Community and Reciprocity in Conservation Aquaculture of Pacific Lamprey and Western Freshwater Mussels: Species of Importance to Native American Tribes of the Columbia River Basin

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AUTHORIZATION TO SUBMIT DISSERTATION

This dissertation of Alexa N. Maine, submitted for the degree of Doctor of Philosophy with a Major in Environmental Science and titled "Community and Reciprocity in Conservation Aquaculture of Pacific Lamprey and Western Freshwater Mussels: Species of Importance to Native American Tribes of the Columbia River Basin," has been reviewed in final form. Permission, as indicated by the signatures and dates below, is now granted to submit final copies to the College of Graduate Studies for approval.

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ABSTRACT

Columbia River Plateau tribes, such as the Confederated Tribes of the Umatilla Indian Reservation (CTUIR), have developed sustainable relationships with communities of food resources throughout the Pacific Northwest (PNW) since time immemorial (i.e., as far back as time is recalled or recognized). These relationships are evident in tribal creation stories, where plants and animals offered themselves to sustain humans, and in turn humans were given the responsibility of reciprocity—to take care of the foods that take care of them. Today, indigenous organizations like the CTUIR fulfill the promise of reciprocity by developing research programs that are focused on restoring ecological and cultural services. For many indigenous groups, traditional ecological knowledge (TEK) drives these programs and restoration objectives. Policies and management frameworks that are based on TEK promote the restoration of both physical and biological river processes that include important interspecies linkages and community interactions (Chapter 2).

Interactions between First Foods, traditional foods of cultural importance (e.g., water, fish, big game, roots, and berries), and their environments are critical to tribal natural resource management. The populations of two First Foods, Pacific Lamprey *Entosphenus tridentatus* and native western freshwater mussels, are declining in the Columbia River Basin in the western United States. Specific reasons for these declines are not well understood but are linked to changes to the biotic and abiotic features of the system. Physical habitat alterations, declining water quality and quantity, and changes to the biotic community structure of the river are possible factors that are affecting population declines of lamprey, mussels, and other native aquatic species (e.g., salmon, trout, and nongame native fishes). The loss of cultural connection to Pacific Lamprey and native freshwater mussels and the loss of the ecological services that are provided by these organisms are understudied elements of most Pacific Northwest aquatic-restoration projects.

The positive contributions of lamprey and mussels to the river environment have been explored through limited studies. For example, larval lamprey can affect the benthic microbial community structure, promoting more aerobic than anaerobic microbial species. They also contribute to nutrient cycling, processing organic matter and waste products from other river organisms. Freshwater mussels provide numerous ecosystem services to the river

community, including water filtration, nutrient cycling and storage, and food-web connections. For example, macroinvertebrate communities associated with mussel beds are larger and more diverse than those not associated with mussel beds; these macroinvertebrate communities in turn provide food and ecosystem services for other river organisms. Mussels are reliant on fishes to provide nutrients and transportation to their larvae, which transform into juveniles while on the fishes. Reciprocity drives community interactions and flows from the benthos to higher and lower trophic levels.

The community interactions that are observed in the river environment can be further investigated through laboratory study to improve culture techniques for both lamprey and mussels. Lamprey and mussels require artificial propagation and laboratory culture to supply organisms for research and restoration. Providing community interactions during laboratory rearing can benefit cultured organisms. In this dissertation, I use macro- (teleost fishes) and micro- (bacteria) organisms to study community interactions in the context of artificial propagation and rearing of freshwater mussels (Chapter 3) and Pacific Lamprey (Chapters 4 and 5).

A healthy, thriving community in any freshwater system requires that all parts of the system are functional and connected, a sentiment popularized for a western audience by Aldo Leopold's Land Ethic essay in the 1940s. Aquatic restoration projects that are implemented to improve habitat for salmonids can also serve to improve habitat for benthic organisms like larval Pacific Lamprey and native freshwater mussels with little extra effort or expense. To restore populations to harvestable abundances, inclusion of lamprey and mussels in ongoing habitat restoration projects should be a priority for Columbia Plateau tribes, and other agencies. Using Leopold's Land Ethic principles, I discuss ecological benefits of including lamprey and mussels in ongoing salmonid restoration projects, explore a case study of this in action for a tribal project, and present a call to action for regional restoration practitioners to acknowledge and include the benthic community in river restorations—a "benthic ethic" (Chapter 6).

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DEDICATION

To my Dad, Mary, and Gramma, for their love and support.

To Jonathan, for his patience and humor.

To my mom, with love.

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CHAPTER 1: INTRODUCTION

Background

This dissertation considers the role of community and reciprocity in conservation aquaculture of two important Columbia River Basin benthic organisms, Pacific Lamprey and native freshwater mussels. These notions are discussed throughout this dissertation in the context of holistic management principles that are represented in the practices of the Confederated Tribes of the Umatilla Indian Reservation (CTUIR). However, the terms community and reciprocity, as used in tribal restoration frameworks, represent an extension of the traditional belief systems of the CTUIR into the scientific realm, and are discussed throughout this dissertation with that understanding.

Larval Pacific Lamprey and western freshwater mussels were historically two of the most abundant benthic species in freshwater ecosystems of the Pacific Northwest, United States (U.S.). However, populations of both organisms have declined over the last 60-80 years (Kan 1975; Close et al. 1995; Close et al. 2002; CTUIR 2015; Blevins et al. 2017a). Tribal members belonging to the Confederated Tribes of the Umatilla Indian Reservation retain harvest rights to both organisms in usual and accustomed areas under Walla Walla Treaty of 1855 with the U.S. (Treaty 1855), but opportunities to exercise those rights are limited due to declines of lamprey and mussel populations (Close et al. 2002; Brim Box et al. 2006; CTUIR 2015; Hunn et al. 2015). Pacific Lamprey *Entosphenus tridentatus* are a traditional subsistence food for Pacific Northwest tribes like the CTUIR, but in the Columbia River Basin they are currently only harvested in limited quantities at one location (Close et al. 2002). Lamprey and native freshwater mussels are considered First Foods, foods items that historically have been used for subsistence and ceremony (Quaempts et al. 2018). Because of the reduced opportunity to exercise harvest rights, tribal members are losing cultural connections to these foods, including traditional harvest methods and locations, preparation methods, and the stories and legends that are associated with them (Close et al. 1995; Close et al. 2002; Karson 2006; CTUIR 2015; Hunn et al. 2015; Quaempts et al. 2018). The concept of reciprocity obliges the people, specifically the tribal people of the Columbia River Basin, to take care of the resources so the resources (First Foods—water, fish, big game, roots, and berries) can take care of them (Karson 2006; Jones et al. 2008; Quaempts et al. 2018; Endress et al. 2019). Historic reliance on First Foods has promoted holistic management approaches that value landscapes as communities that collectively contribute to healthy populations of foods (Jones et al. 2008; Hunn et al. 2015). This has led to efforts to restore populations of Pacific Lamprey and freshwater mussels (as important contributing members of the river community) to the Columbia River Basin through artificial propagation. However, large-scale propagation efforts for both species have not yet produced the high-density stocks that are needed for out-planting, and the optimization of laboratory techniques is necessary. I investigate the role of supportive faunal communities that are associated with lamprey and mussels to (1) improve laboratory rearing outcomes for the benefit of restoration and conservation efforts for the two organisms, and (2) link ecological services from benthic organisms to other members of the river community.

Given the declines in their populations and resulting lack of harvest opportunities, research to propagate Pacific Lamprey and freshwater mussels was started at CTUIR with the goal to restore harvestable populations to the Umatilla Indian Reservation and surrounding watersheds (CTUIR 2015; CRITFC 2018). Partnerships with other Columbia River Inter-Tribal Fish Commission (CRITFC) tribes, including the Yakama Nation, have benefited Pacific Lamprey restoration actions by increasing knowledge and awareness of this ancient fish (CRITFC 2018). Pacific Lamprey have been produced and reared in the laboratory environment since 2012 at CTUIR facilities, and the techniques to fertilize and incubate them have been summarized (Lampman et al. 2016). However, early life stage grow-out, rearing to out-planting age, and high-density production techniques have yet to be developed and are a focus of part of my dissertation.

Research on native western freshwater mussels is sparse and underfunded throughout the northwest. The CTUIR is the only western agency with dedicated staff to propagate mussels. This lack of active research has hampered progress towards goals and has perpetuated a lack of awareness about their ecological benefits and the reasons for their declines. Western freshwater mussels have been lab-produced since 2013, though grow-out and rearing techniques have not yet been successful (CTUIR 2015). The need for high-density, production-level rearing of lamprey and mussels continues to be a regional and tribal research priority (Quaempts et al. 2018). Restoring populations of lamprey and mussels is important to restoring not only tribal harvest opportunities but also a healthy ecological community in the Columbia River Basin.

The resource management philosophy in the Department of Natural Resources at CTUIR is based on the concepts of reciprocity and community, as discussed in Jones et al. (2008), Quaempts et al. (2018), and Endress et al. (2019). These two concepts are central to this dissertation. Reciprocity, in this context, emphasizes the management of resources, like lamprey and mussels, for long-term ecosystem resilience rather than short-term resource use. This is a principle that is rooted in the idea that the resources will take care of the people if the people take care of the resources (Jones et al. 2008). Population restoration is a means of reestablishing tribal harvest opportunity and resilience to the ecosystem, as a healthy and functioning ecosystem relies on all parts of the community working together. Using a holistic approach to watershed management, the River Vision (Jones et al. 2008) details the importance of the "community" of an ecosystem. The focus on the restoration of key riverine processes, or ecosystem services, values the role each trophic level plays in the long-term health and resilience of an ecosystem (Jones et al. 2008). Articulating this ecosystem approach to management for Euro-American audiences in the 1940s, Aldo Leopold expanded the collective view of the land community (Leopold 1949); and more recently these concepts have been used to guide modern restoration practices (CRITFC 2011; Naiman et al. 2012; Rieman et al. 2015; CRITFC 2018). The importance of a healthy benthic community structure is often overlooked in restoration projects in favor of large-scale structural improvements that are directly beneficial to target fauna (e.g., salmonids). I use CTUIR natural resource policy and management frameworks to investigate the concepts of reciprocity and community in resource management to provide a context for the propagation, research, and restoration of Pacific Lamprey and freshwater mussels (Chapter 2).

Conservation aquaculture techniques for lamprey and mussels must include careful consideration of their unique life histories and habitat requirements. Larval Pacific Lamprey and freshwater mussels spend long sedentary periods in freshwater, typically burrowed in gravel, sand, and fine-sediment habitats. They both filter feed, lamprey from at or below the sediment surface and mussels from the lower water column. As such, both organisms are closely tied to benthic productivity and are reliant on a myriad of services from lower and higher trophic levels. Mussels use specific host fish on which to transform, and studies have shown the majority of suitable hosts to be native fish species (O'Brien et al. 2013; Maine et al. 2016). Nonnative fishes in western rivers could be negatively affecting mussel populations in a number of ways and their role as a fish host for western mussels is not known. To propagate mussels in the lab for restoration and research, suitable fish hosts must be identified. I investigate host use for one mussel species, *Anodonta californiensis*, using both native and nonnative fish species (Chapter 3).

In the laboratory culture of larval lamprey, monoculture and laboratory system sterility provide a homogenous rearing environment, possibly contributing to poor survival and low growth, especially in high-density cultures. Investigating the importance of community in laboratory propagation in lamprey, I test the addition of "communities" in the form of micro- (bacteria) and macro- (teleost fish) organisms (Chapters 4 and 5). Finally, in this dissertation, I use traditional ecological and cultural knowledge, scientific results, and ethical reasoning to advocate for the inclusion of benthic organisms like lamprey and mussels in northwest river restoration projects (Chapter 6). Below, I describe the ecological and cultural significance of Pacific Lamprey and western freshwater mussels in the Columbia River Basin to provide context for the coming chapters.

Life histories and ecological importance of lamprey and mussels

Anadromous Pacific Lamprey spawn in freshwater environments, with both sexes contributing to the construction of gravel and cobble redds in which they deposit and fertilize eggs (Figure 1-1; Kan 1975; Close et al. 1995; Mayfield et al. 2014). Incubation time is temperature-dependent; and generally, at approximately 15°C, hatching occurs 13–15 days postfertilization (Yamazaki et al. 2003; Meeuwig et al. 2005; Lampman et al. 2016). Larvae are thought to reside in the redds until their yolk sac is consumed, approximately 30 days posthatching (Brumo 2006). The larvae, called ammocoetes, then emerge and drift downstream with the water current to an area of low velocity and fine sediment into which they burrow (Brumo 2006). The ammocoetes remain burrowed in the sediment for 3–7 years, filter feeding on organic detritus and algae, including diatoms (Moore and Mallatt 1980; Close et al. 2002; Quintella et al. 2005; Lampman et al. 2016).

Unknown signals trigger ammocoetes to metamorphose into macrophthalmia, a smolt-like life stage that travels from freshwater to the ocean (Close et al. 1995; Close et al.

2002). Macrophthalmia develop a more complex eye than present in ammocoetes and a sucking disc mouth, signifying a change in feeding behavior and food type from filter feeding to parasitic feeding on ocean organisms (Beamish 1980; Close et al. 2002). Adult Pacific Lamprey are not well studied in the ocean environment, but it is known that they spend several years in a parasitic phase, using fish like Pacific Hake *Merluccius productus* for food and transportation (Kan 1975; Beamish 1980; Close et al. 2002; Murauskas et al. 2013; Weitkamp et al. 2015). Unknown signals or genetic traits trigger adult lamprey to cease feeding and travel from the ocean back into freshwater in search of suitable spawning habitat. Recent genetic discoveries suggest that Pacific Lamprey display two distinct life histories when returning to freshwater: they will either migrate upstream immediately for spawning or overwinter in the river and spawn the following year (Parker 2018). Unlike salmonids, lamprey do not travel back to their natal streams; instead, they target larval lamprey pheromones that indicate spawning areas that are already successful (Spice et al. 2012).

Larval Pacific Lamprey and freshwater mussels of all life stages occupy similar habitats: those with low velocity and stable sediment. While lamprey are usually burrowed completely under the sediment, mussels may be only partially buried, filter feeding from water near the sediment–water interface. Adult mussels take in nutrients from the water column but also use that space to distribute gametes and larvae. Freshwater mussels employ a unique and complex strategy to complete their life cycle: they use fish to host their obligate parasitic larvae (Figure 1-2; Haag 2012). In general, males release sperm into the water column while females internally fertilize eggs with sperm that is obtained during normal siphoning activity (Haag 2012). Generally, embryos are then incubated in the gill chambers, using two or four gills for this purpose, until they are fully developed as larvae—called glochidia (Haag and Staton 2003; Haag 2012). Mussels employ a variety of larval-release strategies, generally developed evolutionarily through relationships with certain fish hosts (Haag and Warren 1998). Glochidia in mussels of the western United States attach to either the gills of the fish (in the case of *Margaritifera* and *Gonidea*) or the fins of the fish (*Anodonta* species), and their larval-release strategies reflect those adaptations. *Margaritifera falcata* and *Gonidea angulata*, as host specialists, produce conglutinates, condensed packets of larvae, targeted at specific predatory host fish (juvenile salmonids for *M. falcata* and sculpins for *G. angulata*; O'Brien et al. 2013). All western *Anodonta* species are considered host generalists, releasing larvae in loose, sticky masses, that target a wide variety of fish families as hosts (O'Brien et al. 2013).

As two of the most historically abundant benthic species, Pacific Lamprey and native freshwater mussels have significantly contributed to healthy riverine processes for millennia, especially food-web interactions, nutrient cycling, and water quality improvement (Kan 1975; Simpson and Wallace 1978; Close et al. 2002; Vaughn 2017). The loss of abundant populations of lamprey and mussels from the Columbia River Basin has reduced the beneficial ecosystem services contributed by these organisms, thereby negatively affecting watershed health and resiliency (Southwick and Loftus 2017; CRITFC 2018; Quaempts et al. 2018).

Pacific Lamprey in the Columbia River Basin

Pacific Lamprey, as members of the order Petromyzontiformes, are one of the most ancient vertebrates in existence in the Columbia River Basin, having occupied regional rivers for over 350 million years (Renaud 1997; CRITFC 2011). With few morphological changes in that time, lamprey are distinctive in their primitive form; they lack a jaw, scales, and paired fins. Pacific Lamprey have been used by western United States tribes for subsistence, ceremonial, and medicinal purposes since time immemorial (Close et al. 2002). Historically, populations were sufficiently robust to support harvest by area tribes in many main-stem and tributary locations (Close et al. 1995).

The abundance of Pacific Lamprey in the Columbia River Basin has been severely reduced at all life stages (Close et al. 1995; Moser and Close 2003; Murauskas et al. 2013). Spawning lamprey and sedentary benthic larval lamprey in tributaries are faced with poor quality habitat, shifting sediments, low water quality, and unsuitable regulated flows, effects of river management practices of the early- and mid-1900s (Close et al. 2002; Torgersen and Close 2004). Significant losses to migratory Pacific Lamprey populations can be attributed to main-stem and tributary passage barriers, including physical damage from screens or turbines, exhaustion from navigating salmonid-type fish ladders, or complete inability to pass structures (Jackson and Moser 2012). The upstream movement of adult lamprey from the ocean to many freshwater tributaries is blocked by numerous dams. For example, lamprey returning to the Umatilla River in southeastern Oregon navigate four major dams and numerous lowhead dams on their journey to the ocean as juveniles or returning back to freshwaters as spawning adults. Prior to and shortly after construction of dams on the main-stem of the Columbia River (Rock Island Dam: 1933 – John Day Dam: 1971, for the U.S.) adult lamprey numbered upwards of 1,000,000 but plummeted to just 20,000 in the mid-2000s (CRITFC 2011). Where present, fishways at dams are typically designed for salmonids and are inadequate for adult lamprey passage (Jackson and Moser 2012; Keefer et al. 2013; Mesa et al. 2014). A reduction in the abundance of host species in the ocean has also been cited in relation to declining populations of adult lamprey returning to the Columbia River Basin (Murauskas et al. 2013).

Juvenile lamprey that are out-migrating to the ocean must pass the same impounded systems faced by their parents. Entrainment in irrigation diversions, impingement on screens, and turbine blade strikes result in mortality for juvenile lamprey that navigate impounded river systems, and these challenges are not adequately mitigated with traditional salmonid-focused fish passage improvements (Moser et al. 2015). In 1999, extensive electrofishing surveys for larval lamprey conducted in southeastern Washington and northeastern Oregon showed a lack of recent recruitment and very low populations in upper reaches, possibly a result of severely limited adult passage through main-stem and tributary dams (Moser and Close 2003). The installation and improvement of lamprey passage structures, as well as adult translocation, has led to increased survival through Columbia Basin impoundments, and in 2018 a record number of adult lamprey returned (since lows in the 1990s and 2000s) to the Umatilla River to spawn (CTUIR, unpublished data; Keefer et al. 2013; Flatt 2018). Though passage issues are ongoing but improving, additional recovery efforts need to be explored for Pacific Lamprey in the Columbia River Basin (CRITFC 2018).

Populations of Pacific Lamprey are not yet sufficiently robust to sustain a harvest on the Umatilla Indian Reservation or other tribal lands, a long-term goal of Columbia Basin restoration programs. The recent increase in returning adult lamprey shows promise for population recovery in the region (CTUIR, unpublished data; Flatt 2018). Supplementation of depleted fish populations through artificial propagation has been widely used in salmonid management strategies, and research has been initiated for Pacific Lamprey production (CRITFC 2011; CRITFC 2018).

Artificial propagation of Pacific Lamprey

To supplement declining wild populations and provide opportunity for early life history research, Columbia basin agencies and tribes began artificial propagation in 2012. Adult lamprey migrating upstream to spawn are collected at main-stem Columbia River dams and transported to holding facilities on tribal lands (Ward et al. 2012). Adults are generally overwintered in flow-through holding chambers using ambient spring water and checked for spawning readiness in the spring (Close et al. 2009). Males and females are sorted in the late spring (typically late April for CTUIR facilities) and graded for reproductive characteristics (Mesa et al. 2010; CTUIR, unpublished data). Lamprey that are chosen as broodstock for artificial propagation are hand-stripped of gametes (see Lampman et al. 2016). The eggs and milt are mixed and water-hardened (as described in Lampman et al. 2016). Fertilization and incubation techniques have been well studied and currently practiced methods yield nearly 100 percent fertilization and hatching success (Lampman et al. 2016; Maine et al. 2017). The survival rate of larvae from hatching to “first feeding,” indicated by the disappearance of the yolk sac and the development of the gut to anus connection, is variable depending on laboratory rearing conditions (Jolley et al. 2012; Lampman et al. 2016). Typical stocking densities range from approximately 50,000/m^2 for larvae up to 30 days posthatch to 500/m^2 for nine-month-old larvae (Lampman et al. 2016). Larval feeding in the laboratory has also been well studied, and a standard feeding protocol uses 250 mg of a mixture of active dry yeast (80%, RedStar Baking Yeast, Lesaffre Yeast Corp. Milwaukee, WI) and larval fish food (20%, Otohime A1, Marubeni Nisshin Feed Co. Ltd., Tokyo, Japan) per liter of water (Mallatt 1983; Barron et al. 2016). Food is generally introduced to laboratory-reared larvae at approximately 30 days posthatch, coinciding with the exhaustion of the yolk sac and the start of burrowing behavior (Lampman et al. 2016). Metamorphosis from ammocoete to macrophthalmia has been observed recently in limited numbers in propagated individuals (R. Lampman, Yakama Nation, Prosser, Washington, personal communication), but it has also been observed in one wild-caught ammocoete that was held in a laboratory environment for three years (CTUIR, unpublished data). These observations suggest that controlled or laboratory holding conditions do not negatively affect normal developmental processes, an important point with respect to eventual out-planting and supplementation of wild populations.

Numerous research questions remain concerning the biology of Pacific Lamprey during the early life stages. Survival in lab cultures, in both flowing (flow-through and recirculating) and static conditions, is low by production standards for fish. However, comparisons with survival rates in the wild at those life stages have not been evaluated and could reflect naturally high larval mortality. Larvae that are reared in static cultures have shown improved survival and growth compared with those that are raised in recirculating systems. Recent studies have also shown preliminary success in improved growth and survival by using a commercial aquatic probiotic product in addition to standard feed for larvae in static cultures (CTUIR, unpublished data; Maine et al. 2017). Further, the addition of a co-reared organism, Speckled Dace, with larval Pacific Lamprey has improved survival in a recirculating aquaculture system (Chapter 4). Limm and Power (2011) also show that cohabitation with freshwater mussels can improve food availability in the form of organic matter retention, thereby increasing growth in larval Pacific Lamprey. These aspects are explored in greater detail in this dissertation.

Freshwater mussels in the Columbia River Basin

North America boasts the greatest species diversity of freshwater mussels (298 currently recognized species), with much of that concentrated in hotspot locations like the southeastern United States (Lydeard et al. 2004; Williams et al. 2017). Western North America's mussel fauna is considerably less diverse, with only seven currently recognized species (Williams et al. 2017). The Columbia River Basin is host to six of the seven species, belonging to three genera: *Margaritifera*, represented by one species—*M. falcata*, *Gonidea*, represented by one species—*G. angulata* (Lea, 1838), and *Anodonta*—represented by two distinct clades, currently designated as four species—Clade 1: *A. nuttalliana* (Lea, 1838) and *A. californiensis* (Lea, 1852) and Clade 2: *A. oregonensis* (Lea, 1838) and *A. kennerlyi* (Lea, 1860) (Chong et al. 2008; Williams et al. 2017).

Worldwide, freshwater mussels are one of the most imperiled faunal groups (Lydeard et al. 2004; IUCN 2020), and recent surveys in the northwestern United States indicate that the rate of decline is increasing (Blevins et al. 2017a). Both in numbers and diversity (species richness and genetic diversity), freshwater mussels are negatively affected by degraded environmental quality that is perpetuated by increasingly warm water temperatures, increased sedimentation, and regulated flow regimes (Cope et al. 2008; Galbraith et al. 2010; Ganser et al. 2013; Blevins et al. 2017b). Declines in mussels across North America, particularly over the last century, have been well documented (FMCS 2016), but mussel declines in western North America have been explored only recently, showing a 35% decline in richness by area and a significant loss in geographic range (Blevins et al. 2017a). These declines can be linked to human-derived disturbances including the following: impoundments (Vaughn and Taylor 1999), which cause selective extinction of reservoir-intolerant species in addition to changes in sedimentation (Brim Box and Mossa 1999; Hastie et al. 2003; Poole and Downing 2004), warming temperature regimes (Galbraith et al. 2010), and reduced host-fish availability (Kelner and Sietman 2000); competition from introduced species such as *Dreissenid* mussels (zebra and quagga mussels) and the Asian clam (*Corbicula fluminea*), both of which have highly successful reproductive strategies (McMahon and Bogan 2001; Strayer 2010), and proliferation of invasive game fish (e.g., bass and carp), which outcompete native fish species and directly feed on young native mussels (Haag 2012); and habitat and water quality degradation resulting from poor land management, point and non-point-source pollution, dredging, siltation, and in-stream construction activities (discussed in Haag 2012). The declines that are specific to the Columbia River Basin have reduced populations of native freshwater mussels such that harvest (a retained treaty right for area tribes) is no longer possible (Brim Box et al. 2006; CTUIR 2015). Moreover, significant cultural and ecological losses have resulted from these declines (Brim Box et al. 2006; CTUIR 2015). Columbia Plateau tribes have experienced a loss of their cultural connection to freshwater mussels, and ecosystems are negatively affected by the loss of important ecological services that are delivered by mussels (CTUIR 2015; Vaughn 2017). For these reasons, CTUIR initiated propagation efforts for all three genera of western mussels in 2013, with the goal of restoring populations throughout the region (CTUIR 2015).

The genus *Anodonta* in the western United States is of particular research interest because of the uncertainty regarding correct naming convention and taxonomic placement. Members of this genus in the west are the subject of ongoing taxonomic, morphological, and genetic review to determine the exact number and placement of species within the family Unionidae (Zanatta et al. 2007; Chong et al. 2008; Mock et al. 2010; O’Brien et al. 2019). Recently, the western species *Anodonta beringiana* (Middendorf, 1851)—the Yukon

Floater—was determined to be more closely related to mussels from Russia and Asia than to those in the United States, resulting in a reclassification to *Sinanodonta beringiana* (Chong et al. 2008; Lopes-Lima et al. 2017; Williams et al. 2017). Additionally, morphometric differences in glochidia (mussel larvae) of the four western *Anodonta* support species- and clade-level distinctions (O'Brien et al. 2019). An incomplete list of fish species that are suitable hosts for western mussels has been compiled, and several nonnative species have been excluded as hosts through laboratory and field studies (Haley et al. 2007; O'Brien et al. 2013). Therefore, mussel production is thought to be negatively affected by the increasing populations of nonnative fishes that have been observed in the Columbia River Basin (O'Brien et al. 2013). Accurate species designations are important in any conservation context, and even more so in light of recent investigations into artificial propagation for population restoration (CTUIR 2015).

Artificial propagation of native western freshwater mussels

For laboratory propagation of mussels, gravid females are obtained from wild populations. Fully developed larvae are extracted by using standard methods (Neves 2004). Briefly, the gill chamber of a gravid female is ruptured by using a probe and the cavity is flushed with water to collect glochidia (either in a loose mass or in conglutinates). The glochidia are checked via microscope for viability (as described in Zale and Neves 1982)—viable glochidia are actively "snapping" valves open and closed in search of attachment to fish tissue. *M. falcata* and *G. angulata*, by using conglutinates to entice fish to "eat" their glochidia, attach to the gill filament tissue of their hosts (O'Brien et al. 2013). *Anodonta* glochidia attach to the fins of their hosts, with fish swimming through or near the sticky masses of released glochidia (O'Brien et al. 2013). The full suite of host fish that is used by each western mussel is still unknown, though some have been identified (O'Brien et al. 2013; Maine et al. 2016). The confirmation of a fish species as a host for a particular mussel species must involve a twofold investigation that involves both the laboratory and the field (Neves et al. 1985; O'Brien et al. 2013; Maine et al. 2016). First, a fish species must produce viable juvenile mussels from glochidia in the laboratory. Second, a fish species must be identified in the wild with attached mussel larvae. Identification of host-fish–mussel relationships are highly important in conservation planning for any mussel restoration actions, as reproductive success is entirely dependent on availability of host fish (O'Brien et al. 2013).

Another important consideration in the artificial propagation of native western freshwater mussels includes investigations into genetic diversity and broodstock selection, especially in light of declining populations and limited abundance (Jones et al. 2006; Blevins et al. 2017a). Of the three genera in the western United States, genetic diversity has been determined to be lowest in *Margaritifera falcata*, likely a result of host-fish specificity and hermaphroditism (Mock et al. 2013). Genetic variability in the genus *Anodonta* appears to be highly dependent on geographic location, generally increasing in diversity downstream (Mock et al. 2010). *Gonidea angulata*, belonging to the monotypic genus *Gonidea*, is relatively understudied, but anecdotal information suggests that populations are increasingly and negatively affected by unknown stressors throughout the Pacific Northwest. The species is currently being petitioned for listing as endangered under the Endangered Species Act (E. Blevins, Xerces Society for Invertebrate Conservation, Portland, Oregon, personal communication), and they should be considered a high priority for conservation efforts in the region (CTUIR, unpublished data; Blevins et al. 2017a). A sound conservation plan for any western mussel species will include watershed-level considerations of genetic variability as well as careful broodstock selection, to minimize loss of within- and among-population genetic variation (Jones et al. 2006; Haag 2012).

Study area

The Columbia River, the fourth largest river in North America, drains over 670,000 km^2 from Canada through Washington, Oregon, and Idaho to the Pacific Ocean (Figure 1-3). The Columbia River Basin has over 400 dams providing controlled river flows for hydroelectric power generation, irrigation, flood control, barge navigation, and water storage (Williams et al. 2006; BOR 2016). The Columbia Plateau, encompassing Eastern Washington, north-central Oregon, and part of Northern Idaho, is home to four main Native American tribes who have historically shared the use and care of common resources—the Nez Perce Tribe, the Confederated Tribes of the Warm Springs Reservation of Oregon, the Confederated Tribes and Bands of the Yakama Nation, and the Confederated Tribes of the Umatilla Indian Reservation.

Of particular focus in this dissertation are the innovative resource management policies of the CTUIR, as it is currently the only area tribe with restoration-focused projects for both Pacific Lamprey and native freshwater mussel. The CTUIR retain use rights to harvest and gather traditional foods, including lamprey and mussels, on ceded territory, 6.4 million acres in northeastern Oregon and southeastern Washington (Quaempts et al. 2018). According to CTUIR's Comprehensive Plan (2010),

> [t]reaty-reserved rights of the Confederated Tribes are interconnected to the Tribe's land base, to their culture, traditions and religion and to their languages. The rights reserved in the Treaty of 1855 and the land base identified capture both the geographic area critical to the economy and the life-ways learned over thousands of years of where and when to find salmon, elk, bighorn sheep, camas and berries. Treaty rights are not just statements or words. They are an expression and a definition of a people and their relationship to the land and rivers that is many thousands of years old. The Treaty rights of the Confederated Tribes represent the lives lost, the blood and tears shed, and all that has transpired in the complex history of a nation and its people.

Tribes today work to conserve and restore resources across their culture area (southeastern Washington and northeastern Oregon) so that treaty rights to hunt, fish, and gather traditional foods are exercisable. The culture area of the CTUIR contains eco-cultural systems, which are defined as "the set of cultural, religious, nutritional, educational, psychological, and other services provided by intact, functioning ecosystems and landscapes" (Walker 1998; Harris and Harper 2000). Healthy, functioning habitats across a culture area are vital to the development and maintenance of harvestable populations of resources for tribal people (Harris and Harper 2000).

Populations of both lamprey and mussels are depleted throughout the Columbia River Basin, but are particularly imperiled in the Umatilla River, a Columbia River tributary that is considered the home river of the CTUIR people (Jones et al. 2008). In 1995, a status report on Pacific Lamprey concluded that lamprey no longer occurred in the Umatilla River above Threemile Dam on the lower river (Close et al. 1995). Surveys of the Umatilla River in 2003 showed very low abundance and severely limited distribution of *Anodonta* and *Gonidea*, while *Margaritifera* appeared to have been extirpated from the system (Brim Box et al. 2006). Archeological records show that *Margaritifera falcata* occurred in abundance in the system historically (CTUIR, unpublished data). CTUIR resource management guiding frameworks like the River Vision promote holistic restoration of abiotic and biotic components of the Umatilla and other subbasins on ceded lands to restore First Foods and their ecological communities.

Summary

Pacific Lamprey and western freshwater mussels contribute valuable ecological services to the river community, but populations of both organisms are declined from historic levels. Conservation aquaculture has been recently implemented as a restoration strategy to restore ecological and cultural services derived from these two benthic organisms. Chapter 2 will detail tribal resource management policies and actions, using traditional ecological knowledge and the concepts of reciprocity and community to develop holistic watershed and resource management strategies. Chapter 3 investigates the community of fishes used by a native freshwater mussel, *Anodonta californiensis*, and explores negative interactions of increasing nonnative fish populations for native mussels in the Columbia River Basin. Chapters 4 and 5 explore the supplementation of a community in conservation aquaculture and laboratory rearing of larval Pacific Lamprey, using macro- (Speckled Dace) and micro- (bacteria) organisms. Chapter 6 calls on Pacific Northwest river restoration professionals to include the benthic community in restoration practices, to the benefit of target restoration organisms like salmonids. Finally, a general discussion reiterates the ecological and cultural importance of lamprey and mussels and draws connections between these two organisms, the river community, tribal human communities reliant on these resources, and offers directions for future research.

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Figures

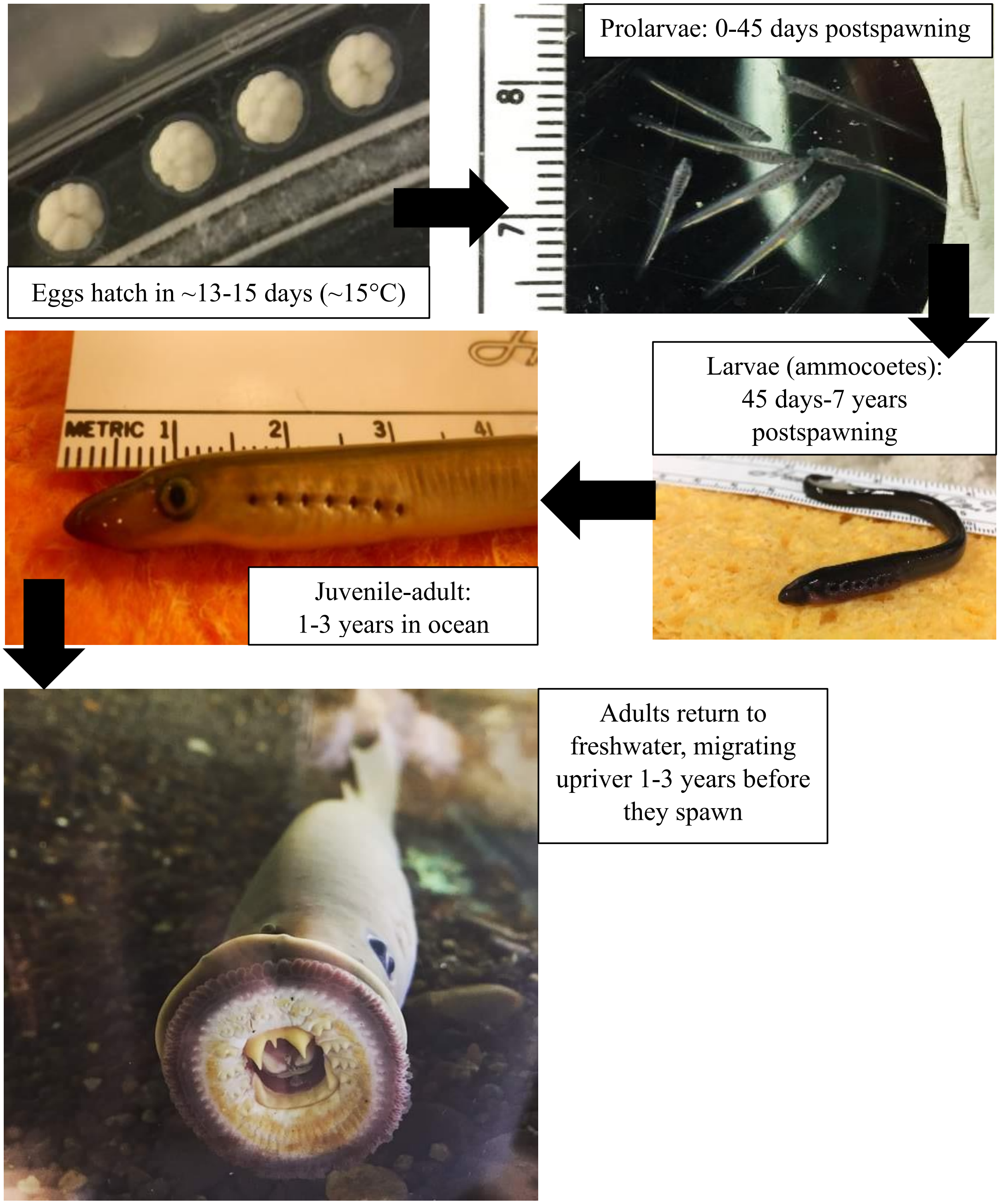

Figure 1-1. Pacific Lamprey life cycle. Adults spawn in freshwater, then larvae burrow in fine sediment for 3–7 years as filter feeders. After metamorphosis into juveniles, lamprey out-migrate to the ocean where they parasitically feed on ocean fishes for 1–3 years. They return to freshwater and migrate upriver to spawn then die shortly after. Photographs by the author.

