Pacific lamprey, *Entosphenus tridentatus*, have shown recent and rapid declines in abundance. These anadromous fish return to streams where they mature, spawn and die. It has been inferred that Pacific lamprey enter freshwater and reside for ~ 1 year before spawning. This long exposure to the freshwater environment may affect the plasticity of the maturation process and the migration timing of Pacific lamprey. Diversity in run times and body size has been observed for Pacific lamprey, yet it is unknown if this diversity is induced by the freshwater environment or if it is genetic. My first goal was to describe the maturation and migration characteristics of adult Pacific lamprey during their freshwater migration. My second goal was to use these data to make an estimation of the run diversity in Pacific lamprey. I conducted three complementary studies, in the laboratory and the field, to achieve these goals.
I held immature adult lamprey (non-ripe fish that had ceased parasitic feeding in the ocean and had returned to freshwater) in the laboratory at temperatures that mimicked what these fish would experience in the wild, during the summer (mean: 21.8 °C), and another group of lamprey at cooler temperature (mean: 13.6 °C) to compare maturation timing and characteristics. The warm water group of lamprey showed significantly greater proportional decreases in body mass following temperature exposure than fish in the cooler water. All fish exposed to the warm water matured the following spring (8-10 months later) whereas only about half of the fish from the cool water exposure matured.

To understand the migration distances and timing of adult Pacific lamprey, I tracked radio-tagged fish throughout the Willamette Basin above Willamette Falls, Oregon, by airplane and recorded their location. Fish migrated primarily during the spring to early summer period before stopping during the remainder of summer, when peak river temperatures (≥ 20°C) occurred. These fish tended to remain stationary through the fall and winter. However, at least a few fish continued to migrate upstream after September.

I monitored maturation characteristics of adult Pacific lamprey, over time at Willamette Falls, Oregon and compared these fish with recent migrants collected from the Pacific Ocean as they entered freshwater. The results suggest a unimodal spawn timing between April and June, at water temperatures < 20°C. Between July and mid-September, as water temperatures peaked at ~ 25 °C, relatively immature fish for both sexes prevailed. Warm summer temperatures coincided with an increase and prevalence of testicular atrophy in males, and I also observed a large die-off of lamprey during this
time. The immature fish had maturation stages and phenotypic characteristics similar to recent migrants collected at the mouth of the Klamath River, suggesting that the immature fish at Willamette Falls would spawn the following year, and spawners in any given year may have been recent migrants during the previous year. However there is a temporal overlap in the spring of immature and mature fish, and I found evidence from gonad histology of maturing fish as they entered the river from the ocean, suggesting that a cohort is comprised of recent migrants that spawn within several weeks of entering freshwater, and another cohort is comprised of recent migrants that mature and spawn at least 1 year later. I hypothesize that the recent migrants that would likely spawn shortly after entering freshwater are akin to a winter or “ocean maturing” steelhead, *Oncorhynchus mykiss*, that optimizes feeding and growth in the open ocean for a few years before entering freshwater to spawn low in the river system shortly afterwards. Alternatively, these lamprey may be similar to coastal cutthroat trout, *O. clarki clarki*, that feed and grow in the coastal areas of the ocean for a few months before entering freshwater to spawn. There could be other less apparent explanations as well. I also hypothesize that the lamprey that would likely spawn within ~ 1 year of entering freshwater are akin to a “stream maturing” steelhead that foregoes feeding and growth opportunities, enters freshwater during the summer – fall, and accesses spawning grounds to spawn at temperatures that promote evolutionary fitness via successful spawning the following spring.

Based on the results of my research, I hypothesize that warm summer temperatures (> 20 °C) can act as a strong selection factor against stream maturing Pacific
lamprey in two ways. First, these temperatures may expedite their maturation, while at the same time slowing their migration. If these hypotheses are true, then I predict an uncoupling of spawn timing with optimal habitat characteristics, that would promote fitness, in the upper watershed. Second, summer temperatures may cause gonad atrophy and death prior to spawning. This scenario may select for ocean maturing Pacific lamprey.
The Physiological Ecology and Run Diversity of Adult Pacific Lamprey, *Entosphenus tridentatus*, During the Freshwater Spawning Migration

by

Benjamin J. Clemens

A DISSERTATION

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degree of

Doctor of Philosophy

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APPROVED:

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Major Professor, representing Fisheries Science

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Head of the Department of Fisheries and Wildlife

___________________________________________________
Dean of the Graduate School

I understand that my dissertation will become part of the permanent collection of Oregon State University libraries. My signature below authorizes release of my dissertation to any reader upon request.

___________________________________________________
Benjamin J. Clemens, Author
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I wish to express my gratitude to my family for their patience, love and support. Courtney, thank you for your encouragement and love. I still remember back in 1999 when you suggested I earn a Ph.D. I love you and Lucas. Graduate school has been one roller coaster ride….

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Appendix A: Stacia Sower and Carl Schreck assisted with interpretation of gonad stages and intersex.

Appendix B: Carl Schreck assisted with the sample design, protocol, data acquisition and interpretation.
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DEDICATION

To the late Bernard Lee Miller, and to Lucas Asher Clemens, my father-in-law and my F1. I love you both....
The Physiological Ecology and Run Diversity of Adult Pacific Lamprey, *Entosphenus tridentatus*, During the Freshwater Spawning Migration

**General Introduction**

PERSISTENCE THROUGH GEOLOGIC TIME: STABLE CHARACTERS, COMPLEX AND DIVERSE BIOLOGY

Lampreys, Petromyzontiformes, are some of the oldest vertebrates on earth, and they have changed very little since they appeared in the fossil record over ~300-500 million years ago (Dawkins 2004; Janvier 2008; Helfman et al. 2009). This morphological similarity between extant and extinct lampreys is why Janvier (2008) calls lampreys “living fossils”: “…because they have gained some highly derived characters that remained extremely stable throughout time”.

Despite or perhaps because of this morphological stability, lampreys exhibit complexity and diversity of life histories, including variability in larval periods among species (Potter 1980), diverse trophic ecology as adults (non-feeding vs. different modes and extents of parasitization), and a wide range of body sizes, fecundities, and migration strategies. Lampreys range from very small (~100 mm in total length), low fecundity, non-feeding, resident adults to very large (> 800 mm), high fecundity, parasitic feeding, anadromous adults that return to freshwater to spawn (Beamish 1987; Gill et al. 2003; Salewski 2003; Docker 2005; Clemens et al. 2010), with variations on these biological themes (e.g., see Beamish 1987, Lorion et al. 2000, and Docker 2009).
Lampreys also exhibit tremendous diversity in the endocrinology of the reproductive, osmoregulation, and metamorphic axes. This diversity in physiology can explain the distribution of freshwater resident and anadromous forms, which have arisen from a marine ancestor (Youson and Beamish 1991; Youson and Sower 2001). Finally, lampreys show incredible metabolic adaptations to prolonged fasting in freshwater (Larsen 1980; Whyte et al. 1993). For example, Pacific lamprey, *Entosphenus tridentatus*, can live up to ~3 years in freshwater without feeding in the laboratory (van de Wetering and Schreck, unpubl. data). This complexity and diversity in the behavior, ecology, and physiology of lampreys may explain why they have persisted for so long.

**BIOLOGY**

There are approximately 38 species of lampreys, 18 of which are parasitic (Gill et al. 2003). One of these parasitic lampreys, the Pacific lamprey, *Entosphenus tridentatus*, is a relatively large, parasitic, anadromous fish that is found throughout river drainages that have access to the Pacific Ocean, from Baja California to Alaska and Japan. Pacific lamprey reside as burrowing, filter-feeding larvae in soft, fluvial substrates for approximately 3-8 years before undergoing a metamorphic transition into parasitic macrophthalmia juveniles that outmigrate to the ocean. Marine phase Pacific lamprey spend approximately 3.5 years in the ocean, where they feed as external parasites on teleost fishes and even whales before returning to streams to spawn and then die. Pacific lamprey cease their parasitic lifestyle in the ocean, return to freshwater during the spring (April – June), and begin their initial upstream migration during the summer (July –
September) before their pre-spawning holding during October – March. Pacific lamprey then mature, spawn, and die during April — July, approximately 1 year after having entered freshwater. This spawning period, however, is contingent upon temperature, photoperiod, and likely also with the migration distance to spawning grounds. The spawning migration and spawning vary with latitude, being earlier in California than in the time periods noted above, which tends to hold for British Columbia and Oregon (reviewed in Clemens et al. 2010). Their prolonged freshwater residency, along with their capacity for extensive migration distances ($\leq 700$ km) and incredible climbing abilities, compared to Great Lakes and anadromous sea lamprey, Petromyzon marinus (Clemens et al. 2010), raises many questions about the maturation characteristics and run diversity of the enigmatic Pacific lamprey.

**IMPORTANCE**

Pacific lamprey is an important and valuable species to the ecology of the Pacific Northwest and to the Native American Tribes that use this fishery for food, medicine, and ceremony (Close et al. 2002; CRITFC 2008). Key ecological associations of Pacific lamprey include the potential inter-relatedness of declines among Pacific salmon (Oncorhynchus spp.) and Pacific lamprey; predation buffers for salmonids, and nutrient addition to, and cycling within, watersheds (Close et al. 2002; Moyle et al. 2009; Petersen Lewis 2009).

Pacific lamprey may act as predation buffers to outmigrating salmonids (Close et al. 2002) and returning adult salmonids (Close et al. 1995). The energy-rich body
composition of adult Pacific lamprey (range = 5.92-6.34 kcal/g wet weight; Whyte et al. 1993) relative to salmon (average = 1.26-2.87 kcal/g wet weight; Stewart et al. 1983) may render them more susceptible to predation from pinnipeds (Roff and Mate 1984). In addition to acting as potential predation buffers for salmonids, the energy-rich Pacific lamprey carcasses likely also means that their carcasses may provide a substantial source of marine-derived nutrients to the watersheds in which they spawn, something that has long been recognized in salmon, but often overlooked with regards to lamprey. Finally, filter-feeding juvenile lamprey cycle nutrients within watersheds (Kan 1975; see also brief review in Close et al. 2002), between the water column and the benthos.

**POPULATION DECLINES**

Despite the persistence of lampreys through geologic time, lampreys worldwide have been undergoing severe declines. Lamprey populations in the northern hemisphere are imperiled, and river habitat degradation and barriers to spawning sites have been implicated (Renaud 1997). In North America, 10 of the 20 lamprey species are imperiled, likely from the same causes (Jelks et al. 2008).

