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<https://doi.org/10.15760/etd.2355>

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Investigations of Larval Pacific Lamprey *Entosphenus tridentatus* Osmotic Stress
Tolerance and Occurrence in a Tidally-Influenced Estuarine Stream

by

Gregory S. Silver

A thesis submitted in partial fulfillment of the
requirements for the degree of

Master of Science
in
Biology

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Portland State University
2015

ABSTRACT

Pacific lamprey is a culturally valuable species to indigenous people, and has significant ecological importance in freshwater and marine ecosystems. Over the past several decades, constrictions in range and reductions in Pacific lamprey abundance have been observed in Western North America, and may be indicators of range-wide declines. In the face of declining populations, the U.S. Fish and Wildlife Service has partnered with tribal, state, federal, and local entities to implement a regional Pacific lamprey conservation agreement aimed at reducing threats to Pacific lamprey and improving their habitats and population status. Research needs identified in the conservation agreement include assessing larval Pacific lamprey occupancy and distribution, habitat requirements, and the limiting factors of larval distribution in the freshwater ecosystem. As part of the effort to address these knowledge gaps, we investigated the potential for larval lampreys to occur in tidally-influenced estuarine environments. Research of this type may be valuable for future conservation, management or recovery efforts of Pacific lamprey throughout its range.

We employed a two-phased approach, consisting of laboratory and field components to address our aims. We first conducted a series of controlled laboratory experiments to evaluate osmotic stress tolerance and osmoregulatory status of larval Pacific lamprey exposed to a range of (1) fixed salinity in various dilutions of saltwater and (2) oscillating salinity treatments designed to simulate tidal activity. Tolerance was assessed by monitoring and comparing survival of larvae in various treatments through 96 h. Osmoregulatory status was assessed by quantifying and comparing total body water content, plasma osmolality, and plasma cation (i.e., sodium) concentrations among

larvae surviving various treatments. In fixed salinity experiments, 100% survival was observed in 0‰, 6‰, 8‰ and 10‰ through 96 h, while 0% survival was observed through 48 h in 12‰, 30 h in 15‰, and 12 h in 25‰ and 35‰. In oscillating salinity experiments, on the other hand, a significant increase in survival (100%) was observed through 96 h in treatments that oscillated between 12‰ and 0‰ (freshwater) at about 6 h intervals versus fixed 12‰ salinity experiments. A significant increase in survival also occurred in oscillating 15‰ treatments (60%) versus fixed 15‰ through 96 h. Linear regression analysis indicated higher environmental salinity in laboratory experiments was significantly related to increases in plasma osmolality and plasma sodium (the most abundant osmotically active plasma cation) concentrations, and concurrent decreases in total body water content among larvae that survived various treatments. Tidal oscillations in salinity appeared to temper the desiccating effects of salinity, as changes in body water content and sodium ion concentration were less abrupt than fixed salinity treatments. These results suggest larvae cannot osmoregulate in hyperosmotic environments, but are able to tolerate some fixed and oscillating hyperosmotic salinity exposure. Consequently, larvae may be able to occur in certain areas of estuaries, such as oligohaline habitats that are characterized by low levels of salinity. Experimental results were used, in part, to guide larval sampling in a tidally-influenced habitat.

Occurrence of larval Pacific lamprey and *Lampetra* spp. (western brook and river lampreys) was subsequently investigated across a gradient of salinity in Ellsworth Creek (Pacific County, Washington) by electrofishing. Larval Pacific and *Lampetra* spp. were detected within an approximately 300 m long tidally-influenced segment of the study area. Salinity monitoring was conducted in six tidally-influenced reaches where larvae

were detected for up to 14 d following electrofishing. Maximum tidal cycle salinity exceeded 15 ppt during 52% to 80% of tidal cycles within tidally-influenced reaches where larvae were detected. These results suggest potential for larval lamprey to occur in certain portions of tidal estuaries. However, long-term residence of larvae in tidally-influenced habitats and whether larvae are able to subsequently survive, grow, transform, and out-migrate is not known and requires further study. Given the potential for tidally-influenced habitats to be occupied by larvae, assessments of larval occurrence in other areas, such as the lower Columbia River, may be warranted. Knowledge of larval lamprey distribution in estuarine environments may be valuable for habitat restoration, and mitigating potential impacts from dredging and other human disturbances.

ACKNOWLEDGEMENTS

This project was a collaborative effort between Portland State University and the U.S. Fish and Wildlife Service, Columbia River Fisheries Program Office (CRFPO) where I have worked on lamprey-related research for over 10 years. I have been privileged to work with many talented people on both sides of the aisle, who were integral in making this partnership work, and to whom I owe much gratitude for this opportunity. I would like to thank my advisor Jason Podrabsky, in particular, for taking on a non-traditional student such as myself and for his patience with my extended residence in his lab! Thanks also for your vision, ideas, wisdom, and support during this process. It has truly been a pleasure to be a part of your team, and the time I've spent as a Podrabsky lab member will be remembered with fondness and pride. Thanks also to Stan Hillman for your contributions and assistance on this project and for the comparative physiology lessons. Your wisdom and experience are unmatched, and it was an honor to have you as a committee member. At my office, I owe special thanks to Tim Whitesel for his leadership and willingness to invest time and effort planning and executing this project through countless brainstorming sessions over the years. It could not have been done without your help. I would also like to thank Howard Schaller for suggesting this opportunity, and for his support of graduate education for employees of the CRFPO. I owe many thanks to Jeff Jolley for his vital contributions to this work.

Thanks to the Elemental Analysis Core at OHSU for conducting elemental analyses using ICP-MS. Much gratitude also to the many friends and colleagues at PSU, the Podrabsky lab, and the CRFPO whom I've had the pleasure to work with over the last few years and for your help along the way. Finally, special thanks to my wife Brook who

has been so helpful over the years, your contributions to this work were many, varied, and vital to my success in this endeavor. Your assistance on things ranging from 3 a.m. runs to the lab for data collection, to fieldwork in Ellsworth Creek, to providing comments and ideas for figures, graphs, and thesis chapters was an immeasurable help. I am forever grateful for your unending support and patience during this process, I couldn't have done it without you!

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CHAPTER 1

GENERAL INTRODUCTION

Conservation Background

Patterns of ecological distribution of a species are influenced by biotic and abiotic interactions and physiological adaptations for survival within a particular range of environmental conditions. Deviations from optimal conditions within the niche of a particular species can lead to stress and require an organism to undergo metabolic, physiological, or behavioral compensation to maintain homeostasis (Bozinovic et al. 2011). In the aquatic environment, for example, tolerance of a fish species to variations in abiotic factors such as water temperature and salinity may in part determine what habitats are suitable for occupancy and survival (Deaton and Greenberg 1986; Peterson and Ross 1991; Wagner 1999). In this study, I investigated the physiological and ecological effects of water salinity on the protracted larval life history stage of the anadromous parasitic Pacific lamprey *Entosphenus tridentatus* (Pletcher 1963; Kan 1975; Hammond 1979). Our aims were to first evaluate the tolerance of larval Pacific lamprey to osmotic stress induced by a suite of salinity exposures in the laboratory. We then used the results of laboratory experiments, in part, to guide field investigations of the ecological distribution of larval Pacific lamprey at the interface of freshwater and saltwater habitats.

Pacific lamprey are primitive jawless fishes and a member of the oldest lineage of extant vertebrates (Agnatha). The ancestry of Pacific lamprey dates back in the fossil record over 350 million years to the Middle Paleozoic era (Bardack and Zangerl 1968).

As such, Pacific lamprey are thought to have survived four major extinction events (Wang and Schaller 2015). Historically, anadromous Pacific lampreys were widely distributed in river systems draining into the Eastern and Northern Pacific Ocean, from Punta Canoas, Baja California, Mexico northward around the Pacific Rim to Hokkaido Island, Japan (Swift et al. 1993; Ruiz-Campos and Gonzalez-Guzman 1996; Yamazaki et al. 2005; Wang and Schaller 2015). Throughout their native range, Pacific lamprey have been an important component of aquatic communities that may have influenced the evolution of many co-occurring species (Wang and Schaller 2015). Thus, the importance of Pacific lamprey to aquatic ecosystems in which they occur may be difficult to overstate.

Pacific lamprey is a culturally valuable species to indigenous people throughout its range, and has significant ecological importance in freshwater and marine ecosystems. For Native American tribes of the Pacific Northwest, Pacific lamprey continues to be an integral part of tribal culture, and is one of many subsistence foods that have been harvested in the region for generations (Pletcher 1963; Close 2004). Pacific lamprey also holds ceremonial, medicinal and spiritual values to tribal peoples which remain to this day (Close et al. 2002). Ecologically, Pacific lamprey is thought to contribute to freshwater ecosystem health in a number of ways. Larval Pacific lampreys represent a large portion of the biomass in streams in which they occur, and are important in processing, cycling and storing nutrients (Kan 1975). Pacific lamprey is also an important component of aquatic food webs (Kan 1975; Close et al. 2002). Outmigrating juvenile Pacific lamprey are consumed by many fishes and birds, and are thought to buffer predation on salmonid smolts during seaward migrations. Similarly, upstream

migrating adult Pacific lampreys are preyed upon by fish, birds, and marine mammals, which again may significantly reduce predation rates on salmonids and other co-occurring fishes (Close et al. 1995; Close et al. 2002; Luzier et al. 2011). Given the potential ecological significance of Pacific lamprey within aquatic ecosystems, reduction or extirpation of this species is likely to negatively affect trophic relationships in both the freshwater and marine ecosystems (Close et al. 2002).

Over the past several decades, constrictions in range and reductions in abundance of Pacific lamprey have been observed in Western North America, and may be indicators of range-wide declines in Pacific lamprey populations (Luzier 2011). Extirpation risk of Pacific lamprey is considered high in the majority of watersheds in Western North America (Wang and Schaller 2015). The causes of declines are not explicitly understood, but are thought to parallel those adversely impacting sympatric Pacific salmonids, given the general similarities in range, habitat requirements, and life history (Moyle 1996; Close et al. 2002; Kostow 2002; Moser and Close 2003; Nawa 2003; Luzier et al. 2011). Until relatively recently, conservation and management of Pacific lamprey have not been a priority in Western North America. Consequently, unlike fishes with significant economic value to sport and commercial fisheries (like Pacific salmon, for example), the biology, ecology, status and distribution of Pacific lamprey in Western North America have not been extensively studied (Luzier et al. 2011). The increasing appreciation of the ecological value and importance of Pacific lamprey to Northwest tribes has led, in part, to a greater understanding of the need to conserve this declining species. Research targeting gaps in knowledge relative to threats, distribution, and limiting factors of Pacific lamprey may be critical for their persistence.

The apparent declines in abundance and distribution of Pacific lamprey have raised concern about the status of Pacific lamprey in Western North America among tribal, federal, state, and local agencies, and have catalyzed development of several conservation measures, including the Pacific Lamprey Conservation Agreement (PLCA) by the U.S. Fish and Wildlife Service (USFWS). The PLCA was signed in 2012 by 12 tribal governments, four state and eight federal agencies, and several local governments that cover much of the range of Pacific lamprey in the continental U.S. The ultimate goal of the PLCA is securing long-term persistence of Pacific lamprey and supporting traditional tribal cultural use throughout their historic range in the U.S. (Wang and Schaller 2015). To date, conservation and management efforts have been hampered by a paucity of demographic information, as well as limited information on Pacific lamprey biology and ecology (Wang and Schaller 2015). The PLCA outlines many research needs and knowledge gaps that must be addressed to ensure the persistence of Pacific lamprey in the Pacific Northwest. Included among these is developing a better understanding of occupancy and distribution of the larval life stage, habitat requirements of larvae, and the limiting factors of larval distribution. This information may be a critical component in identifying and prioritizing threats to Pacific lamprey, implementing regional or basin-specific conservation plans, and designing and implementing habitat restoration.

Pacific Lamprey Life History

Pacific lamprey has a complex life history, in which adults spend an estimated 18 to 40 months in the Pacific Ocean feeding parasitically on a variety of teleost fishes and

elasmobranchs before returning to freshwater streams and rivers to spawn (Kan 1975; Beamish 1980; Beamish and Levings 1991). Highly migratory adults may seek out spawning grounds located in tributary rivers and streams many hundreds of kilometers upstream of the ocean (Torgerson and Close 2004). Spawning adult Pacific lamprey deposit embryos into nests (redds) constructed in gravel with well-oxygenated flowing water, often overlapping spatially and temporally with steelhead (*Oncorhynchus mykiss*; Pletcher 1963). Newly-hatched larvae (ammocoetes) emerge from nests and drift downstream, settling in slow moving depositional areas. Microphagous larvae burrow into soft benthic sediments, feeding on organic detritus and microorganisms such as algae and diatoms that are filtered from the substrate and water column (Moore and Mallat 1980; Sutton and Bowen 1994). The larval stage of Pacific lamprey is protracted, lasting an estimated three to seven years (Pletcher 1963; Kan 1975; Hammond 1979). Over the course of the protracted larval phase, downstream dispersal occurs as the results of both passive displacement during high velocity periods and active redistribution to seek out favorable new rearing habitats (Wagner and Stouffer 1962; Hardisty and Potter 1971; Potter 1980; Sjoberg 1980; White and Harvey 2003). The larval phase culminates in a true metamorphosis from larval to juvenile (macrophthalmia) form (Kan 1975; Richards and Beamish 1981). During metamorphosis, marked anatomical and physiological changes occur both internally and externally including the development of the oral sucking disc and associated dentition, prominent functional eyes, as well as modifications to organ systems required for parasitic feeding and seawater osmoregulation (Mathers and Beamish 1974; Youson 1980; Richards and Beamish 1981; McGree et al. 2008). Macrophthalmia enter the marine environment, and undergo a period of rapid growth to

adult size during the ensuing parasitic feeding period (Beamish 1980; Richards and Beamish 1981; Beamish and Levings 1991).

Although the longitudinal extent of downstream movement during the protracted larval phase is not known, observations of Pacific lamprey larvae occurring significant distances from known spawning grounds indicate the potential for considerable and consistent downstream dispersal (Silver et al. 2007; Jolley et al 2011; Jolley et al. 2012; Jolley et al. 2014). In the Columbia River basin, for example, observations of larval Pacific lamprey routinely occur at collection facilities at the John Day Dam salmonid juvenile bypass system (Fish Passage Center data query; fpc.org), as well as during surveys of deepwater habitats of the mainstem Columbia and Willamette Rivers (Jolley et al. 2011; Jolley et al 2012; Jolley et al. 2014). Cumulatively, movements over multiple years may result in significant dispersal of larvae out of tributary habitats, and continual recruitment of older larvae into lower portions of river basins (Kostow 2002; NPCC 2004). A potential consequence of downstream movement of larvae in river systems that enter the ocean (for example coastal river basins, and the lower Columbia River) includes larvae ultimately encountering saline waters (Potter 1980; White and Harvey 2003). In the Columbia River mainstem and tributaries in the lower river, downstream dispersal of larval lampreys results in occurrence of larvae throughout the tidal freshwater portion of the upper estuary (NPCC 2004). Distribution of primarily freshwater fishes, such as larval lampreys, in tidally-influenced habitats, is known to be governed by tolerance to environmental salinity (Deaton and Greenberg 1986; Neves et al. 2011; Whitfield 2015). Occurrence of such species in lower regions of estuaries is generally unlikely given high salinities occurring in proximity to the ocean. However, the ability of larval Pacific

lampreys to tolerate low or variable levels of salinity and occur in some areas of tidal estuaries is not well understood and has not been extensively investigated. Given the significant geographic extent of low salinity, estuarine habitat types, particularly in river systems with large freshwater inputs (such as the Columbia River, or the Sacramento-San Joaquin Rivers for example; Odum 1988; Norris et al. 2010), as well as the abundance of suitable larval burrowing substrates, these areas may represent important rearing habitats for larval lampreys and potentially be occupied by large numbers of larval lampreys.

Goals and Objectives

As part of the effort to conserve and restore Pacific lamprey populations in western North America, we proposed to address gaps in knowledge of relative to larval Pacific lamprey salinity tolerance, osmoregulatory capacity, and ecological distribution. The overall goal of this project was to determine the potential for larval Pacific lamprey to occur in tidally-influenced estuarine environments that have variable salinity. A two-path approach was used to address this goal. Controlled laboratory experiments were first conducted to evaluate hyperosmotic stress tolerance and osmoregulatory status of larval Pacific lampreys that survive a range of fixed and oscillating salinity exposure regimes. Based on experimental findings, field sampling was then conducted to investigate and describe occurrence of larval Pacific lamprey across habitats in a natural stream characterized by a gradient of tidally-influenced salinity. Our formal objectives were to:

1. Evaluate and compare osmotic stress tolerance of larval Pacific lamprey subjected to (1) fixed salinity exposures, and (2) oscillating salinity exposures that simulate tidal activity in the natural environment
2. Evaluate and compare osmoregulatory ability of larval Pacific lamprey from various salinity exposure treatments through quantification of (1) plasma osmolality, (2) total body water content, and (3) plasma elemental/cation concentrations.
3. Assess larval lamprey occurrence in tidally-influenced habitats across a gradient of salinity conditions
4. Quantify and characterize salinity profiles in tidally-influenced areas where larval lampreys are detected.

Chapter 2 discusses laboratory experiments conducted to address objectives one and two above. Tolerance of larval Pacific lamprey to osmotic stress was determined by assessing survival of larvae exposed to fixed or oscillating salinity treatments.

Osmoregulatory ability was assessed by quantifying total body water content, plasma osmolality, and plasma cation concentrations of larvae that survived in fixed or oscillating salinity treatments. Chapter 3 discusses field sampling for larval lampreys in a tidally-influenced segment of Ellsworth Creek, a tributary of the Naselle River.

