spanned the channel. Water velocity at the substrate was much faster at South Elma than the other two sites during the low flow period. Although not significant, the average substrate velocity follows a trend of increasing speed within mussel beds as stream size increases. It is interesting to note that although the annual discharge at Satsop and the Chehalis River at Porter are very similar, the water velocity at substrate varies greatly (Fig. 17).

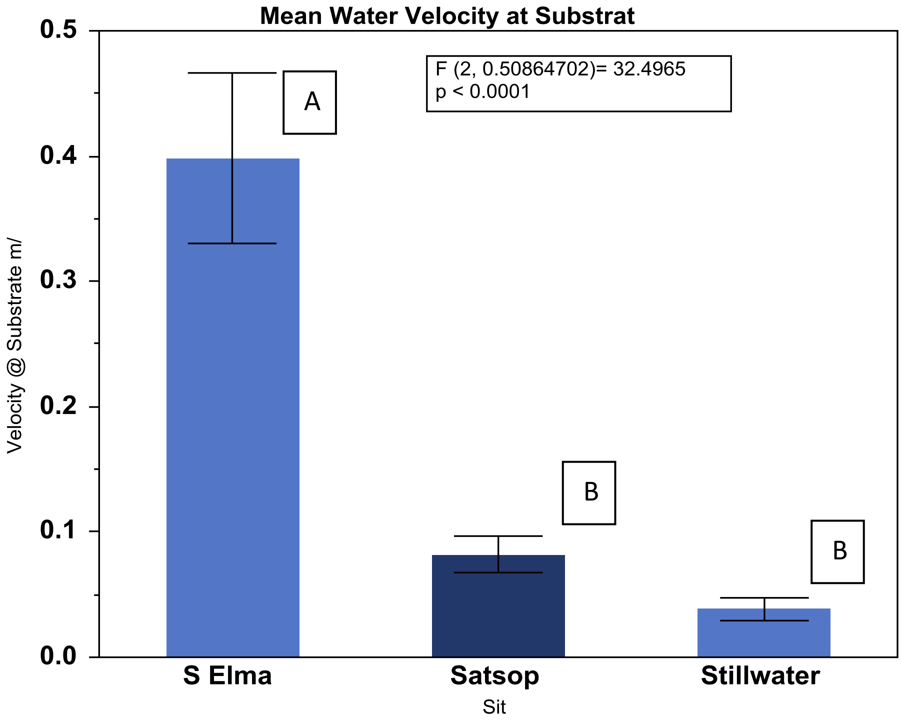


Figure 17: Mean velocities ($^{+}/_{-}$ 1SE) at the substrate level compared in an ANOVA for sites containing pearlshell mussels in the Lower Chehalis watershed in 2012.

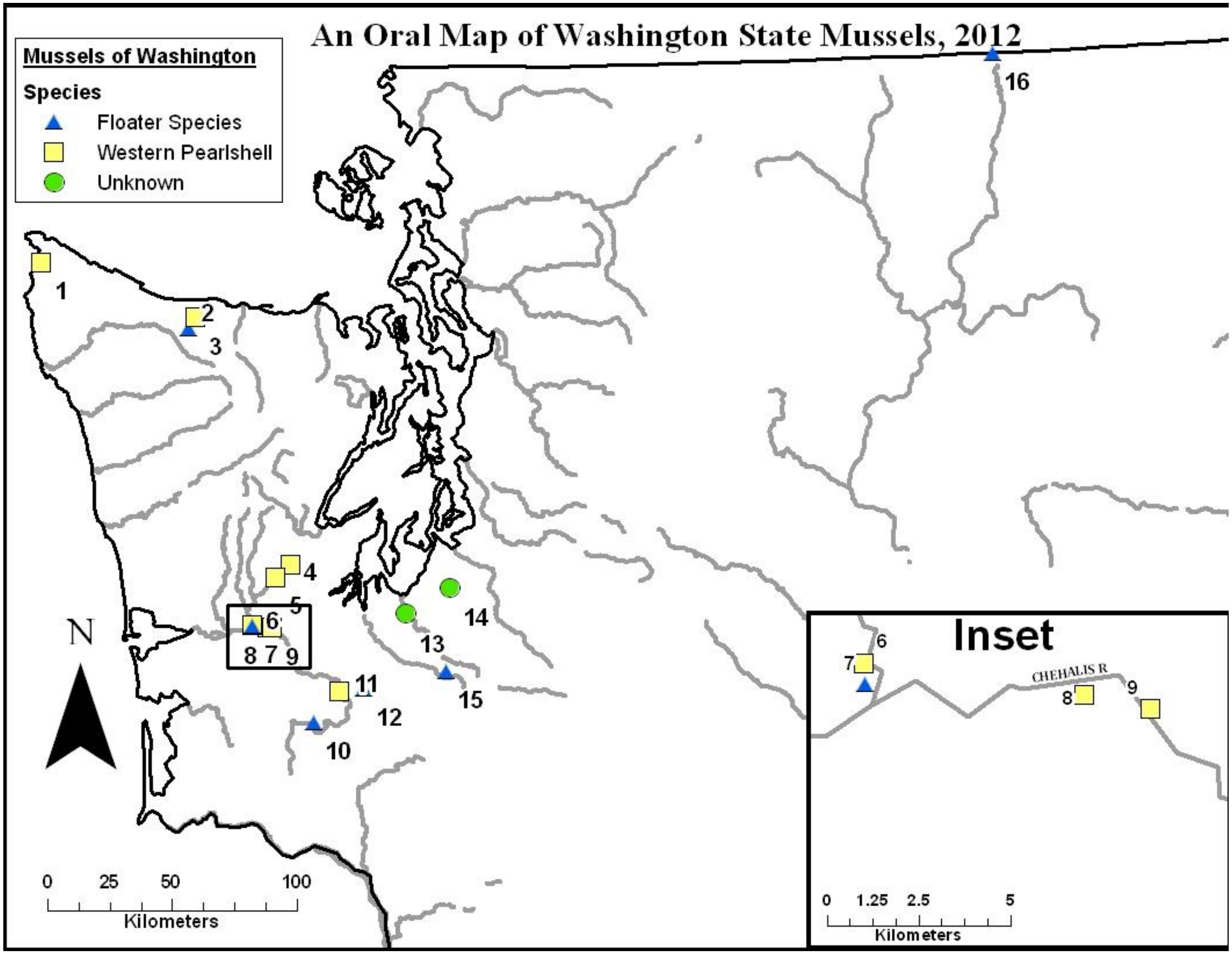


Figure 18: This map depicts locations were freshwater mussels where reported to exist during conversations with professional biologists and individuals encountered during surveys or over the course of everyday life in 2012 and 2013.