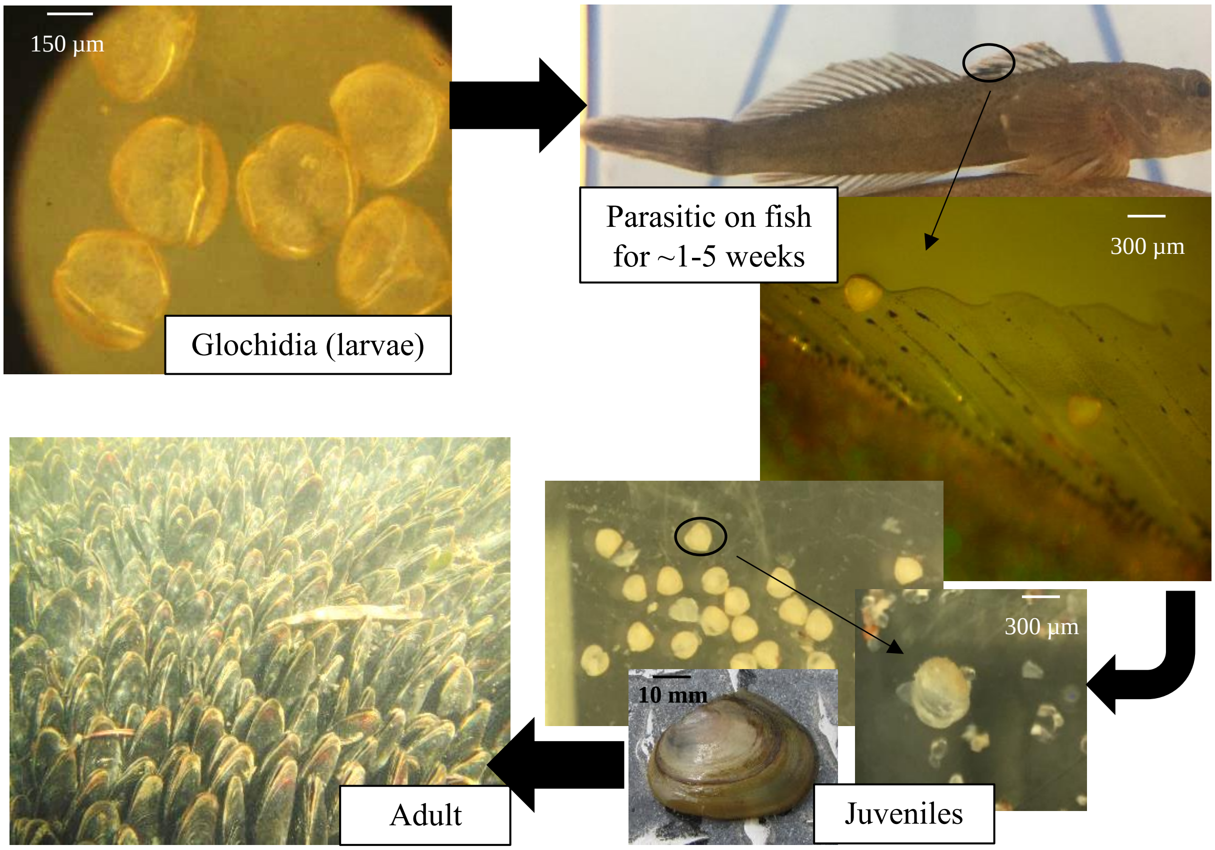

Figure 1-2. Freshwater mussel life cycle. Mussels release larvae, called glochidia, which then attach to a suitable host fish and transform into juveniles. Juveniles drop off fish and settle into a low velocity, fine sediment habitat to burrow and grow into adults. Photographs by the author.

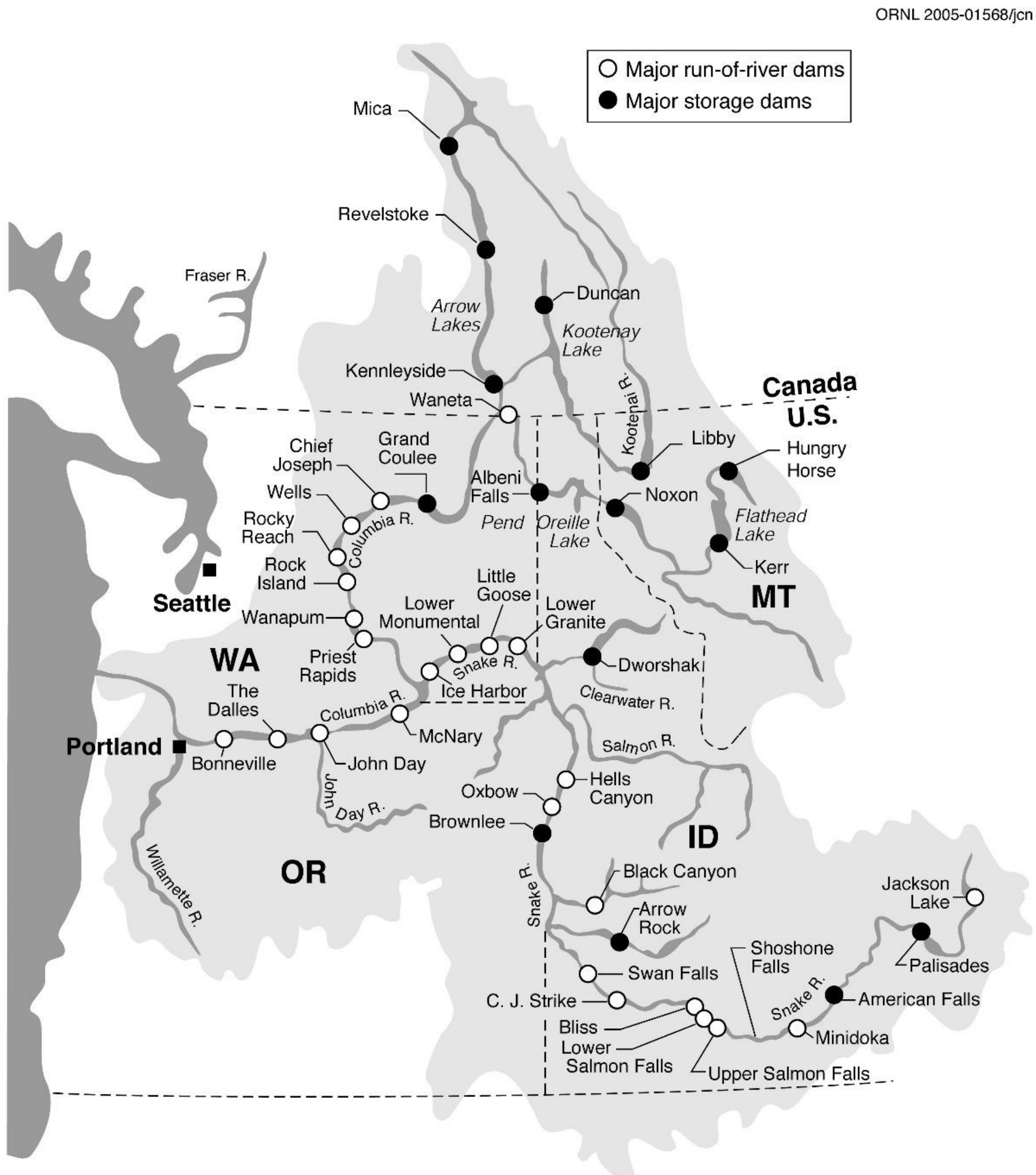

Figure 1-3. The Columbia River Basin and its major dams. Map from Williams et al. (2006), used with permission.

CHAPTER 2: COMMUNITY AND RECIPROCITY IN RESOURCE MANAGEMENT, EXAMINED THROUGH TRADITIONAL ECOLOGICAL KNOWLEDGE AND TRIBAL MANAGEMENT POLICY

Abstract

Columbia Plateau tribes, such as the Confederated Tribes of the Umatilla Indian Reservation (CTUIR), have developed sustainable relationships with communities of food resources, First Foods, throughout the Pacific Northwest (PNW) since time immemorial. These relationships are evident in tribal creation stories, where plants and animals offered themselves to sustain humans, and in turn humans were given the responsibility of reciprocity—to take care of the foods that take care of them. For millennia, tribal people have managed resources for continued and long-term use. Today, indigenous organizations fulfill the promise of reciprocity by developing research programs that are focused on restoring ecological and cultural services. For many indigenous groups, traditional ecological knowledge (TEK) drives these inquires and restoration objectives. The CTUIR's Department of Natural Resources (DNR) actively participates in multi-scale regional projects in the PNW that are focused on the restoration of both physical and biological processes that include important interspecies linkages and community interactions, to reconnect cultural and ecological services.

In aquatic ecosystems in the Columbia River Basin, the restoration focus remains on salmon, an important tribal First Food. To restore this First Food to historic levels, the DNR relies on the restoration of intricate and complex relationships with all of the organisms in their community—a holistic approach. Some of these other organisms are also traditional First Foods (e.g., lamprey, mussels). While less commonly consumed today for a variety of reasons, these resources have also experienced population declines. In food-dependent (i.e., foods and culture are intricately linked) cultures such as the CTUIR, the perpetuation of reciprocity relies on both access to those food resources and sustainable, harvestable populations. When First Food populations decline in the Columbia Basin, so does the ability of tribal members to teach and learn about harvest, preparation, consumption, and celebration of those foods—their culture. Restoring such cultural and ecological services in aquatic ecosystems, for example, requires careful investigation of physical (geomorphology,

hydrology, connectivity, riparian vegetation) and biological (biota) structure and function. The CTUIR use First Food policies and guiding frameworks, such as River Vision, to conduct restoration and resource management practices in ways that reconnect foods, food communities, and the humans who depend on them. These types of policies focus resource management on the restoration of ecological function, cultural services, and community structure in each restoration project. The use of TEK combined with western science produces holistic conservation and management outcomes that honor the values of reciprocity and community.

Introduction

Since time immemorial, Columbia Plateau tribes like the Confederated Tribes of the Umatilla Indian Reservation (CTUIR) have used and managed diverse populations of First Foods for subsistence and ceremony. These First Foods include water, fish, big game, roots, and berries that have significant historical, cultural, and ecological value for indigenous people (Quaempts et al. 2018). Lack of availability or limitations on access to First Food resources has greatly impacted many aspects of the CTUIR's food-dependent culture, including sharing and celebrating foods through the traditions that are associated with harvesting, preparing, and serving (Hunn et al. 2015). When populations of First Foods decline, so does the ability for tribal members to teach and learn about harvest, preparation, consumption and celebration of those foods, in essence their culture (Karson 2006; Hunn et al. 2015).

An indigenous natural law, called *tamánwit* or *tamálwit* in the CTUIR culture, urges reciprocity, in that humans are entrusted with the responsibility of caring for their resources so the resources can sustain them (Karson 2006; Hunn et al. 2015; Quaempts et al. 2018). Armand Minthorn describes this natural law in Karson (2006:224),

> [t]here is so much to this word or this way, this *Tamánwit*. It's how we live. It's our lifestyle. There is so much that we as Indian people are governed by, through our traditions, our culture, our religion and most of all, by this land that we live on. We know through our oral histories, our religion, and our traditions how time began. We know the order of the food, when this world was created, and when those foods were created for us. We know of a time when the animals and foods could speak. Each of those foods spoke a promise. They spoke a law— how they would take care of the Indian people and the time of the year that they would come. All of those foods got themselves ready for us—our Indian people who lived by the land. It was the land that made our lifestyle. The foods first directed our life. Today, we all have these traditions and customs that recognize our food; our first kill, first fish, first digging, the first picking of berries. All of those things are dictated to us because it was shown and it directed our ancestors before us.

> The songs we sing with our religion are derived from how we live on this land. Our cultural way of life and the land cannot be separated. Even though we recognize that our life is short, it all goes back to that promise that was made when this land was created for us Indian people, the promise that this land would take care of us from the day we are born until the day that we die.
>
> When we recognize our foods, we recognize our ancestors, we recognize the language. It's all within the same context and teachings that we live day by day. The promise that this land made and the promise that we made as Indian people to take care of this land, to take care of the resources, and to live by those teachings is the grander principle of the bigger law that was put down on this land when this world was created. This is the law that we recognize on Sundays, that we recognize when we lose a family member, and that recognize when the seasons changes. When we can live by those traditions and customs, then we're fulfilling that law, we're living by that law.

The First Foods serving order in the tribal longhouse is one way that the CTUIR honors the reciprocity from foods to human. Water is served first and last, before and after the other foods, to signify its important role in the perpetuity of the human and food community that rely on it (Quaempts et al. 2018). The other foods are then served in a ritual order of salmon, big game, roots, and berries to honor the gift of nourishment that they provide.

Another way that CTUIR fulfills reciprocity is through prioritizing ecosystem resilience over resource extraction in tribal resource management. The CTUIR's First Foods resource management approach emphasizes the restoration of First Foods through the restoration of ecosystem processes and function (CTUIR 2010; Quaempts et al. 2018). Specifically, the Department of Natural Resources (DNR) of the CTUIR states that its mission is to "protect, restore, and enhance the First Foods for the perpetual, cultural, economic, and sovereign benefit of the CTUIR" and that it will accomplish this by "utilizing traditional ecological and cultural knowledge and science" (CTUIR 2010; CTUIR 2015; Quaempts et al. 2018). Incorporating traditional ecological and cultural knowledge (TEK) into tribal management actions and policies promotes awareness and respect in the nontribal management community for indigenous methodology and long-term ecosystem management by tribes in the region (Hunn et al. 2015). The term TEK is a westernized understanding of what tribes consider, simply, knowledge. I acknowledge that the use of the term TEK does not fully represent the depth of tribal knowledge resulting from the coexistence of humans and foods since time immemorial. Traditional ecological knowledge and indigenous knowledge systems provide a context for using and relying on natural resources, and they provide a foundation for the integration of cultural health into resource management practices, both tribal and nontribal. My objective was to explore the concepts of reciprocity and community in tribal resource management, and the way these concepts shape management policies and actions.

Traditional ecological knowledge in resource management

Traditional ecological and cultural knowledge (TEK) refers to aboriginal understanding and belief surrounding the connections between all living beings to each other, and to their environments (Berkes 1999; Pierotti and Wildcat 2000). The term TEK is a westernized simplification of deeply cultural and place-based knowledge systems of Native Americans. Knowledge systems described by TEK are the ecological and cultural practices of such knowledge in use and care of local resources (Kimmerer 2002). Traditional ecological knowledge is collective, long-term observational ecological data, rooted in place and community. Oral traditions in TEK provide observational information, with storytelling and place-naming providing context and valuable information about how to navigate in and coexist with the natural world (Basso 1996; Cajete 2000). Traditional ecological or environmental knowledge is “a rich vein of indigenous intellectual property” and “is in large part by nature inalienable. It is a work of art, a symphony of understanding, and a scientific contribution to human knowledge of nature” (Hunn et al. 2015).

Traditional ecological knowledge is rooted in the belief that everything is inter-connected. The First Foods serving order of CTUIR longhouse traditional meals describes an order between people and their foods (E. Quaempts, CTUIR, Pendleton, Oregon, personal communication). Water is of great importance and this is signified through its place both first and last in the serving order during First Food ceremony and celebration. Water is honored in this way to show the connection between water and people, as well as water and other foods (Quaempts et al. 2018). Other First Foods are served following water to show their important connection with, and reliance on, water.

> Water was created first, life and land were created next, the land promised to take care of all life, all life promised to take care of the land. A long time ago the Indian people also promised to protect the land and have the responsibility to care for her. Water represents an integral link in a world view where water is sacred and extremely important in preserving precious balance. Water is the origin of, and essential, for the survival of all life. [From CTUIR 2010:20].

The serving order of First Foods represents the ecological products of healthy, functioning environments, and the relationships between First Foods, people, and their environments are those of interdependence and reciprocity (Figure 2-1).

Time-honored traditions and the stories that are associated with the harvest, preparation, and consumption of First Foods show reciprocity in methodology. For example, in the CTUIR, when harvesting huckleberries, gatherers leave berries remaining on the bushes to feed other animals and to perpetuate regrowth of the resource for the future (Quaempts et al. 2018). A CTUIR pollinator exclusion study also captured reciprocity from pollinator to plant. The study showed that huckleberry bushes that were exposed to pollinators produced more fruit than did bushes that were excluded from pollination (Shippentower 2019). These acts of reciprocity affirm the connection between humans and their environment, serve to honor and respect the gifts that are given by plants and animals, and bring awareness of the value of TEK to a wider audience (Hunn et al. 2015; Figure 2-2).

The CTUIR and other Columbia Plateau tribes (Yakama, Warm Springs, and Nez Perce) also implement management approaches for their traditional foods and resources, where interconnection, community, and reciprocity play key roles. An important feature of this style of resource management is the continuum of cultural and natural resources. Traditional ecological and cultural knowledge is the incorporation of subsistence practices with spiritual customs, and the CTUIR, for example, does not separate the religious, cultural, and ecological importance of First Food resources because they are interdependent; without the food, the religion and culture could not be practiced (Quaempts et al. 2018; Wicks-Arshack et al. 2018). Schure et al. (2013) found TEK to be a unifying thread in participants' responses to a discussion on changes in the natural environment. The CTUIR members that were interviewed for the study reported that lack of access to traditional foods acted as a barrier to good health. The participants further reported that the incorporation of traditional methods and values, especially for youth, may induce positive health effects in the tribal community. The current limited access and limited abundance of First Food resources has adverse effects on the spiritual, cultural, and physical well-being of the CTUIR people.

Nonindigenous, Euro-American resource management style has often sought to control and use rather than to coexist with or reciprocally care for nature (Pierotti and Wildcat 2000), and it has lacked the longer-term understanding and ecological order of North American landscapes and processes. Nonindigenous resource management styles have promoted resource extraction for short-term maximum economic benefit, often to the detriment of the connections and inter-connections of natural, native communities and long-term ecosystem resilience (Quaempts et al. 2018). This example of a reductionistic approach to resource management, in which parts of a system are treated as units separate from the whole, can be harmful to ecosystems, landscapes, and especially eco-cultural systems, on which tribes like the CTUIR rely (Walker 1998; Harris and Harper 2000). As a result of reductionism in nontribal resource management, the availability of and access to First Foods has greatly declined in the Columbia Basin from historic levels. For example, in the 1960s and 70s, rotenone treatments were used in the Umatilla River to remove fish species that were thought to compete for resources with commercially prized steelhead *Oncorhynchus mykiss*. The management practice for this single species killed over one million native fish, including Pacific Lamprey (Close et al. 1995).

Nontribal approaches to salmonid management, in particular, provide other examples of a single-species focus to the detriment of the ecosystem. Salmonid populations, already depleted by the late 1800s from commercial overfishing pressures and dam-related river alterations, were further affected by the implementation of large-scale hatchery facilities. By the late 19th century, hatcheries were producing millions of fish per year, without knowledge of the salmonid life cycle or an understanding of the ecological relationships of salmon and their environment (White 1995; Taylor 2001). The singular focus on salmonids in this period led to the hatchery-born issues of salmonids today, including domestication-selection, inadequate genetic management, and poor survivorship (e.g., Johnsson et al. 2001; Fleming et al. 2002; Huntingford 2004). The single-species management approach unbalances a system, like tinkering with one leg of three-legged-stool. By solely focusing on a single leg (species), we fail to recognize the instability passed on to the entire system.

Where a First Foods approach to resource management looks to the biotic connections and relationships between parts of the ecosystem (Jones et al. 2008; Endress et al. 2019), nonindigenous resource management actions have treated species singly, often inadvertently damaging the ecosystem on which the target species relies. For example, wolves were eradicated from much of the United States to alleviate predatory pressures on big game like elk. Elk overpopulation quickly became a larger ecosystem-wide problem than the wolves' predation pressures. Wolves were reintroduced to Yellowstone National Park in the 1990s and their presence had cascading effects across ecosystems and trophic levels (Ripple et al. 2001; Ripple and Beschta 2006). With wolves present, riparian vegetation (willows, aspens) increased in abundance and health (trees grew taller, canopy cover increased) and river bank stabilization increased (Beschta and Ripple 2006; Ripple and Beschta 2006). Further, the indirect effects of wolf predatory pressure increased populations of bison and beavers as interspecies competition with elk for food resources was reduced (Ripple and Beschta 2012). Aldo Leopold, notable wildlife ecologist of the 1930s and 40s, interestingly advocated for wolf extirpation before gaining a deeper understanding of their intricate role in the balance of an ecosystem through his own observations (Leopold 1949; Ripple and Beschta 2005). Place-based ecological knowledge, like Leopold's short-term observations or long-term TEK of indigenous people, can have profound effects on management actions. Tribal resource management practices, in contrast to nonindigenous management actions, prioritize the sustainable management of collective native populations, such as leaving a portion of roots and berries in place during harvest to perpetuate continued production and to leave food for other ecological community members (e.g., bears) to use (Kimmerer 2013; Quaempts et al. 2018). While TEK principles are incorporated into some nonindigenous socioecological practices (i.e., an ecosystem approach to management), the general separation of western culture from the environment (e.g., foods processed beyond recognition as plant or animal, foods from nonlocal areas and out of season) discourages ecological or cultural connections between humans and resources. This perpetuates disrespect for the environment because western culture is disconnected from food and natural resources.

Where indigenous resource management places humans as an intricate member in the environmental and ecological community, nonindigenous resource management has historically placed the human above all else, often separated from the environment. Early American settlers set out to mimic that which they saw in nature (i.e., glaciers once dammed the Columbia River; therefore, building a dam at Grand Coulee was simply a re-creation of that natural phenomenon), but instead they exploited that sentiment into region-wide control of the entire Columbia system (White 1995). Nonindigenous management decisions prioritized individual parts of the environment that most benefited their population, purposefully or inadvertently exterminating other parts along the way (e.g., suction-dredge mining activities destroy benthic communities and their ecological functions). Placing individuals within the membership of a community ties each member to a collective, intertwined interest (Pierotti and Wildcat 2000), and indigenous management weaves resources and humans collectively into policies and actions. Holistic resource management, as practiced by the CTUIR through multiple management frameworks and policies, calls for restoration actions to exert broad, substantial effects on traditional resources, both aquatic and terrestrial, for cultural and ecological reconnection (Jones et al. 2008).

Weaving together TEK and scientific investigation produces a comprehensive foundation on which to make management and conservation decisions for First Foods. Each of the three strands that are used to braid sweetgrass in Potawatomi traditional methods (a North American indigenous group originally of the Great Lakes region, now Oklahoma) represents knowledge from a separate but related source (Kimmerer 2013). The first strand represents the indigenous knowledge that is passed from ancestors through stories, the second represents scientific knowledge that is obtained through education and culture-sharing, and the third is the knowledge that organisms still hold to be given over time through mutual respect. Indigenous natural resource management incorporates these three ways of knowing together into holistic ecosystem management in which each part is important in the understanding of the whole.

Reciprocity and community in resource management

Reciprocity is conferred as a responsibility of the human community to care for their resources to the perpetual benefit of the CTUIR people. Reciprocity can also be understood in the linkages between species in ecosystems that provide First Foods. In CTUIR creation belief "the Creator asked the foods, 'who will take care of the Indian people?' Salmon was first to promise, then the other fish lined up behind salmon. Next was deer," and so on (Jones et al. 2008; Quaempts et al. 2018). The portion of the phrase, "then the other fish lined up behind salmon" signifies the importance of reciprocity to the CTUIR's creation belief, which is verified by the inclusion of those supporting organisms on which the salmon has relied for millennia. A healthy and functioning river ecosystem, with a diverse, native biotic community, will provide First Foods in abundance for future generations as it has for many previous generations (Quaempts et al. 2018).

As an example of the importance of reciprocity between trophic levels in a river ecosystem, we look to the symbiotic relationships between freshwater mussels and salmonids. To complete their life cycle, salmon return to their natal streams and search out stable, clean substrates to create redds in which to lay their eggs (Quinn et al. 2018). These spawning substrates are located in habitats called glides that are located upstream of deep pools. The glides have a slope that forces water into the interstitial spaces between the gravel and oxygenates the eggs during incubation (Quinn et al. 2018). Freshwater mussels use similar complex habitats, including glides, that remain stable over a wide range of water levels and discharge changes (Haag 2012). Freshwater mussels can also form dense beds of hundreds or thousands of mussels, filtering water and stabilizing smaller substrates around them (Strayer et al. 2004). This mussel—salmon association is also noted in tribal place names, where cultural use sites named for mussels were also locations where fishing was common (Hunn et al. 2015). For example, a site on the Columbia River near Rock Creek was named *Išáaxuyi*, or "covered with mussel shells" (Hunn et al. 2015). This site was named for its mussel population but it also provided fishing opportunities for salmon and lamprey as well as opportunities to gather plant foods (Hunn et al. 2015). It is likely that the ecosystem services provided by freshwater mussels (i.e., water quality improvements, substrate stabilization, food-web connections) improved conditions for other First Foods, both aquatic and terrestrial (Vaughn 2017).

The acts of reciprocity between mussels and fish also flow the opposite way. Freshwater mussels use fishes of all types to transform their parasitic larvae into juveniles and to move their offspring/population up or downstream through this fish-transport mechanism (Haag 2012). Mussels in the western United States have developed species-specific relationships with certain types of fishes, suggesting coevolution (i.e., fish become better able to host mussel larvae, mussels develop adaptations to attract their preferred hosts) between mussels and native fish (Mock et al. 2013; O'Brien et al. 2013). Identification of host-fish—mussel relationships are highly important in conservation planning for any mussel restoration actions, as reproductive success is entirely dependent on the availability of host fish (O'Brien et al. 2013; see also Chapter 3). The same should be the case for salmonid-restoration practices: supportive community organisms like freshwater mussels should be considered for restoration in areas where they occurred historically and co-occur/co-occurred with salmonids.

The understanding of important connections and interconnections of these communities are one reason that CTUIR resource management can be understood through the principles of the River Vision and Upland Vision (Figure 2-3, 2-4; Jones et al. 2008; Quaempts et al. 2018; Endress et al. 2019). The River Vision, a management framework that is focused on riverine First Foods like water and fish, provides five distinct touchstones as pillars of restoration focus for tribal projects. One touchstone specifically, “Aquatic Biota,” refers to acknowledging and restoring species linkages in the aquatic community (Jones et al. 2008). The Upland Vision is a management framework that expands the principles of the River Vision to upland ecosystems to “serve as a foundation for natural resource management and restoration activities to ensure healthy, resilient and dynamic upland ecosystems” (Endress et al. 2019). The Upland Vision calls for management actions and objectives to align with tribal resource needs. Abundance and accessibility of First Foods is intricately linked to tribal community health and cultural well-being (Schure et al. 2013; Endress et al. 2019). The physical and spiritual practices that are associated with First Food procurement have direct positive effects on the health and culture of the community by using and strengthening TEK (Endress et al. 2019).

Linkages between First Foods and related native organisms are understood in opposition from tribal and nontribal perspectives. Elder tribal members who were interviewed about the changes to current abundances of aquatic First Foods recalled how mussels disappeared as salmon populations declined, suggesting that the two are intricately linked (CTUIR 2015). The single-species focus of hatcheries and salmonid restoration of the early and mid-1900s ignored the TEK of regional tribes. Still today, many salmonid habitat restoration projects follow nonindigenous resource management principles that narrowly target salmonids without acknowledging their food-web and aquatic-community connections (e.g., Golightly 1999; Roni et al. 2018; but see ISAB 2011). Traditional ecological knowledge offers a holistic perspective, and an understanding that organisms rely on and help each other. Using TEK, tribal restoration fulfills the promise of reciprocity and prioritizes community restoration.

Traditional ecological knowledge can also be explored through the Pacific Lamprey, another important First Food of the CTUIR people. Miller (2012) noted that TEK of different Pacific Northwest tribes recognized two “kinds” of lamprey. One a longer, lighter-colored lamprey that moves primarily at night and the other a shorter, darker lamprey that moves farther upriver. The two forms of lamprey were harvested and used in different ways by various regional tribes, depending on their home river and proximity to the ocean (Miller 2012). These two types of Pacific Lamprey are now understood to be two genetically distinct life forms, ocean-maturing (longer, lighter) and river-maturing (shorter, darker) (Parker et al. 2018). Traditional ecological knowledge, in this case, preceded western science by many decades in understanding the ecological differences in this culturally-valued species.

From reciprocity to management action

Using reciprocity to understand the importance of a community of supportive organisms in sustaining First Food resources, and to understand the importance of a community of First Foods in sustaining populations of humans, the CTUIR DNR has developed management actions that align closely with the principles that are set forth in policy and the frameworks described in this paper (Figures 2-1, 2-3, 2-4). Population restoration as means to restoring tribal harvest opportunity is implemented in a way that also restores ecological resilience. Quaempts et al. (2018) describe how the concept of reciprocity and the value of community importance, as framed through the River Vision, led to changes in tribal restoration methodology. Tribal restoration practitioners use the five touchstones of River Vision to design reach-scale projects that are directed at reconnecting processes and restoring systemic linkages (Figure 2-3, 2-5).

The river community is not well understood, in terms of ecological roles and feedback loops between trophic levels and organisms. Jones et al. (2008) list a number of critical data needs in describing the aquatic biota touchstone of the River Vision, including information regarding the ecological roles of river community members (e.g., mussels, lamprey, whitefish, trout, and suckers). Few studies have investigated the ecological role of nonsalmonid organisms in western United States rivers compared with those that have focused on salmonids (e.g., Close et al. 2002; Brim-Box et al. 2006; Howard and Cuffey 2006; Lance and Baxter 2011; Limm and Power 2011; O'Brien et al. 2013; Swain et al. 2014; Ismail et al. 2014, 2015; Maine et al. 2016). Where western science has not yet provided a full suite of evidence linking ecological components using defined parameters and finite experimentation, TEK provides alternate pathways to develop a knowledge base for use in conservation, restoration, and resource protection (Petersen 2006).

The focus on restoring key riverine processes, ecosystem services, and community structure emphasizes the role that each trophic level plays in the long-term health and resilience of an ecosystem, even if those values are not yet scientifically understood (Jones et al. 2008). Valuing the organisms and processes that have little or no direct economic or consumptive value outside of tribal culture is one way that the CTUIR DNR honors and fulfills the promise of reciprocity through management action. The CTUIR habitat-restoration projects have focused on salmonids, but they also acknowledge the supportive community on which salmon rely for subsistence and ecosystem services. Similarly, tribal people rely on this community to provide essential ecosystem services so that populations are robust and healthy enough to sustain tribal harvest (Figure 2-6).

In the 1980s, the CTUIR implemented an aggressive program to restore salmonids to the Columbia River system, but they also sought to restore other organisms like Pacific Lamprey in the 1990s and freshwater mussels in the early 2000s as well as other resources of cultural importance (Quaempts et al. 2018). At the time, CTUIR DNR staff worked against regional policy that had been set in place to eliminate “rough fish” (i.e., anything not a salmon or steelhead). The Oregon Department of Fish and Wildlife poisoned lower sections of the Walla Walla and Umatilla rivers in efforts to increase habitat and resources solely for salmonids. This practice highlights a lack of understanding of community relationships between salmonids and other river organisms. First Foods-based management and the River Vision are among tribal policies that have been set in place to remedy this type of myopic resource management. Other regional tribes have also garnered support for the management of culturally important resources. The Wanapums, a mid-Columbia River tribe, were successful in petitioning Grant County, Washington to develop a lamprey management plan as part of the 2008 license renewal for the Priest Rapids dam project, ensuring protection and conservation of this culturally-important resource.

At an international scale, the Columbia River Treaty, ratified and implemented in 1964, is currently under renegotiation. In the latest development of talks on the treaty, tribal organizations are advocating for the inclusion of ecological function as a third objective of the treaty in addition to the current objectives of flood control and power production. The inclusion of ecological function as a pillar of the Columbia River Treaty will set a new standard for collaboration and co-management of shared resources. The narrowness of the original treaty mirrors the narrowness of restoration efforts in the Columbia Basin to date (Hirt and Sowards 2012). Widening the focus can help restoration efforts to achieve more far-reaching goals than harvest numbers (Williams et al. 2006; Rieman et al. 2015). For example, hatcheries produce large numbers of fishes to reach production and harvest goals. However, we have not seen the successful return of fish from those programs into natural production (Lichatowich 2001; Taylor 2001; Rieman et al. 2015). Including habitat restoration into species conservation programs widens the focus; but, it could still be expanded further to include education and cultural knowledge (Quaempts et al. 2018).

Strengthening the connections between TEK, science, and management through adaptive management techniques provides flexible boundaries for management inputs and restoration outcomes (Rieman et al. 2015; Quaempts et al. 2018; Figure 2-1, 2-5). Including and prioritizing TEK in conservation plans and resource management plans, tribal and nontribal, as well as integrating indigenous knowledge and education into the context of Columbia River Basin restoration will result in comprehensive management and collaborative efforts between co-managing agencies. As Kimmerer (2002) calls for the incorporation of TEK into scientific education, I call for the incorporation of TEK, and the concepts of community and reciprocity, to be integrated into the greater context of resource management, habitat and species restorations, and ecological conservation so that “we will all be richer for the effort.” The following chapters serve to demonstrate the integration of community, reciprocity, and TEK in conservation aquaculture, resource management, and restoration action.

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Figures

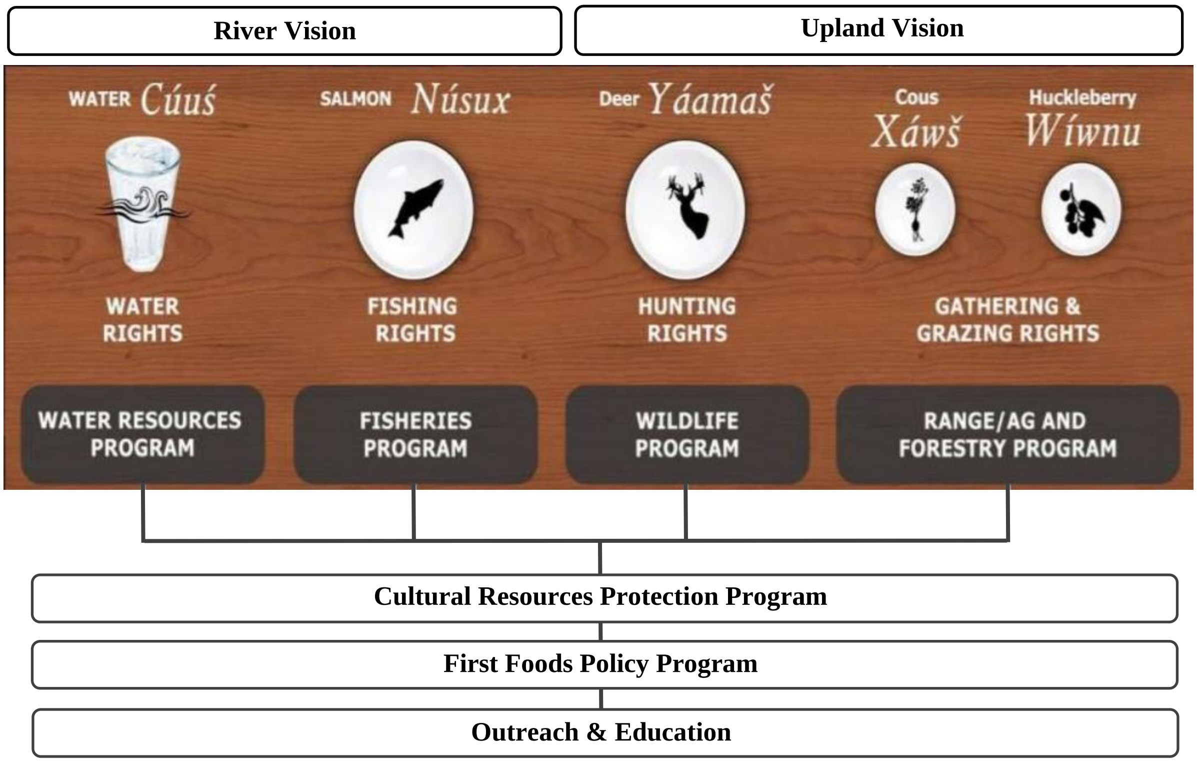

Figure 2-1. Confederated Tribes of the Umatilla Indian Reservation (CTUIR) First Foods serving order (water, salmon, deer, cous, and huckleberry) and supportive policies and programs. River Vision and Upland Vision serve as guiding management frameworks for the aquatic and terrestrial communities, respectively. Below the wood panel, two umbrella programs (Cultural Resources Protection, First Foods Policy) support and provide overarching guidance so resource-specific programs can work collectively toward resource management goals. Outreach and education are used to facilitate sharing traditional methods, stories, and ceremonies within the CTUIR and to increase awareness of the importance of First Foods to the general public. Figure modified from Shippentower 2019.