Distribution of larval lampreys across a gradient of salinity was investigated using a backpack electrofisher, and salinity was characterized following electrofishing in tidally-

influenced reaches in which larvae were detected. Chapter 4 summarizes these findings and discusses potential conservation and management implications for Pacific lamprey.

CHAPTER 2

EVALUATION OF LARVAL PACIFIC LAMPREY OSMOTIC STRESS TOLERANCE AND OSMOREGULATORY CAPACITY

INTRODUCTION

Osmoregulatory Biology

Lampreys are the oldest extant vertebrate known to maintain relatively constant internal fluid composition and concentration through the processes of osmoregulation (Morris 1972; Beamish 1980; Bartels and Potter 2004; Reis-Santos 2008). Although lampreys are not closely related to teleost fishes, the overall mechanisms of osmoregulation are similar in which the gills, gut, and kidneys are thought to be the primary organs responsible for maintaining internal salt and water balance (Hardisty 1956; Morris 1972; Beamish 1980; Bartels and Potter 2004; Evans et al 2005). As discussed in Chapter 1, anadromous species of lampreys, including Pacific lamprey and sea lamprey, migrate from freshwater to seawater and back to freshwater during the course of one lifetime. Differences in concentration between internal fluids and the external environment produce strong osmotic gradients that drive movement of ions and water in one direction in freshwater and the opposite direction in saltwater. Maintaining constant internal fluid composition and concentration in environments with opposite osmotic pressures requires efficient mechanisms for regulating internal fluid concentrations during all life stages.

In the freshwater environment, in which microphagous larvae of anadromous lampreys occur for three to seven years, concentrations of the internal milieu are

maintained well above that of the surrounding environment (Morris 1972; Morris 1980). Consequently, osmotic gradients drive continuous influx of water and efflux of ions across skin and gill epithelial membranes of larval lampreys (Morris 1972; Bartels and Potter 2004). Osmotic uptake of water is countered by production of copious dilute urine. Ion balance is thought to occur by both active recruitment of ions from the ambient environment through mitochondrial rich cells (MRCs) in the gill epithelium (although the precise mechanisms are not yet completely understood), as well as absorption of ions from ingested food (Morris 1972; Beamish 1980; Bartels and Potter 2004; Bartels et al. 2009). The larval phase of anadromous lampreys culminates in a period of true metamorphosis that includes anatomical and physiological changes needed for life in the marine environment (Youson 1980; Richards and Beamish 1981). The resultant juvenile lamprey migrates to the ocean and transitions from hyperosmotic regulation to hypo-osmotic regulation as it enters the marine environment.

For adult lampreys, which occur in the marine environment for an estimated 18 to 40 months, the osmotic driving forces are the reverse of those in freshwater. Adult lampreys in the ocean function as hypo-osmoregulators, where internal fluid concentrations are maintained below that of the seawater environment (Hardisty and Potter 1971; Morris 1972; Beamish 1980; Richards and Beamish 1981). As a result osmotic gradients drive the continuous efflux of water and influx of ions, again across the skin and at the gills (Morris 1972; Bartels and Potter 2004). Osmotic water loss is countered by the swallowing of sea water, coupled with excretion of small volumes of concentrated urine (Morris 1972). Excess monovalent ions present in seawater (including sodium and chloride ions) are thought to be absorbed from the digestive tract and

secreted by MRCs at the gills, while divalent ions (including magnesium and calcium ions) are excreted in feces (Morris 1972; Beamish 1980; Bartels and Potter 2004; Bartels et al. 2009). Upon adults re-entering the freshwater environment during spawning migrations, marine osmoregulatory systems degenerate and mechanisms of freshwater osmoregulation are again regained.

The capacity for larval lamprey to osmoregulate in and tolerate (survive) various concentrations of saltwater has been evaluated on a number of lamprey species.

Generally, in these experiments, larvae were abruptly transferred from freshwater to media with fixed salinity for a given time interval or until death (Hardisty 1956; Mathers and Beamish 1974; Beamish et al. 1978; Richards and Beamish 1981; Reis-Santos et al. 2008). In the case of anadromous Pacific and sea lampreys, such experimental salinity challenges indicated larvae are unable to maintain ion and water balance through the mechanisms of osmoregulation in solutions that exceed the osmotic concentration of their internal milieu (225-260 mOsm/kg or about 8 to 10 ppt; Hardisty 1956; Mathers and Beamish 1974; Beamish et al. 1978; Beamish 1980; Morris 1980; Richards and Beamish 1981). In turn, survival (tolerance) of larvae in hyperosmotic media is generally low. For larval Pacific lamprey, the 96-h LC₅₀ (concentration at which 50% mortality occurs) was found to be 12.2 ppt (Richards and Beamish 1981), while sea lamprey 24-h LC₅₀ was found to be 14.8 ppt (Beamish et al. 1978). Overall, these and other studies suggest larvae of all lamprey species are largely stenohaline, obligate freshwater organisms (Hardisty 1956; Mathers and Beamish 1974; Beamish et al. 1978; Beamish 1980; Morris 1980; Bartels and Potter 2004).

Studies of the effects of abrupt transfer of larvae into various dilutions of saltwater have provided valuable insights into the lethal salinity exposure thresholds of various lamprey species. However, the abrupt transfer experiments previously conducted are not likely to reflect conditions encountered by larvae as they disperse downstream into estuarine habitats in the natural environment, and provide little insight on the types and magnitudes of salinity exposures larvae may be able to tolerate in the wild. In particular, little is known about the ability of larvae to tolerate variable or fluctuating hyperosmotic salinity exposures, such as those that may occur in tidal estuaries, for example. Additional experiments to evaluate tolerance of larvae to variable or oscillating salinity may be useful, as differences in survival may be observed versus abrupt transfer experiments. Although variation in salinity is typically considered to be harsh (Deaton and Greenberg 1986; Peterson and Wagner 1991), freshwater periods during tidal cycles may allow for osmotic recovery of water lost when transitioning from hyperosmotic to hypo-osmotic (freshwater) environments (Hardisty 1956). Osmotic recovery of water during freshwater periods may act to buffer the desiccating effects of hyperosmotic environments, and in turn lead to higher survival of larvae versus fixed salinity in abrupt transfer experiments. If larval lamprey are able to tolerate oscillating salinity with freshwater periods, larval occurrence in certain areas of tidally-influenced estuaries, such as oligohaline environments, may be possible.

Experimental Design

To better understand the potential for larval lampreys to inhabit tidally-influenced estuarine areas that experience low or occasional salinity intrusion, we conducted a series

of laboratory experiments designed to evaluate and compare osmotic stress tolerance and osmoregulatory status of larval Pacific lampreys that survived exposure to a suite of fixed and oscillating salinity exposures. Osmotic stress tolerance was assessed through quantification of survival of larvae to various salinity exposures, while osmoregulatory status was evaluated according to several common, organismal-level physiological metrics. Tolerance thresholds of larvae to oscillating salinity in the laboratory will be used to help identify and predict areas in the natural environment that may be suitable (according to known salinity data) for larval lampreys, and guide sampling efforts for larvae in a tidally-influenced estuarine environment. The following two objectives are discussed in detail in this chapter:

1. Evaluate and compare osmotic stress tolerance of larval Pacific lamprey subjected to (1) fixed salinity exposures, and (2) oscillating salinity exposures that simulate tidal activity in the natural environment.
2. Evaluate and compare osmoregulatory status of larval Pacific lamprey from various salinity exposure treatments through quantification of (1) plasma osmolality, (2) total body water content, and (3) plasma cation concentrations.

METHODS

General Husbandry

Larval Pacific lamprey were collected from Eagle Creek, a tributary to the Clackamas River (Clackamas County, Oregon), and Cedar Creek, a tributary to the Lewis River (Clark County, Washington) using an AbP-2 backpack electrofisher (ETS

Electrofishing, Verona, Wisconsin). Identification of larvae as Pacific lamprey was done through visual inspection of caudal pigmentation characteristics (Goodman et al. 2009). Larvae were transported in aerated buckets of fresh creek water to an aquatics laboratory at Portland State University-Center for Life in Extreme Environments, (Portland, Oregon). In the laboratory, larvae were anesthetized in a buffered solution of tricaine methanesulfonate (MS-222; 150 mg/L), and measured for total length (TL; mm) and wet weight (grams). Following measurement of length and weight, larvae were haphazardly distributed among experimental test chambers (described below), four to five larvae per chamber.

During laboratory experiments, larvae were held in thin, clear acrylic test chambers (60 cm wide x 35 cm high x 1.5 cm thick) containing approximately 600 mL of clear quartz sand burrowing substrate to a depth of 6 to 8 cm (Bowen et al. 2003). The thin test chambers and light colored quartz sand improved observability of larvae while burrowed within the substrate (Bowen et al. 2003). The chambers were open on the top, and sealed on all sides. Each test chamber contained about 1600 mL of dechlorinated city of Portland (Multnomah County, Oregon) tap water and was aerated with an air pump and air stone. Test chambers were housed within a large recirculating freshwater holding tank equipped with a water chiller, which maintained water temperature at constant 8°C (+/- 1°C) for the duration of acclimation and experimental periods. Larvae were acclimated to the laboratory for 24 h to 48 h in freshwater prior to initiation of salinity experiments. Larvae were not fed during acclimation or salinity experiments. Saltwater solutions were prepared by dissolving Instant Ocean® Sea Salt (United Pet Group, Blacksburg, Virginia) into dechlorinated tap water. Salinity was measured during

solution preparation using calibrated Yellow Springs Instruments (YSI; Yellow Springs, Ohio) Model 30 and Model 85 handheld water quality meters. All experiments were carried out in accordance with IACUC guidelines.

OBJECTIVE 1. EVALUATING OSMOTIC STRESS TOLERANCE

Survival in Fixed Salinity

Experimental challenges of larval Pacific lamprey tolerance to fixed salinity exposure were initiated by replacing freshwater in the test chambers with water of the following salinity concentrations: 0 ppt (freshwater control), 6 ppt, 8 ppt, 10 ppt, 12 ppt, 15 ppt, 25 ppt, and 35 ppt salinity. Freshwater used for acclimation was slowly decanted from chambers to prevent disturbing burrowed larvae, and the appropriate volume (1600 mL) of saltwater (or 0 ppt freshwater controls) was added. Each salinity treatment was replicated in two test chambers (N = 4 or N = 5 larvae per test chamber). Mean TL and wet weight of larvae were similar among all experimental treatments (ANOVA; $P > 0.05$; Table 2.1). To minimize nitrogenous waste accumulation within test chambers, water replacements occurred every 48 to 72 h.

Monitoring of survival and mortalities was done at 6 h intervals over the first 30 h of salinity exposure, then 24 h intervals (from time 0) through 96 h. At each observation, salinity and temperature were monitored with YSI Model 30 and Model 85 water quality meters. Obvious mortalities, in which larvae were generally found out of the substrate, were removed from each chamber. Condition of larvae burrowed in the substrate at each observation interval was first evaluated by visual inspection for ventilation while within the substrate. If ventilation could not be detected, the substrate near a larva was gently

disturbed (Richards 1980). Larvae that responded to the disturbance (i.e., tail movement) were left burrowed in the substrate, whereas unresponsive larvae were removed. Larvae showing responsiveness (i.e., movement) after removal from the substrate were returned to the test chamber, while unresponsive larvae (i.e., no movement) were deemed mortalities and removed from the chamber.

Larvae that survived 96 h fixed salinity treatments were held for an additional 10 d (14 d total) at the same level of continuous salinity to evaluate potential acclimation to salinity over a longer time interval. After 14 total d in fixed salinity treatments, surviving larvae were sacrificed and metrics of osmoregulatory status (plasma osmolality, total body water content, and plasma cation concentrations) were quantified. Assessments of osmoregulatory status of these larvae are described in objective 2 below.

Survival in Oscillating Salinity

Based on results of larval survival in fixed salinity experiments, three oscillating salinity experiments were conducted, with the following salinity ranges: 0-12 ppt, 0-15 ppt, and 0-25 ppt. Peak salinity in each of the three experiments oscillated with freshwater at approximately 6 h intervals. Simulated tidal cycle oscillations (period and amplitude) were generally patterned after salinity data collected at two water quality monitoring stations in upstream regions of the Columbia River Estuary in the fall of 2012 (Figure 2.1; stccmop.org). The stations are part of the Columbia River Estuary observation network operated by the Center for Coastal Margin Observation and Prediction (CMOP). One station (Saturn 4) is located near the Oregon shore of the Columbia River just upstream of Tongue Point while the other (Grays Point) is near the

Washington shore at the mouth of Grays Bay (Figure 2.2). Laboratory tidal profiles were intended to simulate average high and low tide conditions that may be found in potentially suitable larval lamprey habitat in an estuarine environment.

Experimental configuration of tidal oscillation salinity challenges was generally similar to fixed salinity challenges (as described above); however minor modifications were made to the acrylic test chambers. Test chamber thickness was increased from 1.5 cm to 3 cm, to accommodate placement of Onset HOB0® conductivity data loggers (Model U24-002, Onset Computer Corporation, Bourne, Massachusetts) in chambers during tidal simulations. Test chamber length and height remained unchanged from previous experiments. Secondly, one hole was drilled on each end of the test chambers, approximately 3 cm from the top to allow water drainage during tidal simulations. Holes were small enough to prevent larval escape. These modified test chambers contained approximately 1200 mL of clear quartz sand burrowing substrate at a depth of approximately 6 to 8 cm, and were initially filled with approximately 3 L of dechlorinated tap water. During tidal simulations, water was pumped from one of two 208 L (55 gallon) holding containers, one containing freshwater and one containing saltwater of the appropriate concentration. Peristaltic pumps equipped with plastic tubing and drip irrigation manifolds and were used to deliver water to the test chambers. Pumps were adjusted to maintain water flow rates of 50 to 70 mL/min. During oscillation experiments, conductivity, salinity, and temperature were logged at 10 min intervals.

Each experimental treatment was replicated in two test chambers, N = 5 larvae per test chamber. Mean TL and wet weight of larvae were similar among groups of larvae from oscillating salinity experiments, as well as fixed salinity experiments described

above (ANOVA; $P > 0.05$; Table 2.1). Observations of larvae were made to monitor survival and mortality at 6 h intervals over the first 24 h, then at 24 h intervals through 96 h. Procedures for monitoring larval survival and mortality were similar to those of fixed salinity challenge experiments described above. Surviving larvae from oscillating 12 ppt salinity treatments were sacrificed after 24 h and 96 h, and osmoregulatory status metrics (as described below in Objective 2) were quantified.

To allow for comparison of survival and osmoregulatory status metrics two additional experimental treatments were conducted, fixed 0 ppt, and fixed 12 ppt salinity (partial water changes occurred every 24 h to 48 h). These treatments were each replicated in two test chambers, $N = 5$ larvae per test chamber. Surviving larvae in these treatments would be sacrificed at the same time intervals (24 h, and 96 h) as oscillating salinity treatment larvae to allow for comparisons of osmoregulatory status.

Statistical analysis

Differences in survival between 96 h fixed salinity and 96 h oscillating salinity challenge experiments were tested for significance using Fisher's exact tests in 2×2 contingency tables. Significance level was set at $\alpha = 0.05$.

OBJECTIVE 2. EVALUATING OSMOREGULATORY STATUS

Experimental Protocol

Three physiological parameters were quantified to evaluate and characterize osmoregulatory status of larvae that survived fixed and oscillating salinity experiments described above, (1) plasma osmolality (mOsm kg^{-1}), (2) total body water content ($\text{g H}_2\text{O}$

g⁻¹ tissue dry mass, and (3) plasma elemental/cation concentrations (mmol L⁻¹ or μ mol L⁻¹). The metrics were first quantified from a group (N = 10) of larvae that were sacrificed in the laboratory within several hours of collection in Cedar Creek (henceforth referred to as ‘fresh-collected’ larvae) to establish baseline stream-type values for the three parameters. From experimental larvae that survived 14 d fixed salinity treatments, osmoregulatory status was evaluated to investigate potential acclimation to salinity exposures. In oscillating salinity challenges, osmoregulatory status of larvae from oscillating 0-12 ppt experiments, fixed 12 ppt, and fixed 0 ppt salinity were evaluated and compared at 24 h and 96 h intervals to investigate the potential for homeostatic recovery within a freshwater tidal cycle.

To collect blood plasma, larvae were removed from test chambers, and sacrificed by overdosing in a concentrated solution of buffered MS-222. Sacrificed larvae were patted dry to remove excess water, and measured for TL and weight. A razor blade was used to sever the tail near the vent, and blood was collected from caudal vessels with heparinized microcapillary tubes. Plasma was separated by centrifuging for 5 minutes at 5000 g, hematocrit was recorded, and plasma samples were sealed in microcapillary tubes and stored at -80 °C. .

Plasma Osmotic Concentration

Plasma osmolality of sacrificed larvae was measured on a Wescor 5100B vapor pressure osmometer (Wescor Inc., Logan, Utah). The osmometer was calibrated prior to each run of analyses and prior to beginning each new salinity treatment group using 1000, 500, and 150 mOsm kg⁻¹ calibration standards. A 5 μ L sample was pipetted onto

filter paper disks which were inserted into the osmometer for analysis. Osmotic concentration of each salinity treatment (Cedar Creek water, 0 ppt laboratory freshwater, 6 ppt, 8 ppt, and 10 ppt) was also quantified through vapor pressure osmometry and used in subsequent regression analyses.

Total Body Water Content

Total body water content was calculated from the change in wet weight (in grams; at time of sacrificing) versus dry weight of each larva. Dissected carcasses were oven dried at 35°C until no change in weight was observed, generally 72 to 96 h. Desiccated carcasses were then reweighed and total body water content determined by calculating the change in weight. The weight difference was then divided by the weight (in grams) of dry tissue to compute g water gram⁻¹ tissue dry mass.