Informal Interviews and opportunistic sampling

As a result of informal conversations with both professional biologists and individuals encountered on mussel surveys, 15 confirmed mussel localities were identified that were not located in the WDFW Priority Habitat Species (PHS) database (Fig. 18). Six conversations identified the South Elma site, used in this study and recorded in the WDFW PHS database, as having an abundance of mussels. Four of those conversations were with sport fishermen and two were with highschool students participating in educational outreach events. Two locations, Lower Nisqually and Spanaway Creek, are unconfirmed, but probable, based on descriptions of the site and the mussels referenced during the conversation. Two sites were identified by myself while conducting routine fish surveys in the Upper Chehalis Watershed (WRIA 23) for USFWS. One instance of contempary human consumption of mussels was recorded. The individual stated that "he ate them raw all the time" and had very detailed and accurate information about locations and abundances of mussels in the Chehalis River betweeen Porter and Satsop, Washington. In this case the consumption of raw mussels is specuated to be a partial subsistance measure. Other instances involved fishermen that recounted their knowledge of the river and its faunal assemblages happily and Washington residents recalling observations of mussels earlier in their lives. These converstaions allude to interest and information about freshwater mussels within the general public, recreational, and scientific communities, and suggest that knowledge of mussel species and their abundance may be widespread, but remains undocumented. Appendix III detailslocations and information used to generate the map and will reference the site numbers in Figure 18.

Chapter 5: Discussion

There is evidence that freshwater mussels in Washington State and throughout the Pacific Region are decreasing in abundance and diversity. The work here has been an attempt to increase our knowledge of freshwater mussel populations in western Washington State and to better understand what conditions influence their morphology and growth rates. Earlier studies in Washington have revealed findings that furthered our understanding of the distribution and life history of pearlshell mussels (Stock 1996, Toy 1998, Stone et al. 2004, Krueger et al. 2007, Hastie and Toy 2008, Helmstetler and Cowles 2008), but this is the first to examine mussels in the Chehalis Watershed.

Demographics of Mussel populations

Mussels showed variation in densities where they were sampled. On average Stillwater Creek contained 14 mussels/m^2, Satsop 2 mussels/m^2, and South Elma over 90 mussels/m^2.The average densities found at South Elma are comparable to that of Battle Creek (80 mussels/m^2; Toy 1998) and much higher than those found in Nason Creek during the late 1990's (Stock 1996). The recorded abundance of mussels estimated at these sites is the result of targeting populations and should not be used to estimate populations outside the study areas.

At the Stillwater Creek and South Elma sites a subset of the entire area visually confirmed to have mussels was sampled, but at the Satsop site the population was found to be restricted to an area smaller than the initial survey area assumed. After the Satsop site was randomly surveyed, a complete visual census of the same sample area was undertaken. Two snorkelers completed individual visual surveys of the area and determined that the visual surface population of pearlshell mussels was 252 -254 mussels, roughly half of the estimated population of 487 individuals. Even with the gross mismatch, relative abundance ratios may remain similar and populations Satsop: Stillwater Creek: South Elma may remain roughly 1:15:120. Juveniles were not targeted when sampling and are likely un- or under- represented in all populations. Methods for sampling for juveniles include sifting through the sediments as outlined in Stayer and Smith (2003). These would be important future surveys because inclusion of juvenile mussels would provide evidence of reproduction and provide shells that could help expand the growth and aging estimates that were limited in the dataset for this study.

The South Elma site is the most populous and diverse site in this study and has the greatest range of sizes, ages, growth rates, and overall numbers in a continuous spatially restricted large bed. Small individuals (25 mm) indicate recent recruitment and the large range of ages and sizes are indicative of reoccurring reproductive events. Sampling at South Elma reveled 10 western ridged mussels (*Gonidea angulata*) of small size (38 -72 mm length), interspersed with the western pearlshells.

The Satsop population was the least numerous with 254 individuals and no small or visually identifiable juvenile mussels found. Satsop also had the most restricted area of mussel occupancy. This population may represent a population sink in WRIA 22 that became established during favorable conditions but has not expanded due to either lack of reproduction within the bed or from transport of glochidia on host fish beyond the bed (Toy 1998).

Stillwater Creek has a large estimated population of over 8000 individuals. Direct observation of glochidial release from mussels at this site, along with very small individuals (14.4 cm measured and smaller observed) likely indicate a successfully reproducing population.

Stillwater Creek has the overall smallest individual mussels of any site. Mussels at this site are arranged in patchy aggregations throughout the reach, indicating strong micro-habitat preferences within the study reach.

Concerns and discussions about how to best estimate mussel populations are common in the literature (Strayer and Smith 2003, Strayer 2008). Because of the very great range of densities encountered in sampling plots even one meter apart (0 to 99 mussels/0.25m$^{2)}$ it is difficult to use a small randomly selected sample area to extrapolate to a larger areas. Earlier accounts of mussel abundance and increasing the number of plots would help improve abundance estimates. A systematic sampling strategy would have improved the accuracy of the abundance estimates, but was not applied to this study due to the heterogeneity of the Stillwater Creek system. A better approach to long-term monitoring for population trends may be to resample fewer larger plots in exact locations over many years.

Morphological patterns in mussels along a river spectrum

Valve characteristics

The mussels found at the study sites are situated along a spectrum of increasing watershed area, stream length, stream order, and were observed to increase in size as watershed size increased. This observation concurs with the hypothesis set forth in the

River continuum Concept that: “*In developing a theory of biological strategies along a river continuum, it should also be possible to observe a number of patterns that describe various processing rates, growth rates, growth strategies, metabolic strategies, and community structures and functions”* (Vannote et al. 1980). Is the observed increase in mussel size a response to some function of increasing stream length and or width? Some ecological gradients have been tested for *Margaritifera margaritifera* patterns along changing latitudes and temperatures (Bauer 1992) and for broad patterns in mussel shell morphology (Ortmann 1920).