Pacific lamprey abundance has declined significantly over the last 50 years in the Pacific Northwest of North America (PNW). This decline has been attributed to the aforementioned problems with lamprey populations in the northern hemisphere: declining habitat quantity and quality, and barriers to upstream passage for spawning (Close et al. 1995; CRITFC 2008; Cochnauer and Claire 2009; Moyle et al. 2009) and barriers to downstream migration of larvae and juveniles (CRITFC 2008).
Native American tribes that use Pacific lamprey as a food, medicine and ceremonial and cultural resource have expressed great concern about the persistence of these fish (Close et al. 2002; CRITFC 2008; Petersen Lewis 2009). The state of Oregon, USA, has listed Pacific lamprey as a ‘sensitive’ species at risk of extinction (Kostow 2002; ODFW 2006). In 2003, a petition to list the Pacific lamprey as threatened or endangered under the Endangered Species Act was considered by the U. S. Fish and Wildlife Service (USFWS), which concluded that insufficient evidence on biology, ecology, habitat needs and specific threats was available to list this fish (USFWS 2004).

Populations of Pacific lamprey at Willamette Falls are some of the largest in the Pacific Northwest, and Willamette Falls is a pre-eminent harvest location for Native Americans in this region (Kostow 2002). Estimated harvest from the falls was ~94,000 kg in 1943 and 4,000 in 1979 (Ward 2001). Despite this apparent drop in the numbers of fish harvested, Pacific lamprey were still characterized as highly abundant in the Willamette River Basin as recently as 35 years ago (Huff et al. 1976). This time span is about equal to a mere 3 – 4 generations of Pacific lamprey (assuming ammocoetes for 3-8 years + ~3.5 years in the ocean + ~1 year as returning adults in freshwater [Clemens et al. 2010]). In the last couple of years, tribes have not been able to meet their harvest needs as easily as they have historically.

The number of Pacific lamprey in the Columbia River also appears to have declined greatly: total annual abundance of lamprey passing Bonneville Dam ranged from ~225,000 adults in 1938 to only ~25,000 adults in 2005 (Cochnauer and Claire 2009). More recently researchers at Bonneville Dam have experienced difficulty getting
enough fish to study. These shocking declines of Pacific lamprey in the Columbia River underscore the importance of Willamette River populations, which could act as a seed for the Columbia.

DIVERSITY
Several studies suggest that parasitic Pacific lamprey cannot thrive in freshwater, yet populations of freshwater-resident Pacific lamprey may exist. The lineage and taxonomy of five species of *Entosphenus* in freshwater deserves further study, as Pacific lamprey may speciate rapidly in fresh water (reviewed in Clemens et al. 2010). In his review of other studies, Peter Moyle noted, “*It is possible that Pacific lampreys within one stream system have more than one run….***” (Moyle 2002).

Two “runs” of adult Pacific lamprey have been observed in some California rivers (Moyle et al. 2009), and a similar observation has been made in Cedar Creek, which enters the lower Columbia River just below the Willamette River. Two distinct pulses of returning adult *E. tridentatus* were observed over several years of monitoring: 1) an early pulse, occurring April — July and 2) a late pulse, occurring late August — November (Stone et al. 2001; Stone et al. 2002; Lê et al. 2004; Luzier and Silver 2005). It is not known whether these “runs” or “pulses” of Pacific lamprey represent “…*a spring run that spawns immediately after the upstream migration and a fall run, which holds over and spawns the following spring***” (Moyle 2002) or a spring run that was last years migrants and this years spawners and a fall run that would spawn the following spring.
Native Americans from the Confederated Tribes of the Umatilla Indian Reservation noted a single migration lasting from spring through fall, of which two morphotypes of Pacific lamprey were readily observed in the Umatilla River of northeast Oregon: 1) ‘short, brown eels’ called ‘day eels’, and 2) ‘long, dark eels’ called ‘night eels’ (Close et al. 2004). Close et al. (2004) speculated that the day eels were animals that had overwintered and possibly spawned, whereas the night eels may have been recent migrants from the ocean; however, they were unable to rule out the possibility of two different life history types. Moyle (2002) also noted, “In the Trinity River (northern California), for example, there may be two distinct forms of Pacific lamprey, one smaller and paler than the other, that represent either separate runs or resident versus migratory individuals.”

GENETICS AND MORPHOLOGY: IS THERE STOCK STRUCTURE?

In addition to the observations on the differences in run timing, general body size and color, the nature of the diversity within Pacific lamprey has been assessed on a broad geographical scale by genetics and by observations on body length. From a genetic standpoint, and across a very wide geographical scale, there is conflicting evidence for population structure. This suggests either that population structure does not exist or that there is some form of weak population structuring (reviewed in Clemens et al. 2010). Differences in adult body size exist across and within lamprey species, and body size typically correlates directly with migration distance (Potter 1980). For example, coastal runs of Pacific lamprey have smaller body sizes than Pacific lamprey that migrate up the
Columbia and Snake Rivers to eastern Washington and Idaho (reviewed in Clemens et al. 2010). It is not known whether or how these differences in body size of Pacific lamprey relate to the small, pale, ‘day’ eel or the large, dark, ‘night’ eel. It is also not known if these differences in body size represent a genetic correlate of locally-adapted populations. An alternative hypothesis to the apparent adaptation of body size to migration distance in streams and rivers is phenotypic plasticity determined by differences in the energy density of the prey that the Pacific lamprey feed on, with resultant growth rates of the lamprey. Two obvious limitations of these studies include: 1) differences in the genetic analyses and sampling methods, and 2) the lack of an attempt to correlate genetic analyses with body morphology. As a result, geography has not been controlled for, and therefore the biology of run characteristics has not been examined at a given location, over the course of time.

PROBLEM

Pacific lamprey was first collected and described from the Willamette Falls area (Richardson 1836). In the 175 years since they were described as a species, much remains unknown about the basic biology and ecology and run diversity of Pacific lamprey, and this information will be essential for informing management, conservation and tribal restoration initiatives of these fish (USFWS 2004; CRITFC 2008; Luzier et al. 2009; Clemens et al. 2010).

RESEARCH GOALS AND OBJECTIVES
The primary goal of this research is to describe the physiological ecology of adult Pacific lamprey during their freshwater residency at Willamette Falls, Oregon. This research will provide information on the physiological ecology (maturation, reproductive biology, proximate analyses), and basic biology (morphology, run timing, sex ratios, fecundity) of Pacific lamprey in relation to their environment. A secondary goal is to use multivariate analyses to explore the data for the presence of character modes with respect to sample date and thermal and flow regimes for evidence of different life histories or run times. The holistic approach presented in this proposal will fill information gaps in the biology of *E. tridentatus* and thus inform imminent conservation and management decisions.

I have five research objectives that I will elaborate in the ensuing paragraphs:

1) conduct a controlled laboratory test to investigate the maturation timing and biology of adult Pacific lamprey in freshwater, in relation to thermal regimes (Chapter 2);

2) to explore and describe the migration biology of radio-tagged adult lamprey in their natural environment, in the Willamette Basin (Chapter 3) in relation to date, discharge, and temperature;

3) to monitor the morphology and physiology of adult lamprey over time at Willamette Falls in comparison with fresh migrants taken from the sea/freshwater interface (at the Klamath River mouth) to describe the maturation timing and characteristics of adults (Chapter 4);

4) to use the data acquired from monitoring lamprey at Willamette Falls to discern whether there is evidence for diversity of maturation times and associated characteristics and interpret that aspect of biological diversity (Chapter 4); and
5) to formulate a predictive model for reproductive maturation and spawning of Pacific lamprey in relation to the thermal and flow regimes of the Willamette River (Chapter 5: Conclusions).

In addition to the studies presented in this dissertation, I have included two appendices that have arisen from the monitoring study presented in Chapter 4. The first appendix (Appendix A) involves some interesting observations of gonads that describe the occurrence of Pacific lamprey that are male intersex. The second appendix (Appendix B) is a test of the validity of the methods used to sample a reproductive hormone from the blood plasma of Pacific lamprey.

In Chapter 2, I present the manipulative, controlled research that I conducted on adult Pacific lamprey at the Fish Performance and Genetics Laboratory at Oregon State University. I examine the effects of relatively warm, summer water temperatures (>20 °C) on the maturation status (mature = spermiating or ovulating), maturation timing, survival rates and maturation characteristics (body size and qualitative measures of morphology) of Pacific lamprey over time (immediately after treatment and 10 months later) to ascertain whether there is evidence for a cause-and-effect relationship between body size and maturation timing. Because high temperatures increase metabolism and activity in lampreys (Johansen et al. 1973; Lewis 1980) as in other fishes, I predicted that shrinkage in body size (length and mass) during freshwater residency would be significantly increased at relatively warm water temperatures experienced during the summer. I also predicted that if summer temperatures led to significant decreases in body size of Pacific lamprey, then a higher percentage of fish would mature early in
comparison with lamprey held at cooler water temperatures. I subjected sexually immature, adult Pacific lamprey to water at relatively warm summer temperatures (>20 °C; ‘warm water group’) that mimicked the diel fluctuations of temperatures that occur in the Willamette River Basin during the summer and compared these fish with fish reared at the temperatures (13.6 °C) that occur in the upper tributaries of the Willamette River Basin (cool water group).

In Chapter 3 I present the results from tracking, locating and monitoring of radio-tagged lamprey in the Willamette River Basin above Willamette Falls over the course of several months during 2005. The falls are ~204 km from the Pacific Ocean and the lamprey are found there prior to moving upstream or back downstream to spawn. Nothing is known of the migration behavior or spawning locations of Pacific lamprey that pass Willamette Falls. I undertook this study to describe the characteristics of the initial or pre-spawning migration of adult Pacific lamprey (migration phases defined in Clemens et al. 2010). Specifically, I was interested in describing upstream migration timing, rates and distances traveled by lamprey that had passed Willamette Falls. I tested four predictions. Specifically, I predicted that adult Pacific lamprey would: 1) distribute evenly with respect to the distance migrated in the Willamette basin, based on a priori assumptions of an even distribution of spawning habitat throughout the basin, with no passage barriers; 2) cease their migrations during the summer, as described for these fish in another river basin (e.g., see Robinson and Bayer 2005) as a result of increasing water temperatures; 3) show a positive correlation between upstream migration distance and body size, as has been noted for this and other species of lampreys (briefly reviewed in
Clemens et al. 2010); and 4) show a correlation between upstream migration distance and date of passage of Willamette Falls.