Plasma Elemental/Cation Concentrations

Concentrations of larval plasma elements/cations were quantified by inductively coupled plasma mass spectrometry (ICP-MS). Analyses were conducted in the Elemental Analysis Core at Oregon Health and Science University (OHSU) using an Agilent 7700x ICP-MS equipped with an ASX 250 autosampler. The system was operated at a radio frequency power of 1550 W with an argon (Ar) plasma gas flow rate of 15 L min⁻¹ and an Ar carrier gas flow rate of 1.08 L min⁻¹. Concentrations of the following ten elements (ICP-MS does not discriminate between different ionic or oxidation states of the same element) were quantified: sodium (Na), magnesium (Mg), phosphorus (P), potassium (K), calcium (Ca), manganese (Mn), iron (Fe), copper (Cu), selenium (Se), and zinc (Zn),

although not all elements were expected to have important osmoregulatory functions. Measurements of Na, Mg, P, K, Ca, Mn, Fe, Cu, and Zn were made in kinetic energy discrimination (KED) mode using helium gas (4.2 mL min⁻¹); Se was measured in hydrogen gas mode (3.5 mL min⁻¹). For measurement, 101x-201x dilutions of plasma samples were prepared in 1% HNO₃ in acid treated tubes (via incubation in 1% HNO₃ [trace metal grade, Fisher Scientific] for at least 24 h). Data were quantified using a 11-point calibration curve (0, 1, 2, 5, 10, 50, 100, 1000, 2000, 10000 and 15000 ppb (ng/g)) with external standards for Na, Mg, P, K, Ca, Mn, Fe, Cu, Zn, and Se (Common Elements Mix 2 Multi-Element Aqueous Standard and Selenium Single Elemental Standard, VHG Labs) in 1% HNO₃. For each sample, data were acquired in triplicate and averaged. A Ge-72 internal standard (Internal Standard Multi-Element Mix 3, VHG Labs) introduced with the sample was used to correct for plasma instabilities, and frequent measurements of a 100 ppb all-analyte solution was used to determine the coefficient of variance. To assess recovery rates of elements and probe background contamination from containers the following controls were prepared and analyzed by the same method as the samples: certified NIST standard reference materials 1598a (Animal Serum) and 1643e (Trace Elements in Water).

Statistical analyses

All data are presented as mean $\pm$ standard error of the mean (s.e.) unless specified otherwise. One-way ANOVA was used to test for significant differences in plasma osmolality, total body water content, and plasma elemental/cation concentrations among larvae surviving (i) 14 d fixed salinity treatments of 0 ppt, 6 ppt, 8 ppt, and 10 ppt (12 d),

and (ii) oscillating 12 ppt salinity, fixed 12 ppt salinity, and fixed 0 ppt salinity treatments at 24 h and 96 h intervals. Comparisons of metrics to those from Cedar Creek fresh-collected larvae (baseline natural condition) were also made. Tukey's *post hoc* test was used to identify where significant differences occurred. Significance level was set at $\alpha = 0.05$.

Linear regression analyses were used to evaluate the relationship between osmotic concentration of fixed salinity experimental treatments and larval plasma osmolality and total body water content. Linear regression analyses were also used to evaluate the relationship between the concentrations of sodium, potassium, magnesium, and calcium ions in fixed salinity experimental treatments and respective larval plasma ionic concentrations. Linear regression analysis was again used to evaluate the relationship between larval plasma osmolality and plasma sodium ion concentrations using data pooled from both fixed and oscillating salinity experiments. The relationship between larval total body water content and plasma osmolality and plasma sodium ion concentration was also evaluated using linear regression analysis of data pooled from both fixed and oscillating salinity experiments.

To illustrate magnitude and direction of driving forces for flux of water in respective salinity treatments, osmotic gradients between fixed salinity treatment solutions and larval plasma were quantified by subtracting the measured osmotic concentration of each larva (mOsm kg^{-1}) from the measured osmotic concentration of each experimental treatment. The results were graphed to illustrate osmotic concentration gradients. To illustrate magnitude and direction of driving forces for flux of sodium ions in respective salinity treatments, sodium ion gradients between fixed

salinity treatment solutions and larval plasma were quantified by subtracting the measured sodium concentration of each larva (mmol L^{-1}) from the measured sodium concentration of each experimental treatment. The results were graphed to illustrate sodium ion concentration gradients.

RESULTS

OBJECTIVE 1. EVALUATING OSMOTIC STRESS TOLERANCE

Survival in Fixed Salinity

In general, higher salinity concentrations resulted in lower survival of larvae in fixed salinity experiments. Through 96 h, 100% survival occurred in 0 ppt, 6 ppt, 8 ppt, and 10 ppt fixed salinity challenges (Figure 2.3). In 12 ppt fixed salinity, 100% survival occurred through 36 h, with 0% survival ultimately observed at hour 48. In 15 ppt fixed salinity, 0% survival was observed at hour 30. In 25 ppt and 35 ppt fixed salinity, 0% survival was observed at 12 h (Figure 2.3).

Experimental treatments extended through a total of 14 d (0 ppt, 6 ppt, 8 ppt, and 10 ppt) resulted in 100% survival in 0 ppt, and 8 ppt fixed salinity, while 87.5% survival occurred in 6 ppt fixed salinity (Figure 2.3). Larvae in these three treatments generally remained in the substrate and exhibited no external indications of stress. In 10 ppt fixed salinity, beginning on day 6, all larvae were out of the substrate and exhibited signs of stress (lethargic behavior, branchial mucus accumulation). As a result, larvae in the 10 ppt treatment were sacrificed on day 12 (while 100% were alive) to allow for blood plasma collected and quantification of osmoregulatory status.

Survival in Oscillating Salinity

In oscillating 0-12 ppt salinity, 100% survival occurred through the 96 h experiment (Figure 2.4) and was significantly higher than survival in fixed 12 ppt salinity (0%) over the same time interval ($P < 0.0001$). In oscillating 0-15 ppt salinity, 60% survival occurred through the 96 h experiment (Figure 2.5) and was significantly higher than survival in fixed 15 ppt salinity (0%) over the same time interval ($P = 0.013$). In oscillating 0-25 ppt salinity, 20% survival occurred through 12 h and was not different from survival in fixed 25 ppt salinity (0%) over the same time interval ($P = 0.477$; Figure 2.6). Given the rapid mortality in oscillating 25 ppt, the experiment was terminated after 12 h.

OBJECTIVE 2. EVALUATING OSMOREGULATORY STATUS

Plasma Osmolality

Linear regression analysis indicated increasing osmotic concentration of the 14 d fixed salinity experimental treatments was significantly related to increases in larval plasma osmolality ($P < 0.001$, $r^2 = 0.89$; Figure 2.7). Plasma osmolality (mean mOsm kg⁻¹ $\pm$ s.e.) of larvae in 6 ppt fixed salinity (224.6 ± 8.7) was similar to Cedar Creek fresh-collected larvae (216.6 ± 4.6 ; ANOVA; $P > 0.05$), but was significantly higher than 0 ppt larvae (148.9 ± 7.1 ; ANOVA; $P < 0.05$; Table 2.2). Plasma osmolality of larvae in 8 ppt (297.3 ± 4.7) and 10 ppt fixed salinity (335.8 ± 11.5) was significantly higher than all other treatments (ANOVA; $P < 0.05$; Table 2.2).

In oscillating salinity experiments, plasma osmolality of larvae after 24 h in oscillating 12 ppt salinity (213.9 ± 5.8) was similar to both 0 ppt larvae (197.6 ± 2.8) as

well as Cedar Creek fresh-collected larvae (216.6 ± 4.6 ; ANOVA; $P > 0.05$), but was significantly lower than fixed 12 ppt salinity larvae (332.9 ± 6.1 ; ANOVA; $P < 0.05$; Figure 2.8). Plasma osmolality gradually increased in oscillating the 12 ppt salinity treatment over the course of the experiment and after 96 h of oscillation (259.1 ± 6.8) was significantly higher than all other treatments (except fixed 12 ppt salinity after 24 h) as well as Cedar Creek fresh-collected larvae (ANOVA; $P < 0.05$; Figure 2.8).

Osmotic gradients between experimental treatments and larval plasma indicate larval internal fluids were more concentrated than their treatment concentrations, favoring osmotic influx of water (Figure 2.9). Sodium ion gradients, on the other hand, show concentrations in larval plasma were higher than treatment concentrations, favoring osmotic efflux of sodium ions (Figure 2.10).

Total Body Water Content

Linear regression analysis indicated increasing osmotic concentration of the 14 d fixed salinity experimental treatments was significantly related to decreases in larval total body water content ($P < 0.0001$, $r^2 = 0.52$; Figure 2.11). Mean total body water content (g H₂O g⁻¹ tissue dry mass $\pm$ s.e.) of larvae in 6 ppt salinity (3.6 ± 0.3) was similar to Cedar Creek fresh-collected larvae (4.4 ± 0.2 ; ANOVA; $P > 0.05$), but was lower than the 0 ppt treatment larvae (4.8 ± 0.2 ; ANOVA; $P < 0.05$; Table 2.2). Mean total body water content of larvae in 8 ppt (3.4 ± 0.3) and 10 ppt salinity treatments (3.2 ± 0.2) was lower than both 0 ppt, and fresh-collected larvae (ANOVA; $P < 0.05$; Table 2.2).

In oscillating salinity experiments after 24 h, mean total body water content (g H₂O g⁻¹ tissue dry mass $\pm$ s.e.) of larvae in oscillating 12 ppt salinity (4.0 ± 0.2) was

similar to 0 ppt, 24 h (4.5 ± 0.2) and Cedar Creek fresh-collected larvae (4.4 ± 0.2 ; ANOVA; $P > 0.05$), but was significantly higher than fixed 12 ppt salinity (2.7 ± 0.1 ; ANOVA; $P < 0.05$; Figure 2.12). Mean total body water content gradually decreased in oscillating 12 ppt salinity over the course of the experiment, and after 96 h of oscillation (2.8 ± 0.2) was significantly lower than after 24 h (ANOVA; $P < 0.05$). In addition, mean total body water content of oscillating 12 ppt larvae after 96 h was lower than 0 ppt, 96 h (4.5 ± 0.2) as well as fresh-collected larvae (ANOVA; $P < 0.05$; Figure 2.12)

Plasma Elemental/Cation Concentrations

Although ICP-MS does not differentiate between difference ionic states of elements, it was assumed that sodium, potassium, calcium and magnesium primarily occurred as cations. The remaining six elements of phosphorus, iron, zinc, selenium, manganese, and copper may have occurred in multiple elemental, ionic, and oxidation states. In all experimental larvae, sodium was the most abundant plasma element quantified. In addition to sodium, five other elements occurred in millimolar (mmol L^{-1}) concentrations: potassium, magnesium, calcium, phosphorus, and iron. The remaining four elements quantified: copper, manganese, selenium, and zinc were present in micromolar ($\mu\text{mol L}^{-1}$) concentrations in larvae from all experiments.

Fixed Salinity Treatment Cation/Elemental Concentrations

Linear regression analysis indicated increasing sodium ion concentration of the 14 d fixed salinity experimental treatments was significantly related to increases in larval plasma sodium ion concentrations ($P < 0.0001$, $r^2 = 0.78$; Figure 2.13). Mean larval

plasma sodium ion concentrations ($\text{mmol/L} \pm \text{s.e.}$) in fixed 6 ppt salinity (97.1 ± 6.5) was significantly higher than 0 ppt (55.7 ± 4.4 ; ANOVA; $P < 0.05$), but was similar to Cedar Creek fresh-collected larvae (85.0 ± 3.3 ; ANOVA; $P > 0.05$; Table 2.3). From larvae in fixed 8 ppt (110.6 ± 6.0) and 10 ppt salinity (130.0 ± 5.6) plasma sodium ion concentrations were higher than both 0 ppt and fresh-collected larvae (ANOVA; $P < 0.05$; Table 2.3). Larvae in 0 ppt had significantly lower plasma sodium ion concentration than fresh-collected larvae (ANOVA; $P < 0.05$).

Linear regression analysis indicated increasing potassium ion concentration of the 14 d fixed salinity experimental treatments was significantly related to increases in larval plasma potassium ion concentrations ($P < 0.0001$, $r^2 = 0.46$; Figure 2.13). Mean larval plasma potassium ion concentrations were significantly lower in all 14 d treatments than Cedar Creek fresh-collected larvae (5.1 ± 0.3 ; ANOVA; $P < 0.05$; Table 2.3). Plasma potassium ion concentration was higher in 10 ppt fixed salinity (4.0 ± 0.3), than the three other treatments: 0 ppt (1.7 ± 0.3), 6 ppt (2.3 ± 0.2) and 8 ppt (2.5 ± 0.2 ; ANOVA; $P < 0.05$; Table 2.3).

Linear regression analysis indicated increasing magnesium ion concentration of the 14 d fixed salinity experimental treatments was significantly related to increases in larval plasma magnesium ion concentrations ($P < 0.0001$, $r^2 = 0.75$; Figure 2.14). Mean larval plasma magnesium ion concentrations in 6 ppt (4.1 ± 0.3), 8 ppt (5.1 ± 0.3), and 10 ppt (9.4 ± 0.8) salinity treatments were all significantly higher than both 0 ppt (1.0 ± 0.1), and Cedar Creek fresh-collected larvae (1.0 ± 0.0 ; ANOVA; $P < 0.05$; Table 2.3). Larval plasma magnesium ion concentration in the 10 ppt salinity treatment was higher than all

other groups (ANOVA; $P < 0.05$; Table 2.3). No difference in magnesium ion concentration was detected in 0 ppt and fresh-collected larvae.

Linear regression analysis indicated increasing calcium ion concentration of the 14 d fixed salinity experimental treatments was significantly related to increases in larval plasma calcium ion concentrations ($P = 0.001$, $r^2 = 0.29$; Figure 2.14). However, larval plasma calcium ion concentrations generally showed the least amount of variation among all elements quantified. Mean larval plasma calcium ion concentrations were not different in fresh-collected (1.8 ± 0.2), 0 ppt (1.6 ± 0.1), 6 ppt (2.1 ± 0.1), and 8 ppt treatment larvae (1.8 ± 0.1 ; ANOVA; $P > 0.05$; Table 2.3), but all were significantly lower than calcium concentration of larvae from 10 ppt treatments (2.9 ± 0.3 ; ANOVA; $P < 0.05$; Table 2.3).

Plasma phosphorus concentration was higher in 10 ppt larvae (4.5 ± 0.3) than all other treatments (ANOVA; $P < 0.05$; Table 2.3). No differences in mean plasma phosphorus concentration were detected among the remaining treatments as well as Cedar Creek fresh-collected larvae (2.1 ± 0.2 ; ANOVA; $P > 0.05$; Table 2.3). Plasma iron concentration was higher in 0 ppt larvae (3.9 ± 0.5) than all other experimental treatments as well as fresh-collected larvae (1.7 ± 0.3 ; ANOVA; $P < 0.05$; Table 2.3). No differences in mean plasma iron concentration were detected among the remaining treatments as well as fresh-collected larvae (ANOVA; $P > 0.05$; Table 2.3).

The remaining elements that were quantified: copper, manganese, selenium, and zinc, were present in minute micromolar concentrations (mean $\mu\text{mol L}^{-1} \pm \text{s.e}$) in larval plasma and generally did not exhibit obvious trends among 14 d fixed salinity treatments. Concentrations of plasma copper were highest in 6 ppt (18.8 ± 3.7) and 10 ppt ($17.6 \pm$

2.0) salinity treatment larvae, which were higher than 0 ppt and fresh-collected larvae (5.3 ± 1.3 ; ANOVA; $P < 0.05$; Table 2.3). No differences in both plasma manganese and selenium were detected among salinity treatment larvae and Cedar Creek fresh-collected larvae (ANOVA; $P > 0.05$; Table 2.3). Mean plasma zinc concentration was highest in 0 ppt larvae (260.0 ± 16.9) and was significantly higher than all other treatments as well as fresh-collected larvae (121.8 ± 12.8 ; ANOVA; $P < 0.05$; Table 2.3). No differences in zinc concentration were detected among the remaining treatments groups as well as fresh-collected larvae (ANOVA; $P > 0.05$; Table 2.3).

Oscillating Salinity Treatment Cation/Elemental Concentrations

In oscillating salinity treatments, mean larval plasma sodium ion concentration after 24 h of oscillating 12 ppt salinity (72.6 ± 5.7) was not different from 0 ppt (80.6 ± 3.8) and Cedar Creek fresh-collected larvae (85.0 ± 3.3 ; ANOVA; $P > 0.05$), but was significantly lower than larvae from fixed 12 ppt salinity (109.3 ± 2.1) over the same time interval (ANOVA; $P < 0.05$; Figure 2.15). Plasma sodium ion concentration gradually increased in oscillating 12 ppt salinity treatments over the course of the 96 h experiment. After 96 h of 12 ppt oscillation, mean plasma sodium ion concentration (101.2 ± 6.1) was higher than after 24 h of 12 ppt oscillation (ANOVA; $P < 0.05$), but was not different from 0 ppt, 96 h (79.0 ± 4.7) or fresh-collected larvae (ANOVA; $P > 0.05$; Figure 2.15). Larval plasma potassium ion concentration significantly declined after 24 h in oscillating 12 ppt salinity (2.2 ± 0.2) and was lower than fixed 12 ppt (4.5 ± 0.3), 0 ppt (4.5 ± 0.4), and fresh-collected larvae (5.1 ± 0.3 ; ANOVA; $P < 0.05$; Figure 2.15). After 96 h, plasma potassium concentration in oscillating 12 ppt salinity (3.5 ± 0.5) increased to

levels similar to 0 ppt (3.5 ± 0.5 ; ANOVA; $P > 0.05$), but remained lower than Cedar Creek fresh-collected larvae (ANOVA; $P < 0.05$; Figure 2.15).