Pearlshell mussels in this study were found to follow a gradient of increasing size in all three dimensions (length, width, and height) as watershed area increased. The ratio of measurements of shell dimensions did not change from the headwater population to the downstream population. This concurs with other findings that the pearlshell mussel (*Margaritifera margaritifera*) does not follow Ortmann’s Law and provides evidence that this trend is absent from the five species in the family *Margaritiferidae* as was previously found for other species in the family Margaritiferidae (Ortmann 1920, Hornbach et al. 2010, Haag 2012). The western pearlshell may use other morphological or behavioral adaptations, such as shell thickness, to improve their ability to remain in place, resist erosional forces in the stream, or for other undescribed needs.

A significant difference in length to weight ratio was measured between the Stillwater and South Elma sites. Width and height of the shell were not considered in this analysis because they were already found to be proportionally the same among populations. This finding is attributed to denser or thicker average mussel shells at the South Elma site, but neither a standard thickness nor shell density were measured during this study. Heavier shells may indicate a response to one or several environmental conditions. The much larger volume and speed of water moving through the Chehalis River may require a more “armored” mussel shell to withstand the erosive scouring by suspended sand, gravel, and perhaps cobbles that occur to a much lesser degree in the more stable and smaller Stillwater Creek discharge and velocity rates. Additionally a denser per unit length (heavier) mussel may stay in place better during high flows that could scour out a less heavy mussel of equal size. Alternatively this increase in weight at size could be the result of mussels at South Elma living in a more productive site than the narrow, shaded, and cooler Stillwater Creek allowing South Elma mussels to dedicate energy to growing shells.

Age and growth patterns

The comparisons of mussel age and growth rates were limited to the Stillwater Creek and South Elma populations because of sampling that allowed only for collections empty and intact shells. Mussels were found to be growing at similar rates, and on average, mussels are older at South Elma than Stillwater Creek. The mussels range in ages from <10 to 40 y.o. at Stillwater and <10 to 60 y.o. at South Elma. This indicates that the initial hypothesis that the observed mussels are smaller because they are slower growing was erroneous and agrees with the supposition that the small size is an indication of a younger population.

Toy (1998) found that western pearlshell mussels become sexually mature at a given size and not at a given age. Based on those findings we should see evidence of reproduction at the same minimum size at the South Elma and Stillwater Creek sites.

Gravid females were seen at the Stillwater Creek site confirming that the minimum reproductive size has been obtained. To further validate Toy's finding minimum reproductive size could be investigated at each site and should be roughly the same.

Three hypothetical reasons for the absence of larger, older mussels at Stillwater Creek would be highly interesting to investigate. First, and with little evidence to support it, is that a fish barrier existed until roughly 40 years ago excluding host fish from Stillwater Creek. Second, evidence of predation on mussels was observed at Stillwater and South Elma, but because of the high flows mussels are likely not accessible to predators year round at South Elma as they are at Stillwater Creek. Mammalian predators likely target the largest mussels because the caloric rewards are greater with larger individuals. There could be selective removal of large mussels at the Stillwater site year round eliminating them from population. And third, early logging practices were not historically protective of water quality and high loads of sediment could have smothered and killed previous mussel populations at Stillwater creek. Higher flows at the Satsop and South Elma sites may have removed fine sediments and allowed for the retention of the older mussel populations there (Vannote and Mishall 1982, Howard and Cuffey 2006). As logging practices improved or the regeneration of the forest improved in-stream conditions, the mussels may have populated Stillwater Creek.

Comparison of environmental conditions

In this study the South Elma mussels were studied within parallel riffle and glide habitats and the Satsop population was found within a single glide. The Stillwater site was complex and contained a large number of habitat units, but there was little annual variation (with the exception of canopy cover not reported here) in physical variables in the stream. Vannote and Minshall (1982) stated that pearlshells may benefit from habitats that do not aggrade, scour, or become turbulent annually, but maintain a constant laminar flow bringing seston particulates to the mussels. Alternatively, Howard and Cuffey (2006) hypothesized that segments of rivers that underwent periods of scour provided a benefit to larger mussels by flushing the fine sediments that inhibit respiration, feeding, survivorship and otherwise maintaining suitable mussel substrate conditions. The Chehalis has the largest and oldest mussels and greatest stream discharge agreeing with Howard and Cuffy's (2006) hypothesis that high discharge events may remove fine sediments and clean out the mussel bed favoring older, larger individuals as was found. It also appeared to have the non-turbulent flow described by Vannote and Mishall (1982) that favors long term and dense occupation by *Margaritifera* in larger rivers. Stillwater Creek has little annual environmental variation. Relatively constant discharge throughout the year may provide a stable long-term habitat if the bed does not aggrade and beavers activity does not alter the reach (Hastie and Toy 2008). The Satsop population was protected by a large fallen tree that shielded the mussels from the destabilizing and erosive effects of faster water velocity, increased turbulence, and greater sediment movement in areas of the river adjacent to the bed.

Substrate preference

Substrate preference by mussels was not different between sites nor was there a preference for one substrate at any site. While no substrate preference among measured variables was found within or among the sites sampled in this study it is important to reiterate that this study targeted segments of the watershed that were already known to contain western pearlshell and these findings should not be interpreted as evidence of nonselective distributions of mussels within streams. Rather, it was hypothesized that if mussels have a morphological response to differing habitat conditions it might be reflected in the substrates in which mussels were found. It is interesting to note the limited presence and use of boulders found at all three sites. Many publications found that refugia from fast turbulent water behind boulders are important to mussel habitat (Vannote and Minshall 1982, Stone et al 2006, Howard and Cuffy 2006). The slow velocities and low discharge rates and Stillwater Creek explain both the absence of boulders in the system as they cannot be moved or exposed, and the presence of mussels without them as they are able to maintain their place in the substrate even at high water. The Satsop site was situated behind a large fallen tree that may act in the same manner as a boulder. South Elma has the highest discharge and fastest water yet little in the way of cover substrate to protect the mussels. The large deep pool upstream of the site may settle out larger substrates before they reach the mussel bed, but that has not been tested.