In Chapter 4 I describe the sexual maturation and other phenotypic characteristics of adult Pacific lamprey that I collected from Willamette Falls, over time (April – September, 2007-2009). I compare these characteristics with those of recent migrants collected from the Klamath River mouth at the Pacific Ocean. The two goals of this research were to: 1) describe the sexual maturation characteristics of lamprey over the course of the run, and 2) characterize any potential run diversity that might be exclusive of obvious spawn timing.

In Chapter 5 I conclude this dissertation with a summary of the results and a working hypothesis that can be used as a basis for further investigations that can deepen and broaden our understanding of the enigmatic Pacific lamprey.

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Do summer temperatures trigger spring maturation in Pacific lamprey, *Entosphenus tridentatus*?

Benjamin J. Clemens, Stan van de Wetering, John Kaufman, Richard A. Holt, and Carl B. Schreck
**Introduction**

Management practices within the commercial forest, agriculture and power generation industries in the Pacific Northwest (USA) have resulted in warming of stream temperatures to > 20 °C during the summertime (Beschta et al. 1987; NRC 1996; EPA 2003; USACE et al. 2007). The effects of these warm temperatures have been most studied in salmonids (*Oncorhynchus* spp.), where warm temperatures have been linked to elevated metabolic rates and energetic expenditures (Brett 1995; Lee et al. 2003; Rand et al. 2006), use of cool water refugia (Goinea et al. 2006; High et al. 2006), altered run times (NRC 1996; Quinn & Adams 1996; McCullough 1999; Hodgson & Quinn 2002), susceptibility to disease (reviewed in McCullough 1999) and mortality for late migrants (Naughton et al. 2005; Keefer et al. 2007). Pacific lamprey (*Entosphenus tridentatus*) co-occur with Pacific salmon in these warming watersheds, and they have a similar life cycle to Pacific salmon (anadromy and semelparity). However little is known about the effects of summer temperatures on maturing adult Pacific lamprey.

After ceasing their parasitic stage in the ocean, Pacific lamprey return to freshwater during the spring (April – June; Beamish 1980), and then begin their initial upstream migration during the summer (July – September; Scott & Crossman 1973), before overwintering during October – March (Scott & Crossman 1973). Like other anadromous lampreys (Kott 1971; Beamish et al. 1979; Larsen 1980), Pacific lamprey do not feed during this prolonged freshwater residency (Beamish 1980; Whyte et al. 1993) and somatic energy reserves fuel sexual maturation (Kott 1971; Beamish et al. 1979;
Larsen 1980). As a result, Pacific lamprey shrink in body size (Beamish et al. 1980; Whyte et al. 1993) prior to maturing, spawning, and then dying the following spring (April – July; Pletcher 1963, cited in Scott & Crossman 1973; Beamish 1980). Most spawning activity in Western Oregon rivers occurs between April and May. However spawning can occur during late May and early June (Brumo 2006; Gunckel et al. 2006) with very little spawning (< 1% of spawning population) occurring during early July in some years (Brumo 2006). Late spawning activity may be associated with high stream flows and associated cool water temperatures (Brumo 2006; Gunckel et al. 2006).

The aims of the present study were twofold: 1) to examine the effects of relatively warm, summer temperatures (> 20 ºC) on the maturation status (mature = spermiating or ovulating), maturation timing, survival rates, and maturation characteristics (body size and general, qualitative measures of morphology) of Pacific lamprey over time (immediately after treatment and 10 months later); and 2) to ascertain whether there was evidence for a cause-and-effect relationship between body size and maturation timing. Because high temperatures increase metabolism and activity in lampreys (Johansen et al. 1973; Lewis 1980) as in other fishes, we predicted that shrinkage in body size during freshwater residency would be accentuated at relatively warm water temperatures experienced during the summer. We also predicted that if summer temperatures led to significant decreases in body size of Pacific lamprey, then a higher percentage of fish would mature early in comparison with lamprey held at cooler water temperatures. We subjected sexually immature, adult Pacific lamprey to relatively warm summer temperatures (> 20 ºC; “warm water group”) that mimicked the diel fluctuations of
temperatures that occur in the Willamette River Basin during the summer and compared these fish with fish reared at relatively cool temperatures that occur in the upper tributaries of the Willamette River Basin (“cool water group”).

Methods

Adult Pacific lamprey were randomly collected by hand from the Willamette River at the base of Willamette Falls near Oregon City, Oregon (USA) on June 14, 2006. Willamette Falls is 43 river kilometers upstream of the confluence of the Willamette and Columbia Rivers, and 206 river kilometers from the Pacific Ocean. Pacific lamprey congregate at the falls prior to ascending during May through October (Mesa et al. 2010) and migrating upstream to spawning grounds. The fish were transported in aerated coolers (66 L) to the Fish Performance and Genetics Laboratory at Oregon State University, where they were transferred to a holding tank (4.9 m long X 0.8 m wide X 0.3 m deep, ~ 1,000 L) with flow-through, pathogen-free well water. Holding tank temperatures averaged ~13.5 ºC. The tank was covered to prevent escapement and to reduce light levels as lamprey are photophobic (Hardisty and Potter 1971). Non-transparent, plastic pipes (~5 cm in diameter and 0.6 – 0.9 m in length) were provided for refuge for the lamprey, and they were frequently observed using the pipes.

During July 10 – July 11 of 2006, 72 healthy-looking fish were selected from a larger group of fish. The fish were anesthetized with 50 mg/L of tricaine methanesulfonate (MS-222) buffered with 125 mg/L of NaHCO₃. Fish were then tagged with Passive
Integrated Transponder (PIT) tags (32 mm long), which were inserted into the body cavity a few millimeters anterior to the cloaca. Six fish were randomly assigned to each of six warm water tanks and six cool water tanks for a total of 36 fish per experimental group. At the start of the experiment, none of the animals showed signs of secondary sexual characteristics that would enable identification of sex, as described by Hardisty & Potter (1971).

Experimental tanks were circular, with a diameter of 0.8 m, a depth of 0.5 m and volume of 295 L. Two plastic, non-transparent pipes (~5 cm in diameter and 0.6 m in length) were provided in each tank for lamprey to use as cover. The tanks were subjected to natural photoperiod and they were covered to reduce light levels. Tanks were supplied with flow-through well water at an average flow rate of 1.25 L * min\(^{-1}\) at different temperatures and the tank temperatures were recorded with automated temperature loggers (Hobos\(^\circledR\) by Onset).

We examined temperature patterns in the Willamette River Basin and used these patterns to guide our warm water and cool water temperature treatments. The gradient of mean summer temperatures in the mainstem Willamette River ranged from 16.5 °C in the upper river to 21.9 °C in the lower river (Table 1). We also examined the range of temperatures in tributaries to the Willamette River with a focus on moderately warm and moderately cold temperature reaches. The mean range of summertime temperatures for moderately warm tributaries = 22.1 – 22.8 °C, whereas moderately cool tributaries had a mean range of 11.5 – 14.5 °C (data for years 2001 and 2002 provided by the Oregon Department of Environmental Quality). Lastly we examined the range of diel
fluctuations in summertime temperatures across the mainstem Willamette River, which were 1.0 – 3.8 °C (data for years 2001 – 2004 provided by the Oregon Department of Environmental Quality). Based on these temperature patterns in the Willamette River Basin, we subjected warm water tanks to a target mean temperature that mimicked the mean summertime temperatures of moderately warm tributaries at ~22 °C with a diel temperature fluctuation resembling the maximum temperature fluctuation of ~4 °C (minimum temperature during nighttime = 20 °C; maximum temperature during the daytime = 24 °C). Cool water tanks were subjected to a mean temperature of 13.6 °C, which is within the range of temperatures occurring in moderately cool tributaries (see above) and also the mean annual temperature in the Willamette Basin (13.3 °C; Stanford et al. 2005). No diel fluctuation was provided for fish subjected to the cool water treatment.

Fish were acclimated to the warm water treatment from a baseline temperature of ~13.6 °C to 20 – 24 °C during July 12 – July 15 of 2006 by an increasing surge-like temperature regime. For example, heated water was supplied to warm water tanks on July 12, allowing a slow rise in temperature from 13° C to 16° C before the water heater was turned off and then again on July 13 from 13° C to 18° C before being turned off. This acclimation regime continued with 2° C increases above the previous day’s maximum temperature until July 16, whereupon the temperature was left to fluctuate between 20 and 24° C. The warm water treatment was terminated on October 1, 2006 at 0720 hours. In this way, fish in the warm water group experienced an average water temperature of 21.8 °C with a mean diel fluctuation of ~4 °C for 94 days, totaling 2,049
temperature units during the summer period, compared with fish in the cool water group, which experienced an average water temperature of 13.6 °C, totaling 1,278 temperature units over the same time period. Upon termination of the warm water treatment on October 1, 2006, both groups of fish were subjected to ambient well water averaging 12.8 °C (range: 11.5 – 13.8 °C) for 220 days for a total of 2,800 temperature units through May 9, 2007. Six fish in one warm water tank experienced ambient temperatures when the inflow of heated water for this tank failed for approximately 3 – 4 weeks; the data from these fish were omitted from the analyses.