Concentrations of larval plasma magnesium ion increased markedly after 24 h in both oscillating 12 ppt (2.8 ± 0.3) and fixed 12 ppt salinity (4.3 ± 0.6), both of which were higher than 0 ppt (1.2 ± 0.1) and Cedar Creek fresh-collected larvae (1.0 ± 0.0 ; ANOVA; $P < 0.05$; Figure 2.16). After 96 h, plasma magnesium ion concentration further increased in oscillating 12 ppt salinity (4.6 ± 0.5), and was higher than all other treatments (except fixed 12 ppt after 24 h) as well as fresh-collected larvae (ANOVA; $P < 0.05$; Figure 2.16). Larval plasma calcium ion concentration showed a similar trend to that observed in 14 d fixed salinity experiments (discussed above), and was less variable in oscillating salinity experiments than other elements evaluated. After 24 h and 96 h intervals, no differences in mean plasma calcium ion concentration were detected among oscillating 12 ppt salinity (1.8 ± 0.2 ; 2.3 ± 0.2 , respectively), 0 ppt (2.0 ± 0.2 ; 2.2 ± 0.3 , respectively), as well as fresh-collected larvae (1.8 ± 0.2 ; ANOVA; $P < 0.05$; Figure 2.16). In fixed 12 ppt salinity through 24 h, concentration of calcium increased significantly (4.2 ± 0.5) and was higher than all treatments, as well as fresh-collected larvae (ANOVA; $P < 0.05$; Figure 2.16).

Phosphorus and iron also occurred in millimolar concentrations in larvae from oscillating salinity experiments. Larval plasma phosphorus concentrations after 24 h were not different among oscillating 12 ppt salinity (2.8 ± 0.4), 0 ppt (2.9 ± 0.3), and fresh-collected larvae (2.1 ± 0.2 ; ANOVA; $P > 0.05$; Figure 2.17), all of which were lower than phosphorus concentration in fixed 12 ppt salinity (6.4 ± 0.6 ; ANOVA; $P < 0.05$; Figure 2.17). After 96 h, plasma phosphorus concentration in oscillating 12 ppt

salinity larvae (4.1 ± 0.3) was similar to 0 ppt (3.8 ± 0.4 ; ANOVA; $P > 0.05$), but was higher than that of fresh-collected larvae (ANOVA; $P < 0.05$; Figure 2.17). Larval plasma iron concentrations after 24 h were not different among oscillating 12 ppt salinity (1.9 ± 0.4), 0 ppt (2.2 ± 0.6), and fresh-collected larvae (1.7 ± 0.3 ; ANOVA; $P > 0.05$), all of which were lower than iron concentration in fixed 12 ppt salinity (3.5 ± 0.4 ; ANOVA; $P < 0.05$; Figure 2.17). After 96 h, larval plasma iron concentration was significantly lower in oscillating 12 ppt salinity (0.9 ± 0.2) than 0 ppt (3.4 ± 0.9 ; ANOVA; $P < 0.05$), but did not differ from fresh-collected larvae (ANOVA; $P > 0.05$; Figure 2.17).

The four elements occurring in micromolar concentrations in larval plasma (mean $\mu\text{mol L}^{-1} \pm \text{s.e.}$) that were quantified: copper, manganese, selenium, and zinc, generally trended similarly to sodium in oscillating salinity experiments, in which concentrations in fixed 12 ppt salinity were higher than oscillating 12 ppt salinity treatments through 24 h (Table 2.4). Larval plasma copper concentration was higher in fixed 12 ppt salinity after 24 h (45.5 ± 12.2) than all treatments as well as fresh collected larvae (ANOVA; $P < 0.05$; Table 2.4). No difference in larval plasma manganese concentrations were detected among all salinity treatments as well as Cedar Creek fresh-collected larvae (ANOVA; $P > 0.05$; Table 2.4). Few differences in larval plasma selenium concentration were detected, however fixed 12 ppt salinity larvae were higher after 24 h (5.0 ± 0.8) than fresh-collected (1.3 ± 0.2), as well as oscillating 12 ppt larvae after 96 h (1.7 ± 0.3 ; ANOVA; $P < 0.05$; Table 2.4). Larval plasma zinc concentration was higher in fixed 12 ppt salinity after 24 h (218.8 ± 24.9) than Cedar Creek fresh-collected larvae (121.8 ± 12.8 ; ANOVA; $P < 0.05$; Table 2.4), but was not different from oscillating 12 ppt (160.1 ± 21.3), or 0 ppt

(179.1 ± 11.1) over the same time interval (ANOVA; $P > 0.05$). In 0 ppt treatments, plasma zinc concentration continuously increased during the experiment and after 96 h (264.1 ± 38.4) was higher than oscillating 12 ppt salinity (128.6 ± 12.6) over the same time interval, as well as fresh collected larvae (ANOVA; $P < 0.05$; Table 2.4).

Larval Plasma Composition – All Treatments

Plasma osmolality (mOsm kg⁻¹) from larval Pacific lamprey was significantly related to larval plasma sodium ion concentration (mmol L⁻¹; $P < 0.0001$; $r^2 = 0.58$, slope = 1.97 ± 0.19) according to data pooled from both fixed and oscillating salinity experiments (Figure 2.18).

Larval Pacific lamprey plasma osmolality (mOsm kg⁻¹; $P < 0.0001$; $r^2 = 0.43$) and plasma sodium ion concentrations (mmol L⁻¹; $P < 0.0001$; $r^2 = 0.23$) were both significantly related to larval water content (g H₂O g⁻¹ tissue dry mass) according to data pooled from both fixed and oscillating salinity experiments (Figure 2.19).

DISCUSSION

Survival

Evaluating survival of larval lampreys to a range of salinity induced osmotic stresses is useful for determining thresholds of tolerance of larvae to salinity exposures. These results can be used to inform and refine predictions of potential occurrence in tidally-influenced habitats in the natural environment. Higher survival of larval lampreys was observed in oscillating hyperosmotic 12 ppt and 15 ppt salinity treatments versus fixed salinity treatments, and is likely a result of tempered osmotic stress incurred by

larvae in these treatments. The inclusion of freshwater periods at about 6 h intervals in these treatments resulted in lower cumulative salinity exposure of larvae versus fixed salinity treatments. In 25 ppt salinity, rapid mortality of larvae occurred in both oscillating and fixed salinity experiments after 12 h, indicating this degree of hyperosmotic salinity exposure is acutely lethal to larvae. The oscillating salinity experiments were designed to mimic tidal variation in water salinity that may occur in estuarine areas in the natural environment. These findings suggest larvae may have some ability to survive in and occupy tidally affected areas, and will be used, in part, to guide larval Pacific lamprey occupancy sampling in tidally affected habitats (discussed in Chapter 3).

Increases in fixed salinity experiment concentrations beyond the iso-osmotic point of larval internal fluids resulted in corresponding decreases in larval survival. According to mean plasma osmolality measured from Cedar Creek fresh-collected larvae, which represent the baseline wild-type condition, larval internal fluid concentration was about 217 mOsm kg⁻¹, equivalent to approximately 7.6 ppt salinity. Thus, the 6 ppt treatment was hypo-osmotic, while the remaining treatments (8 ppt and higher) were increasingly hyperosmotic to larval fluids. Despite being hyperosmotic to larval fluids, high survival was observed through 14 d in 8 ppt and 10 ppt (12 d) salinity. However, above 10 ppt, tolerance to hyperosmotic salinity treatments was low, a finding generally similar to previous experimental evaluations of larval Pacific lamprey, sea lamprey, and anadromous European river lamprey *Lampetra fluviatilis* where survival of larvae in salinities exceeding 10 ppt to 12 ppt was low (Mathers and Beamish 1974; Beamish et al. 1978; Morris 1980; Richards and Beamish 1981; Reis-Santos et al. 2008). Overall, our

results generally parallel previously published salinity tolerance thresholds of larval lamprey species in hyperosmotic media.

Osmoregulation

Organismal-level evaluations of larval osmoregulatory status indicated passive responses of larvae to increases in environmental salinity. As salinity increased in hyperosmotic treatments, progressive concomitant increases in larval plasma osmolality and plasma sodium ion concentrations were observed, while total body water content progressively declined. In larvae that survived 12 d in hyperosmotic fixed 10 ppt salinity treatments, we observed an increase in plasma osmolality of approximately 100 mOsm kg⁻¹, findings that parallel those of previous studies on larval sea lamprey. Mathers and Beamish (1974) reported an increase in plasma osmolality of over 80 mOsm kg⁻¹ after 24 h in 10 ppt salinity. Similarly, Beamish et al. (1978) observed increasing plasma osmolality in larval sea lamprey with increasing ambient salinity. These results provide multiple lines of evidence to suggest that larvae surviving in hyperosmotic treatments were tolerating various levels of salinity, but were not actively maintaining osmotic homeostasis (i.e., osmoregulating). These findings further confirm larval osmoregulatory mechanisms are not adapted for maintaining water and ion balance in hyperosmotic media.

Larval lamprey osmoregulation in hyperosmotic media would require the ability to carry out physiological processes utilized by juveniles and adults to maintain osmotic homeostasis in the marine environment, including swallowing seawater and excreting excess ions at the gills and gut. The most significant limitation of larval lamprey

osmoregulation in hyperosmotic solutions may be their inability to swallow seawater to compensate for the continuous osmotic loss of water to the surrounding environment. Larval lampreys are unable to swallow seawater due to the structure of the filter-feeding adapted gut. It is not until phase six of metamorphosis from larval to juvenile forms that anatomical changes including the opening of the foregut lumen allows the swallowing of water to occur (Richards and Beamish 1981). In turn, development of the foregut appears coincident with the onset of seawater osmoregulation and is thought to be one of several vital steps in the ontogeny of seawater osmoregulation in juvenile Pacific lamprey (Richards and Beamish 1981). In addition, Beamish et al. (1978) concluded larval sea lamprey osmoregulatory mechanisms likely function in the same direction in both hypo-osmotic and hyperosmotic environments. Thus, MRCs in the gill epithelium that uptake sodium ions (and chloride ion) from dilute freshwater environments likewise continue uptake sodium ions in hyperosmotic solutions. Our results suggest osmoregulatory mechanisms of larval Pacific lamprey function in a similar manner, resulting in an inability to osmoregulate in hyperosmotic solutions.

In oscillating 12 ppt salinity experiments, the occurrence of freshwater periods at 6 h intervals appears to have temporarily eased the desiccating effects of hyperosmotic solutions. In general, changes in the three parameters in oscillating 12 ppt salinity treatments were less severe than those observed in fixed 12 ppt salinity experiments. For example, larval plasma osmolality was nearly 120 mOsm kg⁻¹ greater in fixed 12 ppt salinity than oscillating 12 ppt salinity treatments through 24 h. Similarly, Hardisty (1956) found that water lost by larval European brook lamprey *Lampetra planeri* in hyperosmotic solutions could be recovered upon returning to freshwater. The inclusion

of freshwater periods during tidal oscillation experiments here may have facilitated the recovery of water lost during hyperosmotic 12 ppt peaks in salinity. Ultimately however, after 96 h of oscillating 12 ppt salinity plasma osmolality was higher and total body water content lower than 0 ppt freshwater treatments and fresh-collected larvae. Thus, although oscillating salinity treatments led to increased survival versus fixed salinity, physiological metrics in oscillating salinity suggest dehydration was slowly occurring through 96 h. Whether larvae have the ability to tolerate long-term exposure to oscillating hyperosmotic solutions is not known and warrants further investigation. High survival of larvae in oscillating salinity treatments suggests the potential for larvae to occur in estuarine habitats where salinity profiles are similar to those of laboratory experiments. These results will be used, in part, to guide sampling for larvae, discussed in Chapter 3 below.

Plasma Cation Concentrations

Positively charged sodium ion and negatively charged chloride ion are the two most abundant and important osmotic constituents of the extracellular fluid of larval lampreys, and changes in their concentration in experimental salinity treatments provide further understanding of larval osmoregulatory ability (Mathers and Beamish 1978; Beamish 1980; Morris 1980). We used ICP-MS to quantify the concentrations of selected elements, in particular sodium, in larval plasma from various salinity treatments. The ICP-MS approach does not discriminate between different ionic or oxidation states of analytes, but we assume sodium detected in plasma samples occurred as positively charged sodium ion. We found trends in sodium concentrations in fixed salinity experiments generally paralleled changes in plasma osmolality of larvae from various

salinity exposures. As discussed above, relative to the larval extracellular fluids, the 6 ppt salinity treatment was hypo-osmotic, while 8 ppt salinity and 10 ppt salinity treatments were hyperosmotic. As such, sodium concentration of larvae in 6 ppt salinity was similar to Cedar Creek fresh-collected larvae, and flux of sodium ions between larvae and the external media was likely minimal in this treatment. In both 8 ppt and 10 ppt treatments, concentration gradients between the hyperosmotic media and larval extracellular fluids resulted in the influx of sodium ions from the surrounding media. As a result, plasma sodium concentrations of larvae in 10 ppt salinity were over 45 mmol L^{-1} greater than Cedar Creek fresh-collected larvae. Similarly, Beamish et al. (1978) found an increase in larval sea lamprey serum sodium ion concentration of about 38 m-equivalents kg^{-1} between larvae in 0 ppt versus 10 ppt treatments. As discussed above, unidirectional activity of MRC cells in the gills of larval lamprey likely results in continued uptake of ambient sodium ion despite internal concentrations exceeding the homeostatic condition. The increase in larval plasma sodium ion concentration in these treatments further illustrates the inability of larvae to osmoregulate in media that exceed the concentration of their internal milieu.

In oscillating 12 ppt salinity treatments, our results suggest the inclusion of freshwater periods at 6 h intervals slowed the rate of sodium ion accumulation in experimental larvae. Sodium concentration in oscillating 12 ppt was not different from 0 ppt treatments and Cedar Creek fresh-collected larvae after 24 h and 96 h, but was significantly lower than larvae in fixed 12 ppt salinity treatments after 24 h. Despite the lack of statistically significant differences in sodium concentrations in oscillating 12 ppt salinity, however, an increasing trend was observed during the course of the 96 h

experiment. It is not clear whether increases in sodium concentration would continue if oscillation experiments were extended beyond 96 h. Our results suggest that longer term survival of larvae in oscillating 12 ppt may be high in the event of stabilization of sodium concentration near that observed after 96 h.

Concentrations of plasma potassium in larvae from fixed salinity experiments trended differently from sodium, as concentrations in all experimental salinity treatments were lower than Cedar Creek fresh-collected larvae. Lower concentrations of potassium were again likely the result of osmotic and excretory losses to the dilute external environment. In the 10 ppt salinity treatment, however, potassium concentration was higher than 0 ppt, 6 ppt, and 8 ppt treatments. It is likely that the higher concentration of potassium in this treatment is the consequence of osmotic water loss from larvae in 10 ppt resulting in a more concentrated extracellular fluid. In oscillating 12 ppt salinity treatments, trends in potassium concentrations were similar to those in fixed salinity experiments. After both 24 h and 96 h of oscillating 12 ppt salinity, larval potassium concentrations were lower than Cedar Creek fresh-collected larvae, but did not differ from 0 ppt treatment larvae after 96 h. The decreases in potassium concentrations observed through 96 h in both 0 ppt and oscillating 12 ppt salinity are again likely related to osmotic and excretory losses of potassium ions to the surrounding environment.

Concentrations of divalent cations including magnesium and calcium also showed variability in salinity treatments and may further reflect larval osmoregulatory status and function. Unlike monovalent sodium and potassium ion concentrations discussed above, after 14 d in 0 ppt freshwater treatments, concentrations of both plasma magnesium and calcium were not different from Cedar Creek fresh-collected larvae. However, in fixed 6

ppt, 8 ppt and 10 ppt salinity treatments, successive significant increases in larval magnesium concentrations were observed among all treatments. Similarly, in oscillating 12 ppt salinity experiments, magnesium concentration increased after both 24 h and 96 h of oscillation, and was higher than both 0 ppt larvae as well as fresh-collected larvae. The use of Instant Ocean® sea salt mixture to prepare various salinity treatments may partially explain this observation. Instant Ocean® is formulated to contain naturally occurring levels of elements in seawater including magnesium, equal a concentration of about 1.32 g kg⁻¹ at full strength seawater (i.e., 35 ppt; www.instantocean.com). In the Lewis River basin (to which Cedar Creek is a tributary and fresh-collected larval concentrations were determined), maximum values of magnesium are reported to be 1.1 mg L⁻¹ (Fuhrer et al. 1996). Similarly, in the Sandy River (located near the source of City of Portland water used in laboratory experiments) maximum values of magnesium were reported to be 2.3 mg L⁻¹ (Fuhrer et al. 1996). Thus salinity experiments of 6 ppt salinity had concentrations of magnesium (proportionally adjusted from 35 ppt) that were about 20 times higher than Cedar Creek freshwater. Successive experimental increases in treatment salinity would result in higher concentrations of ionic magnesium in salinity treatment solutions. As a result, successive increases in treatment salinity resulted in higher magnesium ion concentrations in larval internal fluids, as an increasing amount of ambient magnesium ion moved down concentration gradients and diffused into larval internal fluids. The increase in magnesium concentrations in salinity treatments may indicate the inability of larval kidneys to effectively excrete magnesium ions occurring in abnormally high concentrations in extracellular fluids.

Concentrations of calcium, while also present in naturally occurring amounts in Instant Ocean® sea salt mixture (about 0.4 g kg⁻¹ at full strength seawater), showed little variation in larval plasma in fixed salinity experiments. Larval calcium concentrations were similar in 0 ppt, 6 ppt, 8 ppt fixed salinity treatments and Cedar Creek fresh-collected larvae, while in 10 ppt salinity, calcium concentration increased and was greater than all other treatments. This finding may be the combined result of higher calcium ion availability in 10 ppt salinity solution (given the quantity found in Instant Ocean®), as well as osmotic water loss in the hyperosmotic 10 ppt treatment leading to higher plasma concentrations. In oscillating salinity treatments, calcium concentration was similarly tightly regulated and no differences were observed among oscillating 12 ppt salinity, 0 ppt, and fresh-collected larvae.