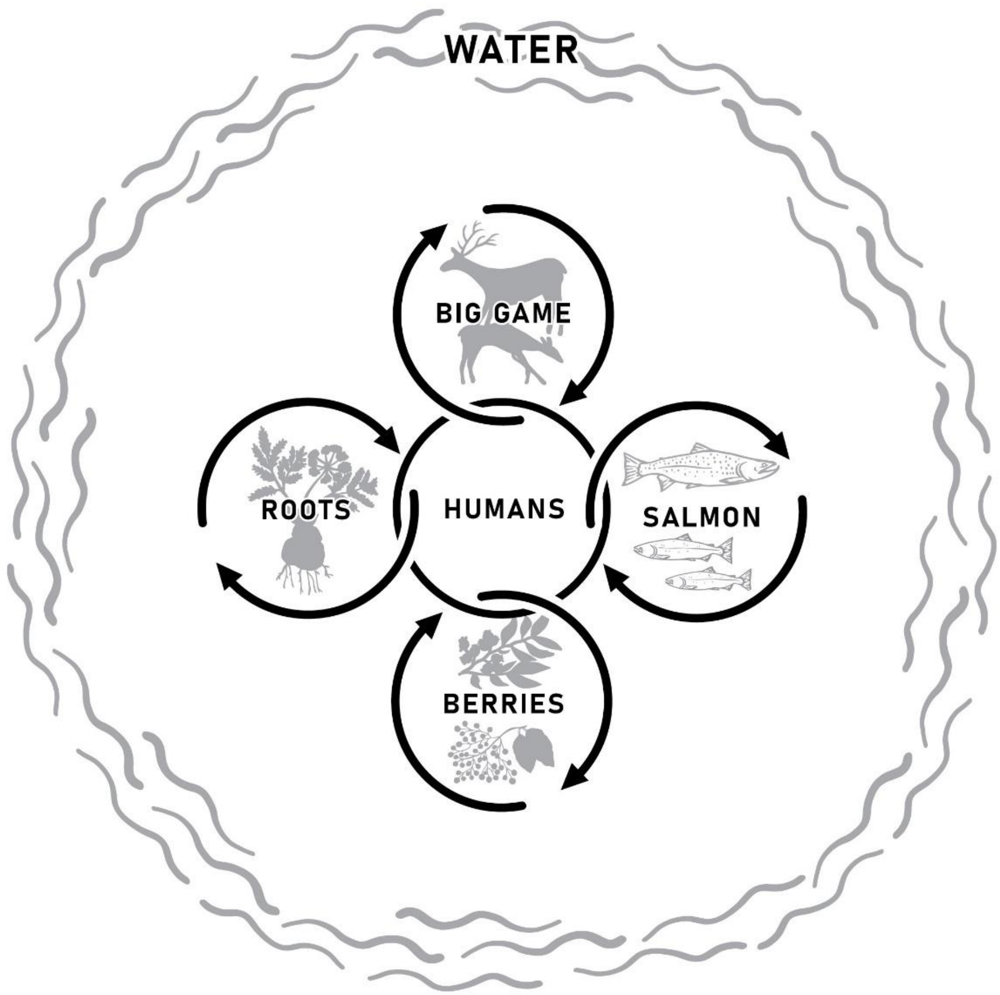

Figure 2-2. Reciprocal relationships between First Foods and humans. Foods provide resources to humans, while humans care for and manage foods for continued use. Figure by the author and J. Hooghkirk.

River Vision

Touchstones

- Hydrology: quality and quantity of water, to provide clean, abundant water for human and food communities
- Geomorphology: flow and dynamic processes that allow the river to move and transport water and sediments
- Habitat and network connectivity: longitudinal and lateral connection of habitats and resources
- Riverine biotic community: protection and restoration for native organisms that serve as or support First Foods
- Riparian vegetation: healthy native vegetation to provide habitat and support First Foods

Reciprocity

- Care for foods that care for the people
- Consideration for the role each First Food plays in the sustainment of the others
- Restoration of processes for a self-sustaining river system

Community

- Acknowledgement of the river food community supportive to highly valued food organisms like salmon and steelhead
- Inclusion of organisms historically but not currently used as food resources
- Valuation of process and function of river community organisms over economic valuation

Figure 2-3. River Vision (Jones et al. 2008). The touchstones of River Vision highlight the connections between each part of the river and the functioning of the whole system. Reciprocity and community in River Vision highlight reciprocal relationships between aquatic organisms and humans that benefit the food–human system.

Upland Vision

Touchstones

- Soil stability: physical, chemical, and biological capacity to provide ecological processes and support food productivity
- Hydrologic function: water storage and provision to sustain diverse food communities across varied landscapes
- Landscape pattern: diversity of ecosystems and ecological processes that allow continuity and movement of food resources
- Biotic integrity: structure and interactions of the biological community to promote resilience and provide ecological services

Reciprocity

- Care for foods that care for the people
- Consideration for the spirit of the resource, to promote respect and mutual support
- Ensure healthy, resilient, and dynamic ecosystems for the provision of First Foods

Community

- Health of the First Foods promotes the health of the human community
- Interconnectedness between each to the health and stability of the ecosystem and the human community
- Management of touchstones toward a functioning and resilient ecosystem to support the relationship between humans and First Foods

Figure 2-4. Upland Vision (Endress et al. 2019). The touchstones of Upland Vision highlight the connections between each part of the upland ecosystem and the functioning of the whole system. Reciprocity and community in Upland Vision highlight reciprocal relationships between plants, animals, and humans that benefit the food–human system.

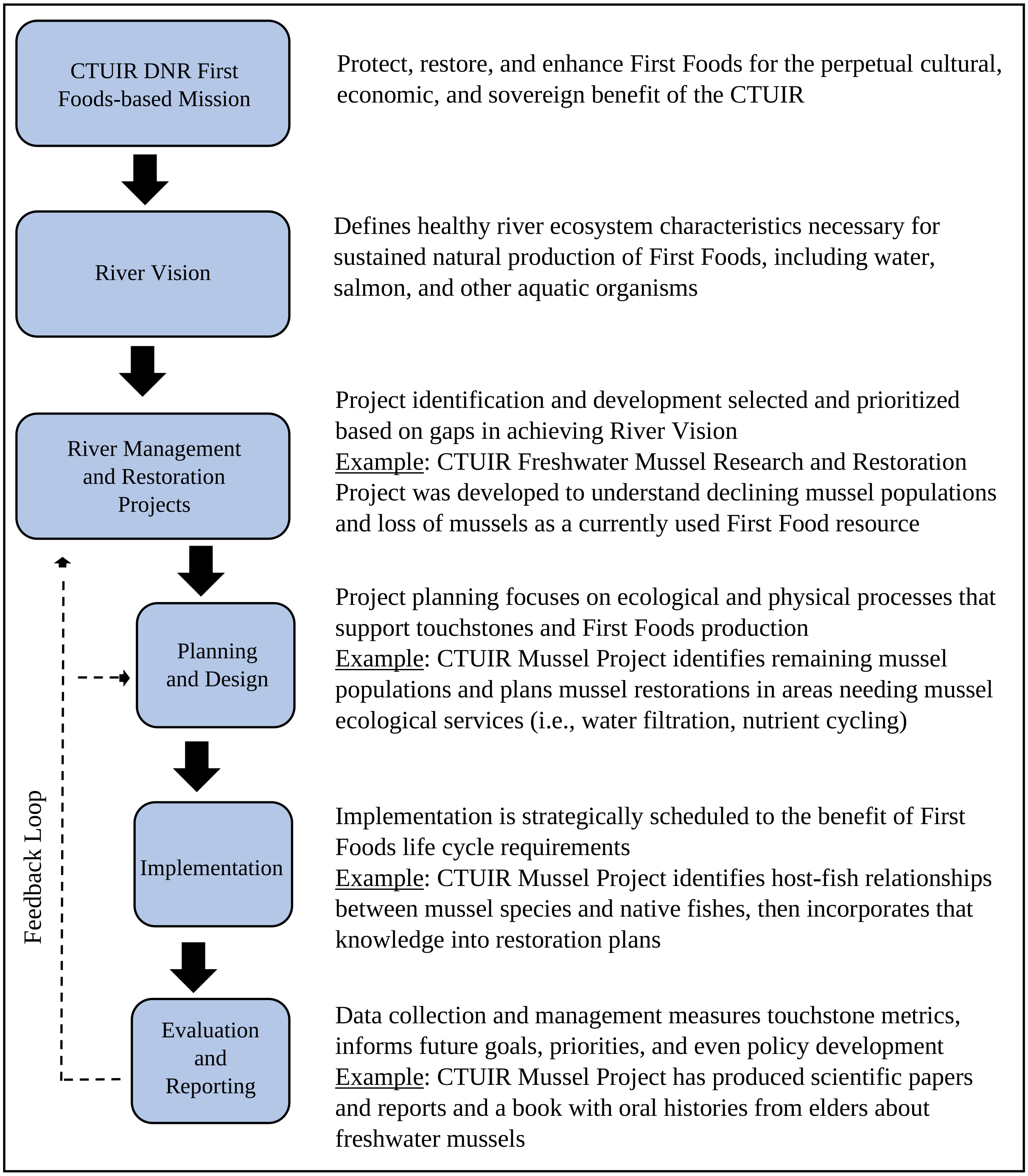

Figure 2-5. Flowchart describing the relationship between the CTUIR Department of Natural Resources mission, vision, and management. The examples show how the CTUIR Freshwater Mussel Research and Restoration Project (CTUIR Mussel Project) management incorporates and fulfills the principles of the mission statement and River Vision principles. Flowchart adapted from Endress et al. 2019, used with permission.

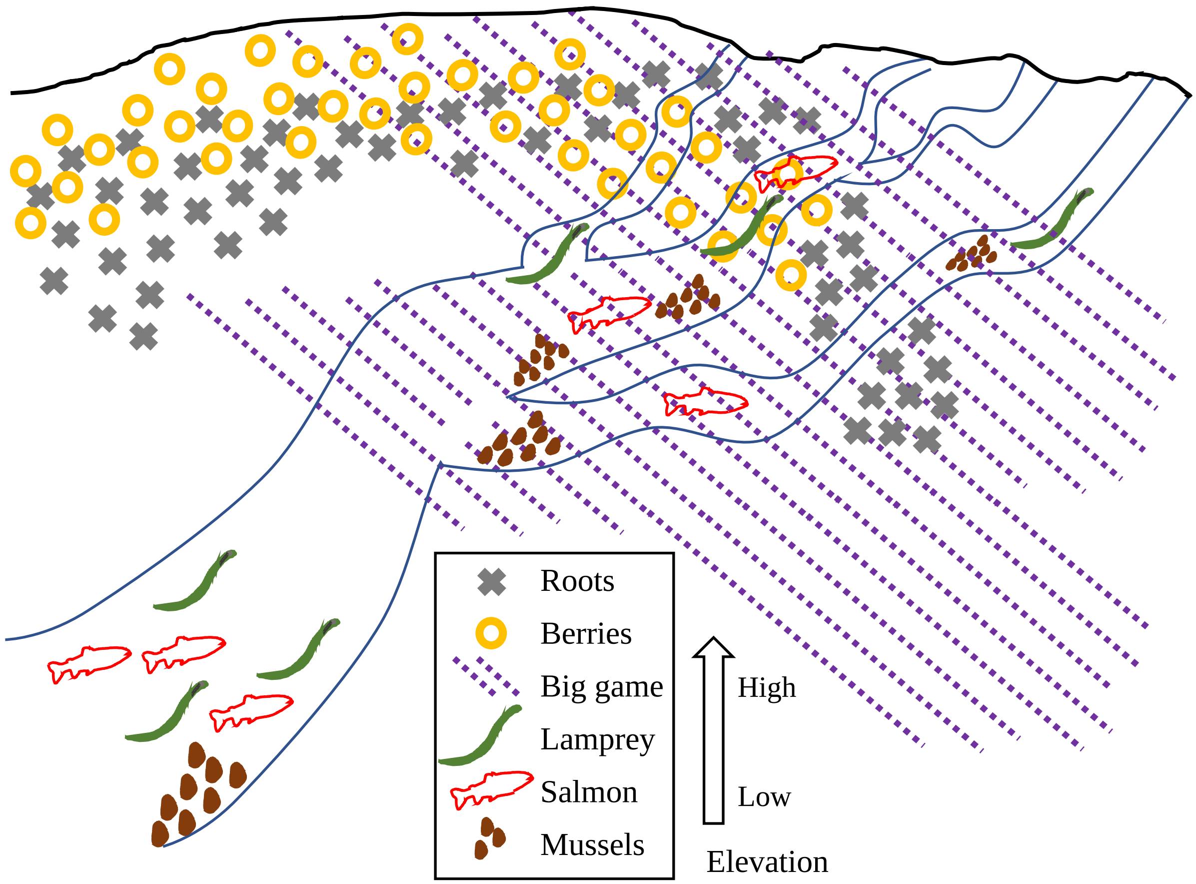

Figure 2-6. First Foods (roots, berries, big game, fish, lamprey, and mussels) across a landscape. A river, outlined in blue, flows from higher elevation to lower elevation. Salmon, trout, lamprey, and other fishes move throughout the river laterally (from the main stem to tributaries) and longitudinally (up and down river, depending on life history and stage). Mussels occupy stable habitats in the river system across a range of elevations. Big game like elk and deer move across the landscape through the full elevation gradient, using various habitats seasonally. Berries are found in riparian areas near streams and rivers, and in forests in mid to high elevations. Plant root foods like cous and celery root are found in higher-elevation grasslands. Foods occur together and separately across the landscape, providing reciprocal services to each other and people as a collective community. Figure by the author.

CHAPTER 3: USE OF NATIVE AND NONNATIVE FISH HOSTS BY THE WESTERN FRESHWATER MUSSEL *Anodonta californiensis* (CALIFORNIA FLOATER) IN THE COLUMBIA RIVER BASIN

Abstract

Many populations of native freshwater mussels such as the California Floater *Anodonta californiensis* are declining in the Columbia River Basin in the western United States. The reason(s) for these declines are unknown, but the increased density of nonnative fish, especially piscivores that displace and reduce the abundance of native fish species, could be negatively influencing the reproductive success of *A. californiensis*, which uses native fishes to complete its life cycle. While *Anodonta* spp. can use nonnative fish as hosts with limited success, the extent of this for *A. californiensis* is not well understood and is the focus of this study. I determined if certain nonnative fishes can host the glochidia (larvae) of *A. californiensis* and quantified differences in host effectiveness (number of juveniles produced) between native and nonnative species.

Overall, native fish species hosted an average of 107.4 ± 39.9 (mean ± SE) juvenile mussels per fish while nonnative species hosted an average of 5.5 ± 4.9 juveniles per fish. This conclusion was unchanged when standardized for fish size; native fishes produced an average of 1.0 ± 0.1 juveniles/mm^2, while nonnative fishes produced an average of 0.16 ± 0.1 juveniles/mm^2 of attachable surface area. Because the nonnative Channel Catfish *Ictalurus punctatus* did not produce any juvenile mussels, it was identified as a nonhost species for *A. californiensis*. The other nonnative fishes tested were determined to be poor or marginal hosts. All native fishes were determined to be primary or secondary hosts for *A. californiensis*. The native fish that produced the highest number of juvenile mussels were sculpin *Cottus* spp. and Redside Shiner *Richardsonius balteatus* with an average of 196 and 151 juveniles per individual of each species, respectively. I conclude that because nonnative fishes are poor hosts, they do not contribute significantly to the reproduction of *A. californiensis* and, because they directly prey on and reduce the abundance of native host fishes and mussels, they contribute to the decline of native mussels in the Columbia River Basin. Future conservation plans for *A. californiensis* must consider the potential negative influence of nonnative fishes.

Introduction

North America boasts the greatest species diversity of freshwater mussels in the world (298 currently recognized species), with much of that concentrated in hot-spot locations like the southeastern United States (Lydeard et al. 2004; Williams et al. 2017). Western North America is considerably less diverse than the rest of the continent, with only seven currently recognized species (Williams et al. 2017). Native freshwater mussels contribute significantly to healthy riverine processes, especially food-web interactions, nutrient cycling, and water quality improvement (Vaughn 2017). However, populations throughout the Columbia River Basin have declined and continue to do so, substantially reducing the ecosystem services that are provided by mussels (Blevins et al. 2017). In addition, native freshwater mussels are an important First Food resource, a traditional food of ecological and cultural importance, for Native American tribes in the Columbia River Basin (CTUIR 2015).

Anodonta californiensis occupies mid- to low-elevation rivers, streams, lakes, and reservoirs in Western North America, and had a historic distribution from Southern California, USA, north to British Columbia, Canada, reaching as far east as Wyoming, USA. However, the species has declined by 9% in extent throughout its historic range and has declined 33% in watershed area occupancy (Blevins et al. 2017), causing the IUCN Red List to update the status to “vulnerable” for this species (IUCN 2020). For example, a recent study summarizing population assessments in the Middle Fork John Day River in 2005 and 2015, showed that *A. californiensis* occupied only 7 of 10 historic sites (Maine et al. 2017). Similar declines have been observed in many mussel populations regionwide, however, there are few agencies in the Columbia Basin that document or monitor freshwater mussels in any capacity.

Because of such declines across the Columbia River Basin, harvest (for meat and shell material), a treaty right that is reserved for area tribes, is no longer possible (Brim Box et al. 2006; CTUIR 2015). This has resulted in significant cultural losses for Columbia Plateau tribes, and ecosystems are negatively affected by the loss of the important ecological services that are accrued from healthy populations of mussels (Brim Box et al. 2006; CTUIR 2015; Vaughn 2017). One Columbia Basin tribe, the Confederated Tribes of the Umatilla

Indian Reservation (CTUIR), began research on western freshwater mussels in 2002 and is, to date, the only agency in the western United States with staff dedicated to research and restoration of native mussel species. The understudied relationships between mussels and native fishes are important to the conservation and recovery of declining mussel populations within ceded tribal territory, a goal of the CTUIR Freshwater Mussel Research and Restoration Project.

The reason(s) for the global decline of native freshwater mussels is currently unknown but may be related to the general decline of global native fish populations (Allan and Flecker 1993). Because mussels require a host fish for the transformation and transport of their juveniles, they can only exist in the presence of their host-fish population. For some mussel species, declines in genetic diversity and recruitment have been linked to declines in the abundance and diversity of host fish species (Ortmann 1920; Watters 1992; Vaughn and Taylor 1999; Bauer 2001; Haag 2012); however, the research is limited and the relationship is not clear in the western United States.

With limited and declining native mussel and fish populations, understanding mussel–host-fish relationships is important to develop effective species-specific conservation and management plans. Critical life history information, especially with respect to host-fish usage, is lacking for most western mussel species. *Anodonta californiensis* is considered to be a host generalist because it uses a variety of fishes to host its glochidia (larvae) (Haag 2012; O'Brien et al. 2013; Maine et al. 2016). However, little research has been conducted on the use of nonnative fishes as hosts by this mussel species. This is important because the Columbia River Basin is home to increasing populations of nonnative fishes, especially centrarchids such as Smallmouth Bass *Micropterus dolomieu* and Bluegill *Lepomis macrochirus*, which may be better adapted than native fishes to warming rivers and also able to outcompete native fishes for space and resources (Naiman et al. 2012; O'Brien et al. 2013; Modesto et al. 2018). In addition, nonnative fishes directly prey on mussels, especially thin-shelled species like *Anodonta* spp. (Haag 2012; Moore et al. 2019). Some host-fish experiments using western *Anodonta* spp. found nonnative fishes to be poor hosts at best; however, the results appear to be region- and species-dependent (d'Eliscu 1972; Lang 1998; Haley et al. 2007; O'Brien et al. 2013). If nonnative fishes serve as poor hosts for *Anodonta* spp. in the Columbia River Basin, future conservation efforts for the mussels may be limited by increasingly fragmented populations of native fishes (Haag 2012; O'Brien et al. 2013; Modesto et al. 2018). For these reasons, I sought to compare *A. californiensis* host-fish use between native and nonnative fish species in the Columbia River Basin. I hypothesized that native fishes would be more effective hosts for *A. californiensis*, producing more juveniles per mm^2 than nonnative fishes.

Methods

To quantify native and nonnative fishes in the reproduction of *A. californiensis*, I conducted a laboratory experiment in which naïve (not previously exposed to mussel larvae) host fish were exposed to mussel glochidia and then incubated and observed for number of juvenile mussels produced. Nonnative fish (Largemouth Bass *Micropterus salmoides*, Bluegill, Channel Catfish *Ictalurus punctatus*, Eastern Mosquitofish *Gambusia holbrooki*, and Black Crappie *Pomoxis nigromaculatus*) were obtained from a local pet store (Channel Catfish: City Zoo LLC, Walla Walla, Washington) or a hatchery (others: Jonah's Aquarium, Delaware, Ohio), while, native fish (Speckled Dace *Rhinichthys osculus*, Redside Shiner *Richardsonius balteatus*, and sculpin *Cottus* spp.) were collected during salmonid screw-trapping efforts in the Walla Walla River, Washington, courtesy of the CTUIR Walla Walla Basin Salmonid Monitoring and Evaluation Project. Rainbow Trout *Oncorhynchus mykiss* were obtained from the Tucannon Fish Hatchery (Washington Department of Fish and Wildlife, Pomeroy, Washington). Because fish are known to develop resistance to mussel larvae after previous exposure (Rogers-Lowery and Dimock 2006), nonnative fish and Rainbow Trout were obtained from sources that were not previously exposed to mussels, while native fish (except Rainbow Trout) were obtained from locations in the Walla Walla River where freshwater mussels are absent (CTUIR, unpublished data). In addition, all of the fish were visually inspected to ensure that they had no existing glochidial infection and were held for a minimum of 5 days prior to the start of the experiment.

Collection of glochidia and inoculation of host fish

Anodonta californiensis mussels were located via snorkeling and collected by hand from the Snake River at New York Island, Columbia County, Washington (46°36'01.8"N 117°52'34.3"W). Five *A. californiensis* mussels were collected on 11 July 2019 and returned to the laboratory for monitoring (Figure 3-1). The water temperature during collection was

22.4°C. On 29 July, I identified two fully gravid mussels from the five that had been collected. Each mussel was carefully inspected for gravidity by gently prying the valves open 1–2 cm to observe the gills. *Anodonta* use the outer pair of gills as marsupial gills, in which fertilized eggs that develop into glochidia are stored. Gravid *A. californiensis* were differentiated from nongravid mussels by the presence of swollen, yellow or orange outer gills (O'Brien et al. 2013; Maine et al. 2016). Under a light microscope (Leica Microsystems, Inc., Buffalo Grove, Illinois), a small sample of glochidia (approximately 20 individual glochidia) from each fully gravid mussel was observed to determine the state of glochidia development. If the glochidia were snapping, they were classified as fully developed (Zale and Neves 1982). To further confirm the viability of the glochidia, a few grains of table salt (NaCl) were introduced to the sample and the reaction of glochidia was observed. Those that were viable snapped shut when they were exposed to NaCl, while those that were not viable remained open (Zale and Neves 1982). For mussels with viable glochidia, the entire contents of both outer gills were collected. This procedure was completed by rupturing the gill membrane with a small scalpel incision and flushing the gill with a squirt bottle. Glochidia were captured in a glass beaker and made into a slurry by bringing the final volume to 900 mL with distilled water.

I tested four native (n = 35) and five nonnative (n = 77) fish species for a total of 112 fish. Fish were held in tanks by species (not individually) on a Pentair Z-Hab AHAB unit (Pentair Aquatic Ecosystems, Apopka, FL) in 9-L polycarbonate tanks held at a constant water temperature of 19.0 ± 1°C for the duration of the experiment. Fish ranged in size between species (mean sizes ranged from 27.5-110.8 mm in length) but fish of a single species were similarly sized (Table 3-1). The number of fish used for inoculation varied between species (N = 6–21 fish) due to differences in availability (Table 3-1).

To inoculate potential host fish with glochidia, 100 mL of the glochidia slurry was added to 10 L of water in a 20-L bucket into which fish were introduced for 10 to 12 minutes. This period was chosen to ensure sufficient contact time but avoid over-inoculation. Fish of one species were removed from their holding tank and inoculated as a batch separately in a freshly prepared inoculation chamber. Given the high density of glochidia used in the inoculation (approximately 600-900 glochidia/mL), the variable fish sizes and inoculation times should not have influenced the glochidial attachment on potential host fish (e.g., Haag 2012; Hart et al. 2018). The inoculation bucket was vigorously aerated with two air stones that were connected to the laboratory air system to ensure that the glochidia circulated throughout the bucket. This was supplemented by using a turkey baster periodically to resuspend any settled glochidia from the bottom of the bucket. Throughout the inoculation process, the fish were closely monitored for signs of stress (e.g., rubbing against bucket sides, and/or labored respiration). None of the fish exhibited these behaviors and all of them completed inoculation and were included in the experiment.

After inoculation, the fish were returned to their original holding tank and tanks were monitored daily for fish mortality. Water quality parameters (temperature, pH, and ammonia) were monitored periodically and adjusted as necessary to remain within the recommended parameters for each fish species as suggested by Losordo et al. (1998) and Ebeling and Timmons (2012).

Collection of juvenile mussels and assessment of effectiveness of host fish

I began monitoring for juvenile mussels daily in each tank starting 3 days after inoculation. Each tank was fitted with a PVC filter cup, designed to collect juvenile mussels dropped from fish hosts. Each filter cup consisted of a 7.6-cm (3”) square of 150-µm mesh sandwiched between 2 pieces of 5-cm diameter (2”) polyvinyl chloride (PVC) pipe. The AHAB tanks provide controlled water flow from the top of each tank to the bottom, where the juvenile mussels collected after dropping from their fish hosts. The juvenile mussels were picked up and transported into the filter cups by increasing the water flow into each tank for 5 minutes daily prior to removing the filter cups. Each filter cup was removed and the contents flushed with water into a petri dish for examination with the aid of a stereomicroscope. The juvenile mussels from each fish species’ tank were identified and counted.

The effectiveness of each host-fish species was assessed by using the following criteria: (1) production of any juvenile mussels, (2) average production of juvenile mussels per fish for each species, and (3) production of juvenile mussels per average area (mm^2) of available fin-attachment surface per fish species. The latter was used as a standardized measure to compare among fish hosts because *Anodonta* spp. primarily attach to the fins of fish (Figure 3-2; Lefevre and Curtis 1910; Haag 2012). I modified the methods from Martel and Lauzon-Guay (2005) to measure the fin surface that was available for glochidial attachment for each fish species by measuring the total fin area for five randomly selected fish from each species to obtain an average available surface area (mm^2). Within each species, fish sizes did not vary widely (Table 3-1) and the sizes used were representative of those that would potentially encounter mussel larvae in the wild (Haag 2012). For each fish species, I then quantified the total number of juveniles produced, divided it by the number of fish tested, and calculated the number of juvenile mussels produced per mm^2 of attachment area.

$$\text{Juveniles/mm}^2 = (\text{total \# juveniles produced per species / \# of fish used}) / \text{average mm}^2 \text{ fin surface available per species}$$

Fish host status

To determine the host status of fish in this experiment, I used criteria modified from Levine et al. (2012) and O'Brien et al. (2013); see Table 3-2 below. Because this experiment was conducted in the laboratory and did not include a field component, the hosts that were identified are considered "physiological" hosts. In contrast, a fish host that is identified in a field study that is not identified in the laboratory would be considered an "ecological" host, while a host that is identified in both the field and laboratory is classified as a "confirmed" host (Levine et al. 2012; Maine et al. 2016).

Statistical Analysis

I used Welch's two-sample *t*-test to compare the number of juveniles per mm^2 attachment surface between native and nonnative fishes (average per species, not per fish) because fish were kept in tanks by species and not individually. The data were normally distributed as identified by Shapiro-Wilk's test ($p = 0.209$). Because Welch's *t*-test is robust to unequal variances, I did not assess homogeneity of variance prior to the analysis (Beckerman et al. 2017). I used R (version 3.5.1, R Core Team 2020) and the following packages to complete the analysis: STATS (version 3.5.1, R Core Team 2020) and GGPLOT2 (version 3.2.1, Wickham et al. 2020).

Results

Juvenile mussels were found on the tank filter screens starting 5 days after inoculation and continued until 18 days postinoculation (Figure 3-3). All of the native fish species produced juveniles (Figure 3-4), while one nonnative, Channel Catfish, failed to produce any. In general, native fish produced far more juveniles (107.4 ± 39.9 juveniles/fish; mean ± SE) than did nonnative fish (5.5 ± 4.9 juveniles/fish). This conclusion was supported when standardized for fish size; native fishes produced an average of 1.0 ± 0.1 juveniles/mm^2, which was significantly greater ($t = 6.11$, df = 7, $p < 0.001$) than the average of 0.16 ± 0.1 juveniles/mm^2 that was produced by nonnative fishes (Table 3-1).

Sculpin produced the highest number of juveniles per fish (196.5/fish), while Speckled Dace produced the lowest number of juveniles per fish (28.7) of the native species tested (Figure 3-3A). For the native species tested, Redside Shiner produced the highest number of juveniles per surface area (1.34/mm^2), and Rainbow Trout produced the lowest number of juveniles per surface area (0.88/mm^2). Of the tested nonnative fishes that did produce juveniles, Black Crappie produced the highest, an average of 25.1 juveniles per fish (0.31/mm^2), while Largemouth Bass produced the lowest average number of juveniles (0.07/fish, 0.01/mm^2; Figure 3-3B).

Based on the criteria in Table 3-2, I identified native species sculpin, Redside Shiner, and Speckled Dace as primary hosts in this study, while Rainbow Trout was identified as a secondary host for *A. californiensis*. Nonnative fishes Black Crappie and Eastern Mosquitofish were identified as marginal hosts, Largemouth Bass and Bluegill were identified as poor hosts, and Channel Catfish was identified as a nonhost (Table 3-1).

Discussion

Native fish were the most suitable hosts for *A. californiensis* larvae although some nonnative fishes also hosted larvae and produced juvenile mussels. Nonnative fishes like Largemouth Bass, Bluegill, Eastern Mosquitofish, and Black Crappie were poor or marginal hosts, producing few juvenile mussels per fish compared with native fish. O'Brien et al. (2013) found that the nonnative Yellow Perch *Perca flavescens* was also a nonhost. Other studies have found similar poor results for nonnative fish hosts. For example, Haley et al. (2007) reported poor attachment or encystment of *Anodonta* spp. glochidia on nonnative fishes (1.0–5.2 average per fish) including Black Crappie, Bluegill, Green Sunfish *Lepomis cyanellus*, and Largemouth Bass but did not observe transformation, except in Green Sunfish which produced 1 juvenile, suggesting that the species is a poor host. Other nonnatives including mosquitofish, Smallmouth Bass, Common Carp *Cyprinus carpio*, and Golden Shiner *Notemigonus crysoleucas* were not observed to have any *Anodonta* spp. glochidial attachment (Haley et al. 2007). The results of one study (d'Eliscu 1972) indicated that Western Mosquitofish *Gambusia affinis* serves as a host for *A. californiensis* [this author did not provide numerical data with which to quantify host status using the criteria presented in this paper]; however, the Eastern Mosquitofish used in this study were identified as a marginal host. The top-feeding behavior of mosquitofishes could limit the possibility that they would encounter *A. californiensis* near the benthos in the wild. Channel Catfish did not produce any juvenile mussels during this study and was determined to be an unsuitable host species for *A. californiensis*. My results are congruent with those of other studies showing that some nonnative fishes can host *A. californiensis* and other native freshwater mussels (Huber and Geist 2019) but are poor producers of juvenile mussels.

Nonnative fishes can displace native fishes, reducing the ability for a mussel to encounter a suitable host. This is problematic—especially for a host generalist like *A. californiensis* because they broadcast their larvae as a host-infection strategy. Furthermore, this species' method of broadcast spawning is called passive entanglement because glochidia are connected within sticky mucous webs or strands that become tangled around the fish as it swims near, providing a means for the glochidia to attach to the fins of the fish (Haag 2012; O'Brien et al. 2013). Glochidia of *Anodonta californiensis* only survive outside the female mussel for several days, at most (d'Eliscu 1972; O'Brien et al. 2013), and once attached to a host, they cannot move to another fish host. This specificity to native fishes and the inability to move to another fish once attached means that as native fish decline so do the reproductive opportunities for mussels.

The mussel–host-fish interaction in the wild, especially for broadcast larval release, is a by-chance process. Though not well studied, the abundance of host fish is thought to be positively linked to mussel recruitment (Vaughn and Taylor 1999; Haag 2012). When the host-fish abundance is low near a mussel population, reproduction will not occur or will be very low. Even under the best circumstances (e.g., an adequate host population around a dense mussel bed), reproduction may still result in low numbers of juvenile mussels. For example, an individual female mussel may only produce 0.1–1.3 juveniles/year, despite releasing hundreds of thousands or millions of larvae (Young and Williams 1984; Haag 2012). Additionally, the passive-entanglement, broadcast larval-release strategy is likely more reliant on high host abundance than on other larval-release strategies like lures or conglutinates, which entice a host to have close interactions with the gravid mussel (Haag 2012). Therefore, it is particularly important for broadcast spawners like *A. californiensis* to co-occur with adequately abundant populations of suitable host fish (Haag 2012).

Nonnative fishes also negatively affect native fish populations through predation or via competition for food (Vander Zanden et al. 1999; Jackson 2002; Sharma et al. 2009). Specifically, in the Columbia River Basin, salmonid production responded positively to the removal of nonnative smallmouth bass, signaling negative interactions between native and nonnative fishes (Harvey and Kareiva 2005). *Anodonta californiensis* co-occurs in the Snake River, Columbia County, Washington, with Smallmouth Bass, carp, and Bluegill, all nonnative fish. This study identified Bluegill as a poor host for *A. californiensis*, and further, some *Lepomis* sunfishes like Bluegill feed directly on mussels, especially small or young individuals (Haag 2012). Populations of Channel Catfish coexist with *A. californiensis* in the lower Walla Walla River, Washington, and their status as a nonhost is significant for conservation and restoration efforts in that area. Additionally, catfish have been shown to consume mussels seasonally, especially thin-shelled species like *A. californiensis* (Haag 2012). Nonnative fishes as poor or marginal hosts do not substantially contribute to mussel reproduction and can further damage populations through predation.

Future research on western freshwater mussels should determine the ecological viability of the weak host relationships that were identified with several nonnative species in this study. A field component can confirm a poor host relationship, or show that certain nonnative fishes are physiological hosts only and cannot serve as hosts in the wild due to timing, habitat, or behavioral incompatibilities (e.g., fish location in the water column may not allow for the mussel-host interaction). Habitat requirements of fish hosts may also limit mussel populations (Haag 2012). Certain nonnative fishes are only associated with the benthos at certain times of the year (e.g., benthic spawning behavior of Bluegill), and if that timing does not coincide with mussel gravidity and larval release, the poor host relationship identified in the lab may be nonexistent in the wild. Slight differences in the timing of peak juvenile drop-off between species were observed during this experiment (Figure 3-4), which could be due to micro temperature differences in holding tanks that result from their location in the array or differences in fish physiology. This would be worthwhile to explore in a future experiment because the relationship between temperature and glochidial development is not well understood in *A. californiensis*, though other mussel species have been shown to exhibit temperature-dependent glochidial development (Haag 2012). Physiological differences between fish species are also not well studied as they relate to the host-fish—mussel relationship.

The results of this study indicate priority fish species to maintain in culture for propagation of *A. californiensis* for research and restoration. Fish hosts that can produce large numbers of juvenile mussels could be kept in the laboratory to readily use for propagation. Further, fish hosts that transform juvenile mussels at rates higher than other species (in this study: sculpin, Redside Shiner, Speckled Dace) should be identified and protected in locations where they co-occur with this mussel. Evaluations of the fish community near *A. californiensis* populations should determine if native fishes are available to serve as hosts before considerable effort and expense are spent on mussel restoration at a location. Anthropogenic changes to western rivers have resulted in widespread degradation of suitable habitat quality and quantity for native fishes, limiting host-fish–mussel interactions in the wild. The CTUIR Freshwater Mussel Project has ongoing efforts to restore populations of mussels to ceded tribal territory (CTUIR 2015). This requires the examination of site-specific host-fish communities and potential negative interactions with nonnative fishes. The identification of poor host relationships between several nonnative fishes that are common in the Columbia Basin and *A. californiensis* strengthens the need for simultaneous restoration of native mussel and fish populations. Native fish declines may precede or co-occur with mussel declines, and the restoration of both organisms should be a priority for restoration practitioners. The results of this study support the importance of community in conservation aquaculture and the understanding of ecological relationships for freshwater mussels. These results also reinforce the holistic TEK approach detailed in Chapter 2.

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Tables

Table 3-1. Average number of juvenile mussels produced per fish between native (N) and nonnative (NN) species.