The fish were transferred to holding tanks for the post-treatment, overwinter to maturation period (mid-October 2006 – May 2007). The holding tanks (1.8 m X 0.6 m X 0.6 m, 648 L;) were supplied with flow-through well water at an average rate of 4.0 L*min^-1. All tanks had cobble substrate (~360 – 1,920 cm³) for refuge. Mortalities during the summer treatment period resulted in fewer than the original 36 fish per treatment group (see Results). Therefore, 11 – 14 fish were held per tank, and two tanks held fish from the cool water group for a total of 24 fish between two tanks and two tanks held fish from the warm water group for a total of 27 fish between two tanks. Aquarium pumps circulated water between each of two tanks in the warm water group and each of two tanks in the cool water group at a mean flow rate of 0.4 – 0.5 L*min^-1. Inflow rates per tank were approximately 0.75 L*min^-1. This exchange of water was done so that mating pheromones and potentially pheromones that induce sexual maturation (see Li 2005) would be similar for all individuals of an experimental group during the spring maturation period.
Experimental and holding tanks were checked for mortalities at least three times per week, and dead fish were removed and identified by tag number. The dead fish were dissected to identify sex and determine maturation status (mature = ovulating or spermiating). Body size of dead fish was not recorded as we had determined previously that the body size of dead fish changes following death (B. Clemens, unpubl. data). To assist in interpretation of the cause of death and to monitor fish health, we screened a subset of lamprey from a spring 2006 collection (fresh fish not held in the laboratory) and also from the spring 2007 maturation period (experimental fish held in the laboratory between June 2006 and May 2007), for common fish pathogens, including *Aeromonas salmonicida*, the etiological agent of furunculosis (Bernoth 1997a; Cipriano & Bullock 2001). Potential key infection sites for *A. salmonicida* were assayed, including kidney and hemorrhagic tissue (Hiney et al. 1997). Samples were placed on tryptic soy agar (TSA) plates and observed for growth after four to eight days at room temperature. Assays were recorded as positive for *A. salmonicida* if brown-pigmented (chromagenic) colonies grew on the TSA and a brown, diffusible pigment was observed in the medium. This is the classic method for diagnosing presumptive *A. salmonicida*, though false negatives (i.e., atypical or achromatic *A. salmonicida*) and false positives (i.e., chromagenic bacteria other than *A. salmonicida*) are minor possibilities with this technique (Bernoth 1997b). Two isolates were collected from lamprey taken from Willamette Falls during May 2006 and these isolates were examined for characteristics of presumptive positive *A. salmonicida*, as described by Shotts (1994).
Six weekly or bi-weekly maturation checks were made on the adult lampreys during the maturation period. During the first two maturation checks, all specimens were examined, and 17 – 31 % of all fish were checked thereafter on the remaining four dates to minimize handling stress on individual fish. Fish within each experimental group were handled identically and all collection gear was disinfected and rinsed thoroughly prior to handling fish from the other experimental group. The experiment was terminated on May 9, 2007.

Body weights and total lengths were measured 1) on anesthetized fish before they were stocked into the experimental tanks immediately prior to initiation of the summer temperature treatment (July 2006), 2) on anesthetized fish following summer temperature treatment (October 2006), and 3) at the termination of the experiment (May 2007). To minimize handling stress during the spring maturation period, only body weight was measured. At the end of the experiment, all fish were euthanized and dissected to identify sex and to determine the maturation status. Fish were noted as mature if the males were spermiating or if the females were ovulating.

Statistical analyses

We tested for significant tank effects ($P < 0.05$) within treatment groups for differences in body size, survival, and sex ratio during the experimental temperature period (July – October 2006). Body size was tested with one-way ANOVAs. The proportion of fish remaining alive, per tank was tested with Chi square at the end of the temperature
treatment. Finally, the sex ratio of each tank was tested with Chi square. Because fish were subjected to the milieu of all remaining fish in their treatment group during the overwinter holding to spring maturation periods (October 2006 – May 2007), either by cohabitation in the same tank or by recirculation of water from an adjacent tank containing fish from the same group, tank effects were controlled for. As previously mentioned this was done so that pheromones would be similar for all individuals of an experimental group during the spring maturation period.

A repeated measures ANOVA was conducted on the body weights of warm water and cool water fish during July 2006 – March 2007, when sufficient numbers of both groups of fish remained alive.

Relative body size (body weight and total length) reductions over time were calculated as proportional reductions in the initial body size from July 2006 to the end of the summer temperature treatment (October 2006) and July 2006 to the early spring maturation period (March 2007). Separate MANOVAs were conducted for body weight and TL for each time period on arcsine-transformed proportions of initial body size. “Treatment type” (warm or cool water treatment), “sex” and the interaction between treatment type and sex were included as model factors in the MANOVA.

We tested whether individuals that were initially large during the summer (July 2006; pre-treatment phase) lived longer. The initial body weight during July 2006 was used as a dependent factor and “treatment type”, “sex”, “status” (dead or alive) and the interactions of these terms were factors in MANOVA models for each of two time periods: 1) the treatment period (July – October 2006) and 2) the early maturation period.
(March 2007). Suggestive trends ($P < 0.10$; Ramsey & Schafer 2002) were further explored within each experimental group with MANOVAs, one-way ANOVAs, and unpaired t-tests, though the effects were not considered significant unless $P < 0.05$. The MANOVAs included July 2006 body weights as the dependent factors and “sex”, “status” (dead or alive) and the interaction of sex and status as model factors.

The proportion of fish remaining alive was calculated for warm water and cool water fish over time. These proportions were arcsine-transformed and analyzed with two ANCOVA models, one for the summer temperature treatment period (July – October 2006) and another for the spring maturation period (March – May 2007). “Day” was included as the covariate (continuous variable) and “treatment type” (warm water/cool water) and the interaction between treatment type and day were independent factors in each of these ANCOVA models.

**Results**

There was no statistically significant tank effect within treatment groups on the body size, survival or sex ratio of Pacific lamprey. The data for all warm water fish were pooled and compared with the pooled data from all cool water fish.

Changes in body size
There was a suggestive, but insignificant trend for warm water fish to decrease in body weight to a greater extent than cool water fish throughout the experiment (July 2006 – March 2007; repeated measures ANOVA, \( P = 0.0679 \)) (Figure 2).

During the summer treatment period (July – October 2006), warm water fish had significantly greater proportional decreases in body weight than cool water fish (MANOVA, \( P = 0.0067 \)) (Figure 3A). There was no significant difference between warm water and cool water fish with regards to proportional decreases in TL during the summer treatment period (July – October 2006; MANOVA, \( P = 0.2295 \)) (Figure 3B).

Throughout the experiment (July 2006 – March 2007), there was no significant difference in proportional decreases in body weight between warm and cool water fish (MANOVA, \( P = 0.9669 \)) (Figure 3A).

Body size, sex and death in cool water fish

Male lampreys were significantly smaller than females immediately prior to initiation of the summer temperature treatment (July 2006; one-way ANOVA, \( P = 0.0247 \)) (Figure 2).

Cool water fish that died by May 2007 tended to have initial body weights smaller than fish that remained alive (MANOVA, \( P = 0.0074 \)). The small fish that died were males (MANOVA, \( P = 0.0040 \)). Weights of cool water males that died by May 2007 were significantly lower than cool water males that remained alive (unpaired t-test, \( P = 0.0358 \)). Similarly, there was a suggestive, but insignificant trend for small, cool water
females to die by May 2007 whereas large females remained alive (unpaired t-test, $P = 0.0845$).

Proportion alive

The proportion of fish remaining alive declined during the summer temperature treatment (July 2006 – October 2006; ANCOVA, $P < 0.0001$), however there was no difference in the proportions of fish remaining alive between warm water and cool water fish during this period (ANCOVA, $P = 0.8209$) (Figure 4). None of the 19 fish (12 cool water + 7 warm water) that died during July 2006 – October 2006 were mature. No mortalities occurred during the overwinter period (November 2006 – February 2007), a time in which the fish were inactive and resided under the cobble substrate.

The proportion of warm water fish remaining alive during March – May 2007 declined at a greater rate than cool water fish (ANCOVA, $P < 0.0001$) (Figure 4), with a 60% spring mortality for warm water fish versus 25% spring mortality for cool water fish. One hundred percent of the warm water fish were sexually mature (i.e., were spermiating or ovulating) during the spring, compared with 53% of cool water fish, and all spring mortalities were mature. Overall, 97% of the warm water fish and 61% of the cool water fish died by the end of the experiment in May 2007. Males survived for a longer duration than females (Table 2).

Aeromonas salmonicida
We detected *A. salmonicida* from the fresh, pre-treatment (spring 2006) collection of adult Pacific lamprey from Willamette Falls and also from the group of experimental fish collected from this same location and held in our laboratory between June 2006 and May 2007. Eight out of 20 immature mortalities (2 from the warm water group and 6 from the cool water group) during the late summer and early fall of 2006 showed gross symptoms consistent with furunculosis (bleeding in the body cavity, and from the gills or anus), as described for other fishes by Hiney et al. (1997), Bernoth (1997b), Cipriano & Bullock (2001). We have also observed an association between this type of internal hemorrhaging and a positive assay for *A. salmonicida* in adult Pacific lamprey (B. Clemens et al. unpubl. data), which further suggests that the eight mortalities described above likely had furunculosis, although we did not assay these particular fish for *A. salmonicida*.

Four warm water fish and 17 cool water fish were assayed during the end of the experiment in May 2007. All of the warm water fish that were assayed were males because the females had all died earlier (Table 2), whereas 12 of the 17 cool water fish were males. Two of the 4 warm water fish tested positive for *A. salmonicida*. Only 2 (1 male + 1 female) of the 17 cool water fish tested positive for *A. salmonicida*. The female that tested positive also exhibited symptoms of furunculosis (nodules/furuncules), as described for other fishes by Hiney et al. (1997), Bernoth (1997b), and Cipriano & Bullock (2001). In summary, all fish that tested positive were sexually mature (ovulating or spermiating), whereas all fish that tested negative were sexually immature.
Maturation characteristics

When palpated, mature females did not emit eggs unless the fish were dead or moribund. Mature males, however, almost always emitted sperm when palpated. Eggs appeared on the bottom of tanks holding warm water fish during mid-April, suggesting that some spawning activity may have occurred during this time.

Spermiating and ovulating lamprey showed a decrease in the space between their two dorsal fins to the point where the fins touched. These mature fish also showed secondary sexual characteristics (Hardisty & Potter 1971) and a liver coloration (Kott 1970) similar to previous descriptions for other lampreys. Approximately 1/3 of all fish were examined for liver color and we found that the liver of immature fish was typically orange in coloration in both sexes. Immature fish less often had a brown, purple or gray liver color. During April – May 2007, the liver of mature fish was typically dark green in both sexes and less frequently a light orange or gray. Changes in somatic tissue were correlated with the maturation status of the lamprey. In general, maturing lamprey had a light pink-to-white coloration in the trunk musculature and a relatively thin body wall, whereas immature fish had a red coloration in the trunk musculature and a relatively thick body wall.

Discussion
Because high temperatures can exponentially increase routine metabolism and motor and ventilatory activity in lampreys (Johansen et al. 1973; Lewis 1980) as in other fishes, we predicted that shrinkage in body size would be accentuated at relatively warm water temperatures experienced during the summer. We also predicted that if summer temperatures led to significant decreases in body size of Pacific lamprey, then maturation would be expedited (i.e., a relatively high proportion of fish would mature the following spring) in comparison with lamprey held at cool water temperatures. Our results supported these predictions.