We quantified larval plasma cation/elemental concentrations using ICP-MS, and thus had the opportunity to quantify concentrations of as many as 10 elements. The four elements discussed above are abundant in larval lamprey plasma and occur in mM concentrations. In addition to these four elements, we elected to measure concentrations of six additional elements that are not known to have significant osmoregulatory function. Two of these elements, phosphorus ion and iron ion, also occurred in mM concentrations, while the remaining four elements, zinc ion, selenium ion, manganese ion, and copper ion, occurred in trace μ M concentrations. In general, these six elements did not exhibit strong relationships among the various salinity treatments and few differences were detected between experimental treatments and Cedar Creek fresh-collected larvae. Given the minute quantities of several of these elements, changes in concentrations may be related to differences such as elemental concentration of

laboratory filtered tap water and Cedar Creek water. As such, it is difficult to draw meaningful conclusions from the few differences in concentration of these six elements.

Conclusions and Implications for Chapter 3

Overall, the results of our study indicate larval Pacific lamprey tolerance to hyperosmotic environments is similar to previous observations of anadromous sea lamprey and European river lamprey larvae. As in previous studies, survival of larvae in fixed salinity treatments was low above 10 ppt salinity. Abrupt, concomitant increases in plasma osmolality and plasma sodium ion concentration, coupled with a decrease in overall body water content occurred in solutions of 10 ppt salinity, a finding in close agreement with that of larval sea lamprey (Mathers and Beamish 1974; Beamish et al. 1978; Reis-Santos et al. 2008). Larvae of anadromous lampreys are highly adapted for life in freshwater and have efficient osmoregulatory mechanisms for maintaining hyperosmotic internal fluids in the freshwater environment. Coincident increases in internal fluid and external media concentration suggest these mechanisms function in one direction in larvae, regardless of changes or increases in concentration of the external environment (Beamish et al. 1978; Richards and Beamish 1981). In hyperosmotic media, larvae are faced with the problem of excess ion accumulation in internal fluids coupled with osmotic water losses to the external environment. Our results further indicate the lack of physiological mechanisms needed for larvae to osmoregulate under these problematic conditions.

We demonstrated an increase in survival of larvae in hypertonic media when oscillations between freshwater and saline water were incorporated into experiments. In

oscillating salinity experiments, hyperosmotic solutions of 12 ppt and 15 ppt alternated with freshwater at 6 h intervals and lead to higher survival of larvae versus fixed exposure to these concentrations. In addition, lower rates of dehydration and ion accumulation were observed in oscillating salinity experiments and suggest an easing of the desiccating effects of hyperosmotic media. Hardisty (1956) reported the ability of larvae to recover lost water after returning to freshwater from hyperosmotic media and our results appear to corroborate this observation. Oscillating salinity treatments were designed to mimic tidal variation in water salinity that may occur in estuarine habitats in the natural environment. The results of our experiments suggest the potential for larvae to inhabit areas in the natural environment that experience tidal oscillation with peaks of salinity in the range of 12 ppt to 15 ppt. Above these tolerance thresholds, our results suggest survival of larvae is likely to be low and occupancy unlikely to occur. Given the results of oscillating salinity experiments, we propose to investigate larval lamprey occupancy in a tidally-influenced environment to address objectives three and four. Ideally, larval occupancy will be evaluated in habitats with salinity profiles similar to those in laboratory experiments in which survival of larvae was high. These objectives are addressed in Chapter 3 below.

TABLES

Salinity (ppt)	Salinity Treatment	Duration (h)	N	Length (mm)	Weight (g)
0	Cedar Creek Fresh	NA	10	103.6±2.6	1.60±0.14
0	Constant	24	10	107.5±3.0	1.96±0.14
0	Constant	96	10	104.6±3.0	1.90±0.17
0	Constant	336	10	100.8±3.0	1.65±0.14
6	Constant	336	8	108.8±3.8	2.06±0.21
8	Constant	336	8	112.3±4.6	2.20±0.22
10	Constant	288	8	110.1±3.9	2.16±0.21
12	Constant	24	10	107.9±3.2	1.91±0.15
12	Constant	<48	10	110.4±3.7	2.16±0.20
12	Oscillating	24	10	106.3±4.4	1.85±0.20
12	Oscillating	96	10	106.2±4.5	1.84±0.23
15	Constant	<48	8	105.6±3.8	1.84±0.20
15	Oscillating	96	10	106.9±4.5	1.86±0.22
25	Constant	<12	8	104.5±4.8	1.95±0.30
25	Oscillating	<12	10	109.1±4.7	1.97±0.23
35	Constant	<12	8	110.1±4.9	2.23±0.28

Table 2.1. Mean length (TL in mm ± standard error) and weight (wet weight in g; ± standard error) of larval Pacific lampreys from various fixed and oscillating salinity experiments. Larval measurements were made the day of collection, prior to haphazard distribution of larvae into various salinity treatments. No differences in mean TL or wet weight were observed among all treatments (ANOVA; $P > 0.05$).

Fixed Salinity Treatment	F.C.	0 ppt	6 ppt	8 ppt	10 ppt
Duration	NA	14 d	14 d	14 d	12 d
Plasma Osmolality (mean mOsm kg ⁻¹ ± s.e.)	216.6 ± 4.6 ^a	148.8 ± 7.1 ^b	224.6 ± 8.7 ^a	297.3 ± 4.7 ^c	335.8 ± 11.5 ^d
Total Body Water Content (mean gH ₂ O g ⁻¹ tissue dry mass ± s.e.)	4.4 ± 0.2 ^{a,b}	4.8 ± 0.2 ^b	3.6 ± 0.3 ^{a,c}	3.4 ± 0.3 ^c	3.2 ± 0.2 ^c

Table 2.2. Plasma osmolality (mOsm kg⁻¹) and total body water content (g H₂O g⁻¹ tissue dry mass) from larval Pacific lamprey in fixed salinity treatments as well as Cedar Creek fresh collected larvae (F.C.). Values across rows with similar letters were not significantly different (ANOVA; $P < 0.05$).

Fixed Salinity Treatment		F.C.	0 ppt	6 ppt	8 ppt	10 ppt
Duration		NA	14 d	14 d	14 d	12 d
mmol L ⁻¹ (mean ± s.e.)	Sodium (Na)	85.0 ± 3.3 ^a	55.7 ± 4.4 ^b	97.1 ± 6.5 ^{a,c}	110.6 ± 6.0 ^{c,d}	130.0 ± 5.6 ^d
	Potassium (K)	5.1 ± 0.3 ^a	1.7 ± 0.3 ^b	2.3 ± 0.2 ^b	2.5 ± 0.2 ^b	4.0 ± 0.3 ^c
	Calcium (Ca)	1.8 ± 0.2 ^a	1.6 ± 0.1 ^a	2.1 ± 0.1 ^a	1.8 ± 0.1 ^a	2.9 ± 0.3 ^b
	Magnesium (Mg)	1.0 ± 0.0 ^a	1.0 ± 0.1 ^a	4.2 ± 0.3 ^b	5.1 ± 0.3 ^b	9.4 ± 0.8 ^c
	Phosphorus (P)	2.1 ± 0.2 ^a	3.1 ± 0.3 ^a	2.7 ± 0.2 ^a	3.0 ± 0.3 ^a	4.5 ± 0.3 ^b
	Iron (Fe)	1.7 ± 0.3 ^a	3.9 ± 0.5 ^b	2.0 ± 0.5 ^a	1.8 ± 0.3 ^a	1.6 ± 0.3 ^a
μmol L ⁻¹ (mean ± s.e.)	Copper (Cu)	5.3 ± 1.3 ^a	8.9 ± 1.1 ^a	18.8 ± 3.7 ^b	12.7 ± 1.5 ^{a,b}	17.6 ± 2.0 ^b
	Manganese (Mn)	4.3 ± 1.7 ^a	1.5 ± 0.2 ^a	1.0 ± 0.2 ^a	1.0 ± 0.1 ^a	1.0 ± 0.2 ^a
	Selenium (Se)	1.3 ± 0.2 ^a	2.2 ± 0.3 ^a	2.2 ± 0.3 ^a	1.8 ± 0.4 ^a	1.7 ± 0.3 ^a
	Zinc (Zn)	121.8 ± 92.2 ^a	260.0 ± 16.9 ^b	153.1 ± 22.5 ^a	127.6 ± 11.9 ^a	110.8 ± 10.8 ^a

Table 2.3. Concentrations of plasma cations (and elements) from larval Pacific lamprey in fixed salinity treatments as well as Cedar Creek fresh collected larvae (F.C.). Measurements were made using ICP-MS. Values across rows with similar letters were not significantly different (ANOVA; $P < 0.05$).

Salinity (ppt)	Treatment	Duration (h)	N	Copper (Cu)	Manganese (Mn)	Selenium (Se)	Zinc (Zn)
NA	Fresh Collected	NA	9	5.3 ± 1.3 ^a	4.3 ± 1.7 ^a	1.3 ± 0.2 ^{a,c}	121.8 ± 92.2 ^{a,c,d}
0	Fixed	24	9	11.5 ± 5.5 ^a	4.3 ± 1.7 ^a	3.3 ± 0.7 ^{a,b,c}	179.1 ± 11.1 ^{a,b,c,d}
0	Fixed	96	5	12.2 ± 3.9 ^a	2.4 ± 0.6 ^a	2.7 ± 0.5 ^{a,b,c}	264.1 ± 38.4 ^b
12	Fixed	24	7	45.5 ± 12.2 ^b	5.1 ± 1.1 ^a	5.0 ± 0.8 ^b	218.8 ± 24.9 ^{b,c}
12	Oscillating	24	9	7.4 ± 1.2 ^a	3.8 ± 1.2 ^a	2.8 ± 0.7 ^{a,b,c}	160.1 ± 21.3 ^{c,d}
12	Oscillating	96	10	14.3 ± 3.7 ^a	1.4 ± 0.6 ^a	1.7 ± 0.3 ^{a,c}	128.6 ± 12.6 ^d

Table 2.4. Concentrations of plasma cations (and elements) from larval Pacific lamprey in oscillating 12 ppt, fixed 12 ppt, and fixed 0 ppt salinity treatments through 24 h and 96 h intervals, as well as Cedar Creek fresh collected larvae. Measurements were made using ICP-MS. Values within columns with similar letters were not significantly different (ANOVA; $P < 0.05$).

FIGURES

Figure 2.1. Bottom salinity data over 96 h periods at two fixed water quality monitoring stations in the Columbia River estuary in September and October 2012. Oscillating salinity experiments conducted in the laboratory with maximum salinities of 12 ppt, 15 ppt, and 25 ppt generally followed similar cycles. Data from the Center for Coastal Margin Observation and Prediction (cmop.org).

Figure 2.2. Locations of two salinity monitoring stations operated by the Center for Coastal Margin Observation and Prediction (CMOP) in the upstream region of the Columbia River estuary. Data collected at these stations are shown in Figure 1 and were used to guide design of oscillating salinity experiments in the laboratory. Map from cmop.org.

Figure 2.3. Survival (%) of larval Pacific lamprey in fixed salinity treatments. Through 96 h, 100% survival of larvae occurred in 10 ppt salinity and below. Above 10 ppt salinity, 100% mortality occurred within 48 h of salinity exposure. Through 14 d, survival of larvae in 0 ppt, 6 ppt, 8 ppt, and 10 ppt (12 d), was high.

Figure 2.4. In oscillating 12 ppt salinity (right y-axis), 100% survival (left y-axis) of larval Pacific lampreys was observed through 96 h. Survival in oscillating 12 ppt salinity through 96 h was significantly higher than in fixed 12 ppt salinity (0%; Figure 3) over the same time interval (Fisher's Exact, $P < 0.05$). Gray hatched bars depict actual salinity data logged in test chambers.

Figure 2.5. In oscillating 15 ppt salinity (right y-axis), 60% survival (left y-axis) of larval Pacific lampreys was observed through 96 h. Survival in oscillating 15 ppt salinity through 96 h was significantly higher than in fixed 15 ppt salinity (0%; Figure 3) over the same time interval (Fisher's Exact, $P < 0.05$). Red checkered bars depict actual salinity data logged in test chambers.

Figure 2.6. In oscillating 25 ppt salinity (right y-axis), 100% mortality (left y-axis) occurred within 12 h. Survival in this treatment was not different from survival in fixed 25 ppt salinity over the same time interval (0%; Fisher's Exact, $P = 0.477$). The orange cross-hatched bar depicts actual salinity data logged in test chambers.

Figure 2.7. Plasma osmolality (mOsm kg⁻¹) from larval Pacific lamprey in 14 d fixed salinity treatments of 0 ppt (FW), 6 ppt, 8 ppt, and 10 ppt (12 d)) was significantly related to the osmotic concentration of experimental treatments ($P < 0.0001$; $r^2 = 0.89$). Darkened symbols show mean concentrations ($\pm$ s.e.) while gray symbols show measurements.

Figure 2.8. Plasma osmolality (mean mOsm kg⁻¹ ± s.e.) of larval Pacific lamprey in oscillating 12 ppt salinity, fixed 12 ppt salinity as well as 0 ppt treatments after 24 h and 96 h experiments. Time 0 value represents Cedar Creek fresh-collected larvae. Values with like characters are not significantly different (ANOVA; $P > 0.05$).

Figure 2.9. Osmotic gradients between larvae from 14 d experimental salinity treatments versus environmental osmolality. Observed concentrations show the

difference between larval plasma osmotic concentration and treatment osmotic concentration. Positive osmotic gradients (i.e., $> 0 \text{ mOsm kg}^{-1}$) would favor osmotic influx of water from the environment into larval internal fluids. The expected osmolality line represents iso-osmotic conformity.

Figure 2.10. Sodium ion concentration gradients between larvae from 14 d fixed salinity treatments versus environmental sodium ion concentrations. Observed concentrations show the difference between larval plasma sodium ion concentration and treatment sodium ion concentration. Positive sodium ion gradients (i.e., $> 0 \text{ mOsm kg}^{-1}$) would favor osmotic influx of sodium ions from the environment into larval internal fluids. The expected sodium ion concentration line represents iso-osmotic conformity.

Figure 2.11. Total body water content from larval Pacific lamprey in 14 d fixed salinity treatments of 0 ppt (FW), 6 ppt, 8 ppt, and 10 ppt (12 d) was significantly related to the osmotic concentration of experimental treatments ($P < 0.0001$; $r^2 = 0.52$). Darkened symbols show mean water content (g water g⁻¹ tissue dry mass $\pm$ s.e.), while gray symbols show measurements.

Figure 2.12. Total body water content (g water g⁻¹ tissue dry mass $\pm$ s.e.) from larval Pacific lamprey in oscillating 12 ppt salinity, fixed 12 ppt, and 0 ppt salinity after 24 h and 96 h experiments. Time 0 value represents Cedar Creek fresh-collected larvae. Values with like characters are not significantly different (ANOVA; $P > 0.05$).

Figure 2.13. Plasma sodium ion concentration (mmol L⁻¹; top figure) and potassium ion concentration (bottom figure) from larval Pacific lamprey after 14 d fixed salinity treatments of 0 ppt (FW), 6 ppt, 8 ppt, and 10 ppt (12 d). Larval plasma sodium ion concentration was significantly related to sodium ion concentration of experimental treatments ($P < 0.0001$; $r^2 = 0.78$). Plasma potassium ion concentration was significantly related to potassium ion concentration of experimental treatments ($P < 0.0001$; $r^2 = 0.46$). Darkened symbols show mean concentrations ($\pm$ s.e.) while gray symbols show measurements.

Figure 2.14. Plasma magnesium ion concentration (mmol L⁻¹; top figure) and calcium ion concentration (bottom figure) from larval Pacific lamprey after 14 d fixed salinity treatments of 0 ppt (FW), 6 ppt, 8 ppt, and 10 ppt (12 d). Larval plasma magnesium ion concentration was significantly related to magnesium ion concentration of experimental treatments ($P < 0.0001$; $r^2 = 0.75$). Plasma calcium ion concentration was significantly related to calcium ion concentration of experimental treatments ($P = 0.001$; $r^2 = 0.29$). Darkened symbols show mean concentrations ($\pm$ s.e.) while gray symbols show measurements.

Figure 2.15. Plasma sodium ion (top figure) and potassium ion concentrations (bottom figure; mean mmol L⁻¹ $\pm$ s.e.) from larval Pacific lamprey in oscillation 12 ppt salinity, fixed 12 ppt salinity, and fixed 0 ppt salinity after 24 h and 96 h experiments. Time 0 values represent Cedar Creek fresh-collected larvae. Values with like characters are not significantly different (ANOVA; $P > 0.05$).

Figure 2.16. Plasma magnesium ion (top figure) and calcium ion concentrations (bottom figure; mean mmol L⁻¹ ± s.e.) from larval Pacific lamprey in oscillation 12 ppt salinity, fixed 12 ppt salinity, and fixed 0 ppt salinity after 24 h and 96 h experiments. Time 0 values represent Cedar Creek fresh-collected larvae. Values with like characters were not significantly different (ANOVA; *P* < 0.05).

Figure 1.17. Plasma phosphorus (top figure) and iron concentrations (bottom figure; mean $\text{mmol L}^{-1} \pm \text{s.e.}$) from larval Pacific lamprey in oscillation 12 ppt salinity, fixed 12 ppt salinity, and fixed 0 ppt salinity after 24 h and 96 h experiments. Time 0 values represent Cedar Creek fresh-collected larvae. Values with like characters were not significantly different (ANOVA; $P < 0.05$).

Figure 2.18. Plasma osmolality (mOsm kg⁻¹) from larval Pacific lamprey was significantly related to larval plasma sodium ion concentration (mmol L⁻¹; $P < 0.0001$; $r^2 = 0.58$, slope = 1.97 ± 0.19). Data are pooled from both fixed and oscillating salinity experiments.