Fish species	N	Status	Host status	Standard length in mm (size range)	Number of juveniles produced/ fish	Juveniles per mm^2 attachment surface	Mean attachment area (mm^2)
Speckled Dace	11	N	Primary	72.9 (69.6–75.4)	28.7	0.92	344.5
Redside Shiner	6	N	Primary	85.2 (80.1–89.1)	151.8	1.34	678.8
sculpin	6	N	Primary	73.7 (68.6–80.9)	196.5	0.95	1238.1
Rainbow Trout	12	N	Secondary	110.8 (106.8–115.9)	52.5	0.88	720.0
Mean ± SE					107.4 ± 39.9	1.0 ± 0.1	525.3± 237.3
Largemouth Bass	15	NN	Poor	43.9 (42.2–45.8)	0.1	0.01	112.1
Bluegill	15	NN	Poor	79.0 (74.1–84.6)	1.1	0.02	1059.5
Channel Catfish	12	NN	Nonhost	51.4 (48.9–53.4)	0.0	0.00	244.7
Mosquitofish	21	NN	Marginal	27.5 (19.1–31.8)	1.4	0.45	67.15
Black Crappie	14	NN	Marginal	92.3 (89.5–96.3)	25.1	0.31	1143.0
Mean ± SE					5.5 ± 4.9	0.16 ± 0.1	745.4 ± 184.5

Table 3-2. Host-fish status criteria, modified from O’Brien et al. (2013) and Maine et al. (2016).

Host designation	**Criteria** (No. of juvenile mussels produced/mm^2 of attachment area)
Nonhost	none
Poor Host	< 0.30
Marginal Host	0.31–0.50
Secondary Host	0.51–0.90
Primary Host	> 0.91

Figures

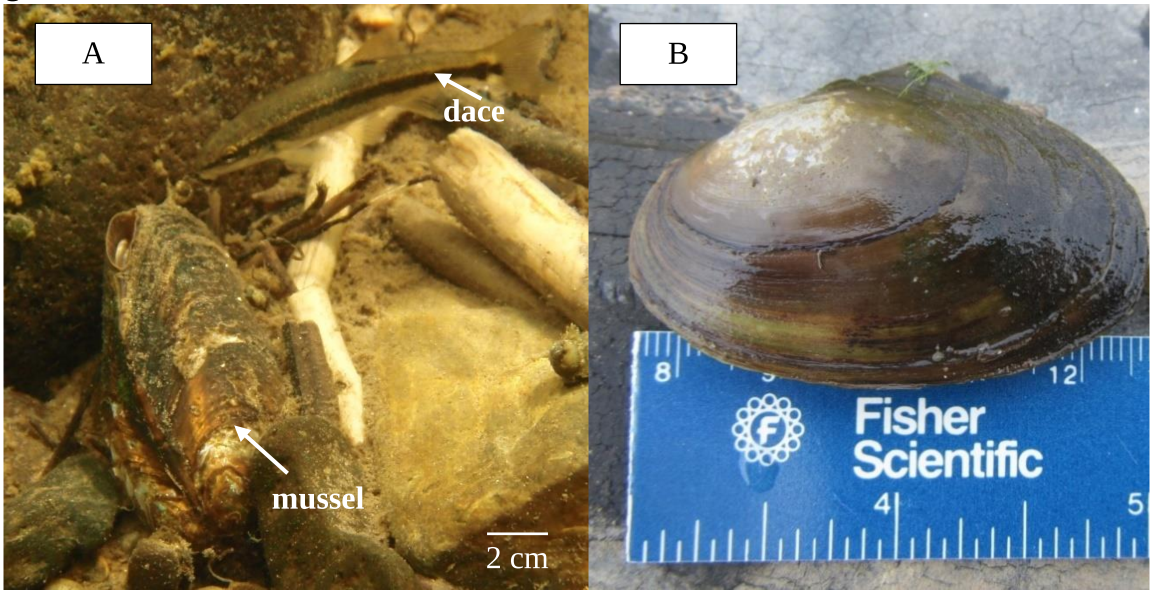

Figure 3-1. *Anodonta californiensis* from the Columbia River Basin; (A) in situ with Speckled Dace and (B) a collected adult. Photographs by C. O'Brien.

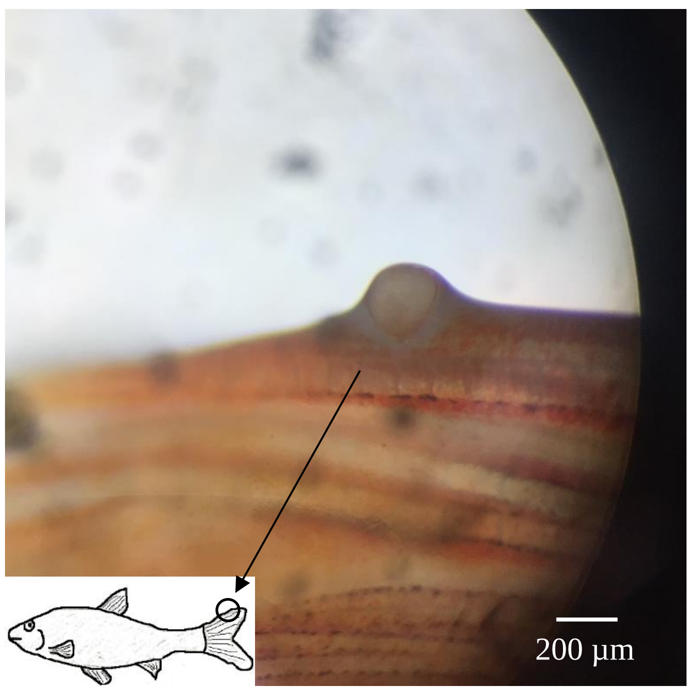

Figure 3-2. *Anodonta californiensis* glochidia attached to Speckled Dace fin. Photograph by the author.

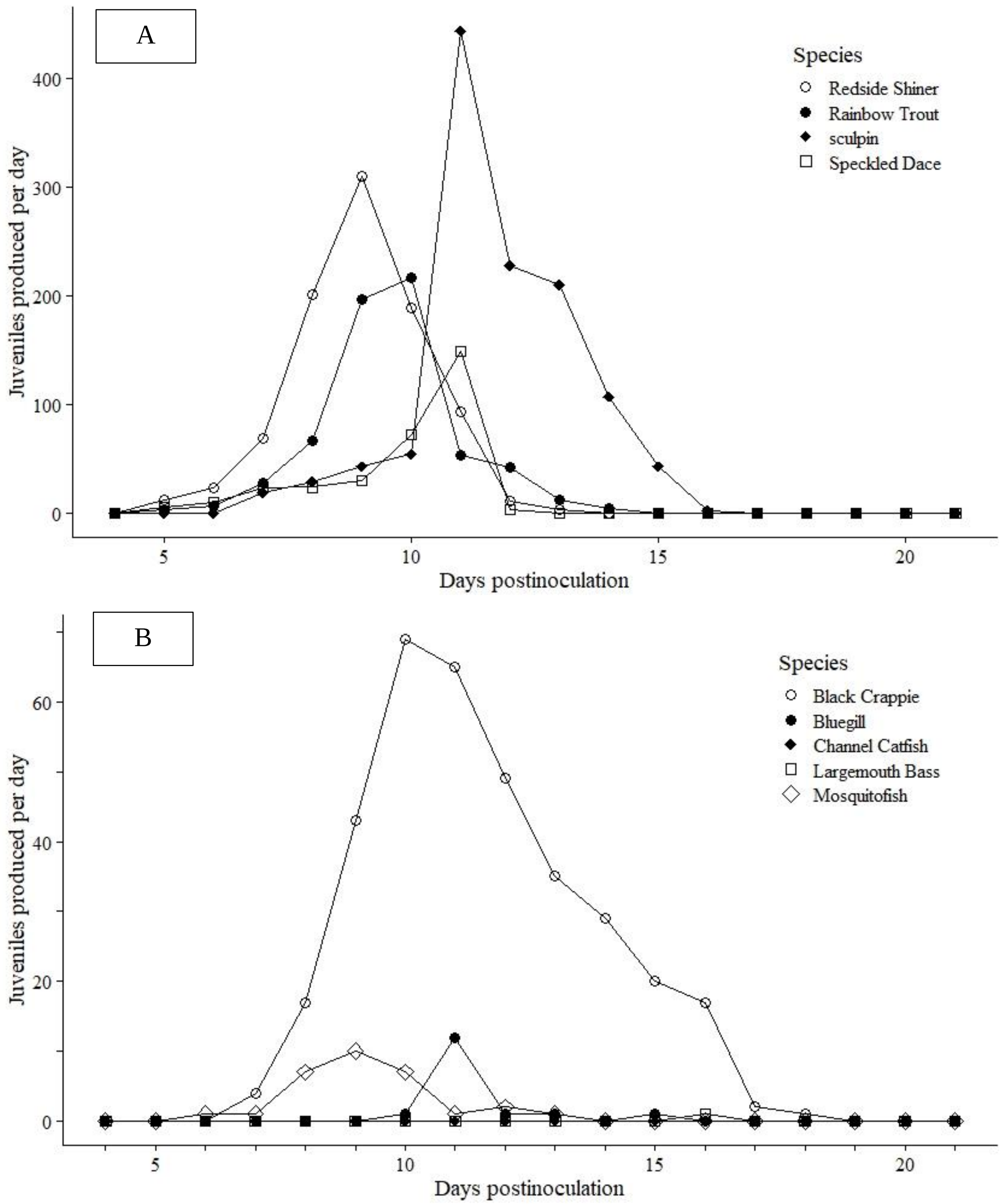

Figure 3-3. Number of juvenile mussels collected per day from (A) native and (B) nonnative fishes that were used during the host-fish experiment. Juvenile collection began at 4 days postinoculation. Note the change of the vertical *y*-axis scale between (A) and (B). Results presented in this figure do not show variation because fish were not held individually, only as species.

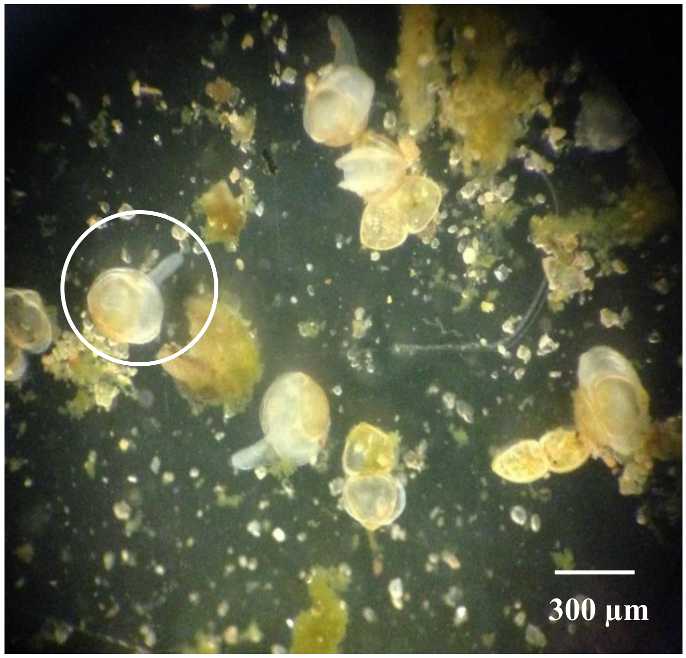

Figure 3-4. Juvenile *Anodonta californiensis* (one in the white circle) produced during this host-fish experiment. Photograph by the author.

CHAPTER 4: POLYCULTURE INCREASES GROWTH IN LARVAL PACIFIC LAMPREY *Entosphenus tridentatus*

Abstract

Healthy river environments sustain diverse communities of fishes, invertebrates, and microorganisms. Conversely, aquaculture environments are typically homogenous, providing little habitat complexity, few interspecies interactions, and a depauperate microbial community. The use of disinfection methods to prevent the development of microorganisms, both harmful and beneficial, further reduces microbial populations in laboratory cultures. Monocultures of Pacific Lamprey *Entosphenus tridentatus* have been reared at the Confederated Tribes of the Umatilla Indian Reservation (CTUIR) facilities since 2012. Because larval lamprey are closely associated with the sediment in their natural environment, they may have an important ecological relationship with the benthic microbial community. To this end, I hypothesized that rearing larval lamprey with other native species would improve growth and survival in a culture setting. To test this hypothesis, I conducted a polyculture experiment in which Speckled Dace *Rhinichthys osculus* were used to increase the diversity of the microbial community in a recirculating system in which lamprey were reared. The size increase of the lamprey larvae was significantly higher (20.52 ± 3.27 mm vs. 16.60 ± 1.25 mm in 274 days, respectively; mean ± SD; $p < 0.05$) in the polyculture than in a replicate monoculture system. This 23.6% higher growth could be a result of direct consumption of microorganisms by larval lamprey or some other synergistic advantage that is conferred by the presence of Speckled Dace. Survival was not enhanced in polyculture; however, the addition of a filter mat to all of the rearing tanks resulted in increased survival relative to previous cultures without the mat. I speculate that the filter mat and polyculture with Speckled Dace likely promoted the development of a larger and more stable microbial community compared with the monoculture system. The improvements in both growth and survival that were observed with the mats support the idea that microbial interactions are important for these benthic, filter-feeding larvae. This work furthers our understanding of the connections between species in river communities and highlights the importance of ecological community linkages among benthic organisms—that is, using a holistic approach in culture techniques.

Introduction

The culture of aquatic organisms is typically conducted in monoculture systems in which chemical disinfection is routinely used to prevent the proliferation of undesirable microbes. These disinfection methods are thought to reduce mortality or production losses due to disease or parasites. Other methods to control pathogens in aquaculture include the use of antibiotics, vaccinations, and biosecurity measures (Moffitt et al. 2004). Beneficial microbial communities in aquaculture systems (e.g., acting as biological filters) can provide environmental bioremediation services, such as the breakdown of waste products (Sabater et al. 2002; Ducklow 2008; Zhou et al. 2009; Ruiz et al. 2019). However, overusing disinfection strategies can render a system susceptible to invasion by opportunistic or antimicrobial-resistant pathogens (Schulze et al. 2006; DeSchryver and Vadstein 2014). Some fish culturists advocate inducing controlled microbial exposures for propagated fish to better prepare them for out-planting to wild environments where they will encounter a wide variety of microorganisms that are both harmful and beneficial (Coutant 1998; Kennedy et al. 1998). For organisms that are closely associated with the benthic environment in the wild, which contains a multitude of microorganisms, laboratory monoculture and typical "clean" practices may not allow for adequate ecological connections with a microbial community, thus suppressing growth and/or survival.

Pacific Lamprey *Entosphenus tridentatus* have suspension/filter-feeding larvae that spend 3–7, or more, years buried in sediments in freshwater (Close et al. 2002) where they rely on flowing water to obtain food and remove waste (Mallatt 1982). Larval lamprey burrow in fine substrates in low-velocity areas to allow for both stable burrow construction and adequate water flow (Torgerson and Close 2004; Dawson et al. 2015). The burrowing activity of larval lamprey can also affect changes in the benthic microbial community, inducing positive effects such as increased nutrient cycling for co-occurring species including salmonids and/or freshwater mussels (Boeker and Geist 2016). Bioturbation, resulting from this burrowing behavior increases oxygen concentrations in the substrate (Shirakawa et al. 2013). Larval lamprey are also known to use carbon and nitrogen from decomposing salmon carcasses at rates that are comparable to two different functional groups (similar ^{15}N uptake as collectors/gatherers and ^{13}C uptake as shredders/grazers), and they do so during times of the year that other organisms do not readily assimilate those resources

(Bilby et al. 1996). Thus, larval lamprey serve a vital role in the aquatic ecosystem and interact with organisms at multiple trophic levels.

The benthic environment is host to a diverse range of macro and microorganisms. In addition to larval lamprey, other fishes use benthic habitats, as do invertebrates like aquatic insects and crustaceans. Bacteria and other microorganisms, existing as biofilm in the benthic environment, play important roles in the river ecosystem by affecting positive changes to the nitrogen cycle, and improving water quality (Winton 2001; Ducklow 2008). Pathogenic microorganisms are also present in healthy river ecosystems, but they are generally maintained at innocuous levels and in balance with beneficial microbes (Coutant 1998; Winton 2001). Pathogenic outbreaks are controlled, in part, through diversity in both microorganisms and fish (i.e., diversity in microbial species/types and diversity in fish immunological responses to pathogenic exposure; Coutant 1998; Moffitt et al. 2004).

Because benthic larval lamprey are filter feeders and part of the meiofauna for a significant length of time, it is not unreasonable to expect them to have important relationships with microorganisms. For example, bacteria, detritus, and diatoms are known to be important food for larval lamprey (Moore and Beamish 1973; Moore and Potter 1976). Although larval lamprey can survive on a diet of only bacteria, no recent in-depth studies have explored this interaction in detail (Moore and Potter 1976; Sutton and Bowen 1994). Larval lamprey likely do not possess or maintain intestinal microbial flora that differ from their environment (Rogers et al. 1980), meaning that the microbial community in a laboratory setting may be even more important for the health and growth of lamprey larvae than for teleosts. In one study, larval lamprey exhibited increased growth and survival when they were supplemented with effluent water from established larval cultures that were thought to be microbially rich (CTUIR, unpublished data; Maine et al. 2017). Other studies also suggest the importance to larval lamprey of a microbial community that results from the presence of other organisms: freshwater mussels, as in Limm and Power (2011), hatchery effluent wastewater from salmonids, as in Barron et al. (2020a), or changes in the substrate microbiome from an anaerobic- to an aerobic-bacteria-dominated community structure (Boeker and Geist 2016). Microorganisms are likely important to larval lamprey for a variety of reasons and developing methods to supplement the microbial community in the laboratory is of increasing research interest.

Recently there has been increasing interest in multitrophic aquaculture and polyculture for many aquatic species (Chopin 2006; Reid et al. 2009; Martinez-Porchas et al. 2010; Ning et al. 2016; Bauer et al. 2019). Such systems have shown remarkable reductions in the environmental effects that are associated with aquaculture practices, such as high nutrient concentrations in effluent water and the development of secondary aquaculture products (e.g., mussels, sea cucumbers; Ahlgren et al. 1998; Kang et al. 2003; Zhou et al. 2006; Slater and Carton 2007). There is evidence that benthic organisms (e.g., sea cucumber and other filter feeders such as bivalves) can use the excess nutrients that result from their feeding activities at finfish or mollusk culture facilities (Jones and Iwama 1991; MacDonald et al. 2011). Thus, larval lamprey as filter feeders that consume detritus as a primary food source could contribute substantial ecological services in polyculture, multi-trophic aquaculture, or aquaponic systems (Dawson et al. 2015). Several recent studies have shown that the larvae of Pacific Lamprey respond positively (increased growth and/or survival) to polyculture (Maine et al. 2017; Moser et al. 2018; Barron et al. 2020a; Moser et al. 2020,) suggesting that bacteria and other microorganisms are important to larval lamprey, though the specific mechanisms remain unknown.

Populations of Pacific Lamprey in the Columbia River Basin have declined precipitously since the installation and operation of main-stem and tributary dams (Moser and Close 2003; Luzier et al. 2011). For restoration and research, larval Pacific Lamprey have been artificially propagated and reared at the Confederated Tribes of the Umatilla Indian Reservation (CTUIR) facilities mainly in monoculture systems, with mixed results. The development of techniques for production-level rearing of larval lamprey is necessary to meet regional research and restoration objectives (CRITFC 2018). The production of large numbers of larval lamprey in recirculating monoculture has not been successful to date. Recent research suggests that lamprey growth and survival improve with the addition of co-reared species (Limm and Power 2011; Moser et al. 2018; Barron et al. 2020a). To investigate this idea, a pilot study was conducted to determine the effects of polyculture on lamprey growth and survival. The inclusion of Speckled Dace *Rhinichthys osculus* as part of a recirculating system improved larval lamprey growth and survival (7.2 µm/day and 57.1% survival) relative to previous years (1.7-2.5 µm/day and 29.0% survival; CTUIR, unpublished data). This pilot study prompted the more robust experiment that is described here. My objective was to test the hypothesis that polyculture significantly increased the growth and survival rates of artificially propagated larval Pacific Lamprey compared with an identical monoculture recirculating system.

Methods

The larval Pacific Lamprey used in this experiment were propagated by the CTUIR from broodstock that was collected at lower Columbia River dams. They were incubated and hatched in a static and temperature-controlled holding chamber with UV-irradiated well water at a density of less than 50,000/m^2 (Lampman et al. 2016). At 32 days postspawning (approximately 18 days posthatching; 9.31 ± 0.22 mm in length [mean ± SD]), 16 groups of 100 larvae were randomly collected from the holding chamber and transferred into 16 replicate 1-L beakers. Each group of larvae was assigned to either a control (monoculture) or a treatment (polyculture) recirculating system. Each beaker contained holding chamber water (irradiated, conditioned well water) and a small section of Spawntex spawning mat (latex coated coconut fiber mat on a polyester net backing; Pentair AES, Apopka, Florida) as cover from light. The beakers were moved to two identical, independent recirculating systems (see details below), each containing nine 10-L Cambro CamWear polycarbonate tanks (53 × 32.5-cm; water depth ~ 7.5 cm; Cambro Manufacturing, Huntington Beach, California), with lids. Each tank held 4-5 cm of washed and sieved sand (63-595 µm) and a tank-sized (~50 × 30-cm) section of spawning mat as substrate and light cover. Water was delivered to each tank from a valved manifold, and temperature for both systems was maintained at 14 ± 1°C (monitored daily). Both systems had a recirculating pump, sump, biofilter, chiller, and weekly water exchange (10% of the system volume). Neither system used a UV-sterilizer.

On each system, eight of the nine tanks were used to house larvae (Figure 4-1). In the polyculture treatment system, one tank was used to house six Speckled Dace with river rocks as substrate. The river rocks were unused and dry for one year prior to use. The flow in this tank was maintained at 18-20 mL/s. The fish were fed 5 days per week with 0.4 g of trout pellet (BioFry, BioOregon, Longview, Washington). Monthly, the Speckled Dace tank was lightly siphoned to remove waste and food debris. The dace were wild-caught (Walla Walla River, Washington, USA) and placed in the recirculating system approximately 2 months prior to the start of the experiment. In the control system (monoculture), one tank was maintained at 18-20 mL/s flow with river rocks as substrate. This tank was "fed" with the same amount and frequency as was the dace tank in the polyculture system starting when the dace were placed into the polyculture system. This tank was lightly siphoned twice monthly instead of monthly because of the increased buildup of uneaten food material. The purpose of this application of food was to control for any excess nutrients that resulted from food that was fed to the dace in the polyculture system.

The larvae were fed a weekly ration of 250 mg/L of yeast (80%; RedStar Baking Yeast, Lesaffre Yeast Corp. Milwaukee, Wisconsin) and Otohime A1 larval fish food (20%; Otohime A1, Marubeni Nisshin Feed Co. Ltd., Tokyo, Japan) (Barron et al. 2016). The feed was blended with culture water and applied as a slurry to each tank. The tank outlets were fitted with a 150-µm mesh filter to prevent the larvae from escaping. These filters were cleaned weekly to prevent clogging after adding food. The filters were changed to 300-µm mesh after the larvae had grown 90 days. The tanks were observed for mortalities that were associated with the water and system transfer for 48 hours after all of the larvae had escaped the acclimation beakers. Any mortalities that were observed during that time were replaced with live larvae from the original holding chamber. All of the larval tanks for both treatment groups began the experiment with a density of 581 larvae/m^2 (100 larvae per tank).

Acclimation

Young Pacific Lamprey larvae are particularly sensitive to water transfer and a shift from static to flowing conditions (Lampman et al. in review). Hence, the larvae were allowed to acclimate slowly from static conditions (in beakers) to flowing conditions (in recirculating polyculture or monoculture systems) by introducing a very low flow (0.5-1.5 mL/s) to each beaker. The larvae swam out of the beakers with the overflow, and the tanks were monitored to observe volitional escape from the beakers under increasing flow conditions. The tanks were also maintained at a low flow for a 14-day acclimation period (2-5 mL/s). Flow was increased in the beakers daily until all of the larvae had escaped the beakers (about 3 days). Flow was increased incrementally in the tanks until it reached 12 mL/s. Flow was then maintained at 12 ± 2 mL/s (checked and adjusted as needed weekly) for the duration of the experiment.

Assessments

The survival of the larvae in the tanks was visually assessed weekly throughout the experiment during feeding, and any obvious mortalities (i.e., larvae dead on sediment surface or on top of filter mat) were counted and removed. Survival in all of the tanks was assessed completely at periodic intervals and a subsample of survivors was photographed to obtain growth data. Survival assessments were conducted by rousting larvae out of the sediment and individually removing them from the tank. This continued until 5 minutes passed without observing a larva. At that time, half of the sediment was removed from the tank, lightly rinsed, and checked for larvae. The sediment remaining in the tank was also lightly rinsed and checked for larvae. If no larvae were present, the removed sediment was returned to the tank. The rinsing confirmed that all of the survivors were counted and also helped reduce the amount of organic matter buildup. This process may have reduced the population of microorganisms that were associated with the sediment, but the filter mats for each tank were not rinsed during this process, preserving part of the microbial community during these assessments. The filter mats were removed during assessments and placed in a bucket with a small amount of water to capture any larvae associated with the mat.

Larval growth in each tank was estimated during the survival assessments by measuring 20-30 randomly-selected individuals from the 1-L glass beaker in which they were briefly held during counting and sediment cleaning. To measure the larvae, they were placed in a petri dish with a ruler in the background and a digital photo was taken. This was repeated three times. I used ImageJ (NIH, version 1.52a; Schneider et al. 2012) to measure 25 larvae from each tank from the three photographs. Only larvae that were straight in the photo (Figure 4-2) were measured. Because of the length of time that it took to assess a single tank, for each assessment period only a few tanks were assessed each day. In general, all of the tanks were assessed in a period ≤ 11 days for any given assessment period (August, October, January). To account for differences in assessment timing, I calculated instantaneous growth rate in addition to the final mean length and percentage of length gain for each treatment.

I used an instantaneous growth rate (G) formula, modified from Wootton (1990), Hopkins (1992), and Crane et al. (2019), to account for measurements that were taken on different sampling dates:

$$G = [\ln(L_2) - \ln(L_1)] / (t_2 - t_1) \times 1{,}000,$$

where G is the instantaneous growth rate (µm/day), $\ln(L_2)$ is the natural logarithm of the average length (mm) of larvae in a given tank at an intermediate or ending assessment period (t_2), and $\ln(L_1)$ is the natural logarithm of the average length of larvae in a given tank at the start of the experiment (t_1).

Water Quality

Water quality parameters (pH, dissolved oxygen, nitrate/nitrite, and ammonia) and temperature were monitored and recorded regularly for the two systems by using a Vernier handheld computer and sensors (temperature, pH, dissolved oxygen; Vernier, Beaverton, Oregon) and colorimetric nitrate/nitrite and ammonia strips (Hach Company, Loveland, Colorado). Water quality parameters during the experiment were within normal limits to maintain suitable conditions for the lamprey and Speckled Dace (Ebeling and Timmons 2012).

Statistical Analysis

The survival rate of the larvae was analyzed by using Welch's *t*-test to determine any differences between treatment groups, with final tank density as the response variable and treatment as the independent variable. The growth of the larvae was analyzed by using Welch's *t*-test with instantaneous growth rate (µm/day) as the response variable and treatment as the independent variable. Individual *t*-tests were used to compare each water quality parameter between the two treatments. The analyses were conducted using R (R Core Team, 2020) with the STATS package (R Core Team, 2020). The datasets were assessed for test assumptions prior to final analysis. The results were deemed significant at $p < 0.05$, and they are presented as means ± SD, unless otherwise indicated.

Results

Although there was a trend of higher survival in the monoculture system (91.0 ± .06%) compared to the polyculture system (84.5 ± 1.4%), the difference was not statistically significant ($t = 1.15$, df = 14, $p = 0.276$). Ending densities ranged from 319 to 581 larvae/m^2 (55-100% survival; Figure 4-3).

Size and growth rates were higher in the polyculture than in the monoculture systems. Larvae reared in the polyculture system grew 23.6% larger (20.52 ± 3.27 mm growth) than those reared in the monoculture system (total growth of 16.60 ± 1.25 mm; Figure 4-4, 4-5). Growth rates differed ($t = -7.76$, df = 14, $p = < 0.001$; Table 4-1) between the two groups, with larvae in the polyculture averaging 5.39 ± 0.04 µm/day compared with those in monoculture at 4.18 ± 0.2 µm/day. A mortality event occurred in one tank in the polyculture system early in the experiment, and this resulted in increased growth among the survivors in that particular tank. When this outlier was removed during the analysis of larval growth, the difference between treatments remained significant.

Water Quality

Water quality parameters did not differ between the polyculture and monoculture treatments ($p > 0.05$ for all parameters; Table 4-2). During the experiment, dissolved oxygen was 8.4–9.9 mg/L and ammonia (NH_3-N) was 0.21–0.51 mg/L.

Discussion

The growth of laboratory-reared larval Pacific Lamprey was improved in the polyculture treatment that included Speckled Dace, a common species found in streams with Pacific Lamprey larvae (Wydoski and Whitney 2003). Other work in co-rearing (with other organisms) or using effluent to rear larval lamprey showed substantial growth and/or survival, even when no supplemental food was provided. Effluent waste water from salmonid hatchery operations and a community fish tank (containing White Sturgeon *Acipenser transmontanus*, Rainbow Trout, and adult Pacific Lamprey) has been used to rear larval lamprey successfully, with and without additional food supplementation (Maine et al. 2017; Moser et al. 2018; Barron et al. 2020a). This provides further evidence for the apparent importance of polyculture and highlights the lamprey's ability to use nutrients that have passed through a higher trophic level. There have also been instances of incidental larval lamprey occurrences in an abatement pond (i.e., hatchery wastewater effluent settling pond), where multiple age classes were observed, including metamorphosed juvenile lamprey (Nelson and Nelle 2007). This is noteworthy because the abatement pond in this case was fed from a salmonid hatchery and the water contained only salmonid food waste, processed nutrients, and likely microorganisms, suggesting that larval lamprey can use nutrients and microorganisms that are associated with teleost fishes in the absence of lamprey-specific supplemental feed. Future research should consider the biomass of microbial communities in treatment and control chambers in corearing or polyculture experiments to better understand causes for observed differences in growth.

As growth to a certain length is a potential trigger of metamorphosis from larva to juvenile, the increased growth in this study suggests that polyculture, or the use of effluent water from teleost fishes, may be a viable and effective option for rearing lamprey of multiple life stages. Polyculture in other species has been shown to improve growth, survival, and immune response (Jones and Iwama 1991; Ahlgren et al. 1998; Kang et al. 2003; Zhou et al. 2006; Slater and Carton 2007; MacDonald et al. 2011). These studies and the results of my experiment suggest that larval lamprey may be a candidate organism for multitrophic aquaculture, polyculture, or for incorporation in existing hatchery programs.

The fish that were used in this study were wild-caught, likely with gut and skin microbes that had been obtained from their natural food and environment. Because the Speckled Dace were placed into the recirculating system two months prior to the larval lamprey, they likely transferred microorganisms from the wild, thereby seeding the biofilter of the recirculating system and inoculating the water and sediment in the system tanks with a “wild-type” microbial community. Inoculation in the laboratory with microbes that are observed in wild populations could help alleviate mortalities that are related to out-planting or transfer to the wild for lamprey that are destined to supplement wild populations. Such inoculations could enhance immunity and increase resistance to invasive pathogens that are encountered in the wild but are not present in the laboratory (reviewed in Kennedy et al. 1998). Additionally, inoculation with microorganisms found at the eventual out-planting site could ease the transition to a more varied food base. This may be especially important for larval lamprey, which are not thought to develop a gut microbiome that is different than that of their environment (Rogers et al. 1980). An internal microbiome develops rapidly in larval fishes and continues to develop throughout that life stage (Nayak 2010). This may also be the case for larval lamprey.

Larval lamprey serve as a unique link in freshwater river ecosystems, providing an essential connection between the benthos and the water column (Shirakawa et al. 2013; Dawson et al. 2015; Boeker and Geist 2016). Lamprey filter feed on algae, diatoms, and detritus during their extended larval phase (Moore and Beamish 1973; Sutton and Bowen 1994). Larval lamprey of various age-classes are a multi-sized food source for Speckled Dace, Rainbow Trout *Oncorhynchus mykiss*, sculpin, and other river predators (Close et al. 2002; Wydoski and Whitney 2003). Larval lamprey also promote aerobic bacterial community changes, increase oxygen concentrations, and increase softness in river sediment (Shirakawa et al. 2013; Boeker and Geist 2016). Further, they can comprise a large portion of the benthic biomass in a river, serving to process, store, and cycle nutrients (Kan 1975; Close et al. 2002; Dawson et al. 2015). This reciprocity, river organisms using larval lamprey as food and for ecosystem services as well as larval lamprey using resources (microorganisms) from other river organisms, is an important example of unseen biotic connections. Quaempts et al. (2018) describe this type of reciprocity in tribal food culture, in that humans have pledged to care for the resources that provide for them (i.e., First Foods: water, fish, big game, roots, and berries). The Umatilla River Vision, a holistic watershed-restoration planning framework that was developed by the CTUIR based on reciprocity, calls for increased understanding of biotic connections and species linkages (Jones et al. 2008; Quaempts et al. 2018). The results of this polyculture study show that larval lamprey derive some benefit from a rearing system that contains a non-lamprey organism (e.g., Speckled Dace).

Though larval lamprey are known to exhibit density-dependent growth, densities in this experiment were well below the threshold densities for this life stage, at which one would expect to see growth variation due to increased or decreased resource competition (Rodriguez-Munoz et al. 2003; Lampman et al. 2016). Survival did not differ between the treatments possibly because of rearing improvements that have been made to standard culture practices in the last few years (CTUIR, unpublished data). Filter mats, which are used to provide alternative substrate and cover from light and to facilitate the development of a microbial community, may have contributed to the improved survival rates over previous culture years before mats were used. Previous cohorts (2016 and 2017) of lab-reared larval lamprey had much lower survival rates (average survival between the 2 years was 29.0%) than did later cohorts that were provided with filter mats. In 2018, filter mats were added to the culture tanks, resulting in an increase in average cohort survival to 57.1%. For the experiment described here, filter mats were used for all of the culture tanks and survival was higher for the larvae in both the poly- and monoculture treatments than in any previous cohort that had been reared in the same systems. Filter mats capture food and particulate matter, allowing a stable microbial community to form that likely contributes to enhance ecosystem services (e.g., nutrient cycling) or provide food (e.g., bacteria) within the system. Kennedy et al. (1998) suggested that larval fish, with their underdeveloped immune systems, could benefit from inoculation with nonpathogenic microbes. Filter mats provide an opportunity for such microbes to develop. However, the growth increases that I observed in the polyculture treatment relative to the monoculture group suggest that larval lamprey derive some nutritional benefit from the presence of a cocultured organism, possibly due to a larger or more diverse microbial community. Further exploration of this would require analysis of the microbial community in these two treatments, but this was beyond the scope of this experiment.

Standard aquaculture disinfection practices attempt to eliminate harmful microorganisms. However, these practices indiscriminately target harmful, innocuous, and beneficial microbes alike (Collins et al. 1976). Those that remain after disinfection may contribute to the rise of antimicrobial-resistant pathogens (Chelossi et al. 2003). Disinfection and biosecurity measures have alleviated many parasite and disease threats in the hatchery environment; however, they may also contribute to unseen effects in the development of the species of interest. Identification of harmful microorganisms can help direct disinfection measures for the microbes that are most detrimental to hatchery production (Kennedy et al. 1998; Jackson et al. 2019). Inoculation with wild-type microbes could also help prevent outbreaks of pathogenic microorganisms in the hatchery environment. Not all host-associated microorganisms are pathogenic, and a majority of species likely have little to no effect on their hosts. Many microbes partly serve to occupy space, preventing invasions of harmful microorganisms (Wong 2016). When disinfection practices eliminate many or all of the microbes that are present in a laboratory or hatchery setting, opportunistic (and potentially harmful) pathogens can recolonize rapidly (Schulze et al. 2006).

Morphological changes in prey species have been linked to exposure to fish kairomones, chemical cues from a predator (e.g., Tollrian 1994). In this case, Speckled Dace, as a natural predator of larval lamprey, could have produced kairomones and induced changes in the growth of the larval lamprey in the polyculture treatment. Research in *Daphnia* has shown variable morphological responses as a result of exposure to predator kairomones including changes in body size and induction of defense characteristics (e.g., helmets and neckteeth) (Tollrian 1994; Sakwinska 2002). Two mechanisms may be at play in kairomone-induced effects on body size. Maturation to a less vulnerable life stage may induce transformation at a smaller body size (Sakwinska 2002) or increased growth to exceed the gape limits of kairomone-releasing predators (Tollrian 1994; Burris 2006). The latter scenario could be a factor in this experiment, as the larval lamprey that were exposed to Speckled Dace grew larger, possibly to outgrow the limitations of their predator. Definitive conclusions await additional research and identification of potential fish kairomones and their interactions. However, recent research has shown that adult Pacific Lamprey did not avoid predator odors (Porter et al. 2017) and that larvae did not significantly alter burrowing behavior when exposed to water containing odors from known predators (White Sturgeon and Rainbow Trout; CTUIR, unpublished data).