European river lamprey, *Lampetra fluviatilis*, subjected to a warm range (12 – 17 °C) of seasonally – varying temperatures in the lab matured and died earlier (mid-March to early April) *within* season than those subjected to a cool range (7 – 11 °C; matured and died late April to mid-May) (Larsen 1965). Pacific lamprey subjected to relatively warm temperatures during the summer mature the following spring (this study).

Our Pacific lamprey matured between March and May and females tended to mature and die earlier than males, most obviously in the warm water group. The warm water group (and several in the cool water group) matured within the seasonal spawning period of wild fish, which typically occurs April – early June (Brumo 2006; Gunckel et al. 2006). The timing of maturation of our lamprey was also identical with the March – May maturation period of Pacific lamprey raised in the lab under seasonally – varying temperatures in another study (Mesa & Bayer 2005). Whereas we do not know whether the remainder of the cool water group would have matured later within the year – the fish
were sacrificed in May so that we could measure and compare body size, maturation status, and presence of the pathogen, *A. salmonicida* before all of the warm water fish died – it seems unlikely that the immature, cool water lamprey would have matured during 2007 for a two reasons. First, the vast majority of spawning activity occurs in April and terminates by late May or early June in Western Oregon Rivers (Brumo 2006; Gunckel et al. 2006). Second, our lamprey did not show morphological/anatomical signs of impending sexual maturation (apparent approximately a few weeks to one month before sexual maturity in Pacific lamprey [B. J. Clemens, pers. obs.] and in *L. fluviatilis* [Larsen 1965]). However, the idea that cool water may prolong immaturity of Pacific lamprey for > 1 year in freshwater remains to be explored. Wild fish may have different energy demands than lamprey in the laboratory, which should be considered when attempting to extrapolate our results to nature. For example, wild fish could experience greater energy demands than those held in the laboratory via high river flows against which they would have to maintain station or swim against. Alternatively, wild lamprey could experience lower energy demands than those held in the laboratory during the cool overwintering period (e.g., compare the winter temperature profile of our laboratory fish with that of the Willamette River at Willamette Falls in Figure 1).

Maturation and mortality were related to a previously existing small body size in cool water fish and also to significant decreases in body size in warm water fish. This suggests that a minimum, threshold body size exists at which maturation must occur or sufficient energy reserves will not be available for reproduction. This conclusion seems
to agree with Larsen’s conclusion (1980) that sexual maturation in L. fluviatilis was related to “…a metabolic signal related to starvation”.

Early maturation timing of adult lamprey exposed to relatively warm summer temperatures raises questions about influences on fitness. Early maturation could minimize the length of time that adult lamprey would be exposed to predation during freshwater residency, which could increase the number of lamprey available for spawning. However for Pacific lamprey experiencing warm temperatures in the lower river, early maturation timing could uncouple spawn timing with optimal habitat characteristics in the upper watershed for spawning, embryonic development and larval emergence, rearing and growth. Clearly there are many facets to the ecology of maturation timing in Pacific lamprey, and more research is needed to ascertain how warm summer temperatures affect reproductive fitness.

Body size

Indirect estimates of body shrinkage suggest that Pacific lamprey shrink by ~18 to 30% in body length between the start of their upstream migrations and spawning (Kan 1975; Beamish 1980; Chase 2001). The direct estimates of our fish, tracked over the course of 10 months, fall within this range of shrinkage in body length for maturing fish. For example, four spermiating male lamprey, including one fish from the warm water group and three from the cool water group, showed maximum reductions in body length of 19 – 22% between July 2006 and May 2007. The overall reduction in body length
between the time these fish first entered freshwater until the end of our experiment was undoubtedly >19 – 22%.

Through laboratory breeding trials, Beamish & Neville (1992) suggested that a difference in total length (TL) > ~20% precluded successful reproduction between the paired species, river (\(L. \ ayresi\)) and brook (\(L. \ richardsoni\)) lampreys, which led them to suggest that such differences in body length were an isolating factor for reproduction. Taken together with estimated shrinkage of 18 – 30% for Pacific lamprey, we wonder whether reductions in TL > 20% would preclude spawning with larger, more recent migrants from the ocean that might mature without overwintering and shrinking substantially in body size. Our question assumes that ocean-maturing races of Pacific lamprey exist; we do not yet have evidence supporting or refuting this hypothesis.

Aeromonas salmonicida and furunculosis

Warm water temperatures (15 – 20 ºC) have been correlated with proliferation of \(A. \ salmonicida\), outbreaks of furunculosis, and increased mortality in salmonids (Wedemeyer 1996; Pickering 1997). In addition to warm water, furunculosis outbreaks have been attributed to hydrographic features that can aggregate infected fish, such as at the base of waterfalls (Mackie et al. 1930, cited in Johnsen & Jensen 1994). Pacific lamprey can be found in aggregations at temperatures > 20 ºC at the base of Willamette Falls (B. Clemens, unpubl. data), and we have detected \(A. \ salmonicida\) in fresh collections of adult Pacific lamprey from this location, and in our experimental fish,
which were also taken from this location. Sexual maturation appeared to be associated with incidence of *A. salmonicida*, although we only directly assayed a subset of mortalities (including euthanized fish at the end of the experiment) during spring of 2007 (mortalities were examined for gross symptoms during 2006). Our research raises questions that warrant further exploration of the nature of the association of *A. salmonicida* with sexual maturation in Pacific lamprey. For example, does *A. salmonicida* proliferate as a result of the sexual maturation process? Does this pathogen kill lamprey before they spawn?

Maturation characteristics

Maturation characteristics have been described for other lampreys (Kott 1970; Hardisty & Potter 1971), and yet no comparable descriptions have been published for Pacific lamprey. In Pacific lamprey, we found that the lack of space between the two dorsal fins (i.e., the fins touch) occurs as a result of body shrinkage, and it appears to be a consistent indicator of sexual maturation in Pacific lamprey (this study) and in other lampreys (Hardisty & Potter 1971). We also observed a correlation between sexual maturity and liver color – immature fish generally had orange-colored livers whereas mature fish generally had dark green-colored livers. This finding is consistent with work by Kott (1970) on Great Lakes sea lamprey (*Petromyzon marinus*): early-migrating fish had orange livers, and late-migrating, mature fish had green-colored livers. The green
coloration arises from the accumulation of the bile pigment, biliverdin, in the liver and an associated degeneration of the liver (Kott 1970).

**Acknowledgments**

Brett Blundon assisted with collection and tagging of lamprey, Rob Chitwood assisted with fish culture, and Peter Sorensen provided advice on maintaining the pheromone milieu between tanks via recirculating water. Jen Bayer provided information on maturation characteristics of Pacific lamprey. David Noakes, Stacia Sower, Matt Mesa, and Doug Markle provided useful comments on an earlier version of the manuscript. Funding was provided by the Confederated Tribes of the Siletz Indians, Oregon Sea Grant and the Department of Fisheries and Wildlife at Oregon State University.

**Literature cited**


Beamish, R.J. & Neville, C.E.M. 1992. The importance of size as an isolating


McCullough, D.A. 1999. A review and synthesis of effects of alterations to the water temperature regime on freshwater life stages of salmonids, with special reference to


Table 1. Mean water temperatures for the mainstem Willamette River for July – September. Data are for year 2001 or 2002 and were provided by the Oregon Department of Environmental Quality.

<table>
<thead>
<tr>
<th>Willamette river kilometer</th>
<th>Mean °C</th>
</tr>
</thead>
<tbody>
<tr>
<td>29</td>
<td>21.7</td>
</tr>
<tr>
<td>64</td>
<td>21.9</td>
</tr>
<tr>
<td>143</td>
<td>20.4</td>
</tr>
<tr>
<td>213</td>
<td>18.9</td>
</tr>
<tr>
<td>291</td>
<td>16.5</td>
</tr>
</tbody>
</table>
**Figure 1.** Temperature profile of the Willamette River at Willamette Falls (grey line; data from Oregon Department of Fish and Wildlife: [http://www.dfw.state.or.us/fish/fish_counts/willamette%20falls.asp](http://www.dfw.state.or.us/fish/fish_counts/willamette%20falls.asp)), where the experimental lamprey were collected. The black, vertical arrow indicates the date when lamprey were collected at the falls (June 14, 2006). Also shown are the mean temperature profiles experienced by the warm water (“WW”; black, long-dashed line) and cool water (“CW”; grey, short-dashed line) lamprey in the laboratory. Mean temperature values experienced by WW and CW lamprey are shown above the brackets for the summer treatment period and overwinter holding to spring maturation periods.
Figure 2. Mean body weight (±SE) of adult Pacific lamprey. Numbers of fish remaining alive, and on which measurements were made, are shown above the bars.
**A.**

<table>
<thead>
<tr>
<th>Month</th>
<th>Warm water males</th>
<th>Warm water females</th>
<th>Cool water males</th>
<th>Cool water females</th>
</tr>
</thead>
<tbody>
<tr>
<td>July to Oct</td>
<td>14</td>
<td>7</td>
<td>13</td>
<td>11***</td>
</tr>
<tr>
<td>July to March</td>
<td>12</td>
<td>7</td>
<td>13</td>
<td>9</td>
</tr>
<tr>
<td>July to May</td>
<td>10</td>
<td>0</td>
<td>4</td>
<td></td>
</tr>
</tbody>
</table>

**B.**

<table>
<thead>
<tr>
<th>Month</th>
<th>% decrease in TL</th>
</tr>
</thead>
<tbody>
<tr>
<td>July to Oct</td>
<td>14</td>
</tr>
<tr>
<td>July to March</td>
<td>13</td>
</tr>
<tr>
<td>July to May</td>
<td>10</td>
</tr>
</tbody>
</table>
**Figure 3.** Mean percent decreases in body weight (±SE) (A) and total length (±SE) (B) of adult Pacific lamprey, between July 2006 and May 2007. Numbers of fish remaining alive, and on which measurements were made, are shown above the bars. No measurements were made on TL during March (see Methods). Asterisks indicate the statistical dissimilarity amongst the fish in that period only (see Results for details).
Figure 4. Percentage of adult Pacific lamprey from the warm water treatment ("WW" = filled circles) and cool water treatment ("CW" = open circles) remaining alive during July – October 2006 and March — May 2007. No mortalities occurred during the November 2006 – February 2007 period, a time in which the fish were inactive and residing within the cobble substrate. All dead fish during the spring period were sexually mature (i.e., ovulating or spermiating).
Table 2. Percentages of males (M) to females (F) of adult Pacific lamprey remaining alive at the start (July 2006) and termination (October 2006) of the temperature treatment, and during the maturation period in May 2007.