Figure 2.19. Larval Pacific lamprey plasma osmolality (mOsm kg⁻¹; $P < 0.0001$; $r^2 = 0.43$) and plasma sodium ion concentrations (mmol L⁻¹; $P < 0.0001$; $r^2 = 0.23$) were both significantly related to larval water content (g H₂O g⁻¹ tissue dry mass). Data are pooled from both fixed and oscillating salinity experiments.

CHAPTER 3

OCCURRENCE OF LARVAL LAMPREYS IN A TIDALLY-INFLUENCED ESTUARINE STREAM

INTRODUCTION

Pacific lamprey are anadromous, parasitic fishes native to Western North America. Pacific lamprey is a culturally valuable species to indigenous people, and has significant ecological importance in freshwater and marine ecosystems (Pletcher 1963; Kan 1975; Close et al. 2002; Luzier et al. 2011). Over the past several decades, constrictions in range and reductions in abundance of Pacific lamprey have been observed in Western North America. Apparent declines in abundance of Pacific lamprey have catalyzed both research efforts to better understand the biology and ecology of this declining species, as well as the development of several conservation measures, including the Pacific Lamprey Conservation Agreement (PLCA) by the U.S. Fish and Wildlife Service (USFWS) in 2011. The PLCA was signed in 2012 by Tribal, state, federal, and local agencies as a cooperative effort to reduce threats to Pacific lamprey and improve their habitats and population status (USFWS 2012). Among the necessary measures identified in the PLCA to ensure the persistence of Pacific lamprey in the Pacific Northwest include improving the understanding of larval Pacific lamprey occupancy and distribution, habitat requirements, and the limiting factors of larval distribution. Research of this type would be valuable for future conservation, management or recovery efforts of Pacific lamprey throughout its range.

To better understand larval Pacific lamprey biology, ecology, and distribution, we propose to investigate potential occurrence of larval Pacific lamprey in estuarine environments characterized by variable salinity due to Pacific Ocean tidal oscillation. Over the course of the protracted larval phase, downstream dispersal of larval lampreys occurs that may be fairly extensive (Scribner and Jones 2002; Derosier et al. 2007; Jolley et al. 2014), and in some cases (such as coastal river basins) result in larvae entering tidally-influenced estuarine habitats. Estuaries are transitional habitats where longitudinal gradients of salinity occur due to seasonally fluctuating freshwater river inputs and daily fluctuations during lunar tidal cycles. Differences in faunal distributions of fishes and invertebrates across estuaries according to salinity tolerances are often used as a basis for subdividing estuaries into different zones (Odum 1988; Bulger et al. 1993; Wagner 1999). These estuarine zones are characterized according to ranges in average annual salinities, and span the conditions from always freshwater at the upstream end, to marine at the downstream end. The most upstream estuarine zone, freshwater tidal, is characterized by average annual salinity of less than 0.5 ppt. The oligohaline zone average annual salinity ranges from 0.5 ppt to 5 ppt. The mesohaline zone average annual salinity ranges from 5 ppt to 18 ppt, while the polyhaline zone average annual salinities exceed 18 ppt (Anonymous 1959; Odum 1988; Wagner 1999).

Unique tolerance of fishes to salinity results in different assemblages of fish species across estuarine habitat zones (Deaton and Greenberg 1986; Odum 1988; Bulger et al. 1993; Neves et al. 2011). Movements and occurrences of some highly mobile teleost fishes and invertebrates across estuarine habitats are common, but are primarily observed to be euryhaline marine fishes moving upstream into lower salinity habitats

(Wagner 1999; Whitfield 2015). Salinity tolerances of primarily freshwater fishes, such as larval lampreys, are generally more constrained by the freshwater interface, and show limited distribution into higher salinity habitats (Deaton and Greenberg 1986; Wagner 1999; Neves et al. 2011; Whitfield 2015). Critical salinity tolerance thresholds ranging from 2 ppt (Deaton and Greenberg 1986) to 6 to 8 ppt (Remane and Schlieper 1971, as cited in Bulger et. al 1993) have been hypothesized to represent physiological barriers for freshwater adapted fishes and thus limit estuarine movements and distribution of such species. However, in some systems, oligohaline estuarine habitats have been found to support an abundance of fishes, including freshwater species (Peterson and 1994; Wagner and Austin 1999; Norris 2010). Whether larval lampreys, which are not highly mobile fishes, may occur in such habitats is not well understood but is likely governed by larval tolerance to salinity.

Tolerance of several species of larval lampreys (including Pacific lamprey and sea lamprey) to various concentrations of seawater has been previously evaluated in laboratory experiments. In general, larvae show an inability to osmoregulate in solutions that exceed the osmotic concentration of their internal milieu (225-260 mOsm/kg or about 8 to 10 ppt; Hardisty 1956; Mathers and Beamish 1974; Morris 1980; Reis-Santos et al. 2008), in turn survival (tolerance) in hyperosmotic environments is generally low. However, some tolerance (i.e., survival) of larval Pacific lamprey to low levels of salinity was observed, resulting in a 96-h LC₅₀ of 12.2 ppt (Richards and Beamish 1981). In Chapter 2 above, we conducted a series of laboratory experiments to better understand tolerance of larval Pacific lamprey to variable hyperosmotic salinity that may occur in some estuarine environments. High survival of larvae was observed through 96 h in

hyperosmotic 12 ppt (100% survival) and 15 ppt (60% survival) salinity treatments in which freshwater alternated with maximum salinity concentration at approximately 6 h intervals to simulate tidal activity. Overall, these results suggest the potential for larval Pacific lamprey to occur in certain regions of tidal estuaries and were used in this chapter to guide design and location of larval estuarine habitat sampling.

Understanding the potential for larval lampreys to occur and survive in regions of tidal estuaries may be worthwhile and provide important information on larval life history. Occurrence and distribution in oligohaline habitats in particular may be important to understand, as these regions are likely the first encountered by downstream dispersing larvae that have periodic increases in tidal salinity. In addition, these habitats may cover fairly substantial geographic areas of large river estuaries (such as the Columbia River, or the Sacramento-San Joaquin Rivers), are known to be productive and nutrient rich (Odum 1988; Norris 2010), and are likely to have an abundance of suitable larval burrowing substrates. Thus, there is potential for these areas to be important larval lamprey rearing habitats in some river systems, and to possibly contain large numbers of larval lampreys. Although observations of larval lampreys occurring in proximity to and drifting into tidal estuaries have previously been made (Richards 1980, Sjoberg 1980, White and Harvey 2003), whether larval disperse into and survive in low salinity regions of estuaries is not known and has not been investigated.

As part of the regional effort to address gaps in knowledge of larval Pacific lamprey biology and ecology, we proposed to investigate occurrence of larval Pacific lamprey in an estuarine environment characterized by a tidally-influenced gradient in salinity. I was likewise interested in evaluating larval western brook (*Lampetra*

richardsoni) and river lamprey (*Lampetra ayresii*) occurrence in an estuarine environment, as larvae of these species commonly co-occur with larval Pacific lamprey in freshwater environments. Western brook and river lamprey are paired species that are morphologically identical as larvae and currently cannot be differentiated, even genetically (Goodman et al. 2009). Thus, these species will be pooled and henceforth referred to as '*Lampetra* spp.'. Occurrence of *Lampetra* spp. in estuarine environments is also not known and has not been investigated. Because larvae of all lamprey species, including Pacific lamprey and *Lampetra* spp. have similar osmoregulatory mechanisms adaptations for life in freshwater and similar tolerance to osmotic stress (Morris 1972; Morris 1980; Beamish et al. 1980; Bartels and Potter 2004), patterns of occurrence of Pacific lamprey and *Lampetra* spp. larvae in tidally-influenced habitats are likely to be similar. To investigate the occurrence of larval Pacific and *Lampetra* spp. in a tidally-influenced estuarine habitat, we will address the following two objectives in this chapter.

3. Assess larval lamprey occurrence in tidally-influenced habitats across a gradient of salinity conditions
4. Quantify and characterize salinity profiles in tidally-influenced areas where larval lampreys are detected.

The results of this work provide a starting point for understanding potential larval lamprey distribution in estuarine environments. Knowledge on the extent of larval lamprey occurrence in estuarine areas could prove important when designing and implementing restoration efforts in these habitats, as well as understanding the effects of

human disturbances channel dredging, streamflow alteration, and climate change on larval lamprey demographics.

METHODS

Study Area

Ellsworth Creek (Pacific County, Washington) is a 3rd order stream that originates in heavily forested coastal mountains in Southwestern Washington State. The entire 7,600 acre watershed is a forestry research reserve owned and managed by the Nature Conservancy. The lower 2 km of Ellsworth Creek (also known as Ellsworth Slough) flows through an approximately 349 acre estuarine emergent marsh typical of coastal estuaries, before emptying into the Naselle River estuary (nature.org; Figure 3.1). This segment of the creek is heavily influenced by tidal activity and exhibits frequent variation in salinity (Tom Kollasch, The Nature Conservancy, pers. comm.). The usual upstream boundary of tidal influence (head of tide) in the creek varies seasonally, but generally extends several hundred meters upstream of the tidal marsh, pushing into forested stream habitat of the creek (Figure 3.3). Exploratory surveys conducted in Ellsworth Creek to evaluate suitability for use in this chapter confirmed the following requirements (i) presence of larval Pacific lamprey and larval *Lampetra* spp. in non-tidal, freshwater habitats in the creek, (ii) tidal salinity oscillations in some portion of the creek generally similar in magnitude and duration to those in laboratory tidal simulation experiments (Figure 3.2), and (iii) ability to sample the stream for larval lampreys with a backpack electrofisher (i.e., wade-able depth and saline areas that experienced periods of

freshwater). It was not known whether the downstream distribution of larval lampreys in Ellsworth Creek extended into or included tidally-influenced habitats.

Sample Area Delineation

To evaluate larval lamprey occurrence in the tidally-influenced portion of Ellsworth Creek where a gradient of salinity occurs, we delineated a 900 m long study area that was approximately bisected by the boundary between forested stream habitat and the tidal marsh. The study area was divided into three sample zones, each 300 m in length. The upstream sample zone was located entirely within forested stream habitat in Ellsworth Creek. The presumptive head of tidal activity in Ellsworth Creek occurred at (or near) the upstream zone boundary (Figure 3.3). Thus, water in the upstream zone was generally always fresh. The middle sample zone was essentially bisected by the forest-tidal marsh boundary, with about half of the zone occurring in each habitat type (Figure 3.3). Significant interaction of freshwater and tidal saltwater was expected to occur in the middle zone. The downstream sample zone occurred entirely within tidal marsh habitat of Ellsworth Slough (Figure 3.3). Water salinity within the downstream zone was expected to be primarily saline, even at low tide. Given that salinity conditions ranged from constant freshwater at the upstream end of the study area to primarily saline water at the downstream end, it was expected that the downstream extent of larval lamprey distribution in the creek would occur within the study area.

To address objective 3 and the above hypotheses, we proposed to investigate larval lamprey occurrence in the three sample zones in Ellsworth Creek through electrofishing. Prior to electrofishing, each 300 m long sample zone was divided into six

contiguous 50 m long reaches, for a total of 18 sample reaches within the study area (Figure 3.3). Reaches were numbered sequentially beginning at the most upstream reach (reach 1), downstream to the lowermost reach (reach 18). A 50 m reach has been used in previous evaluations of larval lamprey occupancy in streams through electrofishing (Silver et al. 2010). Furthermore, sampling for larval lamprey in 6, 50 m reaches within a given unit of interest has been estimated to provide approximately 80% certainty that larvae are absent when they are not detected (Silver et al. 2010; USFWS, unpublished data). Results of larval lamprey sampling would be used to describe patterns of larval occurrence within tidally-influenced and non-tidal zones within the study area. Upon completion of electrofishing of the 18 reaches, salinity monitoring would be conducted in tidally-influenced reaches where larvae were detected.

OBJECTIVE 3. LARVAL LAMPREY OCCURRENCE SAMPLING

Reach Sampling

Larval lamprey occurrence within the 18 sample reaches was assessed through sampling with an AbP-2 backpack electrofisher (ETS Electrofishing, Verona, WI). Electrofisher outputs used here (i.e., voltage, cycle frequency, duty cycle, etc.) were duplicated from previous studies that were found to be effective for collecting larval lampreys in wade-able streams (Bowen et al. 2003; Silver et al. 2010; USFWS 2010). Electrofishing was conducted at or near predicted low tide, beginning at the lowermost reach in the downstream zone (reach 18) and working upstream. At the beginning of each sample reach, water temperature, conductivity, and salinity were recorded prior to electrofishing using a handheld YSI Model 30 water quality meter. Reaches were

sampled in a downstream to upstream direction by a three person crew, one person operating the electrofisher and two people capturing emergent larvae with fine-meshed dip nets. Larval lampreys captured in a reach were placed in a bucket of freshwater and held until sampling of the 50 m reach was complete. Sampling effort focused on Type I larval lamprey burrowing substrates (Slade et al. 2003), with relatively less effort focused on coarse or impenetrable substrates (i.e., gravel, boulders, and bedrock). Each 50 m reach was electrofished until the entire 50 m had been sampled or until larvae of both Pacific lamprey and *Lampetra* spp. had been captured (e.g. larvae occurred in the reach). Upon completion of a sampling reach, biological data was collected from captured larvae before continuing to the next upstream reach. Qualitative assessments of water clarity (good, fair, or poor), and quantity of Type I substrate (abundant, moderate, scarce, or absent) were also recorded at each reach. When practicable, larval capture sites were georeferenced with a handheld Trimble GPS unit (in some reaches larvae were collected at numerous locations, which precluded georeferencing every collection site).

Captured larvae were anesthetized in a solution of buffered MS-222 (150 mg/L), measured for TL and wet weight. Identification of larvae (> 70 mm TL) was made using caudal pigmentation characteristics to differentiate larval Pacific lamprey from *Lampetra* spp. (Goodman et al. 2009). Identification of larvae less than about 70 mm TL cannot be reliably made using morphological or caudal pigmentation characteristics, thus larvae below this threshold were not identified. Fin tissue samples were taken from the caudal fin of larvae that were too small to identify morphologically (< 70 mm TL), larvae identified as Pacific lamprey, and a haphazard sub-sample of *Lampetra* spp. larvae. Tissue samples were preserved in 100% ethanol, and used for subsequent genetic

assignments of genus (see Luzier et al. 2010; Spice et al. 2011) to small, morphologically unidentifiable larvae, and to confirm morphological identifications made in the field. Upon resuming active swimming behavior, larvae were returned to the reach in which they were captured.

Reach Re-Sampling Post Salinity Monitoring

Following reach-specific salinity monitoring (described below), we selected three reaches within the tidally-influenced portion of the creek to re-sample for larval lamprey occurrence. Reaches 6, 7, and 8 were identified as having suitable habitat and a high likelihood of larval occurrence given that larvae had been previously detected in each of the reaches during the electrofishing pass conducted prior to salinity monitoring. Electrofishing was conducted in suitable larval lamprey habitat in each of the three reaches, as described above, until larval lampreys were captured. Biological data was collected from all captured larvae as described above. Upon resuming active swimming behavior, larvae were returned to the reach in which they were captured. Occurrence of larval lampreys in re-sampled reaches would reaffirm observations from previous reach sampling, and conclusions regarding larval lamprey occurrence in a tidally-influenced estuarine area. Salinity data collected in the reach immediately prior to re-sampling would provide a spatially and temporally explicit look at salinity conditions experienced by larvae prior to being detected.

OBJECTIVE 4. SALINITY MONITORING

To address objective 4 in this chapter, salinity monitoring was conducted (1) at the boundaries of the three sample zones prior to electrofishing the study area for larvae, and (2) at reach-specific larval collection sites following reach sampling. These data would be used to characterize salinity broadly across the study area and specifically within reaches where larvae were detected. Sample zone boundary salinity monitoring would characterize the gradient of salinity across the study area, and bracket salinity conditions within each zone. Reach-specific salinity monitoring would characterize salinity conditions within tidally-influenced reaches where larvae were detected by electrofishing. (Salinity monitoring would not be conducted in non-tidal reaches where water was always fresh.) Beyond what could be inferred from zone boundary salinity data, reach-specific salinity monitoring would provide further spatially-explicit and precise estimations of salinity conditions experienced by larvae at sites where they occurred. Salinity monitoring data (in combination with electrofishing results) would provide evidence to suggest natural distribution of larval lampreys may include certain areas in tidally-influenced environments.

Zone Boundary Monitoring

To characterize the gradient in salinity conditions across the 900 m study area, HOBO® conductivity data loggers were deployed at three locations of interest (i) the upstream zone boundary, at the presumptive head of tide (the end of reach 6), above this point water was expected to be always fresh, (ii) the middle zone boundary, predicted to be the approximate lower extent of salinity conditions suitable for larval lampreys (the

end of reach 12), and (iii) the downstream zone boundary, where water was expected to be primarily saline (the end of reach 18; Figure 3.3). Loggers were placed inside an approximately 30.5 cm long by 7.6 cm diameter section of perforated PVC pipe that was affixed to a section of rebar. The rebar was hammered into the substrate until the logger was completely submerged in flowing water, and about 10 cm above the substrate. Water temperature, conductivity, and salinity were logged at 10 min intervals for a minimum of 7 d prior to electrofishing for larval lampreys.

Reach-Specific Monitoring

Reach-specific salinity conditions were monitored in tidally-influenced reaches where larvae were detected during reach sampling. To that end, salinity loggers were deployed within reaches 5, 6, 7, 8, 9, and 10 to monitor salinity in the reaches for up to 14 d following electrofishing (Figure 3.4). Reaches 5 and 6 were within the upstream zone and had been expected to be above tidal influence. However, according to salinity data logged at the upstream zone boundary (discussed below), as well as salinity measurements made while electrofishing, tidal influence was found to extend into in these reaches. Loggers were anchored to the stream bottom as described above, and water temperature, conductivity, and salinity were logged at 10 min intervals. Following salinity monitoring in reaches 6, 7, and 8, the reaches were re-sampled by electrofishing (methods as described above) to evaluate larval lamprey occurrence post-salinity monitoring within these reaches.