Because Pacific Lamprey are a new and emerging species in conservation aquaculture, significantly less funding and fewer resources are directed towards them than towards other fisheries propagation and restoration programs. In light of this, and the need for production-scale rearing of larval lamprey, collaboration with existing finfish hatcheries could increase both awareness and available resources for lamprey research and restoration. Larval lamprey could provide valuable ecosystem services for cultured finfish while gaining necessary microbial connections and becoming conditioned for out-planting via inoculation with watershed- or river-specific microbes. Future research should test containment strategies for temporarily rearing larval lamprey in existing hatcheries. Propagated larval lamprey that are targeted for out-planting in a specific basin could be acclimated and reared in an abatement, effluent, or intake pond at a salmonid hatchery, provided that they are well-contained and can be recovered in predictable numbers and/or sizes (Lampman et al. in review).

Critical uncertainties still remain. For example, although larval lamprey can survive (and thrive) for an undetermined period in salmonid-hatchery-effluent habitat conditions, the long-term limitations or potentially negative effects of effluent nutrient concentrations on larval lamprey are still not well understood. The observation of metamorphosed individuals in effluent (abatement pond, Nelson and Nelle 2007; steelhead effluent wastewater, Barron et al. 2020b), suggests that they are able to assimilate nutrients and grow effectively without disruption to metamorphosis. However, it would be useful to better understand the capacity for larval lamprey to take up certain nutrients and at what concentrations, especially if placement in hatchery systems is of interest for lamprey culturists. Additionally, field studies of early larval lamprey food sources, microbial connections, and trophic interactions would help to develop fundamental knowledge of this poorly understood life stage. Finally, I suggest that genetic analysis of the microbial community in both larval lamprey in the lab and in the field could help (1) better understand larval lamprey biotic connections in the laboratory and the wild, and (2) replicate wild-type conditions for larvae that are reared for population supplementation and out-planting.

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Tables

Table 4-1. Mean (± SD) growth, survival, instantaneous growth rate, and size ranges for polyculture and monoculture larval lamprey during the experimental period.

Treatment	Mean initial length (mm)	Mean growth during experiment (mm)[1]	Size range (mm)	Survival (%)	Instantaneous growth rate (μm/day)
Polyculture	9.31 ± 0.22	20.52 ± 3.27 [b]	20.9-54.8	84.5 ± 1.4 [a]	5.39 ± 0.4 [b]
Monoculture	9.31 ± 0.22	16.60 ± 1.25 [a]	17.4-36.2	91.0 ± .06 [a]	4.18 ± 0.2 [a]

[1]Adjusted to account for differences in assessment date for different tanks (adjusted to 241 days of growth (32-273 days postspawning))
[a, b] Within rows, the values with different superscript letters represent a significant difference (at $p < 0.05$).

Table 4-2. Water quality parameters for polyculture and monoculture of larval lamprey. There were no significant differences between the polyculture and monoculture systems for any parameter.

	Temperature (°C)		**Dissolved Oxygen (mg/L)**		**pH**		**Ammonia (mg/L NH_3-N)**		**Nitrite (mg/L NO_2-N)**		**Nitrate (mg/L NO_3-N)**	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Polyculture	13.91	13.3–14.7	9.10	8.5–9.9	7.6	7.2–7.8	0.36	0.21–0.48	0.1	0.09–0.3	2.0	0–5.0
Monoculture	13.85	13.4–14.6	8.99	8.4–9.8	7.5	7.1–7.9	0.39	0.23–0.51	0.1	0.09–0.3	2.0	0–5.0

Figures

Figure 4-1. The two identical recirculating systems that were used for polyculture (left) and monoculture (right) rearing of larval lamprey. Eight tanks on each system were used to house larval lamprey, and one tank on each system was used to house Speckled Dace (polyculture) or as a feeding control tank (monoculture). Photograph by the author.

Figure 4-2. Subsampled larval lamprey for digital length measurements with ImageJ software (NIH, version 1.52a; Schneider et al. 2012). Photograph by the author.

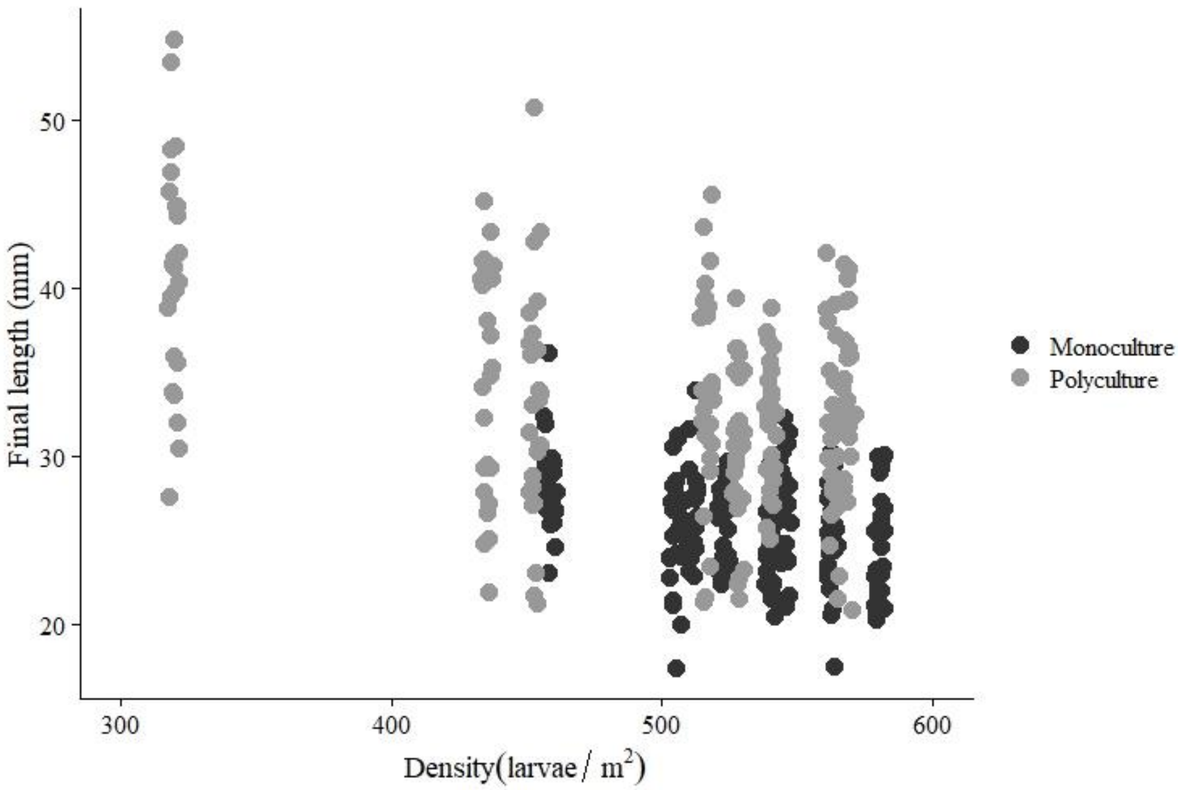

Figure 4-3. Final length (in mm) for larval lamprey in the monoculture (dark grey) and polyculture (light grey) treatments, shown by final tank density (larvae/m^2).

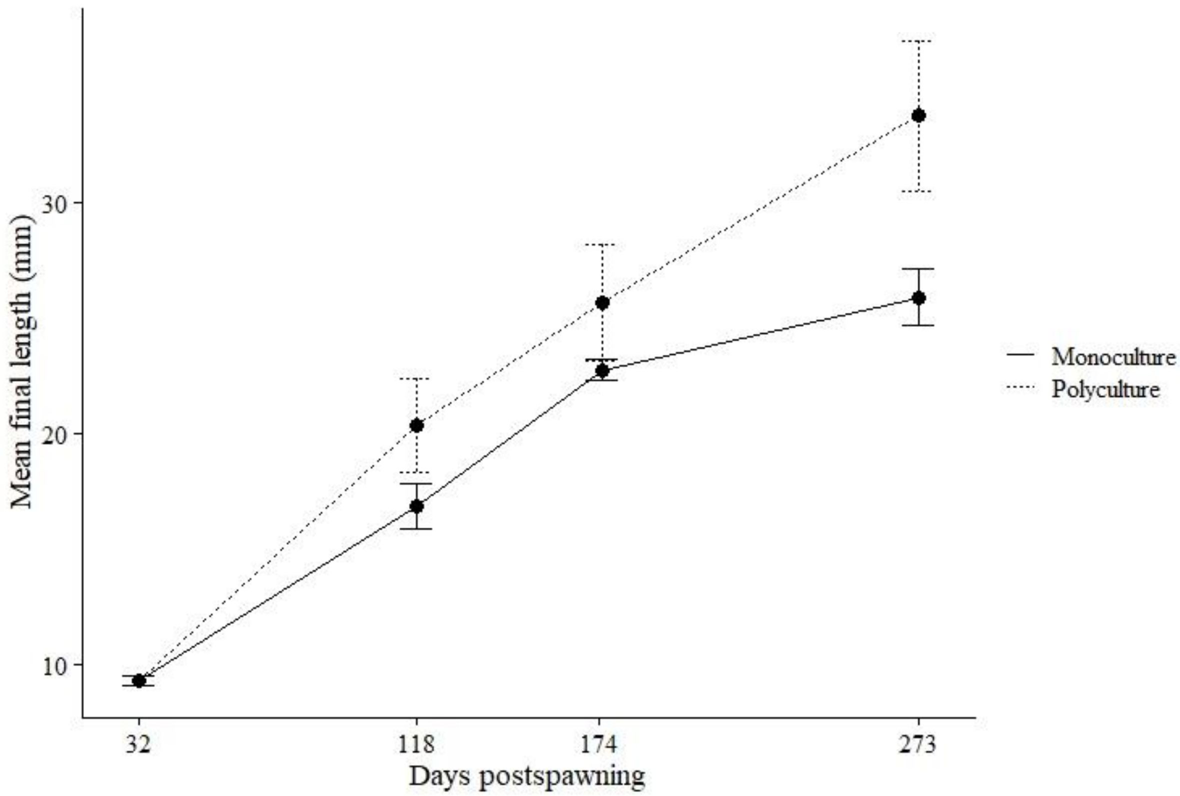

Figure 4-4. Mean length (mm ± 1 standard deviation) for larval lamprey in the monoculture (solid line) and polyculture (dotted line) treatments as a function of days postspawning.

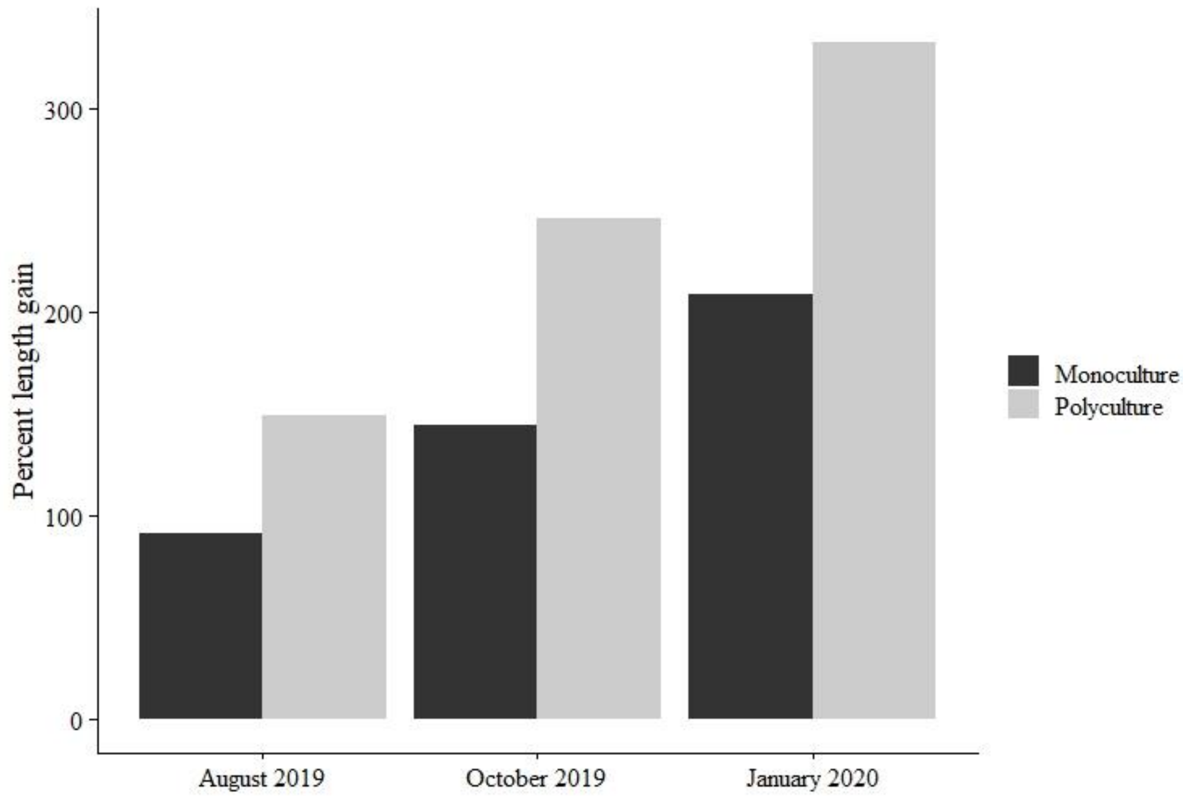

Figure 4-5. Mean percent length gain (%), relative to the start of the experiment, for larval lamprey in the monoculture (dark grey) and polyculture (light grey) treatments at three assessments.

CHAPTER 5: PROBIOTICS IMPROVE GROWTH AND SURVIVAL IN LABORATORY CULTURE OF LARVAL PACIFIC LAMPREY

Abstract

Pacific Lamprey *Entosphenus tridentatus* are a First Food for members of the Confederated Tribes of the Umatilla Indian Reservation (CTUIR) and other Columbia Plateau tribes. The precipitous decline in lamprey abundance has spurred the tribes to focus on restoration efforts such as the River Vision, which prioritizes both biological and physical restoration actions. Part of this multiphase approach is artificial propagation using laboratory culture. The recent development of methods for the laboratory rearing of larval Pacific Lamprey has focused on maximizing survival and growth to conserve resources and increase production. I conducted two experiments to test the hypothesis that bacterial supplements would increase growth and survival of first-feeding larval lamprey in culture. First, I provided a probiotic supplement (EPI-CIN G2, Epicore Bionetworks, Eastampton, New Jersey) at two levels (2 and 5 mg/L) and measured growth after 10 weeks. Larvae that were fed probiotics in addition to a standard ration, at both levels, grew significantly faster (2 mg/L: 11.0 µm/day; 5 mg/L: 13.3 µm/day) than did controls that were fed the standard ration alone (6.6 µm/day). Larvae that received the probiotic supplement also had higher survival (2 mg/L: 36%; 5 mg/L: 44%) than those that were fed the standard ration (24%). Next, I used a different cohort of larval lamprey that was fed the same two levels of probiotic (at the same rate as in the first experiment), but in larger rearing tanks and for 28 weeks. In this experiment, overall growth rates were lower than in the first experiment (2 mg/L: 4.6 µm/day; 5 mg/L: 5.7 µm/day; control: 3.4 µm/day); but, both growth and survival (2 mg/L: 71.4%; 5 mg/L: 78.6%; control: 55.7%) were highest in the treatments with probiotic. For both experiments, I observed the highest growth in the probiotic treatments with higher density than in the control treatments, suggesting that probiotics may be useful to overcome density-dependent growth, a common problem in lamprey culture. Microorganisms provide important services for larval fishes in the laboratory (e.g., survival, growth, nutrition, immune response, and enzyme production). The ecological role of larval lamprey in the river environment (e.g., in food-webs, nutrient cycling, and benthic processes) is supported by microbial communities. This study shows a similar positive relationship between larval lamprey and probiotics in an artificial laboratory culture setting.

Introduction

Pacific Lamprey *Entosphenus tridentatus* are considered a First Food by members of the Confederated Tribes of the Umatilla Indian Reservation (CTUIR) and other Columbia Plateau tribes (Quaempts et al. 2018). Unfortunately, lamprey populations have declined dramatically and harvest opportunities have diminished (Chapter 1; Close et al. 2002). Impoundments, irrigation diversions, and habitat alterations have contributed to decreased habitat for benthic-dwelling larvae and free-swimming, prespawning adults (Close et al. 2002; Murauskas et al. 2013). As part of a multipronged approach to Pacific Lamprey restoration, artificial propagation has been implemented to provide larval lamprey for research and to supplement populations (CRITFC 2018). Propagation research for other lamprey species has been ongoing to supply organisms for evolutionary developmental research (Kuratani et al. 2002; York et al. 2019), to develop control methods (invasive Sea Lamprey *Petromyzon marinus* in the Laurentian Great Lakes; Ciereszko et al. 2005; Wagner et al. 2006; Li et al. 2007; Johnson et al. 2009), and to produce Arctic Lamprey *Lethenteron camtschaticum* (Hokkaido Fish Hatchery 2008), European River Lamprey *Lampetra fluviatilis* (Kujawa et al. 2017), and Yalu River Lamprey *Lampetra morii* (Feng et al. 2018) to supplement populations. Currently, the development of methods for propagating Pacific Lamprey relies on techniques that are used in the culture of other lamprey species. However, low survival and growth are factors that limit production-level laboratory propagation of all lamprey species (Lampman et al. 2016; Lampman et al. 2019; Moser et al. 2019).

Low survival and growth of larval Pacific Lamprey in laboratory culture has led to the investigation of alternative methods to improve rearing success (Lampman et al. 2016; Barron et al. 2020). A pilot experiment in which a microbial supplement was added to the food that was fed to the larvae showed slight improvements in growth, and the addition of spawning filter mats (slowing food degradation and potentially harboring a robust microbial community/biofilm) improved survival over multiple cohorts (Lampman et al. 2016; Maine et al. 2017). Moreover, larval lamprey that received salmonid hatchery effluent grew faster and larger than did those that were raised without such a source of microbes and nutrients (Barron et al. 2020). These observations piqued my interest in testing if a commercially available probiotic supplement could confer any benefits to larval lamprey in culture.

Probiotics are live or dead microorganisms (commonly bacteria) that contribute to intestinal or environmental (in the case of aquatic environments) microbial balance (Nayak 2010; Hai 2015). They are used in aquaculture to improve survival, growth, immune response, or disease resistance, applied with food or directly into the water (Zhou et al. 2009; Nayak 2010). Commercially available probiotics for aquaculture are formulated to perform certain functions in the aquatic environment depending on the individual species or mixture of species present in the product. For this study, I used a commercially sourced probiotic product containing *Bacillus*, *Lactobacillus*, and *Acetobacter* species (EPI-CIN G2, Epicore Bionetworks, Eastampton, New Jersey), genera known to confer benefits in aquaculture (Table 5-1). I used a commercially available probiotic as it was readily available in a shelf-stable container and formulated for use in aquaculture settings. Any non-pathogenic bacteria could potentially be used as a probiotic, especially if the intended effects are not related to specific bacterial species and their products, such as obtaining direct nutrition from the microorganisms (i.e., organism size might matter but not species composition).

Probiotics impart both direct and indirect benefits to cultured organisms. The microbes that are used for probiotic supplementation confer benefits via the following main mechanisms: improving feed conversion efficiency and gut microbiome activity; acting as a direct food source; imparting pathogen resistance; increasing the production of enzymes, antibiotics, and acids; enhancing immune responses; and competitive exclusion of pathogens (e.g., competition with other microbes for resources or space) (Nayak 2010; De et al. 2014). Numerous studies have shown improved growth, survival, and/or increased immune response of adult and larval fishes that are supplemented with commercially available (e.g., commercially mass- or batch-cultured strains) or cultured (e.g., bacteria cultured from adult intestines to be fed to larvae of the same species) probiotics (Table 5-2). Probiotics have also been effective in increasing the growth and survival of other aquatic organisms, such as sea cucumber, marine mussels, seahorses, and shrimp (Table 5-2).

The possibility of enhanced growth and survival from probiotic supplementation is of particular interest in Pacific Lamprey culture. A standard feed for rearing larval Pacific Lamprey has been developed (Barron et al. 2016), but in high-density cultures, larval lamprey exhibit density-dependent growth (Mallatt 1983; Rodriguez-Munoz et al. 2003; Lampman et al. 2016). To improve production capacity, the standard ration can be increased

(Lampman et al. 2016). This practice requires careful monitoring to avoid fouling, especially in static or recirculating systems. The use of probiotics to increase survival and/or growth has a lower risk of water-quality degradation than does increasing the organic food ration (i.e., probiotics can provide supplemental nutrition with additional water quality or competitive exclusion benefits). Probiotic supplements could also provide a more consistent food source through the development and maintenance of a diverse and healthy microbial community compared with providing a food ration only.

Beneficial microbes play an important role in critical aspects of aquaculture such as the absorption of CO_2, oxygen production, the decomposition of organic matter in sediments, and the reduction of nitrogenous wastes (reviewed in Zhou et al. 2009). While they are especially important to maintain high water quality and the cycling of nitrogen, microbes also convey antifungal protection and pathogen control for some fish species (Lowery et al. 2015). Boeker and Geist (2016) found that larval lamprey, through their burrowing activities, play a significant role in structuring the microbial community in river substrate. Because larval lamprey live at the interface between the river substrate and the water column, they likely rely on local benthic microbes to provide food and ecological services. This may be especially important when larvae are unable to filter feed from the water column due to high water velocity or turbidity during high water events or during periods of low stream productivity (Yap and Bowen 2003; Moser et al. 2019). Based on these observations, I hypothesized that the addition of a commercially available probiotic to feed in Pacific Lamprey cultures would increase both the survival rate and growth of first-feeding larvae.

Methods

Two experiments were completed using Pacific Lamprey larvae that were propagated by the CTUIR at the Walla Walla Community College Water and Environmental Center (WEC). Adult lamprey were collected at main-stem Columbia River dams (e.g., Bonneville, McNary, or John Day) and held over winter by the CTUIR Pacific Lamprey Project. In two separate spawnings in 2018 and 2019, ripe adults were hand-stripped for the collection of gametes, and the eggs were fertilized at the WEC (following the methods of Lampman et al. 2016). The embryos were incubated in static cultures with aeration, held in 10-L tanks in a 13.0 ± 1.5°C water bath.

Probiotic supplement experiment in 2018

In 2018, 200 larvae aged 29 days postspawning with an average length of 8.55 mm (± 0.51 mm SD) were randomly collected from a holding chamber and placed into 20, new (never used) 1-L glass beakers (*n* = 10 larvae/beaker) with source water (conditioned well water). The beakers were rinsed with source water prior to use, and they were randomly assigned to one of three treatments: control (no probiotic; *n* = 10 replicate beakers), T1 (2 mg/L probiotic; *n* = 5 replicate beakers), or T2 (5 mg/L probiotic; *n* = 5 replicate beakers), The probiotic treatments used EPI-CIN G2 powdered commercial aquaculture probiotic (Epicore Bionetworks, Eastampton, New Jersey), applied during once weekly feedings. Each beaker (105 mm in diameter) was aerated and contained 1.5 cm (in depth) sieved and autoclaved sediment with particles in the size range of 149–595 µm (sediment volume: 1.27×10^{-4} m^3) and a 5 × 5-cm piece of filter mat (latex-coated coconut fiber spawning mat with polyester backing, Spawntex mat, Pentair AES, Apopko, Florida) for cover. During a 24-hour acclimation period after transfer, I checked the beakers for survival and replaced any mortalities with live larvae so that the densities in all of the beakers were equal (10 larvae/L, 1,154.9 larvae/m^2, and 78,740.2 larvae/m^3) at the start of the experiment.

The larvae were fed a weekly ration of 250 mg/L of yeast (80%; Red Star Baking Yeast, Lesaffre Yeast Corp. Milwaukee, Wisconsin) and Otohime larval fish food (20%; Otohime A1, Marubeni Nisshin Feed Co. Ltd., Tokyo, Japan) (Barron et al. 2016). The food was prepared separately for each treatment each week (control: 2,500 mg food; T1: 1,250 mg food, 10 mg probiotic; and T2: 1,250 mg food, 25 mg probiotic) and emulsified in source water for each feeding using a blender. Weekly, each treatment received the following amounts of food and probiotic mixture: control beakers each received 250 mg of food; for T1 each received 252 mg of food–probiotic mixture and for T2, each beaker received 255 mg of food–probiotic mixture. Feedings were preceded by a 200 mL water change using source water. An additional 200 mL water change was also completed each week that was not associated with feeding.

All of the beakers were held in a randomized order (Figure 5-1) in a water bath to maintain the water at 13.5 ± 1.0°C for the duration of the 10-week experiment. Water temperature was checked daily, but the beakers were left undisturbed otherwise, aside from feeding and water changes. At the end of the experiment, larvae (aged 98 days postspawning) were removed from each beaker by stirring the sediment with a blunt probe and using a dip net to capture them. The larvae were counted to assess survival and final density from each treatment. Surviving larvae were photographed with a calibrated scale (Figure 5-2) and individual body length (to the nearest 0.01 mm) was measured for 20 randomly subsampled larvae from the digital photographs using ImageJ (NIH, version 1.52a; Schneider et al. 2012). I also calculated the final density of larvae/m^2 (sediment area) and larvae/m^3 (sediment volume) for each beaker.

Statistical analysis

I used logistic regression to analyze survival between the treatments (Warton and Hui 2011) and a one-way analysis of variance (ANOVA) to compare body lengths between the treatments at the end of the experiment. This was followed by a Tukey's HSD post hoc test to identify which treatments differed. All of the analyses were completed in R (version 3.5.1, R Core Team 2020) using the STATS package (version 3.5.1, R Core Team 2020).

Probiotic supplement experiment in 2019

In 2019, 210 larvae aged 31 days postspawning with an average length of 9.31 mm (± 0.22 SD) were collected from a holding chamber and randomly placed with source water into three static, aerated 10-L polycarbonate Cambro CamWear pans (53 × 32.5 cm, water depth 9–10 cm; Cambro Manufacturing, Huntington Beach, California). Each pan contained sieved and washed sediment (sized 149–595 µm) to a depth of 7.5 cm (sediment volume: 1.29×10^{-2} m^3) and a pan-sized filter mat (Spawntex mat). After a 24-hour acclimation period, I checked the pans and replaced any mortalities with live larvae so that the densities were equal in all of the tanks (7 larvae/L; 406.4 larvae/m^2; 5,418.5 larvae/m^3) at the start of the experiment.

The larvae in each pan received a standard food ration of 250 mg/L (as in 2018) once weekly. The food was blended with source water and added weekly to the pans after a 2-L (20% of the total pan volume) water change with source water. The probiotic-supplemented treatments used Epicore Bionetworks EPI-CIN G2 powdered commercial aquaculture probiotic, applied weekly with food at the same levels (per volume) as were used in the 2018 experiment. Larvae in the control pan received 2,500 mg of food; those in the T1 pan received 2,500 mg food and 20 mg probiotic; and those in the T2 pan received 2,500 mg food and 50 mg probiotic. An additional 2-L water change also was conducted weekly, not associated with feeding. The pans were held in a water bath to maintain the temperature at 14.0 ± 1.0°C for the duration of the experiment.

The survival and body length of larvae were assessed at 77, 178, 200 (only T2), and 226 (only T1 and control) days postspawning during the 28-week experiment by removing all of the surviving larvae from each pan. The larvae were netted from the water column in each pan after stirring the sediment with a blunt probe. On each occasion, I recorded the number of surviving larvae in each tank and took a digital photograph of larvae from each treatment to measure body lengths for 20 randomly subsampled individuals, as explained above for 2018.

Instantaneous growth rate

I used an instantaneous growth rate (G) formula, modified from Wootton (1990), Hopkins (1992), and Crane et al. (2019), to account for measurements that were taken on different dates:

$$G = [\ln(L_2) - \ln(L_1)] / (t_2 - t_1) \times 1{,}000,$$

where G is the instantaneous growth rate (µm/day), $\ln(L_2)$ is the natural logarithm of the average length (mm) of larvae in a given tank at an intermediate or ending period (t_2), and $\ln(L_1)$ is the natural logarithm of the average length of larvae in a given tank at the start of the experiment (t_1).

Water quality

Water temperature, pH, and dissolved oxygen were monitored and recorded weekly in each system by using a Vernier handheld computer and sensors (Vernier, Beaverton, Oregon) while semiquantitative colorimetric Hach test strips were used to measure nitrate/nitrite and ammonia (Hach Company, Loveland, Colorado).

Results

Probiotic supplement experiment in 2018

Survival was significantly higher (logistic regression, $p = 0.014$, $n = 200$) in the probiotic treatments relative to the control group. The 2 mg/L (T1) and 5 mg/L (T2) probiotic treatments had 36% (415.8 larvae/m^2; 28,346.5 larvae/m^3) and 44% (508.2 larvae/m^2; 34,645.7 larvae/m^3) survival at the end of the trial, respectively (Figure 5-3). The control group had 24% (277.2 larvae/m^2; 18,897.6 larvae/m^3) survival, lower than either probiotic treatment.

Larvae that were supplemented with either dose of probiotic grew significantly larger than did those in the control group (control: 13.6 ± 1.4 mm; T1: 18.5 ± 3.0 mm; and T2: 21.5 ± 2.6 mm, mean final length ± SD; $F_{2, 61} = 65.34$, $p = < 0.001$). The Tukey HSD test indicated that the larvae in the T2 (5 mg/L) treatment grew significantly larger than did the control larvae ($p < 0.001$), and they were significantly larger ($p < 0.001$) than were the larvae in the T1 (2 mg/L) treatment (Figure 5-4). Larvae that were reared in static beakers in 2018 that were supplemented with T1 (2 mg/L) and T2 (5 mg/L) probiotic doses had faster growth rates than did the control larvae that were fed a standard ration only (control: 6.6 µm/day; T1: 11.0 µm/day; and T2: 13.3 µm/day). The larvae in the probiotic-supplemented treatments also did not show density-dependent growth, as is known for lamprey larvae (Mallatt 1983; Rodriguez-Munoz et al. 2013); instead, they grew larger than those in the control group, despite higher densities (Figure 5-5).

Probiotic supplement experiment in 2019

Survival differed between larvae in the control (55.7%; 226.4 larvae/m^2; 3,018.1 larvae/m^3) and the probiotic treatments (T1: 71.4%; 290.2 larvae/m^2; 3,868.8 larvae/m^3; and T2: 78.6%; 319.4 larvae/m^2; 4,258.9 larvae/m^3), as measured during the assessment at 178 days postspawn. A lapse in aeration in the T2 probiotic treatment resulted in mortality to the entire tank at 200 days postspawn when dissolved oxygen dropped to 0.9 mg/L during that period (Figure 5-6).

Because of the mortality in the T2 treatment, I extrapolated from 200 to 226 days using the instantaneous growth rate, to estimate final length for comparison among the control and treatment groups (Figure 5-7). The final lengths of the larvae in either probiotic treatment (T1: 23.2 ± 2.9 mm; or T2: 26.4 ± 5.3 mm) differed from those larvae in the control group (18.4 ± 3.4 mm). The larvae that were reared in static pans and supplemented with T1 (2 mg/L) and T2 (5 mg/L) probiotic doses grew at a faster rate than did the control larvae that were fed a standard ration (control: 3.4 µm/day, T1: 4.6 µm/day, and T2: 5.7

μm/day; Figure 5-8). As observed in the 2018 experiment, larvae in probiotic supplemented treatments grew larger than did those in the control group despite higher densities (Figure 5-9).

Water quality

There were no observed differences among the three treatments for any of the water quality parameters for either the 2018 or the 2019 experiments (Table 5-3).

Discussion

Pacific Lamprey growth and survival were higher in the probiotic treatments than in the control treatments, suggesting that the probiotics provided a benefit in the culture of larvae. Similar results between the two different larval cohorts and rearing environments further strengthens this conclusion. My results also suggest that there is a positive relationship with probiotic dose; the 5-mg/L dose produced faster growth and better survival than did the 2-mg/L dose. It may be that the higher probiotic dose provided more microorganismal food to the larvae or conferred a higher rate of synergistic benefits than did the lower dose. Opiyo et al. (2019) in a study of Nile Tilapia *Oreochromis niloticus* reported a probiotic-dose-dependent growth response, but a threshold of benefit was observed in which the highest dose of probiotic did not result in the highest growth. Bagheri et al. (2008) also found a threshold effect in Rainbow Trout *Oncorhynchus mykiss* that was associated with a higher dose of probiotic. These findings suggest that an intermediate probiotic dose may be optimal, but further research is needed to confirm this for Pacific Lamprey.

While my study used only two levels of probiotic, and showed a relationship between higher dose and higher growth, further research should be conducted to determine if there is a threshold at which the dose becomes sufficiently high to depress feed use and efficiency. I did not measure feed digestibility or nutrient uptake during this study, but it is known that gut microbes can produce amino acids and enzymes to aid in these processes (Burr et al. 2005; Nayak 2010; De et al. 2014; Table 5-1). It is possible that this mechanism resulted in the increased growth and survival that I observed in my study, but further investigation is needed to determine optimal probiotic dose and the pathways that are involved.

The growth in my experiments was similar to that which has been reported for flow-through culture operations for Pacific Lamprey, and it was higher than in previous years of recirculating larval culture at the WEC (for subyearling larvae over similar growth periods as were used in my study; Maine et al. 2019; reviewed in Moser et al. 2019). Larval lamprey that were reared at the WEC facility in 2016 and 2017 had an average instantaneous growth rate of 2.1 µm/day. Changes to the WEC rearing systems in 2018, which included the addition of a filter mat to provide increased surface for microbial attachment in each tank, led to an increase in the average instantaneous growth rate to 5.6 µm/day (Maine et al. 2019). Growth rates between 3.2 and 10.4 µm/day were found in a diet development study that used larvae of similar age and a comparable growth period as were used in my experiments (Barron et al. 2016), with the highest growth rate reported for the diet I used in my study. Barron et al. (2020) reported a growth rate of 5.7 µm/day for yearlings that were fed 500 mg/L over 63 days (which was twice the standard ration that I used) and growth rates as high as 6.5 µm/day for larvae that were reared in effluent water with no supplemental feed. They observed growth rates as high as 8.4 µm/day for larvae that were reared in effluent plus supplemental feed. Similarly, I found growth rates of 4.2 µm/day for subyearling larvae that were reared in a recirculating system and 5.4 µm/day for those that were reared in a polyculture system with a teleost fish (Chapter 4). Growth rates in larval lamprey are clearly influenced by diet and the provision of a probiotic supplement. This suggests that probiotics or other microbial supplementation could be a cost-effective method to improve the growth and survival of lamprey larvae in dense laboratory cultures.

I observed improved survival in the probiotic supplemented treatments in the 10- and 28-week experiments with larvae aged 32–98 (2018) and 29–226 (2019) days postspawning. The survival rates in larval lamprey are not well studied, especially those for larvae that are under 1 year of age. Survival rates of Pacific Lamprey larvae from 30–90 days postspawning varied from 0–50% in various rearing conditions at different facilities (Lampman et al. 2016). Survival typically declines after the first-feeding stage (approximately 45 days postspawning) from over 90% survival before first feeding to an average of 35%, and this period has been identified as a significant bottleneck to lamprey rearing in the hatchery environment (Lampman et al. 2016). Other lamprey species also exhibit low survival at first feeding (Moser et al. 2019), suggesting that this may be an inherent feature of lamprey. For example, Hansen et al. (1974) found that Sea Lamprey larvae survival was between 11.6% and 36.5% in the first year of life. Rodriguez-Munoz et al. (2001) found that the maximum survival rate of larval Sea Lamprey in the lab was 43% during the 3 months after first feeding. Higher survival (55–100%) has been observed in Pacific Lamprey larvae from first feeding to 1 year of age in a polyculture recirculating system with Speckled Dace *Rhinichthys osculus* (Chapter 4). The higher survival rate of the larvae in my study suggests they derived some benefit from probiotic supplementation, and it is possible that microbes are important in overcoming early larval mortality, especially when switching to exogenous feeding.

Pacific Lamprey populations have been negatively affected by anthropogenic changes, and restoration efforts rely on small numbers of broodstock for propagation annually. Maximizing the survival and growth of lab-produced larvae will reduce the number of broodstock needed to produce larvae in the numbers needed for restoration. Previous rearing efforts at the WEC have had mixed success and low survival rates (CTUIR, unpublished data). This may have been linked to the use of UV sterilization and/or chemical disinfection of the culture water, as these practices have been shown to promote low bacterial diversity, control, and stability in other aquaculture settings (de Carvalho 2017; Brugman et al. 2018). My results indicate that the use of a probiotic could improve survival and growth so that the production of large numbers of larval lamprey is possible with relatively little effect on the remaining wild populations.