Temperature

Warmer water temperatures are known to be a factor controlling both mussel size and growth rates (Bauer 1992). Of special interest in this study is that the Chehalis River is not fed by glacial melt and the resulting water temperatures and flow reflect seasonal temperature and precipitation influences and not the later peak discharge events and prolonged cool water periods found in many of Washington’s large glacial rivers.

Unfortunately, a complete temperature profile was not collected year round at the South Elma site due to the loss of a temperature sensor during high flow events in 2012 and redeployment of an additional logger was not conducted until later in the summer. However, data were collected at all three sites July through October 2012 capturing the period of the most dramatic growth in pearlshell mussels in our region (Toy 1998).Temperature was more variable at the South Elma and Satsop sites with South Elma having both the highest and likely lowest temperatures based on historic patterns. Stillwater Creek did not approach the high temperatures found at South Elma which had temperatures reaching as high as 30°C in the 1970's and likely into the present day (United States Geological Survey 2013). Temperature is likely to play a role in influencing the size differences found in pearlshell mussels here as it was in *Margaritifera margaritifera* populations in Europe (Ziuganov et al. 2000; Bauer 1992). Lower annual average temperatures at Stillwater may restrict their annual growth rate, but overall their maximum size and life span could be longer (Bauer 1992). However findings in this study found similar growth rates between populations making it unlikely that the variation in temperatures between sites was influencing growth rates.

Stream discharge

Like temperature, discharge was found to be much more variable at South Elma and Satsop with flows ranging from roughly 200- 300 cfs at low flow periods to over 30,000 during the rainy November – April periods of high precipitation. By contrast the Stillwater site had only a 10 cfs difference throughout the year. South Elma was found to have a much faster water speed at substrate than other sites. The less variable physical conditions found at Stillwater Creek likely make a more stable environment for mussels to exist and grow but warmer water could increase primary productivity and growth for pearlshell mussels (Bauer 1992) and the swifter flows may bring more food sources past the mussels and keep fine sediments from precipitating out of the water column and burying the mussels (Howard and Cuffey 2006).

Water Quality

Water quality parameters measured in this study were within or close to reported levels for most variables, the exception being alkalinity. The data found in the British reports is regarding a separate species of *Margaritifera* so caution must be used when relating them to western pearlshell in the Pacific Northwest. The reports are also from a limited number of rivers with short time periods and may not represent the true range of conditions in which *M. margaritifera* are found (Sime 2005).

All three sites border managed lands and there is a possibility that water could be susceptible to spikes in nitrate levels from fertilization events and animal waste run-off during large precipitation events, but this hypothesis was not tested because of the resolution of sampling and the inability to pinpoint sources of nitrogen.

It is interesting that some *M. margaritifera* populations in Ireland have been found in much harder rivers than summarized in most reports and the range of alkalinity tolerances may be much broader than is commonly reported for *Margaritifera* species (Moorkens et al 1992, Lucey 2006). The mussels in this study are in much harder water than reported for most European populations (20.6 – 35.6 mg/L $CaCO_{3,}$ Table 2), but appear to be establishing large and reproducing populations that have existed for greater than half a century.

The pH of all three streams was within the range found in other theses and studies in the state, but never fell below a neutral level as was found in their investigations

(Stock 1996, Toy 1998). Conductivity fell within the range that Toy (1998) found, but was slightly lower on average.

Finally, when interpreting water quality parameters and tolerances it is critical to address the duration of exposure to different conditions. Water quality ranges that are beneficial or deleterious to mussels at one stage of their life (glochidia, larval, and adult)may change as the mussel matures, and typical conditions of their natal water bodies can influence individual populations (Sime 2005, Preston et al. 2010).

Limitations of this Study

This study was limited to an assessment of mussels found through visual and tactile searches and likely over looked small juveniles and newly settled larvae. The smallest individual encountered was 14.4 mm at Stillwater Creek. Their absence from this study should not be taken as evidence of a lack of recruitment and likely underestimates the true population age range. The methods used to age mussels in this study were primitive at best and the sample size of aged mussels was small. Collection of additional shells from study sites that encompasses a greater range of sizes should be sent to a lab where proper equipment and experienced personnel can better evaluate the age and growth of mussels in the lower Chehalis watershed.

It is likely that mussels in these sites responded to variables not investigated in this study. The levels of primary production, respiration, and nutrient flow at each site were not measured, but are known to influence the growth of freshwater mussels. Quantification of additional variables is highly encouraged in the future. Other sites in the lower Chehalis watershed should also be evaluated to increase the scope of the study and confidence in its findings.

Conclusions

Western pearlshell mussels in this study show an increasing overall shell size in the three metrics measured (length, width, and height) with increasing watershed size. The proportions of the mussel's size do not change concurring with reports that other *Margaritiferidae* do not follow patterns observed in Ortmann's Law (1920).

There is a pattern of increasing shell weight to length ratio between the headwaters and main-stem habitats in the lower Chehalis Watershed, perhaps to protect mussels in large rivers from damaging substrate movements in high discharge events. The growth rate of mussels does not appear to be different between sites despite physical differences between headwater and river habitats. One explanation for similar growth rates could be the equalization of growth rates because of the seasonal variation between sites in discharge, productivity, or temperature. For instance high summer productivity at the South Elma sites may increase summer growth rates, but turbid water and high discharge rates may restrict the mussels' availability to feed and grow in during the rainy winter and spring seasons. The Stillwater site which is cooler and likely less productive in the summer months may have the advantage of having low discharge and warmer waters allowing for feeding and growth in the same period that is restricted at South Elma.

The large populations at both the South Elma and Stillwater Creek sites appear to be stable and have evidence of recent reproduction. Both the South Elma and Stillwater

Creek sites are located in areas of commercial use. The South Elma site is surrounded by plant and animal agricultural practices and the Stillwater Creek site is within an actively managed forest. There has been no long term monitoring of these two sites which were reported to have “lots of mussels” and be a “stronghold of mussels” respectively by individuals visiting the sites before me. It is possible that because of a combination of low levels of land conversion to urban and suburban areas, as well as the Clean Water Act of 1972 and improving forest practices in Washington State both sites are not in current peril. The Satsop population is much smaller and no signs of recruitment were observed. Satsop may represent a sink population that could remain for some time or perhaps disappear completely in a disturbance event such as the removal of the large fallen dead tree that was observed to shelter the population. Long term monitoring of these and other mussel population in the Chehalis River is needed to confirm the stability and longevity of these populations.