Salinity Data Analysis

Tidal Cycle Minimum and Maximum

From continuous zone boundary and reach-specific salinity data, we were interested in making quantitative comparisons of minimum and maximum tidal cycle salinity. To that end, individual tidal cycles were partitioned from continuous salinity data using minimum low tide values as cycle cut points (Figure 3.5). Thus, 7 d zone boundary data were divided into 13 unique tidal cycles, while reach-specific data (12 d to 13 d) were divided into 23 to 25 unique tidal cycles from low tide to low tide. Absolute minimum salinity (lowest recorded salinity within each tidal cycle) and absolute maximum salinity (highest recorded salinity within each tidal cycle) during each individual tidal cycle was determined at monitoring locations. Because tidal cycle absolute minimum and maximum salinities were not normally distributed at each location, (Shapiro-Wilk; $P < 0.05$) median minimum and median maximum tidal cycle salinity were calculated and compared among locations using Kruskal-Wallis ANOVA on ranks. Dunn's Multiple Comparison *post-hoc* test was used to identify where significant differences occurred. Significance level was set at $\alpha = 0.05$.

Tidal Cycle Accumulated Salinity Units

We were also interested in quantifying and comparing the degree/extent of salinity exposure that occurred during each tidal cycle at zone boundaries and reach-specific monitoring sites. To that end, the individually partitioned tidal cycles from zone boundary and reach-specific monitoring sites were plotted and used to quantify the magnitude of salinity exposure during each tidal cycle by adopting quantitative

techniques used to calculate degree-days from air or water temperature data (Baskerville and Emin 1969; Allen 1976). Similar to methods of quantifying degree-days, here the number of salinity-hours during individual tidal cycles was calculated by integrating the area under the salinity curve of each tidal cycle (Savitzky and Golay 1964; Gagnon and Peterson 1998) to yield a quantity termed ‘tidal cycle accumulated salinity units’ (ASU; Figure 3.5). Tidal cycle ASU quantification provides a site-specific, relative index of salinity exposure that accounts for both magnitude and duration of salinity intrusion within tidal cycles. Quantitative comparisons of average tidal cycle ASU at the three zone boundaries and reach-specific monitoring sites could then be used to describe differences in putative salinity exposure of larvae at collection sites in the natural environment. Because tidal cycle ASU data were not normally distributed at each site (Shapiro-Wilk; $P < 0.05$), differences in median tidal cycle ASU among monitoring sites were tested for significance using Kruskal-Wallis ANOVA on ranks. Dunn’s Multiple Comparison *post-hoc* test was used to identify where significant differences occurred. Significance level was set at $\alpha = 0.05$.

RESULTS

OBJECTIVE 3. LARVAL LAMPREY OCCURRENCE SAMPLING

Reach Sampling

Larval lampreys were found to occur in six reaches in Ellsworth Creek that experienced tidally-influenced variation in salinity during the study period, from reach 5 near the head of tide in the upstream zone, downstream into reach 10 in the middle zone

(Figure 3.4). Larval Pacific lampreys were captured in three of the six tidally-influenced reaches [reach 5 (n = 3), reach 6 (n = 3), and reach 7 (n = 1); Table 3.1]. Larval *Lampetra* spp. were captured in each of the six tidally-influenced reaches [reach 5 (n = 9), reach 6 (n = 9), reach 7 (n = 15), reach 8 (n = 5), reach 9 (n = 3), and reach 10 (n = 2); Table 3.1]. The two most downstream larval lamprey detection sites in reach 9 and reach 10 were located near the boundary of forest and tidal marsh habitats (Figure 3.4). In reach 11 and reach 12, no larval lampreys were detected despite the presence of suitable larval burrowing habitat (observations of invertebrates responding to the electrofisher suggested electrical current was being outputted; Table 3.1). In reaches 13 through 18 (the downstream zone), suitable larval burrowing habitat was present in each, however, we were unable to operate the electrofisher in these six reaches. Water conductivity (due to salinity) during the study period exceeded the operating range of the electrofisher ($\sim 9.5 \text{ mS cm}^{-1}$, or about 7 ppt; Table 3.1), which overloaded the unit causing it to shut down. As a result, we were not able to electrofish downstream zone reaches for larvae. Water salinity, conductivity, and temperature at the time of sampling each reach are shown in Table 3.1. The size ranges of larvae (length and weight) captured in each reach is shown in Table 3.2.

The four most upstream reaches in the study area (reach 1 through reach 4) were not tidally-influenced during the study period, and water in these reaches was always fresh. Larval Pacific lampreys were detected in three reaches [reach 2 (n = 4), reach 3 (n = 4), and reach 4 (n = 2); Table 3.1], while larval *Lampetra* spp. were abundant in all four reaches, making it impractical to attempt collection of all *Lampetra* spp. larvae observed. Instead a haphazard subsample of *Lampetra* spp. larvae was collected within these

sample reaches [reach 1 (n = 22), reach 2 (n = 11), reach 3 (n = 9), and reach 4 (n = 14); Table 3.1]. Water salinity, conductivity, and temperature at the time of sampling each reach are shown in Table 3.1. The size ranges of larvae (length and weight) captured in each reach is shown in Table 3.2.

Reach Re-Sampling Post Salinity Monitoring

Subsequent to reach-specific salinity monitoring conducted in tidally-influenced reaches, we re-sampled reach 6, reach 7, and reach 8 for larval lampreys with a backpack electrofisher as described above. Larval Pacific lamprey were detected in reach 6 (n = 1), while larval *Lampetra* spp. were detected in each of the three re-sampled reaches [reach 6 (n = 6), reach 7 (n = 3), and reach 8 (n = 2; Table 3.2)]. Both larvae detected in reach 8 had clipped caudal fins, indicating we had detected them during the previous electrofishing survey of the study area. Through process of elimination according to TL (and assuming minimal growth during the interval between capture and recapture), it was determined that the two larvae were likely initially captured within reach 8 (26 d earlier).

OBJECTIVE 4. SALINITY MONITORING

Zone Boundary Monitoring

Qualitative comparisons of zone boundary continuous salinity logger data indicate an apparent salinity gradient occurred across the 900 m long study area; where higher salinities occurred with increasing distance downstream (Figure 3.6). At the upstream zone boundary, the salinity logger was corrupted and all data collected at this site prior to electrofishing were lost. The logger was redeployed at the site, and monitored salinity for

7 d after electrofishing. The upstream zone boundary was expected to be at (or above) the head of tidal influence. However, continuous salinity data collected at the upstream zone boundary show tidal cycle salinity exceeded 15 ppt (i.e., the upper threshold of survival during laboratory oscillating salinity experiments in Chapter 2) during 8 of 13 tidal cycles (62%) during the 7 d monitoring period (Figure 3.6). Thus, tidal influence extended at least into reach 6 in the upstream zone. At the middle zone boundary, tidal cycle salinity exceeded 15 ppt during 11 of 13 tidal cycles (86%), while at the downstream zone boundary, 13 of 13 tidal cycles (100%) exceeded 15 ppt during the 7 d monitoring period (Figure 3.6). Minimum tidal cycle salinity dropped below 0.1 ppt (i.e., was nearly completely fresh water) during 7 of 13 tidal cycles (54%) at the upstream zone boundary, and during 0 of 13 tidal cycles (0%) at both the middle zone and downstream zone boundaries (Figure 3.6).

Reach-Specific Monitoring

At reach-specific salinity monitoring locations in reaches 5 through 10, qualitative comparisons of continuous salinity logger data in general show higher salinities at the two most downstream reaches (9 and 10). But a clear gradient in salinity across the reaches where larvae occurred was not observed (Figure 3.7). These results are likely related to localized differences in freshwater stream flow and channel morphology among the sample reaches and logger locations. The salinity logger deployed in reach 5 was corrupted and all salinity data from this reach were lost. Reach-specific tidal cycle salinity exceeded 15 ppt during 14 of 23 tidal cycles (61%) in reach 6, 16 of 23 tidal cycles (70%) in reach 7, 13 of 23 tidal cycles (57%) in reach 8, 20 of 25 tidal cycles

(80%) in reach 9, and 13 of 25 tidal cycles (52%) in reach 10 during respective salinity monitoring periods (Figure 3.7). Minimum tidal cycle salinity at reach-specific monitoring locations dropped below 0.1 ppt (i.e., was nearly completely fresh water) during 12 of 23 tidal cycles (52%) in reach 6, 1 of 23 tidal cycles (4%) in reach 7, 2 of 23 tidal cycles (9%) in reach 8, 5 of 25 tidal cycles (20%) in reach 9 and 0 of 25 tidal cycles (0%) in reach 10.

Salinity Data Analysis

Tidal Cycle Minimum and Maximum

Absolute maximum tidal cycle salinity tended to increase with distance downstream in the study area, ranging from 0.14 ppt to 17.98 ppt in reach 6, to 18.40 to 21.44 ppt at the downstream zone boundary (Table 3.4). Similarly, absolute minimum salinity within a tidal cycle tended to increase with distance downstream in the study area, ranging from 0.06 ppt to 0.14 ppt in reach 6, to 1.99 ppt to 6.48 ppt at the downstream zone boundary (Table 3.4). Median maximum tidal cycle salinity tended to increase with distance downstream in the study area, and was significantly higher at the downstream boundary than all other locations (Kruskal-Wallis; $P < 0.05$; Table 3.4), but was similar to reach 9 (Kruskal-Wallis; $P > 0.05$; Table 3.4). Median maximum tidal cycle salinity was similar among all other sample reaches (Kruskal-Wallis; $P > 0.05$; Table 3.4). Median minimum tidal cycle salinity at each monitoring location also tended to increase with distance downstream in the study area, and was significantly lower in reach 6 (0.1 ppt), than the middle zone boundary (1.22 ppt), and the downstream zone boundary (3.5 ppt; Kruskal-Wallis; $P < 0.05$; Table 3.4). In reach 8 (0.21 ppt), reach 9

(0.35 ppt), and reach 10 (0.49 ppt), median minimum tidal cycle salinity was similar to all other salinity monitoring locations (Kruskal-Wallis; $P > 0.05$; Table 3.4).

Tidal Cycle Accumulated Salinity Units

During the 7 d prior to electrofishing, tidal cycle ASU at zone boundaries ranged from 0.3 ASU to 99.3 ASU at the upstream zone boundary, from 58.7 ASU to 131.6 ASU at the middle zone boundary, and from 154.8 ASU to 222.7 ASU at the downstream zone boundary (Figure 3.8). At reach-specific monitoring sites following electrofishing, tidal cycle ASU ranged from 1.04 ASU to 61.60 ASU within reach 6, from 1.51 ASU to 69.38 ASU within reach 7, from 0.99 ASU to 78.33 ASU within reach 8, from 0.57 ASU to 112.5 ASU within reach 9, and from 1.68 ASU to 109.10 ASU within reach 10 (Figure 3.9). Median tidal cycle ASU of reach 6 (47.92 ASU) was similar to reach 7 (56.35 ASU), reach 8 (42.09 ASU), and the upstream zone boundary (56.9 ASU; Kruskal-Wallis; $P > 0.05$; Figure 3.10), but was significantly lower than all other monitoring locations (Kruskal-Wallis; $P < 0.05$; Figure 3.10). No differences in median tidal cycle ASU were detected among reach 7 (56.35 ASU), reach 8 (42.09 ASU), reach 9 (80.09 ASU), reach 10 (88.38 ASU), and the upstream zone boundary (56.9 ASU; Kruskal-Wallis; $P > 0.05$; Figure 3.10), while reaches 9 and 10 were also not significantly different from the middle zone boundary (121.6 ASU; Kruskal-Wallis; $P > 0.05$; Figure 3.10). At the downstream boundary, median tidal cycle ASU (191.8 ASU) was significantly higher than all locations (Kruskal-Wallis; $P < 0.05$), but was similar to the middle zone boundary (121.6 ASU; Kruskal-Wallis; $P > 0.05$; Figure 3.10).

DISCUSSION

To date, few studies of larval lamprey occupancy and distribution in tidally-influenced habitats have been conducted. This may be due, in part, to larvae being regarded as stenohaline, obligate freshwater organisms (Hardisty 1956; Beamish 1980; Morris 1980) that are unable to osmoregulate in and show limited tolerance (i.e., survival) to hyperosmotic media (Mathers and Beamish 1974; Beamish et al. 1978; Richard and Beamish 1981; McVicar and Rankin 1983; Reis-Santos et al. 2008). Thus, larvae would not be expected to occur in estuarine habitats, and those that dispersed into estuaries are generally thought to perish (Potter 1980; Sjoberg 1980). However, stenohaline fishes may be tolerant to some low levels of salinity, and thus would have the potential to occur in certain estuarine areas. For example, stenohaline goldfish *Carassius auratus* have demonstrated normal growth and no signs of stress in experiments up to 6 ppt salinity (Luz et al. 2008). In the case of larval lampreys, previous experiments have demonstrated some tolerance (i.e., survival) of the larvae of several species to fixed salinities up to 10 ppt to 12 ppt (Mathers and Beamish 1974; Beamish et al. 1978; Richard and Beamish 1981; McVicar and Rankin 1983; Reis-Santos et al. 2008). In addition, laboratory experiments we conducted (discussed in Chapter 2) demonstrated high survival of larval Pacific lampreys in treatments where hyperosmotic salinity oscillated between freshwater (0 ppt) and 12 ppt (100% survival), or freshwater (0 ppt) and 15 ppt salinity (60% survival) at approximately 6 h intervals. Overall, these results suggested the potential for larval Pacific lamprey to occur in certain regions of tidal estuaries. However, occurrence and distribution of larval lampreys in tidally-influenced estuarine habitats has been largely unexplored. To better understand the potential for

larval Pacific lamprey and *Lampetra* spp. to occupy estuarine areas, we investigated occurrence of larval lampreys across a gradient of salinity in a tidally-influenced segment of a 3rd order coastal stream.

We detected larvae of both Pacific lamprey and *Lampetra* spp. within tidally-influenced habitats in which tidal cycle salinity generally exceeded 15 ppt to 18 ppt at least once daily during high tide. These results suggest larval lampreys are able to tolerate some exposure to hyperosmotic salinity in the natural environment and occur in certain areas of coastal river basin estuarine habitats. Other accounts of larval lampreys occurring in proximity to and entering tidal estuaries have been published (Richards 1980; Sjoberg 1980; White and Harvey 2003), but whether larvae entered areas with variable salinity was not known. Additional work in other basins is needed and would help put our results into a broader context, as our study was limited to a relatively small tidally-influenced segment of one coastal basin. As such the applicability of our observations to other basins across the range of Pacific lamprey is not known. Quantitative occupancy sampling in other river systems would further elucidate the potential value of tidally-influenced habitats in certain estuarine areas for larval lamprey rearing.

Estuarine habitats in large river systems, such as the Columbia River, may be particularly important to evaluate for larval lampreys for several reasons. River systems like the Columbia River that have significant freshwater inputs into their estuaries may have large areas of potentially suitable oligohaline habitats covering extensive geographic areas (Odum 1988; Norris et al. 2010). Our results suggest these habitats may be suitable for larval lamprey occurrence. In addition, many tributaries of the Columbia River and

lower Columbia support spawning of adult Pacific lamprey. The abundance of potential habitat in combination with multiple tributary sources of larvae in the basin makes the upper Columbia River estuary a potentially valuable larval rearing area. Quantitative sampling with deepwater electrofishing technology may be worthwhile to evaluate larval lamprey occupancy in these Columbia River estuary habitats. Information on larval occupancy and distribution in such areas may be useful to future conservation and management efforts of Pacific lamprey. In addition, knowledge of larval lamprey occurrence in estuarine habitats could be important to help minimize potentially adverse effects of human caused disturbances in these areas, including channel dredging, habitat loss and potential future climate change.

The duration of occurrence and the ultimate fate of larval lampreys that enter tidally-influenced estuarine habitats is an additional uncertainty that warrants further investigation. It is conceivable that larvae may benefit from the highly productive nature of estuarine habitats, as has been documented in other studies of freshwater fishes exploiting nutrient rich estuarine habitats (Odum et al. 1984). As such, tidally-influenced environments may benefit larval lamprey growth. However, larvae in these habitats would at risk from the harshness associated with variation in salinity (Deaton and Greenberg 1986; Peterson and Ross 1991), which in turn may adversely affect growth (Norris 2010). In Ellsworth Creek, the recapture of two larvae nearly 30 d after initial capture suggests potential for larvae to persist in estuarine habitats for extended periods. The two fin-clipped larvae were determined (through process of elimination according to TL) to have been initially captured (during occurrence sampling) and recaptured (during re-sampling) within the same 50 m sample reach (reach 8). This observation suggests the

potential for protracted residence in tidally-influenced habitats. Ultimately, the period of time larvae can survive in tidally-influenced habitats, and the fate of larvae in the study area of Ellsworth Creek are not known. Whether larvae that enter tidal environments are able to survive until metamorphosis and contribute to lamprey productivity within respective river systems requires additional study to determine.