Further, probiotic supplementation was observed to significantly increase growth in larvae that were reared at higher densities and received probiotic treatments compared with those in the control groups in both experiments. I also observed the same response to probiotic use when rearing lamprey in larger tanks, but at lower densities. The larger tank size that was used in the second experiment provides more horizontal surface area for the larvae to use, reducing physical competition for burrow space. The densities that were used in both experiments described in this study (406.4–1,154.9 larvae/m^2) were higher than the densities observed in the wild (< 1–32 larvae/m^2) but lower than the densities recommended for laboratory larval rearing for supplementation production (4,042–6,811 larvae/m^2; CRITFC 2018). Larval lamprey exhibit density-dependent growth (Mallatt 1983; Murdoch et al. 1991; Rodriguez-Munoz et al. 2003), and the production of the large numbers of lamprey needed for restoration efforts have been hampered by this effect. The use of probiotics to overcome density-dependent growth in larval lamprey could significantly increase production and decrease the facility space needed to grow them. Research should focus on the role of probiotics in overcoming a density–growth effect at the densities recommended for production-level rearing of larvae.

Water quality did not differ between the probiotic treatments and controls in either year of study, suggesting that increases in survival and growth were not related to the stability of water quality. Water quality is one criterion that is linked to the development of disease outbreaks in aquaculture (Padmavathi et al. 2012). Improving water quality in the culture environment can be a delicate balance between controlling harmful and promoting beneficial microorganisms (Sayes et al. 2018). Probiotics have been used to improve water quality through mechanisms such as increased nutrient cycling, inhibition of potential pathogens, and decreased build-up of nitrogenous waste compounds (Kim et al. 2005; Lalloo et al. 2007; Padmavathi et al. 2012). The frequent water changes as part of the study protocol likely contributed to stable and suitable water quality. Therefore, I speculate that mechanisms other than that of water quality improvement were at play in the observed increases in the survival and growth of larval lamprey in this study.

It is likely that the larval lamprey obtained some nutritional benefit from supplemented microbes and the fortified microbial community in this experiment. They could have obtained other benefits from the probiotics, such as increased feed digestibility, production of enzymes, or an immune system influence, as has been shown in other fishes (Robertson et al. 2000; Bagheri et al. 2008; Cerezuela et al. 2013; Munir et al. 2016). Larval lamprey are suspension feeders, using primarily bacteria, detritus, and diatoms as food (Moore and Beamish 1973; Moore and Potter 1976; Yap and Bowen 2003). Larval lamprey are known to feed from within their burrows in the lower water column (primarily at night), and they possibly take in nutrients from sediment pore water at other times (CTUIR, unpublished data; Applegate, 1950; Moser et al. 2019). It is unknown how much of their total intake is from subsurface versus surface feeding, and future research should explore this. Investigating feeding behavior may be especially important to determine the optimal method of probiotic application, via food, water, or mixed into the sediment. In this study, I applied the probiotic with food at a rate relative to the total volume of the chamber. Larval densities are presented in terms of water (chamber) volume, sediment surface area, and sediment volume to facilitate comparisons with future studies that examine food assimilation behavior or mechanisms.

Probiotics could provide larval lamprey with the type and size of food that are optimal for growth in the laboratory. Larvae have been shown to survive in cultures with only bacteria or organic detrital material as a food source, though this has not been explored rigorously (Moore and Potter 1976; Sutton and Bowen 1994; Nelson and Nelle 2007; reviewed in Lampman et al. in press). Particularly for first-feeding larvae, small (<50–100 µm) food-particle size is important in growth (Moser et al. 2019). Microorganisms may fit this size requirement, and the development or maintenance of a microbial community in the rearing environment may serve this purpose.

Other mechanisms by which probiotic supplementation conferred benefits to the larvae in this experiment are unknown, but they could include competitive exclusion of pathogens, increased immune response, or directed development of gut or mucosal surface microbial community. Certain microbes can competitively exclude harmful bacteria (Yong 2016), and probiotics could serve this purpose for larval lamprey in the laboratory. Of the three genera that are contained in the EPI-CIN G2 probiotic used in this study, the *Bacillus* and *Lactobacillus* species are known to provide benefits to aquaculture organisms via competitive exclusion including a faster growth rate and higher nutrient uptake rates than are observed in pathogenic bacteria (e.g., Lalloo et al. 2010) as well as the production of antibacterial compounds (e.g., Lash et al. 2005; Table 5-1).

Immune responses are known to occur as a result of probiotic use in aquaculture (e.g., activation of immune defenses and protective effects against pathogens from probiotics containing *Bacillus* or *Lactobacillus*, as reviewed in Balcazar et al. 2006). Jawless fishes like lamprey are thought to require activation of the innate immune system to initiate adaptive immune responses, similar to jawed fishes (Kasamatsu 2012). Giri et al. (2012) found that probiotics improve innate immunity in teleost fishes, and this may have been another benefit of the probiotics in my experiments. Outside of the laboratory, larval lamprey appear to be relatively resistant to disease or infection, as compared with the juvenile and adult life stages (Jackson et al. 2019), However, fungal, parasitic, and pathogenic infections have been reported in dense larval cultures in the laboratory (Lampman et al. 2019; Lampman et al. in review). It is possible that the burrowing behavior of larvae could increase their exposure to pathogens or parasites in the wild, potentially inducing immune responses that lower disease risk at that life stage. It further stands to reason that probiotics could potentially be used to induce such immune responses in the laboratory in the absence of wild microorganisms.

The mucosal surface of fishes is known to host a diverse microbiota, playing an important role in disease control (Lowery et al. 2015), and it has been shown in larval fishes that microbiota in culture water are important to establish an internal microbial community during early development (Egerton et al. 2018; Jiang et al. 2019). Larval lamprey are thought to obtain their gut microbiota entirely from their environment (Rogers et al. 1980). In a culture environment, the use of probiotics may direct the development of the mucosal surface or gut microbiomes in newly hatched and first-feeding larval lamprey. Moser et al. (2020) conducted an early microbial inoculant experiment, which used different water sources to incubate Pacific Lamprey embryos, and they reported no differences in survival or growth between treatments using microbially rich water (from lamprey tanks or from a community fish tank) and those using conditioned or unconditioned well water. The mechanism for the colonization of the lamprey gut by microbes is not well understood, and, while the results from the early inoculant experiment suggested no apparent benefits from the practice, there were also no direct disadvantages of early microbial inoculation. In other cases, the absence of disinfection practices during larval rearing, as was the case in Moser et al. (2020) and this study, induced great risk of fungal infections (Lampman et al. 2016; Jackson et al. 2019). Future studies should assess the ontogeny of the gut microbiome development to identify the time at which exposure to beneficial microbes is most important and to determine the role of disinfection in lamprey incubation and early larval rearing.

Findings from this study could have direct benefit for lamprey culture and management by increasing early survival and growth, which may improve overall survival in a culture setting or in the wild. Identification of lamprey-specific microorganisms could be used to develop probiotic agents to direct gut microbiome development in early larval lamprey, prepare larvae for out-planting through inoculation with wild-type microorganisms, or confer immune benefits prior to release or for research. Further research is needed to investigate differences in gut and mucosal surface microbiomes of wild and laboratory-reared larval lamprey. Identifying and culturing specific bacteria that are isolated from wild larval lamprey could allow for the identification of the microbes that are most important to lamprey and, ultimately, lead to the preparation of lamprey-specific probiotic supplements. This would be especially prudent for production operations that are struggling with low survival rates during the first-feeding bottleneck.

As larval lamprey are a food source for other aquatic organisms, their role in transferring microbial resources to higher trophic levels is particularly important. In systems where lamprey are abundant, they can serve as the energy foundation for stream biota (Stanford and Ward 1993; Craft et al. 2002; Paerl et al. 2003). Particularly of interest in the context of holistic restoration of declining lamprey species, identifying lamprey-specific microbes could elucidate the degree to which larval lamprey link benthic and water-column organisms through trophic connections, broadening our collective understanding of their ecological role in freshwater systems. CTUIR conducts holistic watershed restoration (as in River Vision; Jones et al. 2008) which uniquely targets biological processes such as those conducted by larval lamprey (e.g., food web connections, nutrient cycling). Biotic connections are important in the laboratory for improving conservation aquaculture techniques and for successful habitat and biological community restoration in the field. This study demonstrates the incorporation of the indigenous values of community and reciprocity, using microbial community supplementation, into conservation aquaculture techniques for Pacific Lamprey.

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Tables

Table 5-1. Mechanisms observed in use of probiotics containing genera of bacteria present in the probiotic (EPI-CIN G2) in cultured aquatic organisms.

Genus	**Mechanisms**	**References**
Bacillus	Increased intestinal enzyme activity, competitive exclusion via rapid growth, inhibition of growth of pathogenic bacteria, decreased nitrogenous waste	Wang 2011; Luis-Villasenor et al. 2011; Lalloo et al. 2007; Lalloo et al. 2009
Lactobacillus	Increased intestinal enzyme activity, increased growth performance due to decreased cholesterol and increased fatty acid levels, inhibition of growth of pathogenic bacteria via bacteriocin protein secretion	Wang 2011; Falcinelli et al. 2015; Lash et al. 2005
Acetobacter	Synthesis/fixation of nitrogen, production of acetic acid	Zhou et al. 2009; Zhao et al. 2019

Table 5-2. Studies investigating probiotic use in cultured aquatic organisms.

Species	Life stage	Route of exposure	Metrics improved by probiotic application	Reference
Lumpfish *Cyclopterus lumpus*	Larvae	Water	Survival, growth, disease resistance	Klakegg et al. 2020
Rohu *Labeo rohita*	Fingerlings	Food	Growth, feed conversion	Ghosh et al. 2004
Rohu	Juveniles	Food	Growth, feed utilization, immune function	Giri et al. 2013
Turbot *Scophthalmus maximus*	Larvae	Water	Survival	Ringo and Vadstein 1998
Turbot	Larvae	Food	Survival, growth	Daga et al. 2013
Rainbow Trout *Oncorhynchus mykiss*	Adult	Water	Disease resistance	Gram et al. 1999
Rainbow Trout	Fry	Food	Survival, growth	Bagheri et al. 2008
Channel Catfish *Ictalurus punctatus*	Adult	Water	Survival, growth	Queiroz and Boyd 1998
Atlantic Salmon *Salmo salar* Rainbow Trout	Fingerlings	Food	Disease resistance	Robertson et al. 2000
European Eel *Anguilla anguilla*	Adult	Food	Disease resistance	Chang and Lui 2002
Sea cucumber *Apostichopus japonicus*	Juvenile	Food	Growth, enzyme activity	Ma et al. 2019
Pacific oyster *Crassostrea gigas*	Larvae	Food	Growth	Douillet and Langdon 1994
Greenshell mussel *Perna canaliculus*	Larvae	Water	Survival, disease resistance	Kesarcodi-Watson 2009
Lined seahorse *Hippocampus erectus*	Juvenile	Food	Survival, growth	Lin et al. 2019
White shrimp *Penaeus vannamei*	Larvae	Food and water	Survival, growth	Silva et al. 2011

Table 5-3. Mean (range) of measured water quality parameters during larval Pacific Lamprey survival and growth experiments using two different concentrations of a probiotic (EPI-CIN G2) in 2018 and 2019.

	Temperature (°C)		**Dissolved Oxygen (mg/L)**		**pH**		**Ammonia (mg/L NH_3-N)**		**Nitrite (mg/L NO_2-N)**		**Nitrate (mg/L NO_3-N)**	
	2018	**2019**	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**	**2018**	**2019**
Control (no probiotic)	13.98 (13.5-14.6)	13.59 (13.1-13.9)	8.70 (8.1-9.0)	8.78 (7.9-9.2)	7.6 (7.3-7.8)	7.6 (7.3-7.9)	0.24 (0.1-0.4)	0.25 (0.1-0.4)	0.1 (0.09-0.2)	0.1 (0.09-0.2)	1.7 (0-4.0)	1.7 (0-3.0)
T1 (2 mg/L probiotic)	13.92 (13.7-14.4)	13.61 (13.1-14.2)	8.78 (8.2-9.1)	8.79 (7.9-9.1)	7.6 (7.2-7.9)	7.6 (7.2-8.0)	0.25 (0.1-0.4)	0.22 (0.1-0.4)	0.1 (0.09-0.2)	0.1 (0.09-0.2)	1.7 (0-4.0)	1.7 (0-3.5)
T2 (5 mg/L probiotic)	13.96 (13.4-14.6)	13.6 (13.3-14.6)	8.83 (8.1-9.1)	8.80 (7.9-9.1)	7.6 (7.4-7.8)	7.6 (7.3-7.9)	0.26 (0.1-0.4)	0.24 (0.1-0.4)	0.1 (0.09-0.2)	0.1 (0.09-0.2)	1.7 (0-4.0)	1.7 (0-3.0)

Figures

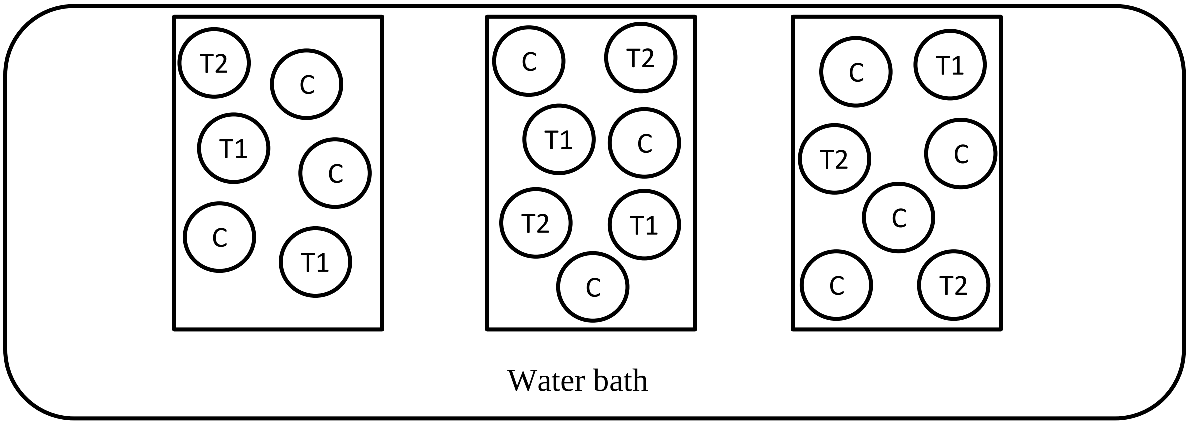

Figure 5-1. Configuration of beakers in the 2018 experiment. A large water bath was used to control the water temperature in three pans holding the beakers. The beakers were randomly assigned to each pan in the water bath.

Figure 5-2. Digital photograph of larval lamprey used to obtain lengths. Photograph by the author.

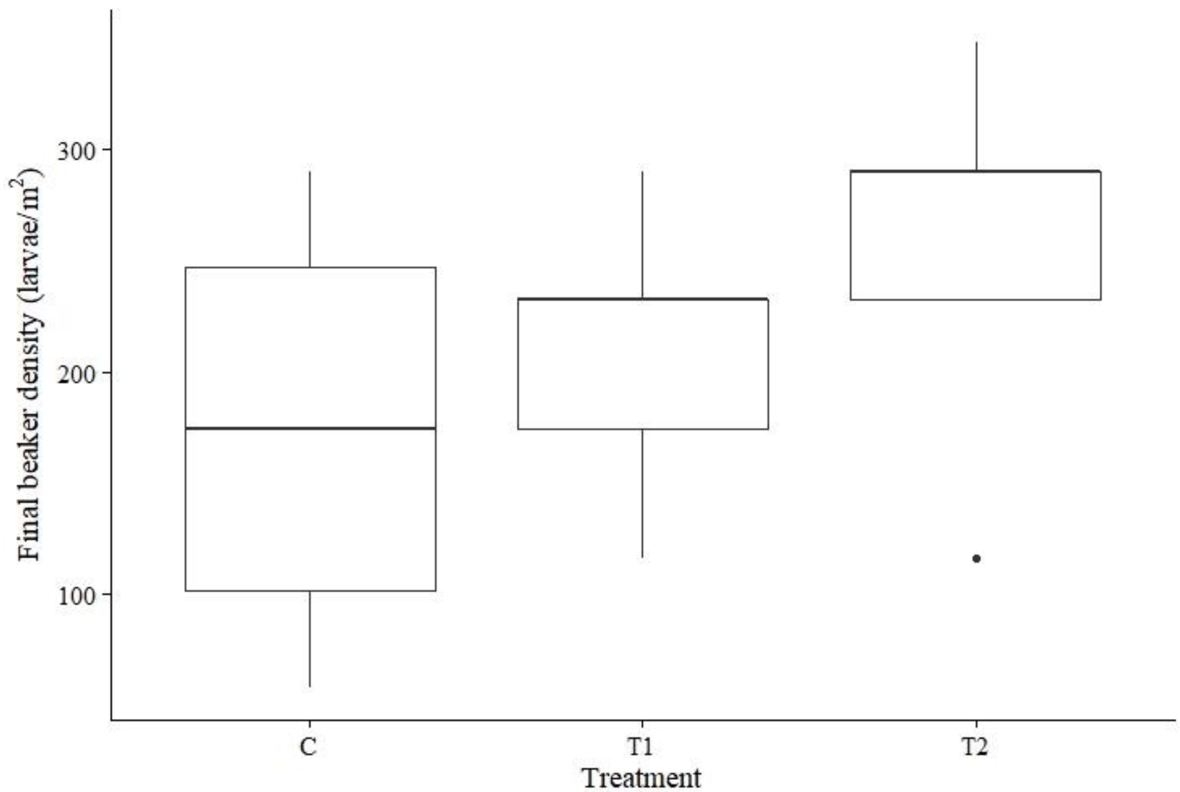

Figure 5-3. Density of beakers (larvae/m^2) at the end of the 2018 probiotic experiment. Densities at the start of the experiment were 1,154.9 larvae/m^2 for each beaker. The 25th and 75th percentiles are defined by the vertical extent of the box, while the thickest line (inside the box for C, top of the box for T1 and T2) represents the mean value. The whiskers mark the maximum and minimum values. Values outside the whiskers are considered outliers. The treatments are as follows: C–Control, T1–2 mg/L probiotic supplement, and T2–5 mg/L probiotic supplement.

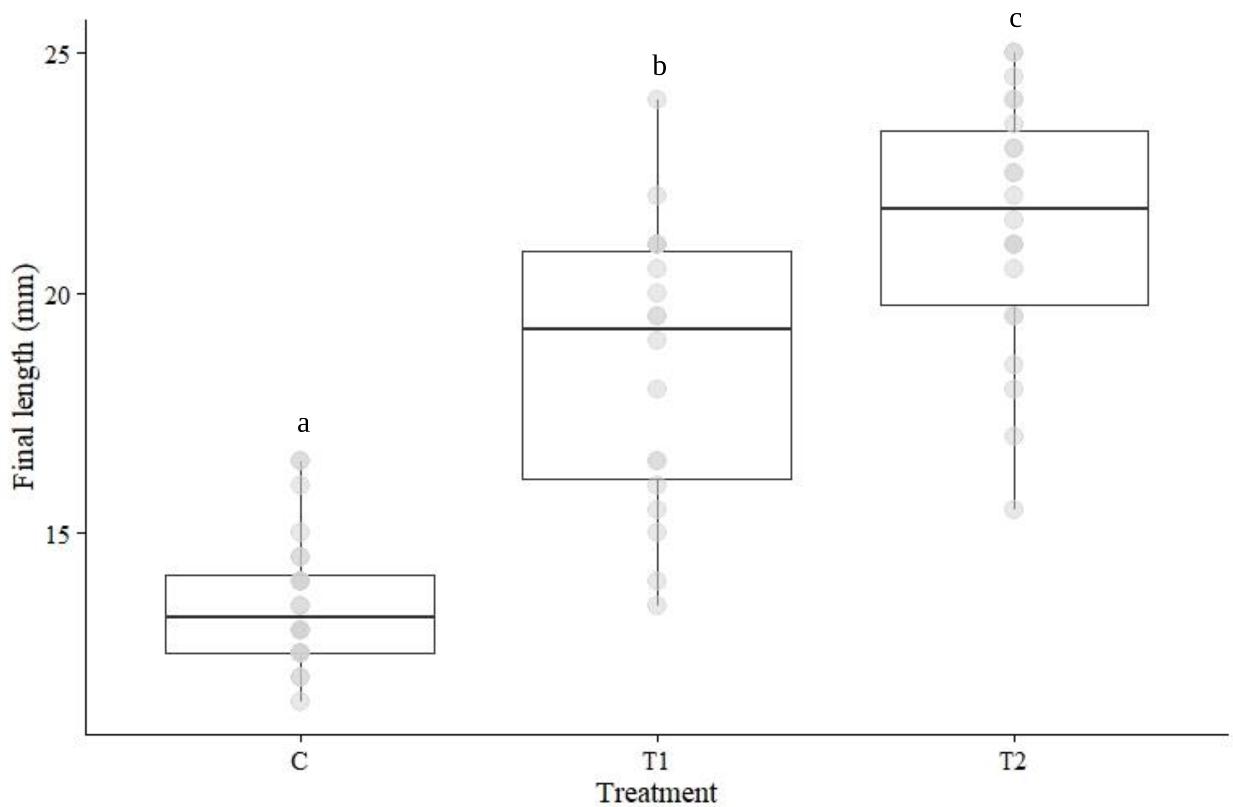

Figure 5-4. Box and whisker plots of mean larval length (mm) as a function of treatment for the 2018 experiment. The 25th and 75th percentiles are defined by the vertical extent of the box, while the line inside each box represents the mean value. The whiskers mark the maximum and minimum values. The treatments are as follows: C–Control, T1–2 mg/L probiotic supplement, and T2–5 mg/L probiotic supplement. Different letters above each treatment note significant differences as a result of the Tukey's HSD post hoc test.

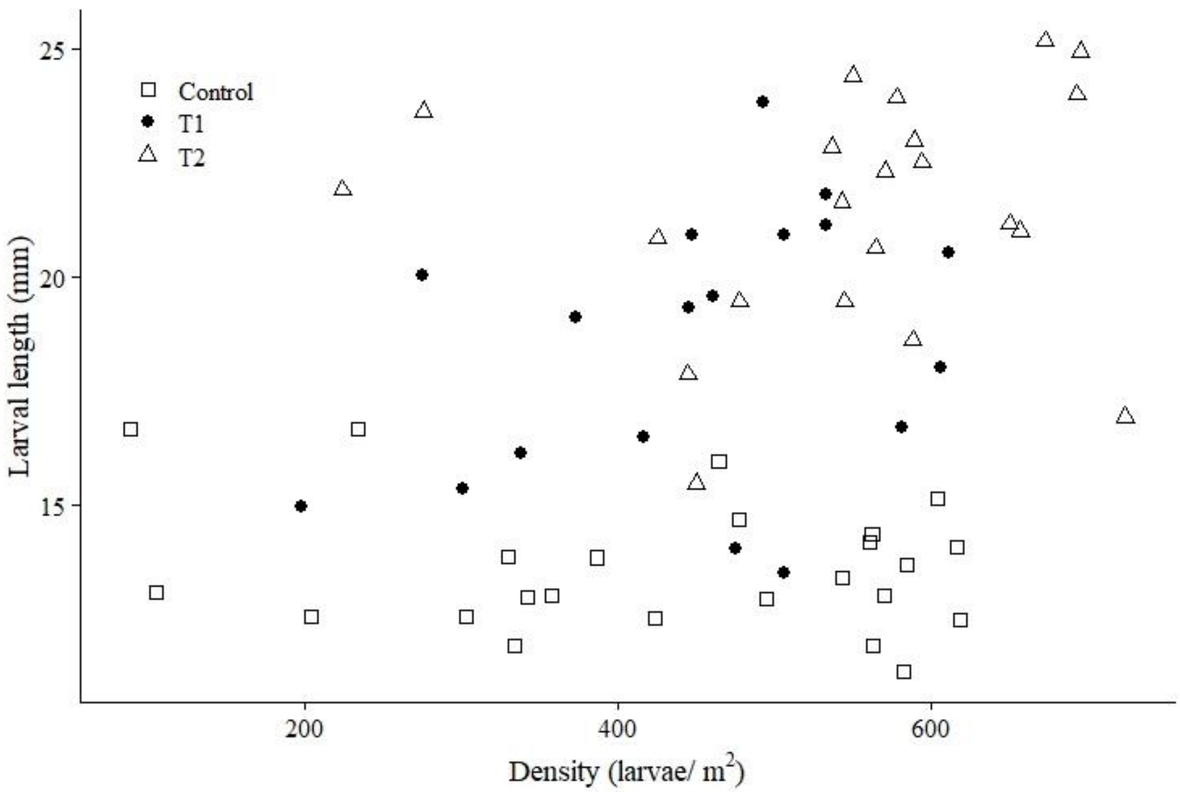

Figure 5-5. Final larval lamprey lengths (in mm) as a function of final larval density (larvae/m^2) at the end of the 2018 experiment. The treatments are as follows: C–Control (open squares), T1–2 mg/L probiotic supplement (solid circles), and T2–5 mg/L probiotic supplement (open triangles). The points are jittered to separate overlapping values.

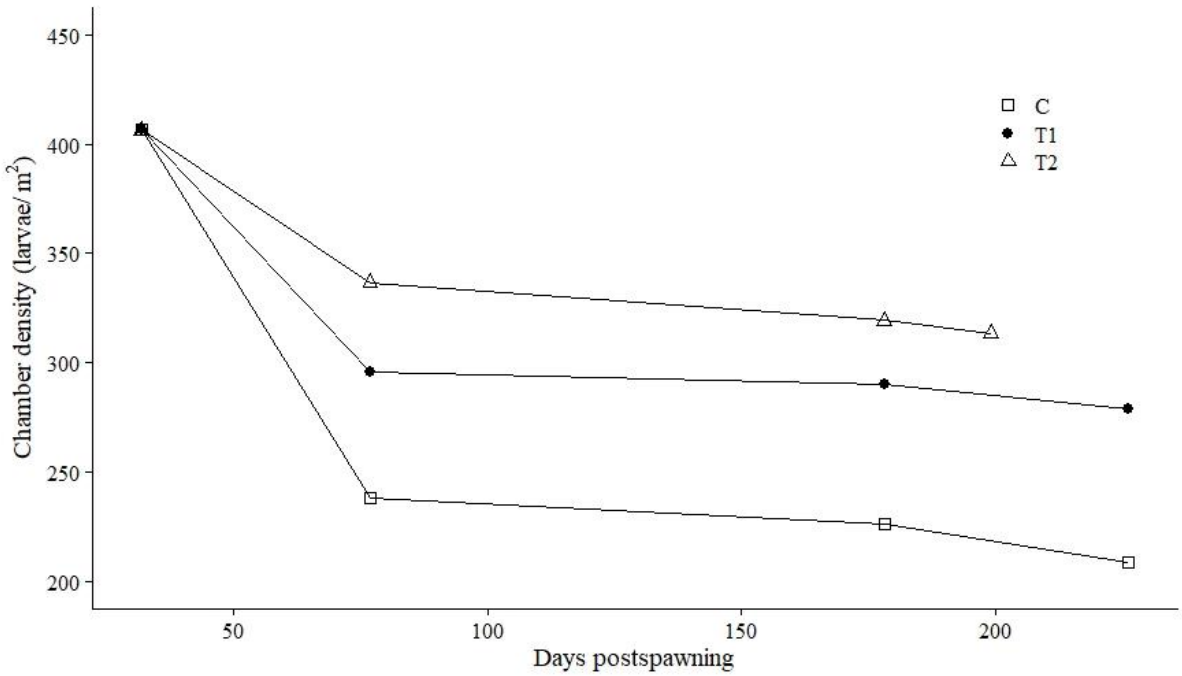

Figure 5-6. Density of tanks (larvae/m^2) during the 2019 probiotic experiment. Densities at the start of the experiment were 406.4 larvae/m^2 (774.1 larvae/m^3) for each tank. Larvae were assessed at 77, 178, 200, and 226 days postspawning during the 2019 experiment. The treatments are as follows: C–Control (open squares), T1–2 mg/L probiotic supplement (solid circles), and T2–5 mg/L probiotic supplement (open triangles). The T2 treatment larvae all died on day 200 postspawning.

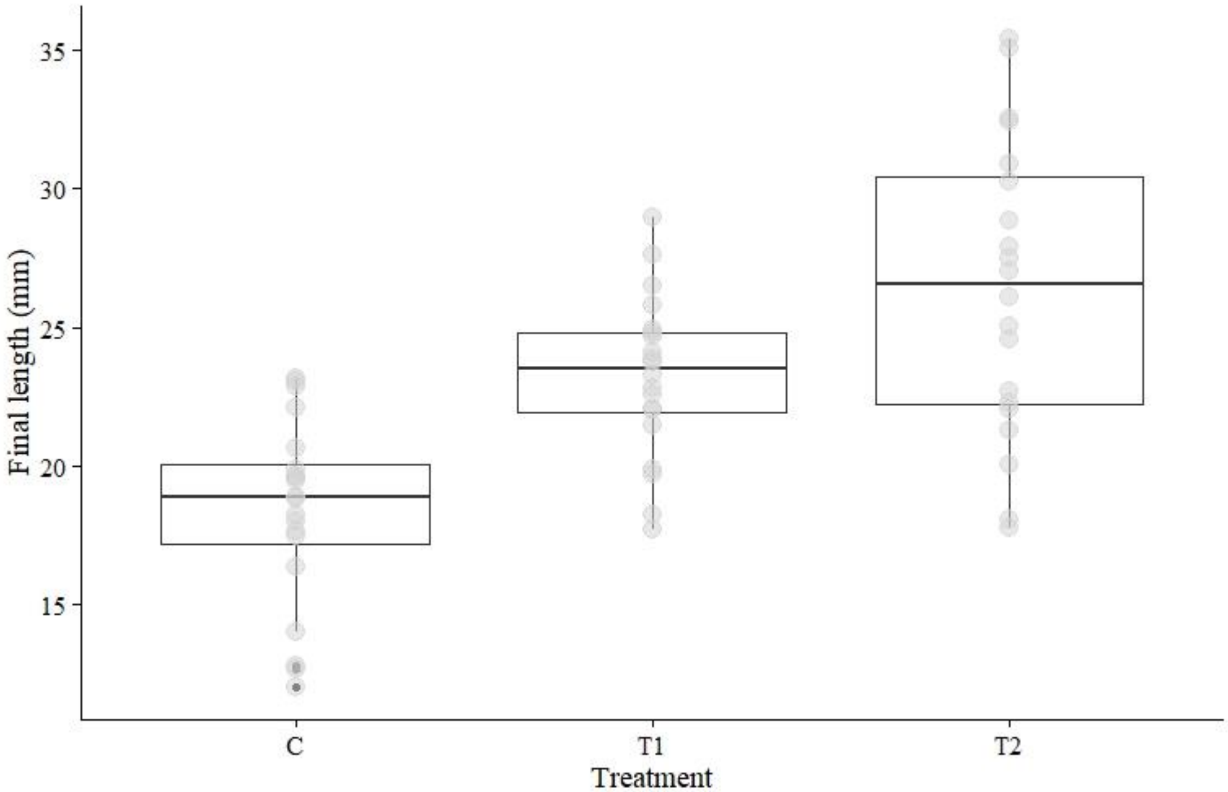

Figure 5-7. Box and whisker plots of mean larval length (mm) as a function of treatment for the 2019 experiment. Higher probiotic dose (T2) lengths were extrapolated from 200 to 226 days using the instantaneous growth rate to estimate final length for comparison due to mortality in the T2 tank prior to the end of the experiment. The 25th and 75th percentiles are defined by the vertical extent of the box, while the line inside each box represents the mean value. The whiskers mark the maximum and minimum values. Values that are outside the whiskers are considered outliers. The treatments are as follows: C–Control, T1–2 mg/L probiotic supplement, and T2–5 mg/L probiotic supplement.

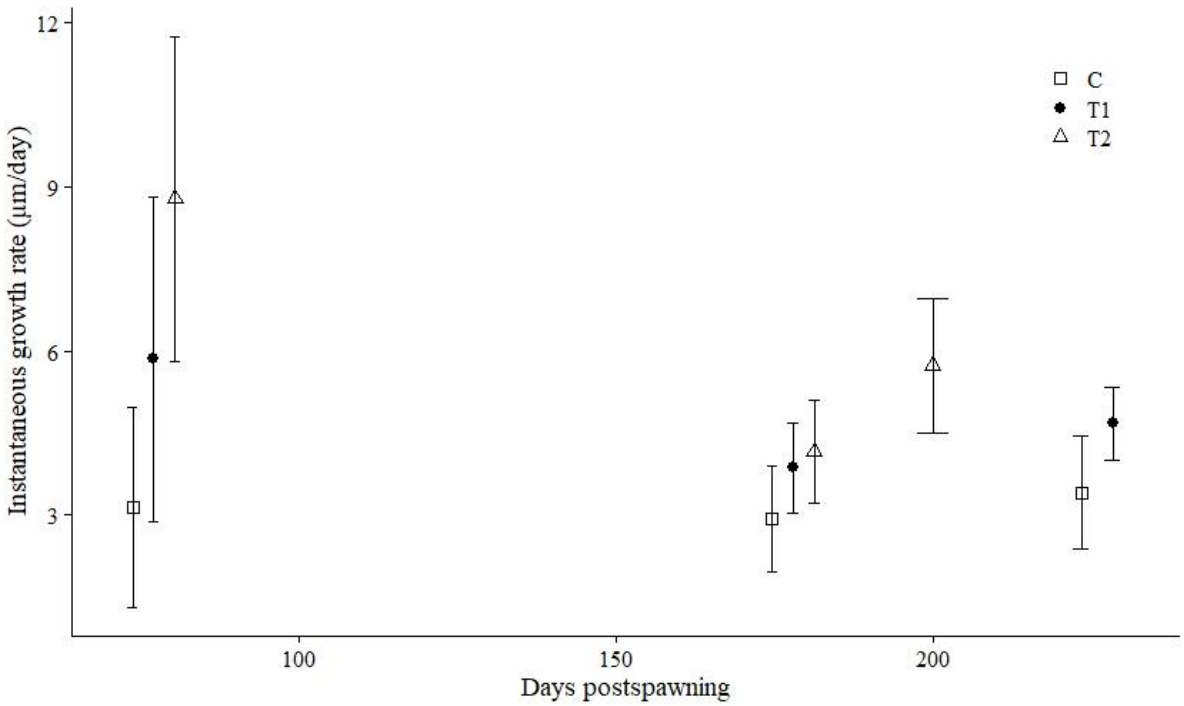

Figure 5-8. Instantaneous growth rate (µm/day) as a function of days postspawning. Larvae were assessed at 77, 178, 200, and 226 days postspawning during the 2019 experiment. The treatments are as follows: C–Control (open squares), T1–2 mg/L probiotic supplement (solid circles), and T2–5 mg/L probiotic supplement (open triangles). The points are jittered at each assessment period to separate overlapping values.

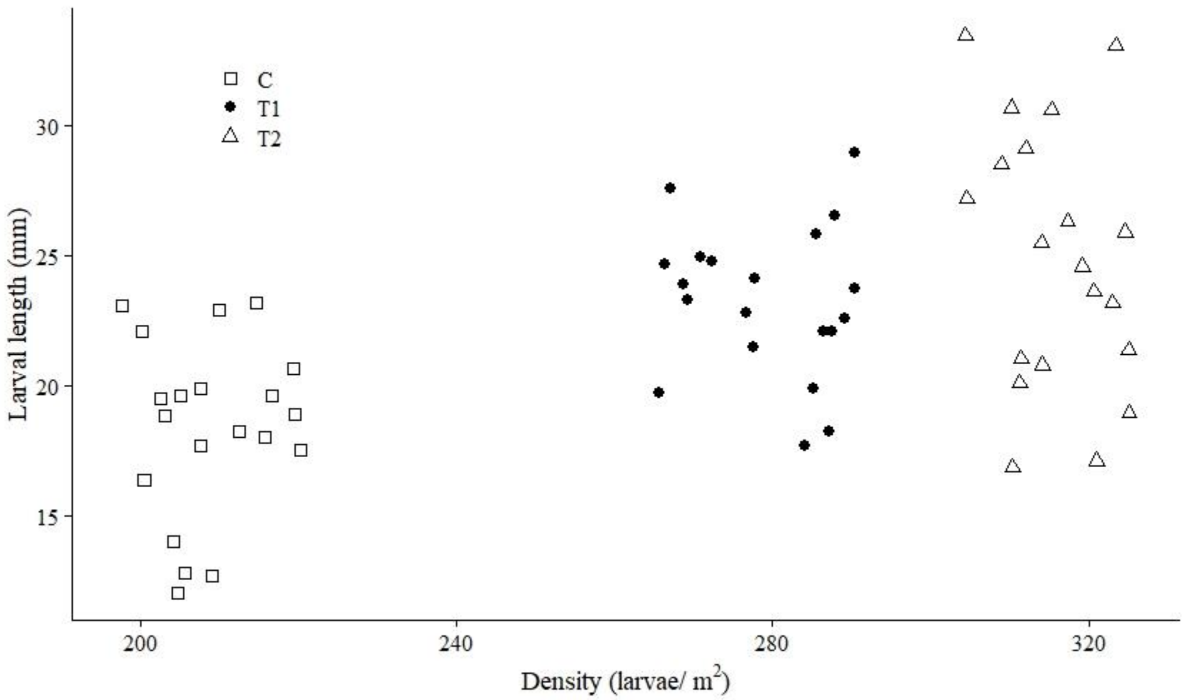

Figure 5-9. Final larval lamprey lengths (in mm) as a function of final tank density at the end of the 2019 experiment. The treatments are as follows: C–Control (open squares), T1–2 mg/L probiotic supplement (solid circles), and T2–5 mg/L probiotic supplement (open triangles). The points are jittered to separate overlapping values.