<table>
<thead>
<tr>
<th></th>
<th>Warm water group</th>
<th>Cool water group</th>
</tr>
</thead>
<tbody>
<tr>
<td>July 2006</td>
<td>57% (M) : 43% (F)</td>
<td>56% (M) : 44% (F)</td>
</tr>
<tr>
<td>October 2006</td>
<td>63% (M) : 37% (F)</td>
<td>54% (M) : 46% (F)</td>
</tr>
<tr>
<td>May 2007</td>
<td>100% (M) : 0% (F)</td>
<td>71% (M) : 29% (F)</td>
</tr>
</tbody>
</table>
Pre-spawning migration of adult Pacific lamprey, *Entosphenus tridentatus*, in the Willamette River, Oregon (U.S.A.)

Benjamin J. Clemens, Matthew G. Mesa, Robert J. Magie, Douglas A. Young, and Carl B. Schreck
Introduction

Lamprey populations in the northern hemisphere are imperiled, and river habitat degradation and barriers to spawning sites have been implicated (Renaud 1997). In North America, 10 of the 20 lamprey species are imperiled, likely from the same causes (Jelks et al. 2008).

Pacific lamprey, *Entosphenus tridentatus*, abundance has declined significantly over the last 50 years in the Pacific Northwest of North America (PNW). This decline has been attributed to the aforementioned problems with lamprey populations in the northern hemisphere: lack of habitat quantity and quality, and barriers to upstream passage for spawning (Close et al. 1995; CRITFC 2008; Cochnauer and Claire 2009; Moyle et al. 2009). Tribes that utilize Pacific lamprey as a food, medicine, ceremonial and cultural resource have expressed great concern about the persistence of these fish (Close et al. 2002; CRITFC 2008; Petersen Lewis 2009). Key ecological associations of Pacific lamprey have also been noted, including watershed nutrient cycling and the potential inter-relatedness of declines among Pacific salmon (*Oncorhynchus* spp.) and Pacific lamprey (Close et al. 2002; Moyle et al. 2009; Petersen Lewis 2009).

The state of Oregon, USA, has listed Pacific lamprey as a ‘sensitive’ species at risk of extinction (Kostow 2002; ODFW 2006). In 2003, a petition to list the Pacific lamprey as ‘threatened’ or ‘endangered’ under the Endangered Species Act was considered by the U. S. Fish and Wildlife Service (USFWS), which concluded that insufficient evidence on biology, ecology, habitat needs and specific threats was available to list this fish (USFWS 2004).
The relative level of information on the biology and migration characteristics of Pacific lamprey is low – moderate (Clemens et al. 2010). Only one study has provided detailed information on migration characteristics of individual Pacific lamprey in freshwater (see Robinson and Bayer 2005). It is been inferred that Pacific lamprey cease their parasitic lifestyle in the ocean, return to freshwater during the spring (April – June), and begin their initial upstream migration during the summer (July – September) before their pre-spawning holding during October – March. Pacific lamprey then mature, spawn, and die during April — July, approximately 1 year after having entered freshwater (reviewed in Clemens et al. 2010).

Pacific lamprey returning from the Pacific Ocean enter the Columbia River and travel ~162 km upstream to the confluence of the Willamette River. Those not continuing up the Columbia can travel another 42 km up the Willamette before encountering Willamette Falls. The 12 m high falls (Stanford et al. 2005) are comprised of basaltic bedrock and boulders, flanked by a hydroelectric dam and paper mill with a fish ladder on the west side of the river. The lamprey congregate at the falls before they attempt to ascend the falls or either pass via the fish ladder and continue their migration to spawning areas or they move back downstream without passing the falls (Mesa et al. 2010).

Populations of Pacific lamprey at Willamette Falls are the largest in the state of Oregon, and they are the source of the largest tribal harvest in the state (Kostow 2002). Researching the migration biology of Pacific lamprey in the Willamette may eventually
uncover why populations here are relatively large (and provide information on what might be done to stem their decline).

Of the Pacific lamprey that pass Willamette Falls, nothing is known about their migration behavior or spawning locations. We undertook the present study to describe the characteristics of the initial or pre-spawning migration of adult Pacific lamprey (migration phases defined in Clemens et al. 2010). Specifically, we were interested in describing upstream migration timing, rates and distances traveled by lamprey that had passed Willamette Falls. The overall goal of the study was to provide basic information on adult Pacific lamprey that could be used: 1) to fill information gaps on their migration; 2) to inform fisheries managers of potential key holding habitats that might be preserved from land development and subsequent habitat degradation; and 3) obtain data that would set the basis for informing more detailed follow-up research on these fish in the Willamette Basin.

We tested four predictions. Specifically, we predicted that adult Pacific lamprey would: 1) distribute evenly (i.e., display a normal distribution) with respect to the distance migrated in the Willamette basin, based on a priori assumptions of an even distribution of spawning habitat throughout the basin, with no passage barriers; 2) cease their migrations during the summer, as described for these fish in another river basin (e.g., see Robinson and Bayer 2005); 3) show a positive correlation between upstream migration distance and body size, as has been noted for this and other species of lampreys (briefly reviewed in Clemens et al. 2010); and 4) show either a positive or negative correlation between upstream migration distance and date of passage of Willamette Falls.
This last prediction was based on the temporal component of migration for adult Pacific lamprey, which raised the question: Do fish that pass Willamette Falls early in the year migrate farther than those that pass later?

**Methods**

**Study area**

The Willamette River basin comprises an area of 29,728 km² within western Oregon, U.S.A. (Fig. 1). The basin spans the Cascade Mountain Range on the east and the Coast Range on the west. Mean annual discharge of the Willamette River is 917 m³ s⁻¹ and mean annual water temperature is 13.3°C. River flow is regulated by 13 tributary dams and another 24 dams are for hydropower generation (Stanford et al. 2005). Only one of these dams — the Willamette Falls project (owned and operated by Portland General Electric) — is on the mainstem Willamette River, at about 205 km from the Pacific Ocean (at river kilometer 42.7 in the Willamette River). The project is incorporated into a natural falls (Willamette Falls), an obstacle to upstream migration by *E. tridentatus*, and has facilities for collection of fish for tagging.

**Fish collection, tag implantation and fish release**
Between April and September of 2005, we collected 136 adult lamprey from the Willamette Falls project and surgically implanted radio tags (Lotek NTC-6-2, 4.5 g in air, ~31 mm X 9 mm, with a whip antenna) into these fish. The radio tags were uniquely coded using eight frequencies, enabling identification of individual fish. Tag transmission rates were 6.8 – 7.2 s and they lasted for about 309 days. Tagged fish were released into the Willamette River 2 km below the dam. Lamprey showing secondary sexual characteristics indicative of sexual maturation (see Hardisty and Potter 1971) were not tagged. Tagged fish were larger than the entire population of individuals (Fig. 2) because we tagged fish that were a minimum of 11 cm in girth to insure there was sufficient space inside the body cavity to hold the radio tags. Additional details of fish capture and tagging can be found in Mesa et al. (2010). We were not able to reliably and consistently sex the immature tagged fish, therefore we did not assess migration behavior in relation to sex.

We conducted flight surveys to track radio tagged fish that had moved upstream of Willamette Falls en route to spawning areas in the upper basin. We recorded the presence of these fish to describe characteristics of their initial or pre-spawning migration (definitions of the migration phases can be found in Clemens et al. 2010), including upstream migration timing, rates and distances traveled.

Temperature monitoring
Mean daily temperature data were acquired from temperature gages from three sites on the mainstem Willamette River: Newberg, Albany and Harrisburg (Fig. 1). These sites are 37.3, 147.2, and 214.9 river kilometers (rkm) upstream of Willamette Falls. This information was used as a general, qualitative comparison by which migration distances and minimum rates of movement of adult lamprey could be compared.

Aerial tracking

We conducted 15 aerial surveys, averaging 4 hours each, from a Cessna 185 aircraft for an overall airtime of about 60 h. Flights occurred on a weekly basis during summer months, when most of the lamprey were available for detection upstream of the falls. Flight surveys were conducted less frequently during the spring when few lamprey were available for tracking and late fall – winter, when fish movements appeared to slow or even stop. The mean altitude was approximately 213 m and the mean flight speed was 148 – 167 km • h⁻¹.

An H-shaped dipole antenna was situated on each wing strut of the aircraft, and an antenna was plugged directly into a radio receiver (Lotek, W32 SRX 400 and W16 SRX 400). The accuracy and precision of detections were checked against radio tags suspended at depths of 3.0, 6.1, 9.1 and 15.2 m in the Willamette River. Antennas and receivers were checked for functionality prior to flights.
Fish location was recorded by GPS within the aircraft and also by aerial photographs. When possible, we collected two GPS locations on each detected fish. After both GPS coordinates for an individual fish were plotted on a map, a location median to each of the two recorded locations was used to delineate the probable location for that fish.

We surveyed the 256 river kilometers (rkm) of the mainstem Willamette River upstream of Willamette Falls. Nine major tributaries, composing 403 rkm, were also surveyed, including the Tualatin, Molalla, Yamhill, Santiam (also North and South Santiam Rivers); McKenzie, Middle Fork Willamette, and the Coast Fork Willamette to Row River (Fig. 1). A mean of 222 km (33% of total distance) was surveyed per flight. The lower to middle Willamette Basin was surveyed most frequently, with 10 dates between June and December, followed by the middle to upper basin, with eight dates between July and November.

The presence of large hydropower dams determined the maximum distance surveyed upstream, with the exception of the Tualatin, Molalla and Yamhill Rivers, which have no large dams. In these latter three rivers, the maximum distance surveyed upstream was 48 km.