Patterns of larval lamprey occurrence in Ellsworth Creek suggest salinity may have been the primary factor limiting downstream distribution of larvae in the creek. Salinity gradients across estuarine environments have been suggested to result in a continuum of physiological stress, and are regarded as the primary factor influencing distribution of fishes within estuarine habitats (Gunter 1956; Peterson and Ross 1991; Wagner and Austin 1999). Differences in species' tolerances to abiotic factors such as salinity act as a 'physiological sieve' resulting in longitudinal changes in distribution (Remmert 1983, as cited in Peterson and Ross 1991). A considerable gradient in salinity was observed across the 900 m study area, from always freshwater above reach 5, to generally always saline at the downstream boundary. In turn, an overall decrease in larval lamprey detections occurred moving downstream, through to the lowest site of larval lamprey capture (in reach 10). Downstream of reach 10, no larval lampreys were detected in the two reaches sampled (reach 11 and reach 12), despite the presence of suitable burrowing substrates. I was unable to sample the lower reaches of the study area (due to high conductivity) and cannot confirm the absence of larvae, however salinity data collected at the downstream zone boundary suggest occurrence of larvae downstream of that point would be unlikely. What aspect of tidal cycle salinity (i.e., magnitude of high, magnitude of low, duration, etc.) exerts the greatest limiting effect on

larval occurrence is not known. However, the duration of tidal cycles exceeding the iso-osmotic point of larval fluids may drive tolerance limits and ultimately patterns of larval lamprey occurrence.

In conclusion, larval lampreys were found to occur in temporal and spatial association with habitats that frequently experienced hyperosmotic peaks in salinity. In Ellsworth Creek, reach-specific salinity data indicated larval lampreys are able to tolerate some exposure to salinity in the natural environment and may occur in certain areas of estuaries and coastal basins. These results provide evidence to suggest larvae to occur in some tidally-influenced habitats. However, additional studies to further address questions of residence time, long term survival, and occurrence of larvae in tidally-influenced habitats of other stream and river basins would help further resolve the potential importance of these habitats for larval lamprey rearing. As such, the relative importance of tidally-influenced estuarine habitats and whether larval rearing in these areas is a significant contributor to lamprey productivity in a particular basin remains unknown.

TABLES

Reach	Sampled	# Pacific lamprey	# <i>Lampetra</i> spp.	Salinity (ppt)	Conductivity (μ S/cm)	Temperature (°C)
1	Yes	0	22	0	36.1	14.1
2	Yes	4	11	0	38.3	15.3
3	Yes	4	9	0	38.3	15.4
4	Yes	2	14	0	38.3	15.7
5	Yes	3	9	0.2	571	15.4
6	Yes	3	9	0.3	564	15.5
7	Yes	1	15	0.7	1,199	15.3
8	Yes	0	5	0.6	1,050	15.5
9	Yes	0	3	1.1	1,681	15.3
10	Yes	0	2	1.8	2,813	15.5
11	Yes	0	0	2.2	3,480	15.9
12	Yes	0	0	3	4,500	15.6
13	No	NA	NA	NA	NA	NA
14	No	NA	NA	NA	NA	NA
15	No	NA	NA	NA	NA	NA
16	No	NA	NA	NA	NA	NA
17	No	NA	NA	NA	NA	NA
18	No	NA	NA	6.7	9,800	16.8

Table 3.1. Results of larval lamprey electrofishing in the 18, 50 m reaches within the study area in Ellsworth Creek. Water salinity, conductivity, and temperature measurements were made at the beginning of each sample reach prior to electrofishing. The reaches within the box were tidally-influenced reaches in which larval lamprey were detected.

	Pacific lamprey		<i>Lampetra</i> spp.	
Reach	TL (mm)	Weight (g)	TL (mm)	Weight (g)
1	0	0	79 - 160	0.9 - 7.6
2	95 - 113	1.4 - 2.2	97 - 195	1.8 - 11.2
3	95 - 122	1.6 - 3.4	63 - 139	0.9 - 4.4
4	107 - 141	1.7 - 4.2	77 - 157	0.8 - 5.6
5	90 - 115	1.4 - 2.4	70 - 167	0.8 - 7
6	85 - 102	1-1.9	68 - 142	0.4 - 5.1
7	84	1	53 - 170	0.3 - 7.5
8	NA	NA	53 - 165	0.3 - 8.9
9	NA	NA	49 - 69	0.3 - 0.7
10	NA	NA	45 - 75	0.3 - 0.8

Table 3.2. Length and weight ranges of larval lampreys collected in each sample reach in Ellsworth Creek. The yellow shaded area denotes tidally-influenced reaches in which larval lampreys were collected.

	Reach 6	Reach 7	Reach 8
	Re-Sampling	Re-Sampling	Re-Sampling
# Pacific lamprey	1	0	0
# <i>Lampetra</i> spp.	6	3	2
Salinity (ppt)	0.1	0.2	0.4
Conductivity (μS/cm)	232	393	750
Temperature (°C)	14.7	14.7	14.6

Table 3.3. Results of reach re-sampling following salinity monitoring in Ellsworth Creek. Salinity was logged in each reach for up to 14 d between first and second electrofishing passes. Water temperature, salinity, and conductivity measurements were made at each habitat unit prior to electrofishing. In reach 8, the two larval *Lampetra* spp. collected had clipped caudal fins indicating they had been collected during the first electrofishing pass prior to salinity monitoring.

Location	Absolute Minimum (ppt)	Absolute Maximum (ppt)	Median Minimum (ppt)	Median Maximum (ppt)
Reach 6	0.06 - 0.14	0.14 - 17.98	0.10 ^a	16.86 ^w
Upstream Zone Boundary	0.01 - 0.20	0.04 - 19.20	0.10 ^{a,b}	16.59 ^{w,y}
Reach 7	0.09 - 0.25	0.21 - 17.68	0.15 ^{a,b}	16.70 ^w
Reach 8	0.01 - 0.27	0.18 - 17.98	0.21 ^{a,b,c}	16.48 ^w
Reach 9	0.07- 0.97	0.10 - 20.26	0.35 ^{a,b,c}	18.04 ^{y,z}
Reach 10	0.17 - 1.28	0.27 - 17.88	0.49 ^{a,b,c}	15.24 ^{w,x}
Middle Zone Boundary	0.36 - 2.38	13.92 - 17.74	1.22 ^{b,c}	17.09 ^{x,y}
Downstream Zone Boundary	1.99 - 6.48	18.40 - 21.44	3.54 ^c	20.66 ^z

Table 3.4. The range in tidal cycle absolute minimum and absolute maximum salinity documented at each salinity monitoring location. Locations in the table are ordered from the most upstream location at the top (reach 6) to the most downstream location at the bottom (downstream zone boundary). Median values with similar letters were not significantly different (Kruskal-Wallis; $P > 0.05$).

FIGURES

Figure 3.1. Ellsworth Creek (Pacific County, WA) is a 3rd order stream located in Southwest Washington State. Ellsworth Creek flows into the Naselle River estuary,

which enters Willapa Bay about 6 km downstream of the confluence with Ellsworth Creek.

Figure 3.2. Salinity data logged near rkm 2 in Ellsworth Creek (blue) showed tidal oscillations that were generally similar in magnitude and duration to laboratory (96 h, 12 ppt) simulated tidal salinity oscillation experiments (black).

Figure 3.3. Downstream-facing view of the study area in Ellsworth Creek, which was comprised of three, 300 m long sample zones. Each sample zone was divided into six contiguous 50 m long reaches, numbered sequentially from upstream to downstream across the study area. Numbered balloons denote the upstream boundary of each reach. White circles (labeled L) denote the locations of salinity logger deployment at zone boundaries. The upstream zone (shaded blue) was entirely within forested stream habitat, the middle zone (shaded green) was essentially bisected by the forest/tidal marsh boundary, while the downstream zone (shaded red) was entirely within tidal marsh habitat. Salinity conditions ranged

from always freshwater at the upstream boundary of the study area to generally always saline at the downstream boundary.

Figure 3.4. Locations of reach-specific salinity monitoring conducted tidally-influenced reaches in Ellsworth Creek in which larval lampreys were captured by electrofishing. Numbered balloons denote the upstream boundary of each reach. White circles (labeled L) denote the locations of salinity logger deployment in each reach. Upstream of reach 5, water was always fresh and larval lampreys were found in every reach. Downstream of reach 10, no larvae were collected in reaches 11 and 12. Below reach 12, water salinity and conductivity exceeding the operating range of the electrofisher and we were not able to sample for larvae.

Figure 3.5. To determine minimum and maximum tidal cycle salinity at each location, we first partitioned continuous salinity data (top left figure) into complete tidal cycles from low tide to low tide. In this example, reach-specific salinity data collected over 13 d in reach 10 was divided into 25 unique tidal cycles (top right figure). Tidal cycle curves were then used to calculate the accumulated salinity units (ASU) of each individual tidal cycle by integrating the area under the salinity curve (AUC) of each cycle. Here the AUC of tidal cycle one (black curve in top right figure) is integrated to calculate tidal cycle ASU (bottom left figure). Integration of each unique tidal cycle yielded a population of tidal cycle ASU values (bottom right), from which statistical comparisons of median tidal cycle ASU among locations could be made.

Figure 3.6. Continuous salinity data collected over 168 h (7 d) prior to electrofishing Ellsworth Creek at the middle zone boundary (top right figure), and the downstream zone boundary (bottom left figure). At the upstream zone boundary (top left figure) the salinity logger was corrupted and all data were lost. The logger was redeployed and collected data at the upstream boundary for 7 d following electrofishing as shown.

Figure 3.7. Reach-specific continuous salinity data collected in tidally-influenced reaches in Ellsworth Creek where larval lampreys were detected through electrofishing. Salinity data was collected for 12 to 13 d following reach electrofishing. Figures are in order of occurrence in the creek, with reach 6 being the most upstream reach, while reach 10 was the furthest downstream. In general, salinity increased with increasing distance downstream.

Figure 3.8. Tidal cycle accumulated salinity units (ASU) calculated from continuous salinity data collected at the three zone boundary sites in Ellsworth Creek. Individual tidal cycles were partitioned from continuous salinity data as described previously (Figure 5) and ASU values were calculated by integrating the area under the salinity curve of each tidal cycle. Thirteen tidal cycles occurred at the zone boundary locations during the 7 d prior to electrofishing.

Figure 3.9. Tidal cycle accumulated salinity units (ASU) calculated from continuous salinity data collected at the five reach-specific monitoring locations in Ellsworth Creek. Individual tidal cycles were partitioned from continuous salinity data as described previously (Figure 5) and ASU values were calculated by integrating the area under the salinity curve of each tidal cycle. Twenty three to 25 tidal cycles occurred at reach-specific monitoring sites during the 12 d to 13 d following electrofishing.

Figure 3.10. From the population of tidal cycle-specific ASU values at each salinity monitoring location (Figure 8 and Figure 9) median tidal cycle-specific ASU was calculated and compared among the locations. Locations plotted on the X-axis are ordered from furthest upstream in the creek on the left, to furthest downstream on the right. The longitudinal distance separating each consecutive location was less than 50 m except between reach 10 and the middle zone boundary (100 m), and the middle zone boundary and downstream zone boundary (300 m). Median tidal cycle ASU values with similar letters were not significantly different. Box and whisker plot shows maximum and minimum tidal cycle ASU values (whiskers), median (mid-line), mean (+), and interquartile range (box).

CHAPTER 4

SUMMARY, CONCLUSIONS, AND MANAGEMENT IMPLICATIONS

The fundamental aim of this thesis was to further the understanding of larval Pacific lamprey biology, ecology, and distribution in the Pacific Northwest. Here we explored the potential for larval lampreys to occur in tidally-influenced habitats at the downstream end of river basins where freshwater and tidal saline waters interface. Larval Pacific lampreys are osmoregulators adapted for life in freshwater. Salinity tolerances of primarily freshwater fishes such as larval lampreys are known to limit distribution into tidally-influenced habitats (Deaton and Greenberg 1986; Wagner 1999; Whitfield 2015). Thus occurrence of larvae in such habitats is likely to be limited (Hardisty 1956; Morris 1972; Richards and Beamish 1981), but has been largely unexplored. Because these habitats occur over a significant area of tidal estuaries of large rivers (such as the Columbia River, or the Sacramento-San Joaquin Rivers), and given the likely abundance of suitable larval burrowing habitats, there is potential for these areas to contain large numbers of larval lampreys.

In the first phase of the approach we employed to address our aims, laboratory experiments demonstrated tolerance of larval Pacific lamprey to fixed salinity exposure decreased sharply once concentrations of the external environment exceeded that of their internal milieu. Tidal simulation experiments in which hyperosmotic 12 ppt or 15 ppt salinity oscillated between freshwater (0 ppt) at about 6 h intervals resulted in increases in larval survival versus fixed salinity experiments through 96 h. Evaluations of osmoregulatory capacity demonstrated that, in general, larvae were unable to

osmoregulate in hyperosmotic solutions. Concomitant declines in total body water and increases in plasma osmolality and plasma sodium ion concentration were observed in larvae held in various hypertonic solutions. These results indicate larvae that survived in these treatments were tolerant to some levels of hyperosmotic salinity, but were not able to osmoregulate. These results suggested the potential for larval Pacific lampreys to occur in certain areas of tidally-influenced environments, and were subsequently used, in part, to guide design and execution of sampling for larvae in Ellsworth Creek, a tributary of the Naselle River.

Investigations of larval lamprey occurrence across a gradient of salinity (from freshwater to mostly marine) in a tidally-influenced segment of Ellsworth Creek indicated larval Pacific lamprey and *Lampetra* spp. co-occurred in some tidally-influenced habitats. Salinity data logged in tidally-influenced reaches where larvae were detected (for up to 14 d following sampling) indicated maximum tidal cycle salinity exceeded 15 ppt during 52% to 80% of tidal cycles. Overall, field sampling results generally corroborate findings of laboratory experiments. In variable salinity exposures in the lab and the field, where maximum tidal cycle salinity of approximately 15 ppt alternated with periods of freshwater (0 ppt) at low tide, larval Pacific lampreys were found to survive and occur. Together, laboratory and field investigations may provide evidence of potential for larvae to occur in some tidally-influenced habitats of coastal stream and river basins.

The findings here may have implications for conservation and management of Pacific lamprey in the Pacific Northwest. Given recent declines in Pacific lamprey

observed, for example in the Columbia River basin, understanding and minimizing the effects human caused disturbances on larval lampreys are generally becoming an increasing priority. In estuarine areas, many potential threats to larval lamprey exist. In particular, activities such as channel dredging and gravel mining, which frequently occur in estuarine areas and coastal basins (Luzier et al. 2011), have direct impacts on river bottom substrates and potentially affect benthic dwelling fauna such as larval lampreys. In the Columbia River estuary, for example, annual dredging since 1976 has averaged the removal of 3.5 million cubic yards of sediment per year (NPPC 2004). The overall extent of impacts of channel and suction dredging activities on larval Pacific lamprey populations are currently not known, but warrants further investigation. Over time, these activities have the potential to negatively impact Pacific lamprey populations in some river basins.

The proximity of many tidally-influenced estuarine habitats to urban areas leads to the potential for a number of human caused environmental disturbances to occur with adverse consequences to larval lampreys, including habitat loss and reduced water quality. For example, urban, industrial, and agricultural practices may result in increased sedimentation, point source and non-point source pollution, significant heavy metal inputs, and hyper-eutrophication of estuarine habitats (Odum 1984). Habitat loss, including diking, draining, and filling of tidally-influenced habitats have been widespread in some areas for over a century and continue today, which may have damaging consequences to aquatic fauna in these habitats (Odum 1984). Many of these factors have previously been attributed to declines in Pacific lamprey abundance in more

upstream areas in river basins. These environmental disturbances would likely have adverse effects on larval lampreys that occur in nearby estuarine habitats.

Modifications to physical habitat may also have significant impacts on estuarine processes. In the Columbia River estuary, for example, altered bathymetry due primarily to development of a deep shipping channel has greatly affected salinity intrusion patterns (NPCC 2004). Distribution of larval lampreys in the mainstem may thus be adversely affected by flow manipulations. Alterations to natural hydrographs, due to freshwater diversions, irrigation withdrawals, and hydropower development have, in some cases, resulted in increased penetration of tidal influence, and higher salinities observed moving further upstream in river systems (Odum 1984). Given the extensive manipulation of the hydrograph in the Columbia River due to hydroelectric power production, water storage, and flood control, upstream penetration of the saltwater wedge is likely to exceed historic levels. Higher salinities in habitats that were historically freshwater tidal or oligohaline in nature are likely as a result, limiting larval lamprey occurrence in many areas. Potential future climate change scenarios may also result in higher upstream salinity penetration into coastal river basins. Sea level rise, as well as frequent and severe droughts, may all contribute to higher salinity penetration into coastal river estuaries. This would again would likely alter fish assemblages across estuarine habitats, and reduce the quantity of habitat available for primarily freshwater species like larval lampreys.

A better understanding of the extent of larval lamprey distribution in estuarine habitats may prove useful to various governmental and local agencies that are involved

with estuarine habitat restoration projects. Salmonid habitat restoration projects, for example, are frequently located in tidally-influenced estuarine habitats in coastal river basins. Given the increasing priority of lamprey conservation and their potential to occur in such habitats, incorporating features beneficial to larval lampreys in future restoration areas may be a worthwhile consideration. In addition, occurrence of larval lampreys in tidally-influenced habitats would indicate salvage operations for larval lampreys may also be required. Removal of larvae prior to dewatering, dredging, or other habitat alterations associated with restoration projects may help avoid unintentional harm to burrowed larvae which may frequently be overlooked. Following the recommendations outlined in the USFWS Best Management Practices document report may aid in minimizing unintended adverse effects to larval Pacific lamprey during habitat restoration activities (USFWS 2010).

In conclusion, the results of our work may provide information useful in the management and conservation of Pacific lampreys. Given the widespread occurrence of tidal environments at the lower end of coastal basins, as well as the large size of these habitats in major river systems throughout the range of Pacific lamprey, these habitats may serve a valuable role in larval lamprey life history in some systems. Additional research to assess the value of these habitats for larval lamprey rearing would further elucidate their range-wide importance for larval Pacific lampreys, while further addressing research needs outlined in the USFWS Pacific lamprey conservation agreement on habitat requirements, and basic biological and ecological information on larval Pacific lamprey (Wang and Schaller 2015). Until further research is conducted, the

magnitude of impacts from human disturbance and alterations of these habitats on larval Pacific lamprey remains uncertain.

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