Recent findings have shown that *Margaritifera* within the same region are not as successful when translocated to other populated streams that have different characteristics than within their natal streams (Valovirta 1998, Preston et al. 2010). Future investigations of *Margaritifera* in the lower Chehalis River should involve experiments translocating individuals from populations to measure rates of mortality and growth in different positions within the watershed.

Chapter 6: The future of Freshwater Mussels in Washington

Freshwater mussels, as often stated in the first lines of nearly every publication I have read during this thesis, are an imperiled group of organisms, and critically so. But are these statements making the impact and creating the reaction that they desperately need if their populations are to stabilize or increase? Documentation of mussel populations in North America have provided protection to 35 species, and at least 10 were given protection under the US Endangered Species Act in 2012 alone (IUCN 2012), but knowledge of mussel distributions and abundances is still lacking for many species and in many watersheds. The challenge is to document populations and their abundances before they decline or disappear from our streams. As there is no known effort to systematically document freshwater mussels in Washington State the conservation of mussels may depend on the ability of an informed public to identify and report mussels. To that end efforts have been made by several organizations to educate and interest the public about native freshwater mussels.

Pearlshell Mussels of Washington: Past, Present, and Future

As I finish this thesis I was greatly aided by Wendall Haag's 2012 publication *Freshwater Mussels of North America*. The author works in Oxford, Mississippi, not too far from my childhood home in Mississippi. In my youth I spent time collecting and observing numerous reptiles, amphibians, fish, and arthropods, ignorant that I was overlooking an even more mysterious group of organisms at the bottom of those muddy waterways, and in the case of the flat pigtoe mussel (*Pleurobema marshalli*) in the Tombigbee River, their demise (Haag 2012). It was troubling to me that if I could grow up, wandering the streams and lakes, in an area rich in freshwater mussels and never hear of them, many other curious individuals may also not even know of their existence.

The two theses on pearlshell mussels in this state were written shortly before my arrival. Amy Stock's 1996 thesis at the Evergreen State College was written as I was turning my thoughts to my future and Kelly Toy's thesis completion at the University of Washington in 1998 coincided with my high school graduation and arrival at the University of Washington just a few short months later. Just several weeks prior to the completion of this thesis I assisted in fish surveys at Bear Creek for monitoring the long-term health of urban streams in King County. I saw, as Toy predicted in 1998 and later verified (Hastie and Toy 2008), piles of empty mussel shells scattered along the banks of Bear Creek. In 2008, Toy called for "effective remedial action with the next 5–10 and 50 years". That five years is up and to the best of my knowledge those populations have continued to decline, despite the activism of impassioned individuals.

In 2012 and 2013 I led a joint US Fish and Wildlife and Educational Service District 113 (www.ESD113.org) project dubbed the "Freshwater Mussel Academy." This program was inspired in part by Celeste Mazzacano's citizen science program to monitor mussels in urban creeks in Portland, Oregon (Dunkle 2012) and my own survey efforts. The academy was composed of a group of middle and high school students from Grays Harbor, Lewis, and Thurston counties and designed to both engage and excite students about uncommon and exciting organisms in their "backyard," as well as cover topics in science and conservation biology. During the first half of this event, students were introduced to the life history and disappearance and decline of mussels. For most of the students, freshwater mussels were an entirely new organism with strange adaptations and transformative cycles and with a clear association to Washington State's iconic salmon. In the second part of the event students went to the South Elma site for a hands-on look at freshwater mussels and to learn about what it means to be a field technician. During the field event students collected data that was included in parts of this thesis.

The Freshwater Mussel Academy served not only as a way to educate youth about the diversity, wonder, decline of freshwater organisms, and their roles in the ecosystem, but also served as evidence of local ecological knowledge within this generation of students and perhaps will cause other students to note mussels in the future. Two of the localities that were identified as undocumented localities of pearlshell mussels were from conversations with this initial group of students. Several of the students who attended high school in Elma were already aware of the presence of mussels at the South Elma site. Since the initial Freshwater Mussel Academy, five other events have been similarly conducted.

I hope that this effort allows some members in the next generation of scientists and conservationists to appreciate both organisms that I was unaware of at that age and to have a more holistic view of Pacific Northwest freshwater ecosystems.

Documentation of Freshwater Mussels in Washington State

Even prior to the 1993 publication by Williams and others documentations of the impending loss of mussel fauna we have been aware of this decline. What life we see in the rivers and lakes around us is not as rich as it once was. The Columbia River has been depleted of its once abundant salmon, sturgeon, brown bears, wolves, grand ponderosa pine and cotton wood-forests, but perhaps only a few note the loss of its once abundant western pearlshell (Helmstetler and Cowles 2008). The overlooking of a species that once fed the people and filtered the waters of our region's largest and arguably most important waterway highlights the great need for a central permanent entity to document and monitor the status of these mussels in the Washington and the Pacific Northwest.

The Pacific Northwest Native Freshwater Mussel Work Group, Xerces Society, and individuals, often self-funded, have dedicated themselves to the conservation of mussels and the education of public about the plight of freshwater mussels. The Xerces Society published a species profile for three Pacific Northwest native mussel species: western pearlshell, western ridged, and California floater mussels which included compiling extensive records of mussel localities throughout the Pacific Northwest Region (Jespen et al. 2010a, 2010b, 2010c) including the records housed in the Washington Department of Fish and Wildlife's Priority Habitat Species database. This excellent database, while exhaustive and detailed, was designed to be static. Much about the locations and abundance of mussels remains undocumented. Field notes are stapled to ziploc bags containing shells and neighbors of rivers and lakes recount stories of mussels they once saw, but much of this knowledge goes undocumented as mussels in the Pacific Northwest continue to disappear. It is acknowledged that a central, maintained, and easily accessible location to document the presence of native freshwater mussels is needed for the preservation conservation, and restoration of native freshwater mussels in Washington and the Pacific Northwest.