The detection efficiency per flight survey was calculated on the basis of the number of fish available for detection on each survey (i.e., the number of fish that had passed Willamette Falls prior to a given survey):

\[(\text{number of novel detections}) \times (\text{number of fish available for detection})^{-1} \times 100\]
Minimum fish velocity was calculated as:

\[ \text{distance migrated} \times \text{days migrated}^{-1} \]

Distance migrated was measured from the upstream precipice of Willamette Falls to the location where lamprey were detected and graphed against two parameters: 1) the frequency distribution of fish migrating upstream; 2) the release date of fish detected two or more times. Days migrated was the time it took the fish to migrate upstream from the precipice of Willamette Falls to the location where they were detected. We use the term “minimum” fish velocity because the fish may have migrated to the particular detection location at some unknown date before we detected them. The significance and strength of association of maximum migration distance was tested with each of two Pearson Product Moment correlation tests: 1) against total body length and 2) against date of fish passage of each fish.

The last location where each fish was detected was compared with habitat features (presence of pools or substrate such as rock revetments, boulders, and bedrock shoals) as a means to associate the last location of detection with potential pre-spawn holding sites. The habitat features were determined via boat reconnaissance surveys during 2009 and from aerial photographs taken during 2005 and published by the Oregon State Parks and Oregon Watershed Enhancement Board (available: [http://www.oweb.state.or.us/OWEB/publications.shtml](http://www.oweb.state.or.us/OWEB/publications.shtml)).
Results

Fish collection, tag implantation and fish release

Tagged fish (N = 136) averaged 658 mm (±3.15 SE) in total length (TL), and 480.8 g (±6.31) in mass, compared with 611 mm (±2.07) and 405.0 g (±3.63) for the population of both tagged and untagged fish (N = 594; Fig. 2). The tags comprised an average of 0.96% (±0.01) of the body mass of tagged lamprey.

Temperature monitoring

Summer temperatures in the lower to mid-Willamette River peaked at ~23°C and were consistently > 20°C during July – August, when river discharge was very low (Fig. 3). From the most upstream to the most downstream site, mean monthly river flows were 200 – 387 m³ * s⁻¹, and mean monthly river temperatures were 14.5 – 16.1°C (Fig. 3) during the survey period.

Aerial tracking
Test tags moored from buoys at 3.0 and 6.1 m depths were detected, whereas tags moored deeper were not detected. Detection accuracy from the aircraft ranged from 0.69 km for the tag at 3.0 m to 0.24 km for the tag at 6.1 m depth. Mean disparity in linear distance for the two coordinate readings for detected fish was 0.45 km, suggesting we could locate fish with an accuracy of ~±0.5 km.

Of the 136 lamprey that were tagged, 43 passed upstream of Willamette Falls (all via the fish ladder; Mesa et al. 2010). Two of the 43 fish that passed Willamette Falls moved back over the falls. One of these two fish was undetected by our aerial surveys and moved back below the falls two days later; the other fish was detected by our surveys (Mesa et al. 2010). We detected 24 of 43 fish (55.8% detection efficiency), including 22 fish in the mainstem Willamette and 2 in tributaries. We detected 17 of the 24 fish two or more times (Figs. 4 – 6). The estimated detection efficiency of lamprey averaged 5.4% (range: 0.0 – 16.7%) per flight for all 15 flights (Fig. 7).

The distribution of the fish approximated a normal distribution (Figs. 4 – 6). Fish tended to migrate during spring – early summer. Fish that slowed or halted their migrations during the summer tended to do so during peak summer temperatures of ≥20°C (Figs. 3 and 6). However, 7 of the 17 fish that were detected multiple times did not slow or halt their migrations until they were further up in the basin where mean daily temperatures were < 20°C (Figs. 3 and 5). Three Pacific lamprey (2 from July releases and 1 from an August release) continued to migrate upstream after September (Fig. 5). In summary, migration behavior ranged from relatively slow movement, followed by
holding for some fish to rapid movements, followed by slower migration further up in the basin by other fish (Figs. 5 and 6).

For the 17 fish that were detected multiple times, mean migration distances were similar to medians, albeit higher (Fig. 6). The mean distance travelled to the first detection location was 127.7 km (range: 47.8 – 239.9 km). The mean distance traveled to the second detection location was farther at 141.2 km (range: 48.1 – 267.3 km).

Minimum fish velocity to the first detection location averaged 7.3 km*d$^{-1}$ (range: 1.3 – 16.4 km*d$^{-1}$). Minimum fish velocity to the second detection location was slower, averaging 4.8 km*d$^{-1}$ (range: 1.2 – 18.6 km*d$^{-1}$; Fig. 5). In summary, the fish traveled farther to the location of their second detection, but at a slower estimated minimum velocity.

The maximum upstream migration distance did not correlate significantly with total body length (Pearson Product Moment Correlation, r = -0.186; P = 0.385, N = 24) or the date the fish passed Willamette Falls (Pearson Product Moment Correlation, r = -0.118, P = 0.582, N = 24).

Holding locations typically occurred in areas of the Willamette that had deep pools (> 10 – 20 m in depth) or rock substrate, including rock revetments and boulder and bedrock shoals. However, the fish that we detected were likely not deep in the pools, given our minimum depth of detection for test tags of ~ 6.1 m.

**Discussion**
Our data supported the hypothesis that adult Pacific lamprey would distribute evenly throughout the basin. However our data did not support the hypotheses that all fish would stop migrating during the summer; that there would be a positive correlation between the maximum distance migrated upstream versus total body length or a correlation (positive or negative) between the maximum distance migrated and the date fish passed Willamette Falls.

Fish were distributed throughout the mainstem Willamette. More detailed research is needed to determine if this distribution is a function of a close proximity to spawning grounds or a preference for pre-spawn holding locations that might be associated with environmental factors (e.g., substrate, river flow and temperature). Clearly more detailed telemetry tracking is needed to address these hypotheses. No fish were detected immediately below barrier dams in tributaries of the Willamette, and so we have no evidence that these dams are preventing access to upstream spawning sites.

Although most lamprey stopped migrating in the fall, three continued to migrate upstream during this time. Robinson and Bayer (2005) conclude that in the John Day River of eastern Oregon, their radio-tagged Pacific lamprey, ‘…halted upstream migration by September, and held a single position for approximately six months…’. A close examination of their Fig. 3 indicates that 4 of their fish from early August – early September releases migrated upstream after mid-September, and they indicate in their results that the median last day of upstream movement was the 12 of September, with a range of 8 August – 14 November. In the Willamette, the median last day of upstream
movement was the 31 of August, with a range of 29 June – 9 November. Accordingly, the Willamette lamprey ceased upstream migration from a few days to a little more than 1 month earlier than the fish from the John Day River of eastern Oregon. It should be emphasized that these two studies occurred in different years. A simultaneous, detailed tracking assessment between river basins is necessary to warrant further conclusions.

Given the positive correlation between body size and migration distance in lampreys (reviewed in Clemens et al. 2010), we expected to see a significant, positive correlation between body size and the distance migrated in the lamprey we tracked, yet this was not the case. The lack of correlation between body size and migration distance in our lamprey might be a function of not tracking the fish to their final spawning destination in the spring. However, Pacific lamprey in eastern Oregon had migrated the majority of their distance towards spawning grounds, with a median of 87% of their total migration distance being completed prior to holding during the winter (Robinson and Bayer 2005). A more likely cause for a lack of correlation between body size and migration distance was that because of tag size, we had to tag and track relatively large fish (see Fig. 2).

Our data suggests that fish that migrated earlier in the year did not migrate a different distance than those that migrated later. Yet fish tagged and released in the summer did appear to migrate faster and a few of these fish migrated further (3 of these fish migrated during the fall). In the John Day River, somewhat similar rapid migrations occurred during the late summer: lamprey tagged and released during late August – early
September had faster maximum and mean migration rates than fish that were tagged and released earlier in the year (Robinson and Bayer 2005).

River temperature correlates strongly with the migration timing of adult sea lamprey, *Petromyzon marinus*, in tributaries to Lake Ontario (Binder et al. 2010) and also in adult Pacific lamprey in the Columbia River Basin (Keefer et al. 2009). Over multiple years in the Columbia River Basin, counts of adult Pacific lamprey at dams indicate that migration occurred earlier in the spring and summer during warm, low discharge years and later during cool, high discharge years. Most adult Pacific lamprey passed Bonneville Dam, the first dam encountered by these fish at river kilometer 223 on the Columbia River, at temperatures of 15 – 23°C (Keefer et al. 2009). Similarly, peak passage of adult Pacific lamprey at Willamette Falls occurred during peak river temperatures of 23°C during 2005 (Mesa et al. 2010). Lamprey that passed Willamette Falls may have done so to avoid warm temperatures, which is interesting in that fish appeared to show the opposite behavior upstream of Willamette Falls: many slowed or stopped migrating during times coinciding with peak river temperatures. Our Pacific lamprey migrated primarily during the summer when river flows were low and most slowed or stopped migrating when mean daily temperatures peaked at ≥ 20°C during the mid-summer. Other possibilities for slowing and stopping migration during the summer include a loss of radio tags from the fish, although this seems unlikely. Loss of radio tags from lamprey may be minimal in natural environments (see Discussion in Mesa et al. 2010).
Holding locations of our Pacific lamprey typically coincided with deep pools or rock substrate, including rock revetments and boulder and bedrock shoals. This is similar to what was found in eastern Oregon, where adult Pacific lamprey held primarily around boulders during the winter (see Robinson and Bayer 2005). Fish that we detected in areas of deep pools were likely within water \( \leq 6.1 \) m, otherwise we would not have detected them.

More research is needed to verify that we detected lamprey frequently enough, and over a long enough time period, to reliably estimate migration rates and to determine if our tracking methods were biased towards detecting fish that migrate and stop whereas others might migrate rapidly and go undetected. Research is also needed on fine-scale habitat use to determine if holding locations are associated with the availability of thermal refugia or habitat structure.