CHAPTER 6: THE BENTHIC ETHIC: LEOPOLD’S LAND ETHIC PRINCIPLES APPLIED TO RESTORATION OF THE RIVER COMMUNITY

Abstract

When benthic river organisms decline or disappear, ecological services are diminished. Benthic organisms like freshwater mussels and larval lamprey provide essential ecosystem services (e.g., water quality, nutrient cycling, and food-web connections) to higher- and lower-trophic-order organisms, but they are understudied and undervalued components of river ecosystems. Although scientists (and resource managers) do not understand many of these relationships well, tribal organizations like the Confederated Tribes of the Umatilla Indian Reservation (CTUIR) value such connections as part of their promise of reciprocity. Reciprocity, in this context, is humans caring for resources (First Foods: water, fish, big game, roots, and berries) that, in tribal creation belief, promised to take care of the people. If benthic communities are damaged, reduced, or lost, tribes may lose cultural connections to organisms such as native freshwater mussels and Pacific Lamprey (e.g., harvest ceremony, traditions, and histories passed between generations) and ecosystems lose the services that are provided by these organisms (e.g., nutrient cycling and storage, sediment bioturbation, and water quality improvements). Managers tasked to restore Pacific Northwest aquatic systems need to understand the role and contributions of ecosystem members, including those that historically have been less valued by western nonindigenous society. Projects to improve salmonid habitat have the potential to also improve habitat for benthic organisms like native freshwater mussels and lamprey with little additional effort. With the goal of restoring populations for harvest opportunities and restoring ecological function, adopting a holistic approach including lamprey and mussels in ongoing habitat and cultural restoration projects should be a priority for those in the Columbia River Basin.

The CTUIR resource management guiding framework (e.g., River Vision) is a holistic approach that prioritizes biotic connections alongside physical habitat improvements, which other agencies should implement to better serve the entire ecosystem more effectively. Aldo Leopold’s Land Ethic essay of the 1940s advocated for resource management to include and keep each piece of the whole, regardless of understanding its role. I present evidence that consideration for and reconnection of benthic organisms to the salmonid food-

web, as part of a functioning and healthy river ecosystem, could result in better outcomes for multiple native freshwater species: a "benthic" ethic. A healthy community in any freshwater system, and the cultures that depend on them, requires that all parts of the system are functional and connected. Without all of these components in place, the community is fragmented and essential processes are lacking. These adverse effects hinder the restoration of healthy river communities and the ecological services that historically have been provided by the Columbia River Basin, and do not foster the cultural reconnection of Columbia Plateau tribes.

Introduction

Balancing human and ecological demands for water and aquatic resources requires vision, compromise, and careful management, especially in light of the ever-expanding human population. Anthropogenic changes to aquatic environments have negatively affected ecosystems and the cultural services that are provided by those systems. To restore cultural and ecological services to rivers of the Pacific Northwest United States, the Confederated Tribes of the Umatilla Indian Reservation (CTUIR) developed an aquatic ecosystem restoration framework, River Vision, which describes five touchstones to guide restoration activities in CTUIR subbasins (hydrology, geomorphology, floodplain connectivity, riparian vegetation, and aquatic biota; Figure 6-1). The inclusion of aquatic biota, including species interactions and linkages between different trophic levels, is a unique aspect of the River Vision that separates it from other regional restoration efforts, which tend to be specifically focused on salmonids listed as threatened or endangered under the Endangered Species Act of 1973 (ESA). Typically, ESA-listed species receive the majority of funding in the Pacific Northwest relative to other aquatic organisms (NPCC 2014). Decades of salmonid restoration have had mixed success with no clear winners or fully recovered stocks (Lichatowich 2001; Taylor 2001; Reiman et al. 2015). The River Vision looks to biotic connections for solutions (Jones et al. 2008; Quaempts et al. 2018). Research and restoration projects for nonsalmonid members of the aquatic community such as freshwater mussels and Pacific Lamprey are ongoing at the CTUIR. The River Vision and CTUIR efforts are embodied in Aldo Leopold's Land Ethic principles of the 1940s in which he espoused that "If the land mechanism as a whole is good then every part is good, whether we understand it or not…to keep every cog and wheel is the first precaution of intelligent tinkering" (Leopold 1949). The holistic restoration approach to include biotic and abiotic elements, as called for in the River Vision, places value on the functioning whole rather than an individual species in the community. I highlight the potential benefits of this method of ecosystem management through a literature review, showing that aquatic community members serve important functional and ecological roles and could improve outcomes in ongoing salmonid habitat restoration projects. My objectives are to: (1) provide examples of reciprocity in the river community, (2) discuss reciprocity in practice in tribal resource management, and (3) advocate for benthic community inclusion (the benthic ethic) in region-wide river restoration, using the ethical foundations that are put forth in Leopold's Land Ethic essay.

Leopold (1949) described an ethic as "a limitation on freedom of action" or "a differentiation of social from anti-social conduct." These restrictions on human action are societally accepted and affirmed by cooperation from other humans who are also acting as such. Leopold (1949) suggested that these human-based ethics be stretched to incorporate land, meaning "soils, water, plants, and animals." The interdependence of these parts of the land mirrors the interdependence of humans on the land. Reciprocity between soils and plants, for example, is not unlike the reciprocity between humans and foods, a central tenet of Pacific Northwest tribal culture. One part of the system is dependent on another part, which is again dependent on the original part. Leopold's Land Ethic (1949) emphasizes that interdependence through the flow of energy between components of a system (or community), an idea widely recognized by ecologists (e.g., Elton 1927; Lindeman 1942; Odum 1968; Polis et al. 1997). As is the case in the land community, aquatic communities also rely on these relationships for ecological resilience and resource continuity (Quaempts et al. 2018).

Historically, the people of the CTUIR used healthy ecosystems with intact trophic relationships to obtain subsistence resources (Hunn et al. 2015). In the traditional CTUIR longhouse serving order (Figure 6-2), certain foods are consumed during specific times of the year (Figure 6-3). The seasonality and geographic distribution of foods allowed people to use resources strategically, traveling to harvesting locations throughout the year as foods became available locally. These traditional foods, called First Foods, are harvested in a way that leaves resources available for other members of the land community (animals and plants) as well as for the food (huckleberry, roots, etc.) to replenish itself. For example, during root harvest by the CTUIR, the seed head of harvested roots may be placed in the hole created by excavating the root to ensure the availability of roots for future gatherers (Quaempts et al. 2018). These acts of reciprocity from human to foods fulfill a promise to care for the plants or animals so that they can care for the humans. They emphasize the role of humans as an important part of the whole rather than the nonindigenous approach in which humans have dominion over all living things.

The River Vision describes a management framework for aquatic ecosystems that emphasizes restoration of not only structural components of habitat but also physical and ecological processes (Jones et al. 2008). This management approach has shifted from site-scale restoration to reach- or landscape-scale restoration to address ecosystem-wide changes that are needed to improve resilience and community stability (Quaempts et al. 2018). Managers attempt to not only protect but also restore resilient and dynamic river habitats to allow reestablishment of First Foods for continued use by the CTUIR.

First Foods management by the CTUIR fosters collaborative relationships with other agencies by allowing the CTUIR to communicate the importance of ecological and cultural resource availability and processes to protect, restore, and enhance such resources (Quaempts et al. 2018). Historic reliance on First Foods has promoted holistic management approaches that value landscapes as communities that collectively contribute to healthy populations of foods (Jones et al. 2008). This has led to collaborative efforts between the CTUIR and other Columbia Plateau tribes to restore populations of Pacific Lamprey to the Columbia River Basin through artificial propagation and other methods, not only to reestablish tribal harvest opportunities but also to restore a healthy ecological community to the Columbia River Basin. Freshwater mussels are valued by many Pacific Northwest tribes, but the CTUIR is the only regional agency with a mussel research and restoration project. The CTUIR offers support and guidance to other regional entities for mussel conservation, and I hope that this paper advances the awareness of their importance and the inclusion of mussels in regional restoration and conservation plans.

The main objective of this study was to examine reciprocity among river organisms, using a tribal resource management context, and to advocate for a shift to a holistic approach by inclusion of community organisms in restorations rather than focus on single species.

After presenting the ethical framework for my argument by using Aldo Leopold’s Land Ethic essay as a foundation, I summarize the biotic connections between two important benthic First Food organisms, mussels and lamprey, and a keystone First Food, salmon (representing salmonid fishes). I use the concepts of reciprocity and community from a tribal resource management perspective, examining the CTUIR River Vision policy and its role in extending restoration efforts beyond salmon and salmon habitat. I argue that the benthic community in the river environment provides essential services to both animals and humans, and restoration practitioners must acknowledge the need for such ecological and cultural services for their restoration efforts to be meaningful.

Ethics in restoration

“All ethics so far evolved rest upon a single premise: that the individual is a member of a community of interdependent parts. The land ethic simply enlarges the boundaries of the community to include soils, waters, plants and animals, or collectively the land.” Leopold’s (1949) stance on the land community can be applied in the context of Pacific Northwest rivers. The restoration of a community is essential for the conservation of functional relationships at higher trophic levels but is also the ethical and “right” way to move forward in freshwater habitat and species restoration. “First, do no harm” (Inman 1860, in Sokol 2013) is an essential part of the Hippocratic oath in the medical profession that emphasizes a code of ethics in which effort is made to prevent further injury or detriment to an individual during treatment for illness or disease. This phrase can, and should, be applied in other facets of our interactions with others and our environment to prevent unnecessary or avoidable damage and destruction. The attitudes and actions of humans, especially those focused on consumptive over use, are largely to blame for widespread ecosystem damages.

Restoration ethics and holistic community inclusion in restoration have only recently been studied in the Columbia River Basin. Naiman et al. (2012) acknowledge that habitat and species restoration projects that focus exclusively on structural habitat restoration miss essential components of complete restoration. Specifically, the authors present food-web connections as important components of holistic restoration in the Columbia Basin (Naiman et al. 2012). Reiman et al. (2015) also remark on the limited effectiveness of habitat restoration actions in the region and call for a more comprehensive approach to restoration.

This would include, in part, a rebalancing of restoration goals to manage for ecosystem resilience and biodiversity, as well as for the continuity of cultural services. This management approach is a shift away from the focus on single-species restorations, which make up the bulk of restoration actions in the Columbia Basin.

The role of biodiversity in ecological resilience has been explored by ecologists under a number of hypotheses. Walker (1992, 1995) describes the role of functional redundancy in an ecosystem, where more than one organism contributes some services to the functional whole. Systemic functionality is weakened by the removal of any one organism and, when we see the removal of more than one organism in a functional group, or observe the removal of entire groups, we see the resilience of a system teeter on the edge of collapse (Walker 1992, 1995). The rivet hypothesis also describes the amplification of species loss on the resiliency of an ecosystem (Ehrlich and Walker 1998). The loss of one species, metaphorically one rivet on the wing of an airplane, might not have an immediate detrimental effect on the ecosystem, but the loss of numerous species (rivets) could cause a complete collapse of the functional integrity of the system (Ehrlich and Walker 1998). Redundancy at each functional level (i.e., lamprey and mussels both contributing to biofiltration and nutrient cycling processes in the benthos) can enhance the resiliency of an ecosystem to respond to and recover from disturbance (Holling 1973; Walker 1992; Peterson et al. 1998). When one organism is removed from the system, others in its functional group will still carry out the biological processes of that group in its absence. This functional redundancy allows regeneration and renewal of ecosystem services and biogeochemical processes after disturbance. Tribally-led resource management focuses restoration strategy on biodiversity, resilience, and functionality rather than just physical restoration of structural components.

Keystone species (i.e., organisms whose activities alter or maintain species or habitat diversity, disproportionate to their abundance; Paine 1969) need to be maintained above a threshold level to maintain ecological effectiveness and functionality (Power et al. 1996; Soule et al. 2005). The ecosystem services that are provided by such strongly interactive species are reduced significantly when populations of these organisms drop below certain levels (Mills et al. 1993; Soule et al. 2005). Freshwater mussels, for example, act as keystone species in river ecosystems by inducing changes to the physical structure of sediments, biofiltration and water clearance rates, and plant and macroinvertebrate abundances which enhance biodiversity and function of the ecosystem at rates disproportionate to their abundance (Vaughn and Hakenkamp 2001; Howard and Cuffey 2006; Geist 2010; Vaughn 2017). However, these types of species are far less studied than are more charismatic organisms like salmon, so their true influence on the ecosystem is not well understood. This makes it important to advance measures to conserve and protect poorly understood native aquatic species. Just as the Land Ethic promoted awareness and a greater understanding of the connections between humans and environment and each part of the ecosystem to another (Leopold 1949), this benthic ethic will serve to push restoration professionals and ecologists to acknowledge and understand the importance of the supportive community of organisms surrounding charismatic organisms like salmonids. This ethic will also support the cultural practices of Columbia Basin area tribes like the CTUIR.

Many restoration projects are undertaken from a self-interested standpoint, in which restoration efforts are directed at organisms of broad economic value. Cairns (2003) lists several ethical issues that are related to restoration in the modern world. One is the homocentric view to restore the commodity of nature rather than an ecocentric view in which the intrinsic value of nature is considered to be equal to the intrinsic value of humans. Leopold (1949) also brings up this perspective in the Land Ethic essay in which he places humans within and among nature rather than superior to or having jurisdiction over the natural world. The CTUIR’s First Foods management programs promote the inclusion of humans as an integral part of the environmental community rather than above or superior to it (Quaempts et al. 2018).

The Millennium Ecosystem Assessment (MEA), a global analysis of the consequences of environmental change for human well-being, used an ecosystem services approach to evaluate biodiversity then and into the future (MEA 2005). Though the MEA’s approach was innovative in using ecological services as social currency, this type of value system still places humans above and separate from the ecosystem in which they reside. Carpenter et al. (2009) call for research to become more interdisciplinary in investigating the effects of reduced biodiversity on ecosystem services. A deeper understanding of socioecological systems as they relate to global and local ecosystem service provision is necessary to relate the work of the MEA to practical application in degraded systems. Carpenter et al. (2009) further call for the use of long-term, place-based ecological research to achieve the goal of locally-applicable socioecological research. Ormarod (2014) also explores the motives of the MEA type of decision-making framework, “If we recognize or value only those species, ecosystems, or processes that benefit our one dominant, resource-hungry species, what incentive is there to protect the many others that provide no such benefit?” Further, if we do not yet understand the role of an organism in a community, do we still have an obligation to protect its existence as a member of the community regardless of our understanding or perceived benefit?

Ecological services from benthic organisms benefit humans and other organisms

Ecosystem services are generally considered benefits to humans that are obtained from the environment (MES 2005). These could be provisioning (e.g., food and water), regulating (e.g., water filtration), supporting (e.g., nutrient cycling and environmental health indication), or cultural. While I discuss the first three services below, I will address cultural services distinctly here. “Religion, culture, and natural resources are…inherently linked” (CTUIR 2015). Because tribal creation stories center on food resources and their connection to humans, it is impossible to separate culture and ecology in this context (T. Farrow-Ferman, CTUIR, Pendleton, Oregon, personal communication). For the CTUIR, ecological services that are provided by organisms are the same as cultural services. This distinction is important because the values that are placed on organisms reach beyond an economic or functional threshold and bridge the gap between human and nature (Folke et al. 2010; Quaempts et al. 2018). The Columbia Plateau tribes have experienced a loss of cultural connection to freshwater mussels in particular, and ecosystems are negatively affected through a loss of important ecological services that are delivered by mussels (CTUIR 2015; Vaughn 2017). Similar to the Land Ethic, tribal culture places humans within the natural world rather than presiding over it (Hunn et al. 2015).

The serving order of First Foods, where water is served first and last, acknowledges a reciprocal relationship between water and all foods, and humans (Quaempts et al. 2018). In the creation story of the CTUIR, “the Creator asked the foods, ‘who will take care of the Indian people?’ Salmon was first to promise, then other fish lined up behind salmon. Next was deer,” and so on (Jones et al. 2008; Quaempts et al. 2018). “Then the other fish lined up behind salmon….”; this particular portion of the quote above deepens the appreciation of reciprocity to include those supporting organisms on which the salmon has relied for millennia.

Current populations of lamprey and mussels are severely reduced from historic numbers (Blevins et al. 2017a; CRITFC 2018). As two of the most historically abundant benthic species, Pacific Lamprey larvae and native freshwater mussels have significantly contributed to healthy riverine processes for millennia, especially food-web interactions, nutrient cycling, and water quality improvement (Kan 1975; Simpson and Wallace 1978; Close et al. 2002; Haag 2012; Vaughn 2017; Figure 6-4). Though at a reduced rate from historic levels, lamprey and mussels still provide essential ecosystem services in freshwater systems. Freshwater mussels contribute to all classes of ecosystem services, as described in Vaughn 2017 and Strayer 2017: biofiltration (regulating); nutrient cycling and storage, habitat modification, environmental monitoring, and food-webs (supporting); and food for humans and other organisms (provisioning) (Figure 6-5). Mussels stabilize substrates, slow erosional processes, increase oxygen penetration into river sediments, and reduce particulates in the water column (Haag 2012; CTUIR 2015; Vaughn 2017). With reduced populations of both lamprey and mussels, ecosystem services are also reduced. The benefits of robust populations of larval lamprey and mussels could help alleviate some ecological disfunction observed in the Columbia Basin (e.g., contaminants, low dissolved oxygen in substrate, etc.), and contribute more significantly to a healthy, functional river community.

As omnivores that feed on planktonic and detrital food sources of < 20 microns in size, freshwater mussels enact control over different trophic levels (Vaughn et al. 2008). Other studies show that mussels have the ability to directly absorb molecules such as NH_3, confirming their vital role in nutrient cycling and their potential as a tool for bioremediation in the lab or in wild systems (Vaughn et al. 2008; reviewed in Vaughn 2017). The feeding activities of both freshwater mussels and larval lamprey serve as a link between the water column and the benthic zone. Macroinvertebrate communities are more diverse and abundant when they are associated with mussel beds, suggesting a cascade effect of mussel-feeding ecology in cycling of particulate matter and dissolved organic matter to the benthos (Howard and Cuffey 2006; Spooner and Vaughn 2006). Mussels also increase growth in larval Pacific Lamprey by producing pseudofeces, undigested material that is siphoned from the water column and deposited on the benthic substrate (Limm and Power 2011). Thus, they are linkages among multiple trophic levels in aquatic ecosystems.

Mussels interact directly and indirectly with other aquatic community organisms. Mussels use fish to transform their parasitic larvae into juveniles and have been shown to do so at higher rates in the lab when they are tested with locally collected fish rather than with fish of the same species from a different watershed (Haag and Warren 2003). Western freshwater mussels, in particular, show preferences for native fishes as hosts over nonnative fishes (CTUIR, unpublished data; O'Brien et al. 2013; Chapter 3). The community on which mussels rely for food, reproduction, and other resources also relies on services provided by mussels. These reciprocal community interactions serve important functional links between the benthos and the water column.

The mussel shell is also an untapped resource in ecological bioremediation services. Made primarily of calcium carbonate, the mussel shell is also comprised of a variety of other materials (Haag 2012). The mussel shell can act as a nutrient sink, in which the mussel uptakes nutrients at a greater rate than it excretes by sequestering those chemicals as shell material (e.g., strontium, zinc, mercury, phosphorus, nitrogen, etc.) (e.g., Vanni 2002; Strayer and Malcolm 2007; Vaughn 2017). After the mussel dies, those materials are released back into the environment at a much slower rate than that of tissue decomposition (Strayer and Malcolm 2007; Vaughn 2017). Because mussels are long-lived (e.g., *Margaritifera falcata* can live 100 years or more; CTUIR 2015), their contributions to nutrient cycling and storage can be significant. Reduced populations of mussels in the Columbia River Basin take up increasing amounts of chemicals and contaminants as human populations increasingly encroach on natural areas. Restoration of mussels to levels more reflective of historic abundance will spread this burden across more individuals, improving individual mussel and ecosystem health.

Pacific Lamprey at all life stages also provide services that benefit freshwater systems. Pacific Lamprey larvae are filter feeders in freshwater for 3–7 years or more: filtering water (regulating); cycling nutrients, contributing to food-web dynamics, and acting as indicators of ecosystem health (supporting); and serving as food for birds, mammals, and other fishes (provisioning) (Kan 1975; Close et al. 2002; Bettaso and Goodman 2010;

CRITFC 2011; Luzier et al. 2011; Hogg et al. 2014; Figure 6-6). As adults, Pacific Lamprey are a key food source for mammals such as seals and sea lions, often buffering migrating adult salmonids from predation (provisioning) (Close et al. 1995; Close et al. 2002; Luzier et al. 2011). Adult lamprey that return from the ocean to spawn, and later die, provide important marine-derived nutrients to the upper reaches of watersheds (supporting) (Kan 1975; Beamish 1980; Close et al. 1995; Ward et al. 2012; Hogg et al. 2014). The loss of abundant populations of lamprey and mussels from the Columbia River Basin has reduced the beneficial ecosystem services that are contributed by these organisms, thereby negatively affecting the health and resiliency of the watershed (CRITFC 2018; Quaempts et al. 2018).

As ecosystem engineers (Jones et al. 1994), larval lamprey can also affect major changes on their environment, triggering cascading effects elsewhere in the trophic structure. For example, larval lamprey induce change in the benthic microbial community structure from anerobic to aerobic bacteria species by increasing interstitial and open water exchange through their burrowing and bioturbation behaviors (Boeker and Geist 2016). Larval lamprey also increase oxygen availability, sediment softness, and nitrate concentrations in interstitial water (Shirakawa et al. 2013; Boeker and Geist 2016). Ecological services provided by larval lamprey burrowing, feeding, and bioturbating activities can positively influence other benthic organisms.

Because larval lamprey occupy benthic habitats for several years as filter feeders, they could serve as ecological indicators for ecosystem health. Similar to freshwater mussels, larval lamprey store contaminants in their tissues (e.g., mercury; Nilsen et al. 2015). Pacific Lamprey have also shown potential in bioremediation during a study that reared larvae using effluent water from a salmonid hatchery (Barron et al. 2020). While these larvae did not significantly contribute to overall improvement in the quality of the effluent water, they were able to use nutrients after they had already passed through salmonids. In the wild, larval lamprey perform similar services in addition to serving as an important food source for other aquatic organisms, often buffering young salmonids from predation pressure (Close et al. 2002).

Lamprey, mussels, and other nonsalmonid aquatic community members serve important food-web connections to salmonids and other river organisms, but the mechanisms behind these are not well studied (Naiman et al. 2012; Bellmore et al. 2013). It is known that stability and adaptive capacity in riverine food-webs are highly dependent on the types and numbers of linkages between species similar to the rivet-redundancy theory discussed above (Ehrlich and Walker 1998; Naiman et al. 2012). Naiman et al. (2012) call for a better understanding of how nonnative organisms interact or change the community structure of native biota and their associated food-webs. The general lack of information regarding the populations, status, and ecological roles of nonsalmonid organisms highlights the need for more comprehensive restoration plans and for this “benthic ethic” to give a voice to ‘quiet’ players in the community.

Reciprocity and community in resource management

The Department of Natural Resources at the CTUIR bases its resource management philosophy on the concepts of reciprocity and community, as discussed in Jones et al. (2008) and Quaempts et al. (2018). Reciprocity, in this context, emphasizes the alignment of the management of First Foods, like lamprey and mussels, with long-term ecosystem resilience rather than short-term exploitation. But beyond that, this principle is rooted in the idea that the resources will take care of the people if the people take care of the resources (Jones et al. 2008). Population restoration is a means of restoring resilience to the ecosystem and restoring tribal harvest opportunities, as a healthy and functioning ecosystem relies on all parts of the community working together. Using a holistic approach to watershed management, the River Vision (Jones et al. 2008) details the importance of the “community” of an ecosystem. The focus on restoration of key riverine processes (i.e., ecosystem services) values the role that each trophic level plays in the long-term health and resilience of an ecosystem (Jones et al. 2008). Outside of indigenous resource management, the ecosystem approach to resource management was presented by Aldo Leopold in the 1940s and has been used to guide some modern restoration practices, in which there has been recent substantial tribal participation (Leopold 1949; CRITFC 2011; Rieman et al. 2015; CRITFC 2018). However, some restoration practices fall short of Leopold’s message by focusing narrowly on a single organism instead of a community of interacting species. The benthic community in a river can influence resilience, productivity, and biodiversity in the river ecosystem.

The Northwest Power and Conservation Council's Columbia Basin Fish and Wildlife Program lists restoration of ecosystem function as an overarching strategy (NPCC 2014). Key principles within this strategy focus on restoring habitat, maintaining native species biodiversity, managing invasive species and predators, protecting ecosystems from future hydroelectric operations, maintaining and improving water quality, mitigating climate change, managing surface and ground water flow, preserving estuary and nearshore areas, and mitigating wildlife losses. Though discussed briefly within existing principles, food-web interactions and native, nongame organisms are not prioritized within this framework. Tribal agencies acknowledge the importance of freshwater mussels, for example, but other regional efforts only weakly consider this or other benthic organisms (NPCC 2014). A healthy benthic community, which supports the food-web connections of target organisms (e.g., salmon), is often overlooked in nontribal habitat restoration projects.

Quaempts et al. (2018) posit that increased ecosystem resilience can be balanced with judicious resource extraction through resource management practices that pay tribute to the reciprocity that is promised in the creation story. Adaptive management, an iterative and structured approach to managing uncertainty in resource management (e.g., McLain and Lee 1996; Walters 1997), and holistic principles (i.e., reciprocity, community, and ecosystem resilience) are used together in the River Vision, a culturally rooted, visionary management framework that combines the use of science and traditional knowledge in pursuit of restoration and conservation goals (Jones et al. 2008; Quaempts et al. 2018). These two approaches work in concert to move restoration forward in the absence of some biological information, while allowing for feedback loops to reincorporate new information as it becomes available. The benthic community is historically not well studied in restoration treatments, but a holistic adaptive management process can incorporate significant findings into ongoing restoration work for a more comprehensive approach.

Increasingly, community organisms like those in the benthos are included in habitat assessments and plans in the Pacific Northwest (Naiman et al. 2012; Reiman et al. 2015; Quaempts et al. 2018). Inclusion of these organisms in salmonid-focused restoration projects has only been implemented recently. Process-based restoration, as practiced through the River Vision in CTUIR habitat-restoration projects, expands efforts beyond heavily engineered approaches that are directed to control river dynamics and focuses on the restoration of critical functions of the ecosystem (Beechie et al. 2010). Process-based restoration includes customizing actions to local biodiversity potential and, though not explicitly named, this could include the identification and restoration of historic benthic aquatic community members like mussels and lamprey (Beechie et al. 2010). Including benthic organisms more clearly into process-based restoration practices could effectively enhance essential ecological services to newly restored river reaches.

Salmonid-focused restoration practices generally benefit larval lamprey and freshwater mussels in the long term by improving habitat conditions (e.g., increasing channel complexity, improving flow refuge areas, and modulating thermal regimes; Gonzalez et al. 2017; Roni et al. 2018). However, without consideration in planning phases, lamprey and mussels can be damaged or killed during instream work like channel reconstruction, dewatering, and from heavy equipment in the river (Blevins et al. 2017b; LTW 2020). The CTUIR's River Vision calls for restoration actions to consider biological processes and ecological functionality (Jones et al. 2008; Quaempts et al. 2018). Including lamprey and mussels in prerestoration planning and during salvages on-site will prevent damage to or the loss of their existing ecological and cultural services as a direct result of restoration practices.

As a case study in the use of River Vision and process-based restoration principles, a CTUIR salmonid habitat-restoration project at Bird Track Springs in the Grande Ronde Basin, Oregon included nonsalmonid organisms in its preconstruction and planning efforts. Freshwater mussels and Pacific Lamprey were specifically targeted for salvage and relocation simultaneous with salmonid salvage efforts. Further, engineering plans for physical habitat restoration work explicitly marked an existing mussel area to protect a particularly dense mussel bed during in-stream work (Figure 6-7). The salvage effort resulted in the relocation of over 10,000 freshwater mussels, 550 Pacific Lamprey larvae, and 7,000 native nonsalmonid fishes, in addition to nearly 200 salmonids (CTUIR, unpublished data). The large numbers of nonsalmonid organisms that were using this < 2-km river reach before the restoration highlights importance of a community of diverse, native organisms in salmonid systems. This < 2-km river reach (in need of habitat-restoration efforts) supported 200 salmonids. The reach also supported thousands of nonsalmonid organisms that were helping to support those 200 salmonids through food-web connections and ecosystem services. The structure and function of this river community, with many nonsalmonid members supporting few salmonids, highlights the importance of holistic restoration that benefits not only a First Food (salmon) but also the community on which salmon relies for its own subsistence. Though performance metrics have not yet been explored from this project, mussels relocated from the in-stream work areas were observed alive, filtering, and burrowed into the sediment when observed several months after the project was complete, signaling continuity of ecological service contributions (CTUIR, unpublished data). Continued consideration for aquatic communities will provide opportunities to research understudied organisms and their role in the ecosystem, as well as provide a diverse and native community for salmonids after structural restoration.

The benthic ethic

Western freshwater mussels and larval Pacific Lamprey were historically two of the most abundant benthic species in Pacific Northwest freshwater ecosystems. Today, populations of both organisms have declined so that tribal harvest opportunities are nonexistent or severely curtailed to limited numbers and areas (Kan 1975; Close et al. 1995; Close et al. 2002; CTUIR 2015; Blevins et al. 2017a). Because of the reduced opportunities to exercise harvest rights, tribal members are losing cultural connections to these foods, including traditional harvest methods and locations, preparation methods, and stories and legends associated with them (Close et al. 1995; Close et al. 2002; CTUIR 2015; Quaempts et al. 2018). The concept of reciprocity obliges the people to take care of the resources so the resources can take care of them (Jones et al. 2008; Quaempts et al. 2018). Reciprocity in resource management is “an elegant connection between humans, foods, and landscapes that is an integral belief in many tribal and indigenous communities” (Quaempts et al. 2018). In this context, reciprocity drives the development of management methods that value the community over the individual. The benthic ethic seeks to represent the underserved members of the aquatic community, especially in projects like aquatic habitat restorations that are focused on salmonids, where human-derived damage is corrected though often at a high cost to the noneconomically valuable members of that ecosystem.

As retold by Thomas Morning Owl, an enrolled CTUIR tribal member, tribal elders recognized the value of every member of the society, “The harbingers of health and ecological vitality are great and small” (CTUIR 2015). Tribal members through time have recognized the connections between one organism and another, particularly in the river ecosystem. Speaking of the loss of Celilo Falls and the damming of the Columbia River, CTUIR tribal member David Wolf stated "It wasn't just the salmon that became lost, but the mussel beds that lined the rapids that ran the course of the Columbia also disappeared" (CTUIR 2015).

As ecological participants, humans modify ecosystems and habitats. Historically, nontribal humans have done this largely for their own benefit. In an effort to control rivers, prevent flooding, produce power, and manage aquatic resources, significant damage has been done to populations of salmonids and other members of the aquatic community in the Columbia River Basin. The damage to habitat and populations is so substantial that extinctions of species have occurred, often without significant concern or study (Pimm and Raven 2000). In more recent times, humans are realizing the need for the restoration of some of these habitats and organisms. Largely these restorations are implemented for economic purposes such as the restoration of populations of salmonids for recreation and revenue opportunities. Restoration of habitats helps support these organismal restorations as well. However, these restorations often fall short. Salmonids do not return in large numbers as they once did, and returning adults are smaller and less fit than those that were present in the area previously (Lichatowich et al. 1999; Williams et al. 1999; Araki et al. 2008). This benthic ethic calls for the restoration of the salmonid support community as a mode of improving effectiveness of existing and ongoing restoration projects. Food-web connections, species linkages, ecosystem services, and cultural significance are all factors that are at play in salmonid restoration that are beyond the historic focus on structural habitat modification (Williams et al. 1999). Including native, historically abundant, benthic community organisms like freshwater mussels and Pacific Lamprey will diversify ecological communities and processes, enhance management understanding, and benefit restoration efforts for river community members like salmon that were historically abundant when aquatic communities and their structures were more diverse.

Ecological services provided by organisms like larval lamprey and freshwater mussels connect the benthos with the rest of the river community in reciprocal community relationships. River ecosystems rely on benthic biodiversity for functions and services that enhance the resiliency of the system. Anthropogenic disturbances increasingly hinder the functional integrity of such systems, and tribal restoration programs work to restore such functionality. The CTUIR's River Vision, in a similar fashion to Leopold's Land Ethic, calls for holistic restoration of all parts of the system to restore the functional whole (Jones et al. 2008; Quaempts et al. 2018). The benthic ethic, as described in this chapter, is the ethical responsibility of river restoration practitioners to recognize and protect the important functional services provided by all benthic organisms. These services benefit traditionally targeted restoration organisms like salmonids but have wide-ranging positive influences on the entire river community and human populations that depend on it. Restoration practices region-wide can be improved through comprehensive management action plans (see Vander Zanden et al. 2006; Naiman et al. 2012; Rieman et al. 2015) that include complex species interactions and functional relationships as part of their performance metrics. Further, funding agencies should emphasize functional restoration over structural restoration, as some of the Columbia Basin Fish and Wildlife Programs do (NPCC 2014). Nontribal entities conducting restoration work should also make efforts to connect their target restoration organisms with the river community as a whole for cohesive restoration outcomes at the community-level (Blevins et al. 2017b; LTW 2020). This benthic ethic calls attention to the interconnectedness of ecological and cultural systems for tribes of the Columbia Plateau, and urges restoration professionals in the Columbia River Basin to acknowledge and understand reciprocity in the river community, take steps to preserve and restore existing benthic biodiversity, and enhance the functional integrity for the improved resilience of the river ecosystem..

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Figures

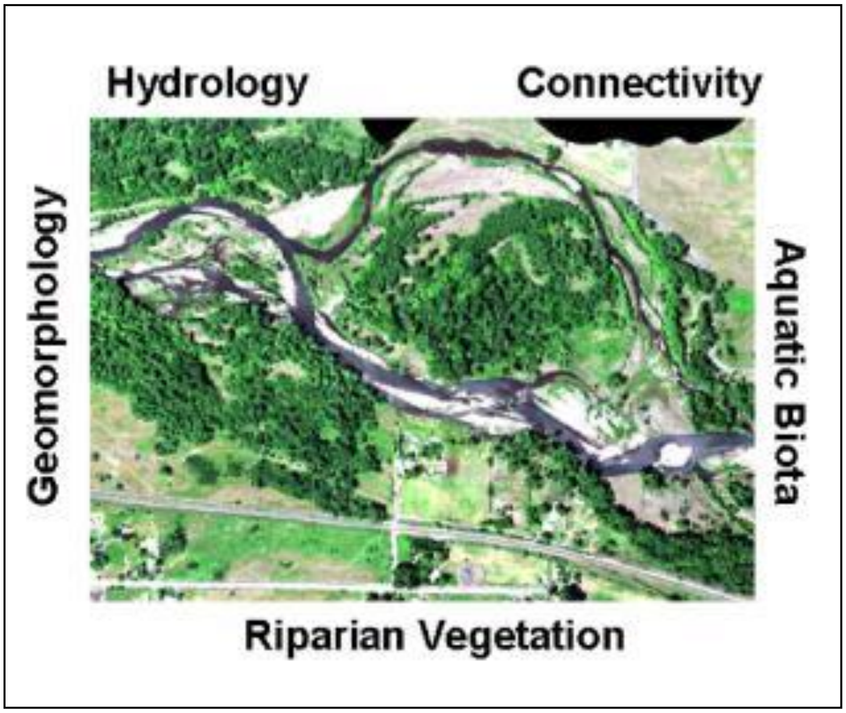

Figure 6-1. The CTUIR River Vision touchstones (Jones et al. 2008). Each touchstone represents management actions that are taken to facilitate the restoration of ecosystem structure and function. In this chapter, I focus on the Aquatic Biota touchstone, in which species linkages and connections between river organisms are valued for their ecological and cultural service provisions. Figure used with permission.

Figure 6-2. Longhouse serving order of traditional First Foods of the CTUIR people. Water is served first and last to signify its importance to both humans and the other First Foods. Figure modified from Shippentower 2019.