Conclusion

Documentation and conservation of freshwater mussels in Washington State should not be left solely to governmental and non-governmental organizations alone but incorporate the knowledge of the public about localities and relative abundances of native freshwater mussel species. The relatively few conversations with individuals not typically considered biologists or conservationists included in this study led to a surprising number of verifications of previously documented mussel populations and of populations that I had not found specifically reported. Providing a central location to easily submit information about mussel localities is acknowledged as a need by many interested in the conservation of native freshwater mussels. In cases where local knowledge of mussel populations may not exist, providing volunteer and curricular opportunities to learn and work with freshwater mussels has been positively received by middle and high school students in the Chehalis and South Puget Sound region. The educational outreach and volunteer events produced informed citizens able to report on the localities of new and future mussel populations and perhaps even sparked the interest of a future malacologist or two.

Appendix I

Noted flora and fauna: This appendix is not a comprehensive list of species found at the study sites, but rather a record extracted from field notes taken to document the presence of organisms encountered while sampling.

*evidence of reproduction

Stillwater Creek:

Fauna		Flora	
Common	***Latin***	**Common**	***Latin***
Mollusca		**Trees**	
Western Pearlshell Mussel*	*Margaritifera falcata*	Red Alder	*Alnus rubra*
Aquatic snails	*Juga spp.*	Western Hemlock	*Tsuga heterophylla*
Asian Clam	*Corbicula sp.*	Douglas Fir	*Psuedotsuga menziesii*
Arthropoda		Western Red Cedar	*Thuja plicata*
Stone Flies	Plecoptera	Willow	*Salix spp*
Mayflies	*Emphemeroptera*	Black Cottonwood	*Poplus balsamifera*
Signal Crayfish*	*Pacifasticus lenticulus*	**Shrubs**	
Petromyzontiformes		Devils Club	*Oplo-panax horridus*
Brook Lamprey*	*Lampetra richardsonii*	Stink Currant	*Ribes bracteosum*
Actinopterygii		Goose Berry	*Ribes lacustre*
Coho Salmon*	*Oncorhynchus kisutch*	Pacific Nine Bark	*Physocarpus capitatus*
Steelhead	*Oncorhynchus mykiss*	Salmon Berry	*Rubus spectabilis*
Coastal Cutthroat Trout*	*Salmo clarkii*	**Herbaceous vegetation**	
Three-spined Stickleback	*Gasterosteus aculeatus*	Horse Tails	*Equistem*
Riffle Sculpin	*Cottus gulosus*	Coontail	*Ceratophyllum demersum*
Aves		Pacific Bleeding Heart	*Dicentra formosa*
Song Sparrow	*Melospiza melodia*	Water-crowfoot	*Ranunculus aquatilis*
Common Merganser	*Mergus merganser*		
Stellars Jay	*Cyanocitta stelleri*		
Amphibia			
Northern Red-legged Frog	*Rana aurora*		
Pacific Chorus Frog	*Psuedacris regilla*		
Reptilia			
Common Garter Snake	*Thamnophis sirtalis*		
Mammalia			
Beaver	*Castor canadensis*		
River Otter	*Lontra canadensis*		
Douglas Squirrel	*Tamiasciurus douglasii*		
Humans	*Homo sapiens*		

Lower Satsop:

Fauna		Flora	
Common	***Latin***	**Common**	***Latin***
Bivalvia		**Trees**	
Western Pearlshell Mussel*	*Margaritifera falcata*	Willow	*Salix spp*
Asian Clam	*Corbicula*	Black Cottonwood	*Poplus balsamifera*
Arthropoda		**Herbaceous vegetation**	
Stone Flies	*Plecoptera*	Japanese knotweed	*Fallopia japonica*
Mayflies	*Emphemeroptera*		
Actinopterygii			
Coho salmon	*Oncorhynchus kisutch*		
Steelhead	*Oncorhynchus mykiss*		
Coastal Cutthroat Trout	*Salmo clarkii*		
Redside Shiner	*Richardsonius balteatus*		
Largescale Sucker	*Catostomus macrocheilus*		
Speckled Dace	*Rhinichthys osculus*		
Aves			
Cedar Waxwing	*Bombycilla cedrorum*		
Common Merganser	*Mergus merganser*		
Chestnut-backed Chickadee	*Poecile rufescens*		
Turkey Vulture	*Cathartes aura*		
Raven	*Corvus corax*		
Crow	*Corvus brachyrhynchos*		
Green Heron	*Butorides virescens*		
Varied Thrush	*Ixoreus naevius*		
Osprey	*Pandion haliaetus*		
Great Blue Heron	*Ardea herodias*		
Ruby Crowned Kinglet	*Regulus calendula*		
Mammalia			
Humans	*Homo sapiens*		

South Elma:

Fauna		Flora	
Common	***Latin***	**Common**	***Latin***
Bivalvia		**Trees**	
Asian Clam	*Corbicula spp*	Red Alder	*Alnus rubra*
Western Ridged Mussel	*Gonidea angulata*	Willow	*Salix spp*
Western Pearlshell Mussel*	*Margaritifera falcata*	Black Cottonwood	*Poplus balsamifera*
Actinopterygii			
Redside Shiner	*Richardsonius balteatus*		
Largescale Sucker	*Catostomus macrocheilus*		
Aves			
Barn owl	*Tyto alba*		
Amphibia			
Northern Red-legged Frog	*Rana aurora*		
Mammalia			
Humans	*Homo sapiens*		

Appendix II: Field Sampling protocols and datasheets

Western Pearlshell Population Monitoring Protocol

This study will measure the abundance, density, size, and environmental conditions targeting populations of the western pearlshell (*Margaritifera falcata*) for the purpose of understanding demographics within and between 3 populations in the Chehalis/Satsop watershed. Three study sites have been identified for sampling: 1) a headwater stream population in Stillwater Creek, 2) the Satsop River, a major tributary, and 3) the main=stem of the Chehalis River.

Data collection order:

1. **Data sheet header information**
2. **Segment delineation and habitat assessment**
3. **Quadrant data (depth, temp, vegetation, wood, substrate, stream type)**
4. **Population counts/mussel measurement**
5. **Shell collection**
6. **Water quality (D.O., Temp, velocity, conductivity)**
7. **Site level**

Header information: Fill out header information on all data sheets prior to starting surveys

Site: three sites will be visited, **Stillwater Creek, Lower Satsop River**, and **South Elma**.
Samplers: Record all sampler initials. Please record your initials first.
Date: Record sampling date: dd-mm-yyyy
Begin and end survey times: use the 24 hr. clock to record your begin and end times (00:00)
Temp: record air temp at 1m above stream and water temp at the stream bed at the beginning of the survey
Weather: coarsely describe weather conditions

Study segment and Habitat assessment:

Segment delineation

100 meters of stream length will be assessed at each stream site location. This 100 m segment will span wetted stream width at some sites (Stillwater Creek and the Chehalis River) and be restricted to the specific habitats where they exist in others (Satsop). All segments will encompass areas in which mussels are known to occur and areas that can be sampled without the need of scuba gear at low flow periods. An average stream azimuth will be taken at the site and used to define the segment end point and divide the segment into regular sampling quadrants.

Stream habitat assessment

Stream habitat assessment will follow the TFW Monitoring program *Method Manual for the Habitat Unit Survey* (Pleus et. al, 1999), and the survey will segment the stream into pool and riffle habitats. Habitats will be designated with RF for riffle and PL for pool and be numbered sequentially from the downstream start point to upstream. Riffles are characterized as shallower faster units with larger substrates in low-gradient areas. This definition of a riffle includes habitat types such as glides, runs, cascades, and rapids. Pools are areas of impounded water within a defined depression in the stream bed. Pools can be formed by features such as changes in water velocity, boulders, LWD, beaver dams and other blockages. Habitat lengths and widths will be measured as per TFW guidelines (see below tables and reference TFW AM9-99-003) and recorded on the habitat data sheet.

Unit Length (m)	**Minimum number of paces per width measurement**
<2.5 to 5	1
≥5 to 10	2
≥10 to 20	3
≥20+	4+

Table 1: [TFW AM9-99-003 - table 5 (p19)] take the given number of paces between each wetted stream width in each identified habitat unit.

Mean segment bankfull width (m)	**Minimum unit size (m^2)**	**Minimum residual pool depth (m)**
0 to <2.5	0.5	0.1
≥2.5 to <50	1	0.2
≥ 5 to <10.0	2	0.25
≥ 10 to < 15.0	3	0.3
≥ 15.0 to <20.0	4	0.35
≥20	5	0.4

Table 2: [TFW AM9-99-003 - table 2 (p10)] Guidelines on determining the minimum parameters that must be met to be classified as a pool habitat. Residual pool depth = max pool depth – depth of water at pool crest

Gradient: will be taken between habitat start and end points while standing on the bed of the river or creek if possible and averaged over the length of the study site. Measurements will be taken to the nearest tenth of a degree (0.0°).

Dominant and subdominant substrates: indicate the inorganic dominant and subdominant substrates as averaged across the habitat unit. See tables in quadrant habitat measurements below for substrate classes and definitions.

% Canopy cover: Using ocular estimates, record all vegetative cover above the water surface to the nearest 10%. Cross verify and calibrate with sampling partner.

% Aquatic Vegetative Cover: Using ocular estimates, record all living vegetative cover below the water surface to the nearest 10%. Cross verify and calibrate with sampling partner. This can include filamentous algae, but not diatoms.

Mussels: Record the presence of mussels observed within the habitat unit. This is a quick presence/absence survey as seen at any time while sampling habitat unit. Record Y (yes) or N (not noted). It is not necessary to return to this sheet if mussels are discovered while sampling quadrants.

Quadrant Placement

For every given habitat unit (RF or PL) in the study segment roughly 20 percent of the area will be sampled for freshwater mussels. The number of quadrants will be determined by multiplying the average width of the habitat unit times the length and dividing by five (# quadrants = $\{L \times W_{average})/5\}$. Quadrant locations will be determined using a random number table for the longitudinal and latitudinal axis position of the stream (see end of protocol for random number tables). Select a number by blindly placing a finger, pencil, stick, or other object on the number table. Repeat the random coordinate selection process for the number of calculated quadrants for each habitat unit. Check to ensure that all coordinates are unique and fit within the habitat area parameter; if not repeat random number selection until useable numbers are generated.

Quadrant Name: Record quadrant as “Site – habitat – x-y” (example: Stillwater-PL3-3-2).

Quadrant habitat measurements datasheet

Depth: Record water depth at the center of quadrant square to the nearest centimeter.

Woody Debris: Measure diameter and length of each piece of woody debris greater than 15 cm in diameter (>15 cm diameter) in the plot. The diameter and length of larger pieces can be estimated if reasonable. Record the function that each piece of wood is providing.

Wood Function	
Bank Stability	Pool
Depositional area	Step
Scour	Turbulence
Loose	Other

Habitat: Record the code that describes the specific stream habitat unit in which the quadrant was located.

Habitat	Code	Habitat	Code
Glide	GL	Riffle	RF
Pool	PL	Other	OTHER

Glide: Deep, swift, and smooth surfaced reach of stream always low gradient.
Pool: A deep, slow to still moving habitat unit often associated with a pool wedge or tailing, and scour area. This includes pools downstream of root balls and debris jams.
Riffle: A rapid moving, shallow, and turbulent reach of stream typically not steep (i.e. <6°).
Other: Any habitat unit that does not fit the above descriptions. Take detailed notes and included sketches as needed.

Substrate: Record the code of the inorganic substrate that mussel was found in/on. If needed indicate dominant and then subdominant substrates (i.e. GR/MUD). If there is no subdominant substrate indicate its absence with **NA**.

Substrate	Code	Substrate	Code
Mud/Silt	MUD	Boulder	BO
Sand	SA	Bedrock	BED
Gravel	GR	Compacted Clay/ Marine Sediment	CLY
Cobble	CO	Other	Other

Mud/Silt: Dense fine particles of sediment <0.06 mm in diameter often associated with areas of little to no flow.
Sand: Fine granular particle of sediment 0.06 – 2 mm in diameter often associated with stream banks and settle areas in pools
Gravel: Small rocks and pebbles 2 – 64 mm in diameter
Cobble: Larger rocks 64 – 256 mm in diameter
Boulder: Any rock larger than 256 mm in diameter.
Bedrock: continuous patches of underlying rock geology
Compacted Clay/ Marine Sediment: Marine sediment or compacted clay that has not turned to sedimentary rock but provides a substrate visually similar to bed rock. You can walk on this substrate without sinking into it, but may leave impressions or footprints.
Other: Substrates other than as defined above, please specify in notes

Mussel measurements

Count: Record each mussel in a quadrant sequentially on the datasheet and measure each individual encountered in the quadrant square. If any portion of the shell is within the inside border of the quadrant, it is included in the total count. Count only live organisms (not empty shells).