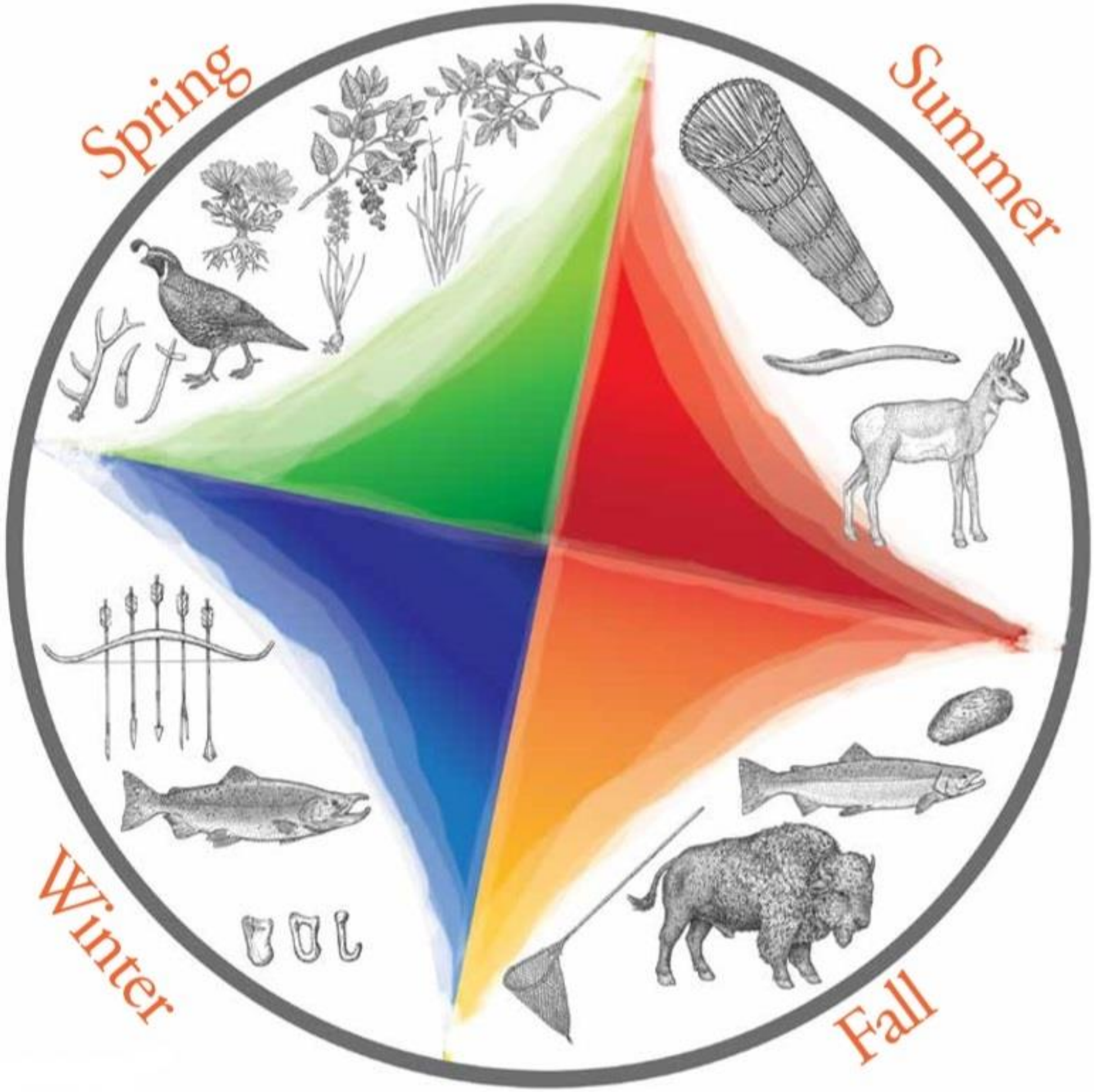

Figure 6-3. Seasonality of traditional foods of the CTUIR people. Image by Donna Nez and Stephanie Kaping, originally published in CTUIR 2015, reproduced with permission.

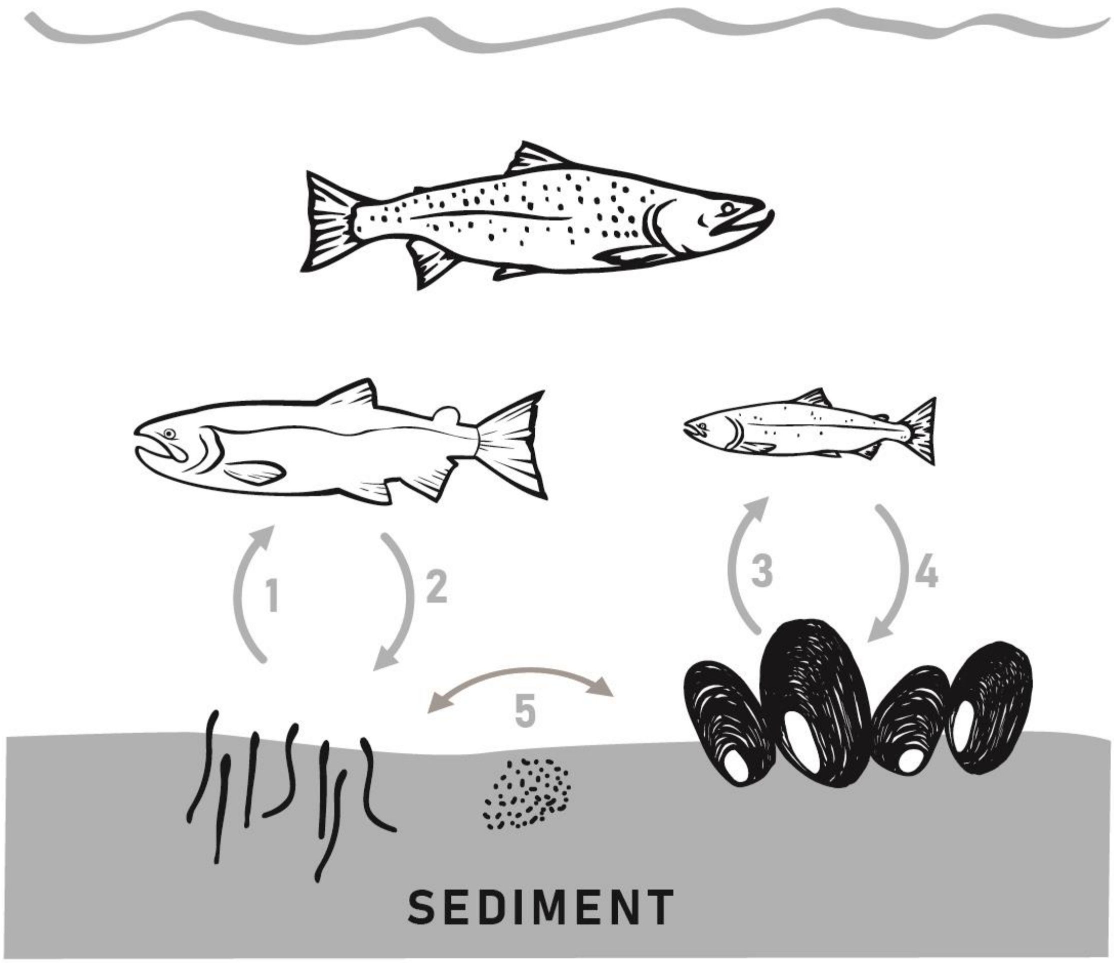

Figure 6-4. Ecosystem service exchange between members of the river community. (1) Food-web connections from larval lamprey to salmonids and other fishes; (2) larval lamprey filter water and breakdown organic matter, providing clean water and nutrient cycling for other organisms; (3) freshwater mussels use fish to transport and transform their larvae, and they also filter water, cycle nutrients, and reduce particulate matter in the water column; (4) mussels support fish food-webs by increasing the abundance and diversity of macroinvertebrate populations and substrate deposition of suspended matter; and (5) mussels and larval lamprey exchange nutrients laterally in the benthos, each supporting the filtering and bioturbation activities of the other, and contributing to the development and maintenance of a benthic microbial community. Figure by the author and J. Hooghkirk.

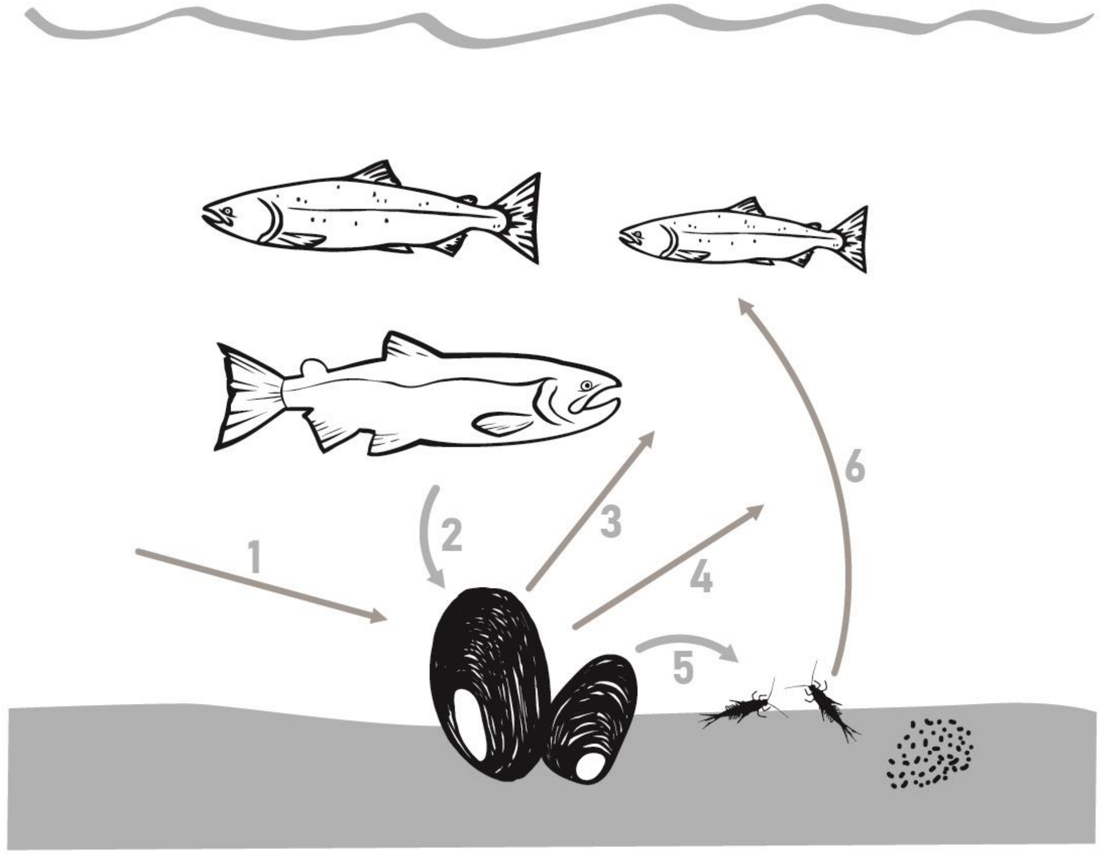

Figure 6-5. Ecological role of freshwater mussels in the river community. Mussels provide a variety of services of benefit to other river organisms like salmonids, including (1) biofiltration, (2) nutrient cycling and storage, (3) the reduction of particulate matter in the water column, (4) the reduction of contaminants and pathogens in the water column, (5) food-web connections by supporting benthic macroinvertebrate and microbial communities, and (6) indirect services such as linking benthic and open water communities. Figure by the author and J. Hooghkirk.

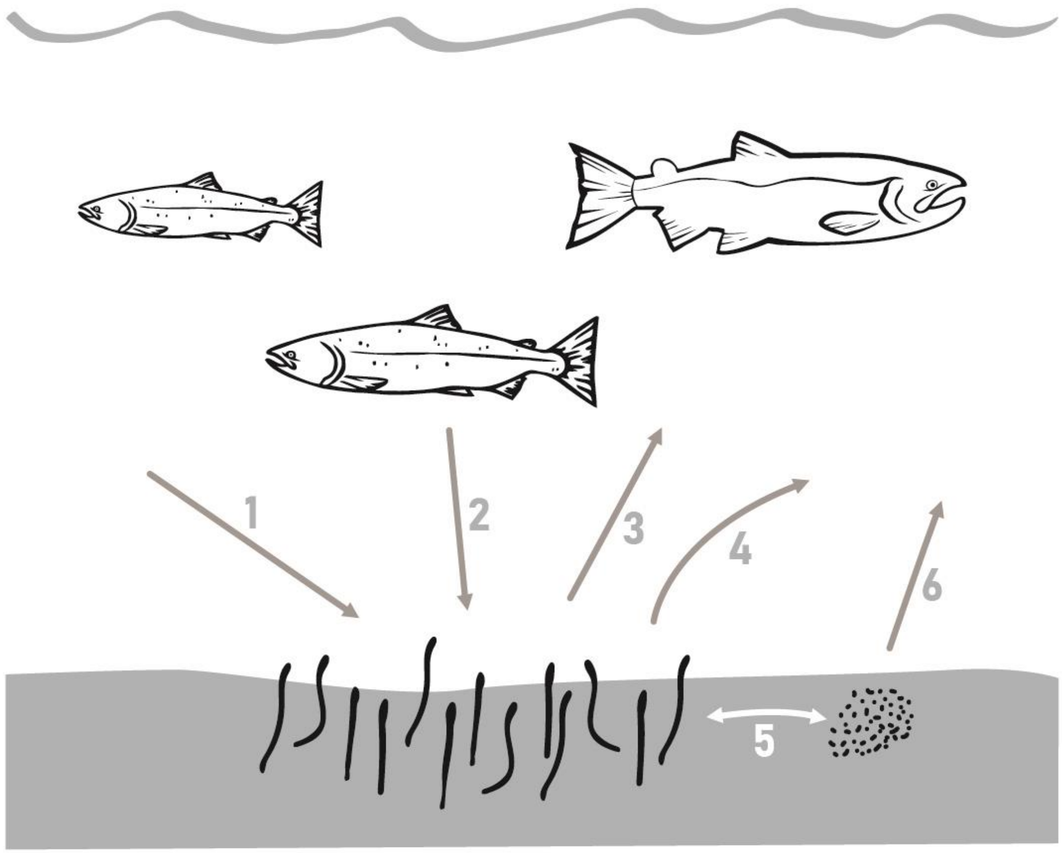

Figure 6-6. Ecological role of larval lamprey in the river community. Larval lamprey provide a variety of services that are of benefit to other river organisms like salmonids, including (1) surface and interstitial water filtration, (2) nutrient cycling and storage, (3) food-web connections (i.e., as a food source for fishes or buffering other benthic organisms from predation), (4) supportive services like acting as ecological health indicators, (5) exchange of nutrients between benthic organisms and microbial communities, and (6) indirect services such as linking benthic and open water communities. Figure by the author and J. Hooghkirk.

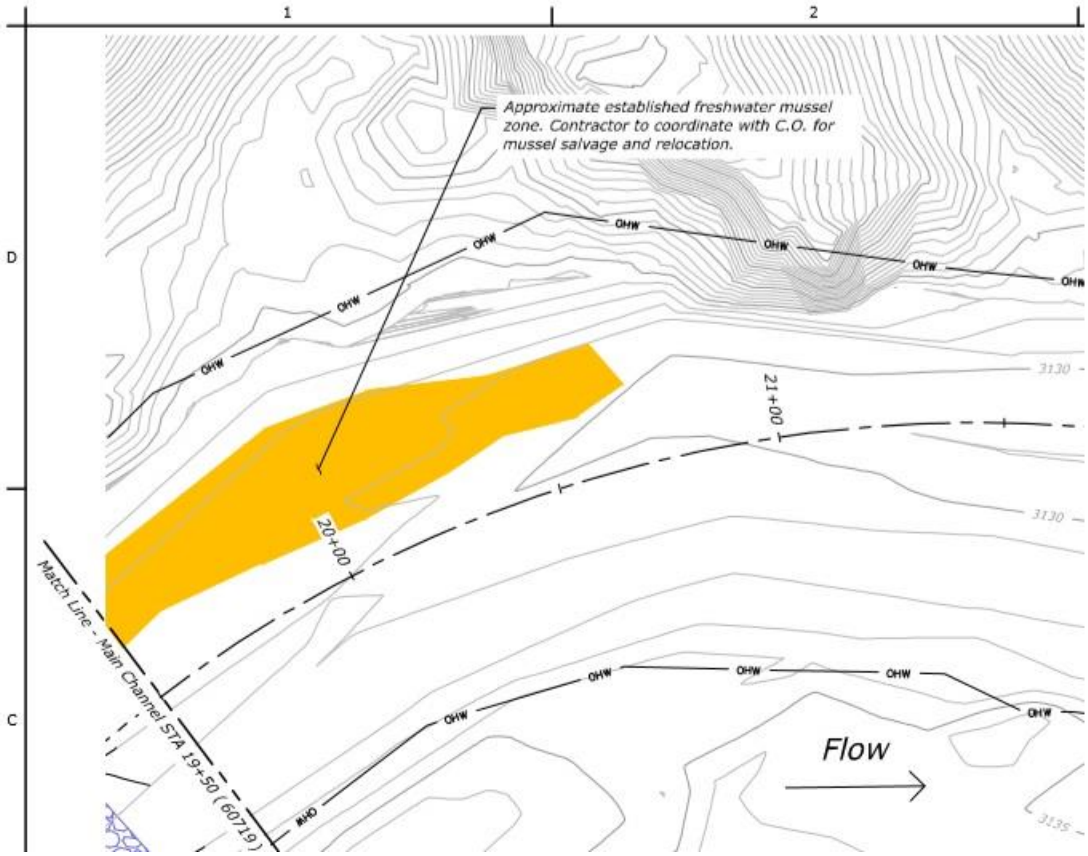

Figure 6-7. Freshwater mussel bed marked (in yellow) on engineering plans for a large-scale salmonid habitat restoration project in Bird Track Springs in the Grande Ronde Basin conducted by the CTUIR.

CHAPTER 7: GENERAL DISCUSSION

This dissertation uses the concepts of community and reciprocity in tribal resource management to guide conservation aquaculture techniques for Pacific Lamprey and native freshwater mussels. Management strategies (e.g., River Vision) and actions (e.g., tribal restoration projects) of the Confederated Tribes of the Umatilla Indian Reservation (CTUIR) provide models for holistic river restoration by acknowledging the important ecological and cultural roles that lamprey and mussels play in the river community (Jones et al. 2008; Quaempts et al. 2018). Tribal populations have developed integral relationships with First Foods, foods holding significant traditional, cultural, or religious value, and a loss of access to First Foods has resulted in a reduced diet diversity for modern tribal communities (Quaempts et al. 2018). Seasonality traditionally drove food choices, compelling a heterogenous diet in congruence with the regional landscape and climate. This suite of First Foods can be considered a “community” on which tribal people have relied for subsistence.

The salmon, as an exemplary First Food, also relies on a community for population sustainability (Quaempts et al. 2018). Salmon is often the target species of restoration and conservation actions because of its connection to human use, especially by nonindigenous communities, but rarely are similar efforts made for members of its community, such as its food sources. Larval lamprey, native fishes, and aquatic invertebrates serve as direct food sources for salmonids in freshwater systems, but they are traditionally undervalued in terms of conservation needs (Naiman et al. 2015; CRITFC 2018). Of even less focus for restoration actions are organisms that serve indirect but highly important functions in an ecosystem, such as freshwater mussels and the microbial community (CTUIR 2015). Freshwater mussels provide myriad environmental services that are of benefit to salmonids (e.g., water quality, nutrient cycling, sediment bioturbation, and ecological health indications), and they, in turn, rely on a diverse community of native fishes for reproduction (Vaughn 2017; Haag 2012). Organisms that are closely tied to the benthos, such as mussels and larval lamprey, likely rely on diverse microbial communities for many functions and processes (Covich et al. 2004). These interactions, from tribal people to foods and from foods to supporting communities of organisms, underscore the importance of community at multiple levels. Recognition of, and inclusion of such interactions may be of high significance in an artificial rearing environment, where natural processes are often lacking perhaps to the detriment of successful rearing progress. The objective of this dissertation was to improve conservation aquaculture techniques for Pacific Lamprey and native freshwater mussels by using tribal resource management concepts of community and reciprocity.

Reciprocity at each level of a community facilitates exchanges of services, nutrients, and resources between trophic levels (i.e., fish to mussels and lamprey and microorganisms to lamprey; Figure 7-1). Tribal resource management emphasizes these community relationships in their management programs and restoration actions (Chapter 2). Reciprocity is a defining theme in tribal resource management, and this dissertation carries that theme forward into conservation aquaculture. The importance of community in conservation aquaculture is evident from the three experiments that were detailed in Chapters 3, 4, and 5. To successfully restore a single organism, there must first be an understanding of the community with which that organism interacts. Aldo Leopold's Land Ethic of the 1940s advocates for the land community in this way, where the whole is strengthened by the health of its individual parts (Leopold 1949). In the final chapter of this dissertation (Chapter 6) I use Land Ethic and tribal management principles to explore ethics in restoration in the Pacific Northwest, advocating for a "benthic ethic" to incorporate community into species restoration projects (Leopold 1949).

Salmonid conservation, including species and habitat restoration, falls short when it does not include restoration or consideration of the community on which salmonids rely (Lichatowich et al. 1999; Lichatowich 2001). Larval lamprey and native freshwater mussels historically comprised a significant portion of the benthic biomass across rivers of the Pacific Northwest, but they are now two of the most critically declining species in the region (Close et al. 2002; Blevins et al. 2017a; CRITFC 2018). Without a functioning benthos, including lamprey and mussels, salmonids lack services like food-web connections, nutrient cycling and storage capabilities, organic matter breakdown, and water quality improvements (see Close et al. 2002 and Vaughn 2017). Where salmonid restoration has not been directly successful, the ecological services of benthic organisms like larval lamprey and mussels could advance biological processes on which salmonids are reliant for food, clean water, and suitable habitat conditions. Tribal organizations recognize these important connections and other agencies could see improved outcomes for salmonids if these connections were considered and incorporated into restoration programs.

In the Pacific Northwest, the loss of ecosystem services that are provided by Pacific Lamprey and freshwater mussels are significantly understudied components in regional species and habitat conservation work. For northwest tribal groups, the loss of cultural connections to Pacific Lamprey and native freshwater mussels is equally significant (Close et al. 2002; CTUIR 2015). For the Confederated Tribes of the Umatilla Indian Reservation (CTUIR), natural resource policy and actions reflect a traditional understanding of the role of lamprey and mussels in a healthy and functioning river system (Jones et al. 2008; Quaempts et al. 2018). The River Vision, a CTUIR guiding restoration framework, promotes understanding and awareness of critical species linkages from a keystone organism, commonly salmon or trout, to its organismal support system (Jones et al. 2008). Other programs, including CTUIR's Department of Natural Resources Fisheries Program, also emphasize relationships between one organism in a community and all of the others, including humans. These relationships, and the reciprocal services that are exchanged between trophic levels in such a community, provide a foundation on which to restore not only a universally valued organism (salmon) but also lesser-known members of the river community.

Reciprocity and community

In this dissertation, I explored the importance of the concepts of community and reciprocity as they relate to the well-being of an ecosystem. Using examples of reciprocity between river trophic levels and different types of river communities, and using holistic tribal resource management principles and policies, I expanded the concepts of reciprocity and community to conservation aquaculture techniques for Pacific Lamprey and freshwater mussels in laboratory experiments. Fish rely on clean, filtered water from freshwater mussels, among other services, and mussels rely on fish to transform their larvae into juveniles and transport populations in a river (Haag 2012). Similar to mussels, larval lamprey provide a variety of ecological services to fishes, including food-web connections and sediment bioturbation (Boeker and Geist 2016). Fish, in turn, improve the growth and survival of larval lamprey in the lab, possibly through the provision of food in the form of a microbial community or organic matter in a polyculture system (Chapter 4). Direct microbial supplementation, via probiotics, also aids the growth and survival of laboratory-reared larvae lamprey, possibly mimicking microbial services that are available to larval lamprey in the benthos of the river (Chapter 5; Covich et al. 2004). Reciprocity between fish and benthic organisms like lamprey and mussels, and between benthic organisms and microbes, help support entire food-webs and ecosystems in the wild. Conservation aquaculture would benefit from including these reciprocal relationships in laboratory rearing techniques.

Without reciprocity from diverse, native communities of microorganisms and freshwater mussels, larval lamprey and other native fishes as well as aquatic invertebrates would not have suitable habitat or environment, which is necessary to sustain populations to serve as a diverse food base for salmonids (Naiman et al. 2012). Quaempts et al. (2018) outline how tribal and related food communities interact across landscapes,

> Water from melting snow pack fed the rivers, connecting them seasonally with their floodplains. Fires ignited by thunderstorms reset terrestrial vegetation communities, and helped to supply rivers with new spawning gravels for salmon and lamprey.

Reciprocity on a landscape-scale, as well-described by Jones et al. (2008), Quaempts et al. (2018), and Endress et al. (2019), can be similarly applied on a micro-scale to better understand integral relationships between First Foods and the biotic communities that support them. Salmon, as a key subsistence First Food, relies on a diverse community of native fishes and invertebrates as foods. Those native fishes, including larval lamprey, and invertebrates rely on relationships with supporting organisms, such as mussels and microbes, as food or as ecosystem engineers (i.e., sediment stabilization via mussel burrowing or biofilm formation; Gerbersdorf et al. 2009; Vaughn 2017). Lack of respect for these reciprocal relationships at each community level has led to a reduction in traditional food availability for Columbia River Basin tribal communities. Tribal resource management can extend the promise of reciprocity to the restoration of entire resource communities in addition to targeting organism restoration efforts (Quaempts et al. 2018). Holistic management for entire ecosystems prioritizes biotic connections and reciprocal relationships that benefit the system as a whole.

Holistic resource management promotes actions that increase ecosystem resilience while balancing thoughtful harvest and resource use. The River Vision, a watershed management framework that combines physical and biological attributes into a cohesive management strategy, and the Upland Vision, an upland ecosystem management framework of similar style, describe the role of reciprocity and community in the maintenance of healthy riverine and upland ecosystems (Jones et al. 2008; Quaempts et al. 2018; Endress et al. 2019). The serving order of First Foods, where water is served first and last, acknowledges a reciprocal relationship between water and all foods, and humans (Quaempts et al. 2018). In this work, I described the reciprocity between tribal human communities and foods, but I also address the reciprocal relationships between levels of communities of foods. Using Jones et al. (2008), Quaempts et al. (2018) and Endress et al. (2019), I described a foundation of reciprocity across landscapes in tribal resource management strategies (Chapter 2).

Community in aquaculture

The same type of reciprocity occurs on a micro scale in freshwater systems and food-webs, which I replicated and studied in the laboratory (Chapters 3, 4, and 5). Healthy wild river environments sustain large communities of fishes, invertebrates, and microorganisms. In Chapter 3, I identified a community of fishes that is used by a mussel species, *Anodonta californiensis*, to transform their obligate parasitic larvae to juveniles and provide a means of their transport over large distances in the river. I further investigated the role that nonnative fishes may play in declining populations of mussels in the region and identify threats that are related to increasing populations of nonnative fishes in the Columbia River Basin. Nonnative fishes are not primary or secondary hosts for this mussel species, though some nonnative fish species demonstrate the ability to transform larvae to a much lesser degree than native species. The work presented in Chapter 3 is important because it identified the fish community on which *A. californiensis* relies for reproduction, and it demonstrated negative interactions with nonnative fishes. This has important implications for the management and introduction of nonnative teleost fishes in the context of holistic system restoration.

Laboratory environments are largely homogenous, providing little complexity and few interspecies interactions, and they are generally maintained in a semi-sterile state with the use of disinfection methods to prevent the development or build-up of harmful bacteria or fungi. Larval Pacific Lamprey have been grown at CTUIR facilities in mainly monoculture systems, with mixed results. The production of large numbers of larval lamprey has not been successful to date, and some research suggests that lamprey growth and survival improves with the addition of another species (Limm and Power 2011; CTUIR, unpublished data). In the laboratory environment, I used a native nongame fish, Speckled Dace, to enrich the environment of larval lamprey (Chapter 4). Larvae that were reared with the Speckled Dace showed significantly increased growth over larvae that were reared in traditional monoculture conditions. These differences were possibly linked to increased microbial activity in larval tanks from the Speckled Dace, increased food availability from the waste products that were produced by them, or other mechanisms (e.g., Jones and Iwama 1991; Slater and Carton 2007; MacDonald et al. 2011). In this experiment, survival did not differ between the two treatments (polyculture and monoculture), which may have been the result of using filter mats in all of the culture tanks. These filter mats capture food particles, potentially slowing delivery, and allow the formation of a robust microbial community (Maine et al. 2017). The addition of a community, in this case Speckled Dace, to the laboratory rearing environment conferred benefits to larval lamprey, suggesting that reciprocity between these two river organisms can be used to improve lamprey production.

Larval lamprey have intricate relationships with a variety of organisms in the river community. They act as a food source for many organisms, including teleost fishes. In the river community, fishes provide nutrient sources for the larval lamprey in the form of organic matter or waste material. Larval lamprey break down these materials into smaller components that are suitable for digestion or further breakdown by microorganisms that are associated with the substrate. The natural habitat in which larval lamprey live hosts a rich microbial community that is rarely replicated in laboratory settings. An unseen component of all freshwater communities, microbes contribute essential services through biogeochemical cycling and food-web interactions (Finlay et al. 1997; Covich et al. 2004). To further test the idea that larval lamprey benefit from exposure to a microbial community, I supplemented larvae in two experiments (10 and 28 weeks in duration) with a probiotic product (EPI-CIN G2, Chapter 5). Both growth and survival were greater in the treatments that received the probiotic. Furthermore, these differences were observed in both experiments using different larval densities and rearing chambers. Larval lamprey use microorganisms and small particles as food (Moore and Potter 1976; Yap and Bowen 2003; Moser et al. 2019). The research presented in Chapter 5 shows that laboratory-reared larval lamprey derive direct nutrition or synergistic benefits from exposure to microorganisms from a probiotic. Probiotics can be used to improve production outcomes for larval lamprey, especially when high-density may play a role in low survival and growth.

Future directions

The information presented in this dissertation demonstrates the importance of community and reciprocal services that are provided at different levels of a community in conservation aquaculture of two declining traditional food resources of the CTUIR people. Through a review of the literature, I identified the cultural importance of a native community of organisms, including lamprey and mussels, to tribal people. In my experiments, I identified this importance in the laboratory environment for the purposes of conservation aquaculture. In my final chapter, Chapter 6 The Benthic Ethic, I suggest that the restoration community include the principles of reciprocity and community in restoration projects in the northwest, specifically to broaden the focus of single-species projects. The incorporation of a healthier community could lead to better restoration outcomes for salmonid habitat-restoration projects. Such projects benefit many more organisms than salmonids, but few other species are included in prerestoration planning or considered during activities such as in-stream construction. Some restoration work could damage existing populations of benthic organisms like larval lamprey and freshwater mussels, so it is important for restoration professionals to plan for the removal or translocation of these organisms prior to major structural work in the river. The ecological services that are provided by benthic organisms benefit salmonids in a variety of ways, but they are often undervalued in restoration projects. A healthy, functioning community at all levels in the river environment has sustained tribal people since time immemorial, and restoring all parts of that community will allow that relationship to continue.

Laboratory propagation of Pacific Lamprey and native western freshwater mussels is only necessary because natural populations have declined such that tribal harvest opportunities are severely limited, if available at all. Artificial propagation is needed to meet restoration goals for Pacific Lamprey and freshwater mussels in the northwest. The research presented in this dissertation provides community-based techniques for producing these organisms in the laboratory wherein reciprocity flows from one organism to another and from organisms to humans. In this dissertation, I used the concepts of community and reciprocity to adjust laboratory culture techniques to overcome data gaps and research bottlenecks. Future studies could focus this line of research on production-scale propagation of lamprey and mussels for restoration research and releases.

As out-planting of larval lamprey is a restoration goal of the CTUIR, the research presented in this dissertation could be used to develop innovative, large-scale rearing techniques to produce millions of larvae for supplementation (CRITFC 2018). Currently, larvae are produced in small numbers (thousands) and are held at facilities on river or well water. Polyculture of larval lamprey with other species could be conducted at existing salmonid hatcheries using effluent water, providing a use for nutrients in fish wastes. Probiotics are increasingly used in hatchery facilities for water quality manipulation and pathogen protection. Larval lamprey benefit from probiotics, deriving direct or indirect advantages from their use. Salmonid hatchery facilities could incorporate cages of larval lamprey in occupied raceways, in effluent ponds, or as downstream components of a flowing system. Lamprey-only facilities could improve larval rearing through the use of effluent water, probiotics, microbial supplementation, or some combination of these methods to maximize cost-savings, survival, and growth. The production of millions of larval lamprey for out-planting is possible using a variety of holistic methods described in this dissertation to overcome survival and growth issues.

Mussels are not currently produced on a large scale for restoration, despite their benefit to the ecological community. Increasing awareness of their ecological benefits by incorporating them into hatchery facilities could advance research and restoration goals. As mussels can filter up to 1 L of water per hour, a population of mussels in an effluent pond could contribute significantly to nutrient processing, cycling, and storage at a hatchery facility. Freshwater mussels are critically understudied in the western United States. Many restoration professionals do not understand the important role a mussel plays in the river community, nor are they aware of the benefits of including their services in restoration projects. Mussels could be reared in hatchery facilities, propagated and grown on site, and used to supplement ecological services damaged by in-stream construction during river restorations.

Awareness of the ecological benefits of benthic organisms like larval lamprey and mussels needs to be increased to facilitate collaborative restoration for both tribal and nontribal projects. Inclusion of lamprey and mussels into future restoration projects, at a minimum in prework salvage or removal, should be a priority for river restoration professionals. To further benefit their target organisms, usually salmonids, restoration practitioners should consider translocating or out-planting wild or propagated lamprey and mussels back into restored reaches after construction is complete. This practice would reestablish essential food-web connections for salmonid fishes, reconnect benthic and open water communities, and contribute to healthy biological processes.

Holistic management techniques, as represented in frameworks like the River Vision, are important in restoration projects both within and beyond the Columbia River Basin. The inclusion of a community in restoration applications could reconnect ecological and cultural services for river organisms and human communities. Ecosystem services provided by benthic organisms like larval lamprey and mussels are often undervalued and unacknowledged during large-scale restorations focused on one target species. Reciprocity in the river community can be replicated in the laboratory to improve conservation aquaculture techniques and propagation strategies. Chapters 3, 4, and 5 provide empirical support for the use of community and reciprocity as management techniques to produce organisms needed for population restoration and conservation research. The benthic ethic presented in Chapter 6 carries this research forward into application, calling upon restoration practitioners to include aspects of community and reciprocity in river restoration projects in the Pacific Northwest.

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Figures

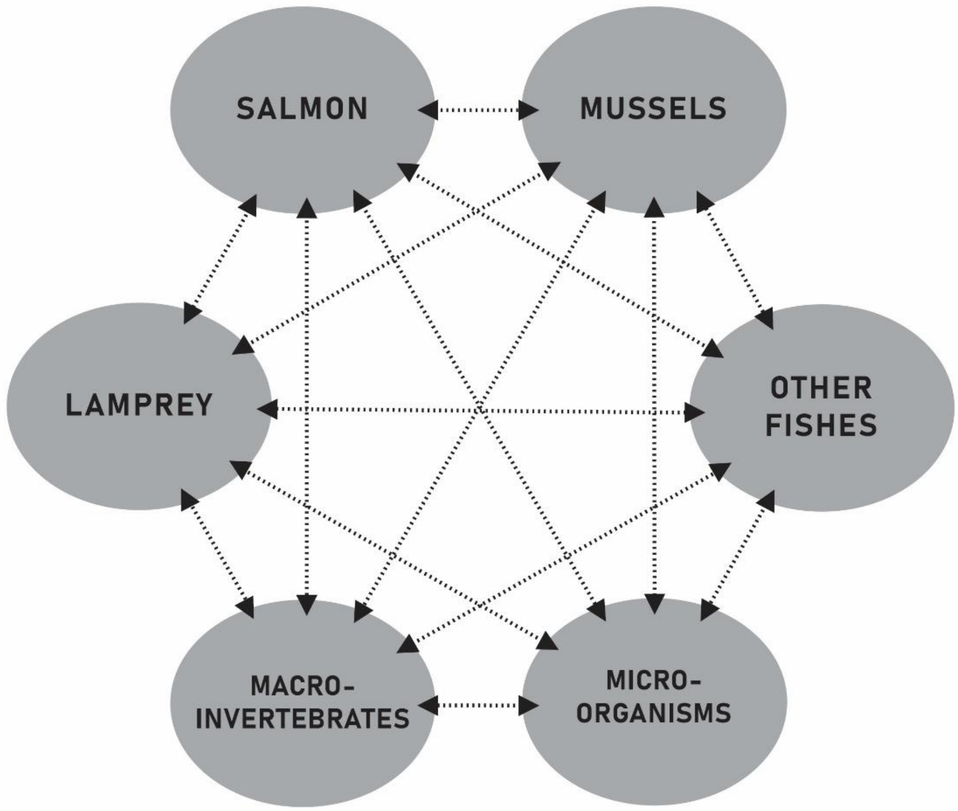

Figure 7-1. River food-web connections. Arrows signify reciprocal relationships and ecological services exchanged between community members. Figure by the author and J. Hooghkirk.