Mussel Name		
Common	Scientific	Code
W Pearlshell	*Margaritifera falcata*	MAFA
W Ridged	*Gonidea angulata*	GOAN
Floaters	*Anodonta sp*	ANSP

Mussel measurements: Record species code and measurements of each individual counted to the nearest tenth of a millimeter (0.0mm) for the length, width, and height (Fig 1) in the population estimate.

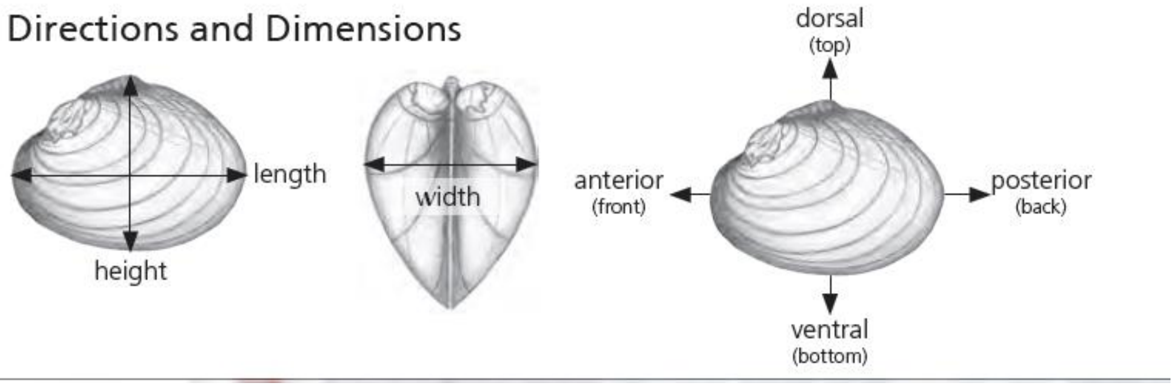


Figure 1: Mussel measurement guidelines: take measurements at widest point in range to tenth of a millimeter.
Image created by Ethan Nedeau, 2009: Freshwater Mussels of the Pacific Northwest. 2nd edition p.16

Aging and growth:

At each population site (Stillwater, Satsop, Chehalis) collect up to 20 complete and intact shells for roughly every 10 cm increment of shell length. (0 – 10, 10 – 20 … 50 – 60 …) Label mussel shells with species and site name.

Water quality

Dissolved Oxygen, conductivity, pH, and velocity:

These measurements will be taken just upstream, just downstream, and near the midpoint of the defined study site at every site visit. Dissolved oxygen, conductivity, and temperature measurements will be taken by calibrated YSI 60 or 85 multimeter. Velocity will be measured with a Flo-Mate 2000 flow meter at the substrate level where mussels would be present.

Site level variables

Mapping and stream typing: Record stream channel type (braided, channelized, sinuous, etc.), underlying geology, stream order, and gradient at each site. Note any roads, the general land-use, stream armoring, and alterations to the riparian area at each site. Please include rough estimates of area affected.

Additional Data: Take notes and create species lists of all flora and fauna in associated lentic, lotic, and riparian habitats with as much detail as possible. Of special interest is a record of fish species seen at sites.

Quadrant microhabitat data

Site: **Date:** **Weather:**

Samplers: **page:** **of**

Quadrant ID	Habitat	Depth (cm)	Dom Substrate	Subdom Substrate	Wood dia	Wood Funct	Notes

1. Mussel datasheet

Site:			**Date:**			**Weather:**		
Samplers:			**Start plot**		**End plot**		**page: of**	
Quadrant ID	**Count**	**Species**	**Length**	**Width**	**Height**	**Gravid**	**Juv (Y/N)**	**Notes**

Appendix III: Detailed information from informal communications and opportunistic sampling

Key	Locality	Species	WRIA	County	Source	Comments	Oral	Field Verified	Live or Shell
1	Sooes River	MAFA	19	Clallam	Biologist	located during salmonid survey	Y	N	
2	Crescent Lake	AN sp	19	Clallam	Biologist/ educator	conversation and interpretive sign revealed these populations	Y	N	
3	Lyre River	MAFA	19	Clallam	Biologist	interpretive signs and conversation with park biologist	Y	N	
4	Stillwater Creek	MAFA	22	Mason	Biologist	Called "stronghold of the Mussels" by biologist	Y	Y	L
5	Lower Stillwater	MAFA	22	Grays Harbor	Biologist	Beautiful site	N	Y	L
6	Satsop WDFW	MAFA	22	Grays Harbor	Biologist	Found on survey for mussels.	N	Y	L
7	Satsop Slough	AN sp	22	Grays Harbor	Biologist	Anodonta Clade 2	N	Y	L
8	South Elma on Chehalis River	MAFA	22	Grays Harbor	Sportsman	four fishermen, and two students identified this site. Very well known.	Y	Y	L
9	Taylors Ferry, Chehalis River	MAFA	22	Grays Harbor	Subsistance	Homeless man "eats them Raw" at this site.	Y	N	
10	Meskill Road Slough Chehalis	AN sp	23	Lewis	Biologist	Complete shells, likely mammal predation. Side channel Slough off Chehalis River	N	Y	S
11	Discovery Site	MAFA	23	Lewis	Educator	brought to attention of educator by land owner on field trip	Y	Y	S
12	South Hanaford Creek	AN sp	23	Lewis	Biologist	Shell Anodonta Clade II	N	Y	S
14	Spanaway Creek	UNKWN	10	Pierce	Sportsman	Seen Racoons eating them	Y	N	
15	Clear Lake	AN sp	11	Pierce	Educator	Anodonta in small pond south of Clear Lake	Y	Y	L
15	Lower Nisqually	UNKWN	11	Thurston	Sportsman	On Lower Nisqually	Y	N	
16	Osoyoos Lake	AN sp	49	Okanogan	Retiree	"mud mussels" 1960s-2013	Y	N	S

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