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**Literature cited**


Figure 1  Willamette River Basin in the state of Oregon, on the northwest coast of the USA (inset). The basin shows the major tributaries surveyed for radio-tagged Pacific lamprey by plane surveys. All surveys were conducted upstream from Willamette Falls. The river flows from South to North. The three sites with temperature gages, Newberg, Albany, and Harrisburg, are shown.
Figure 2 Mean total length (error bars ± SE) versus collection and release date for adult Pacific lamprey captured in a trap in the fish ladder at Willamette Falls (Figure 1).
**Figure. 3** Mean daily river temperatures (A). From the most downstream to the most upstream location, the three temperature gage locations on the mainstem Willamette are Newberg (37.3 km upstream of Willamette Falls), Albany (148.2 km upstream), and Harrisburg (215.3 km upstream) (Figure 1). Mean daily river flows in the mainstem Willamette (B). River temperature and flow data are from USGS gages (http://waterdata.usgs.gov/nwis).
Maximum distance migrated upstream (rkm)

0 25 50 75 100 125 150 175 200 225

Count

0 1 2 3 4 5 6

Maximum distance migrated upstream (rkm)
Figure. 4 Map of the Willamette Basin showing general locations of the maximum upstream migration distance (from the release site below Willamette Falls—see Methods for details) for adult Pacific lamprey detected repeatedly ($N = 17$). Each of the 5 circles on the mainstem Willamette, from top to bottom (North to South) correspond with the count or number of lamprey in each of 5 bins on the adjacent histogram, from left to right (total count for all bars = 17 fish). The data on each fish’s maximum upstream migration distance was lumped into 5 bins, in 25 km increments. On the map, 2 of the locations, corresponding to the ‘125’ and ‘225’ km bins have arrows emanating from them to depict each of 2 fish that migrated 20 and 28 km up the Santiam and McKenzie River systems, respectively (tributary km not included in bins on the histogram).
**Figure. 5** Distance migrated for radio-tagged adult Pacific lamprey that were detected multiple times (N = 17) in the Willamette River Basin upstream of Willamette Falls. Fish are grouped by the month of release. The symbols denote release and detection dates and associated river kilometers (rkm) migrated. One fish from the June releases was detected in the North Santiam River and one fish from the July releases was detected in the McKenzie River (both fish denoted by asterisks). All other fish were detected in the mainstem Willamette River.
Figure. 6 Summary plots for the fish shown in Figure. 5. The symbols denote release and detection dates and the associated river kilometers (rkm) migrated. The fastest and farthest migrating fish was detected in the McKenzie River (denoted by asterices).
Figure. 7 Percentages of adult radio-tagged Pacific lamprey available for detection by plane surveys (i.e., fish had passed Willamette Falls) in relation to novel detections.
Temporal trends in maturation characteristics of adult Pacific lamprey, *Entosphenus tridentatus*, during the freshwater spawning migration

*And*

Maturation characteristics and phenotypic diversity of adult Pacific lamprey, *Entosphenus tridentatus*, provide evidence for temporal intermixing of two cohorts or “runs”

**General Introduction**

One of the precepts of fisheries conservation and management is to understand the adult biology, life cycle, and diversity of the fish to be conserved. For example, when does the animal mature and how long is the spawning season? Is there > 1 spawning group? What are the maturation characteristics? Very little is known about the adult biology of Pacific lamprey (CRITFC 2008; Luzier et al. 2009; Clemens et al. 2010; Chapter 1). For example it is not known what baseline diversity in spawning and maturation characteristics exist in Pacific lamprey populations, or even if such a baseline exists or even existed.

Given the extensive declines in Pacific lamprey in the Pacific Northwest and throughout their range (Chapter 1), it is imperative that the gaps in biological information of adult Pacific lamprey be filled to inform conservation and management scenarios.
Researching adult Pacific lamprey for the purposes of learning when they mature, how long their spawning season is, how many spawning groups and the maturation characteristics are daunting tasks for two reasons:

1. *There are no baseline data.* Monitoring, conservation, and Euro-American values have prioritized research and monitoring of ESA-listed salmonids. Consequently few data are gathered on lampreys and data (Moser et al. 2007).

2. *Lamprey are not tractable animals.* They do not possess tissue that allows aging; they are cryptic, seeking refuge during the day and moving mostly during the night, they have long and complex life histories, they do not home in the classical sense of the definition (e.g., salmon; Dittman and Quinn 1996), and they are poorly understood (Moser et al. 2007; Clemens et al. 2010). Their prolonged (many months to perhaps even years) and extensive freshwater migrations (≤ 700 km) prior to spawning (Clemens et al. 2010) render Pacific lamprey difficult to study. Consequently, the duration of freshwater residency for any given fish (beyond the interface of the ocean and freshwater) is unknown. As an example, the “conventional” wisdom of 1 year of freshwater residency (Clemens et al. 2010) is the result of inferences made by Beamish (1980) on Pacific lamprey that spawned in the laboratory 1 year after entering freshwater (but with an unknown prior history).

Pacific lamprey are anadromous, semelparous fishes (Chapter 1; Clemens et al. 2010). In the Pacific Northwest, USA, it has been inferred that sexually immature Pacific lamprey cease parasitic feeding in the ocean and enter fresh water during April–June in
the year prior to spawning, and begin their initial migration during July–September. Pacific lamprey hide under stones while overwintering during October–March before their final migration, nesting and spawning during April–July, after which they die (Clemens et al. 2010). Like other anadromous lamperys (Kott 1971; Beamish et al. 1979; Larsen 1980), Pacific lamprey do not feed during their prolonged freshwater residency (Beamish 1980; Whyte et al. 1993) and somatic energy reserves fuel sexual maturation (Kott 1971; Beamish et al. 1979; Larsen 1980). As a result, Pacific lamprey shrink in body size, and they also deplete their lipid content (Beamish 1980; Whyte et al. 1993; Chapter 2; Clemens et al. 2009) to fuel maturation.

Details about different runs or cohorts overlap in maturation time and characteristics is not known. Moyle (2002) noted, “It is possible that Pacific lampreys within one stream system have more than one run....” Two “runs” of adult Pacific lamprey have been observed in some California rivers (Moyle et al. 2009), and a seemingly similar observation has been made in a river basin that enters the lower Columbia River just below where the Willamette River enters. In this river system, two distinct pulses of returning adult *E. tridentatus* were observed over several years of monitoring: 1) an early pulse, occurring April — July and 2) a late pulse, occurring late August — November (Stone et al. 2001; Stone et al. 2002; Lê et al. 2004; Luzier and Silver 2005).

It is not known whether these “runs” or “pulses” of Pacific lamprey represent “…a spring run that spawns immediately after the upstream migration and a fall run, which holds over and spawns the following spring” (Moyle 2002) or a spring run that
was last year’s migrants and this year’s spawners and a fall run that would spawn the following spring.

Native Americans from the Confederated Tribes of the Umatilla Indian Reservation noted a single migration lasting from spring through fall, of which two morphotypes of Pacific lamprey were readily observed in the Umatilla River of northeast Oregon: 1) ‘short, brown eels’, and 2) ‘long, dark eels’. These morphotypes appear to be akin to what Native Americans call ‘day’ and ‘night’ eels, respectively (Close et al. 2004). Close et al. (2004) speculated that the day eels were animals that had overwintered and possibly spawned, whereas the night eels may have been recent migrants from the ocean; however, they were unable to rule out the possibility of two different life history types. Moyle (2002) also noted, “In the Trinity River (northern California), for example, there may be two distinct forms of Pacific lamprey, one smaller and paler than the other, that represent either separate runs or resident versus migratory individuals.”

In addition to the aforementioned observations on the differences in run timing, general body size and color, the nature of the diversity within Pacific lamprey has been assessed on a gross geographical scale by genetics and observations on body length. From a genetic standpoint, and across a very wide geographical scale, there is conflicting evidence for population structure. This suggests either that population structure does not exist or that there is some form of weak population structuring (reviewed in Clemens et al. 2010). Differences in body size exist across lamprey species (Potter 1980) and within lamprey species, and body size typically correlates directly with migration distance. For
example, coastal runs of Pacific lamprey show smaller body sizes than Pacific lamprey that migrate up the Columbia and Snake Rivers to eastern Washington and Idaho (reviewed in Clemens et al. 2010). It is not known whether or how these differences in body size of Pacific lamprey relate to the small, pale, ‘day’ eel or the large, dark, ‘night’ eel. It is also not known if these differences in body size represent a genetic correlate of locally-adapted populations. An alternative hypothesis to the apparent adaptation of body size to migration distance in streams and rivers is some sort of phenotypic plasticity determined by differences in the energy density of the prey that the Pacific lamprey feed on and resultant growth rates of the lamprey. Two obvious limitations of these studies are that the genetic analyses and sampling methods differed, and the genetic analyses had not been correlated with body morphology. As a result, geography has not been controlled for, and therefore the biology of run characteristics has not been examined at a given location, over the course of time.

The first goal of this research was to describe the maturation timing and characteristics of adult Pacific lamprey over time in the Willamette River, at Willamette Falls, Oregon, in comparison with recent migrants taken from the sea/freshwater interface. This research provides information on the physiological ecology (maturation, reproductive biology, proximate analyses), and basic biology and life history (morphology, run timing, sex ratios, fecundity) of Pacific lamprey in relation to their environment. This research is covered in Chapter 4.1.

A second goal was to use multivariate analyses to explore the data to discern whether there is evidence for diversity of maturation times and associated characteristics
and interpret that aspect of biological diversity (Chapter 4.2). There are six objectives associated with these two goals. All of these objectives, with a focus on the first five, will be covered in Chapter 4.1. The sixth objective will be treated primarily in Chapter 4.2. The objectives are listed below with brief mentions of the utility of the measures posed.

**Objective 1.** Describe the external body morphology of adult Pacific lamprey to discern sexual dimorphism and reproductive life history types. Morphological differences within Pacific lamprey (body size, the spatial distance between dorsal fins) can be used to infer migration status relative to length of migration and duration of fasting (discussed above; Clemens et al. 2009).

**Objective 2.** Identify the status of reproductive maturation of adult Pacific lamprey via reproductive biopsy, histology, and hormone assays to determine if there are different reproductive life history types. Reproductive status can be assessed by four hormones that regulate reproductive maturation and function in male and female lampreys (Sower 2003; Mesa et al. 2010; Sower et al. 2011): \(17\beta\)-estradiol (\(E_2\)) and lamprey gonadotropin releasing hormones (lGnRHs) –I, -II, and –III.

**Objective 3.** Identify the life history of adult Pacific lamprey — relative to reproductive timing — vis-à-vis the relative proportions of investment in body tissue versus reproductive tissue (relative gonad size and fecundity). Fecundity may also suggest differences in life history tradeoffs with relation to body size, maturation status, and lipid content of the fish. The relative investment of somatic vs. gonadal tissue has implications for the duration of overwintering prior to spawning and also the length of the
migration ‘anticipated’ by the fish (Kan 1975; Beamish et al. 1979; Whyte et al. 1993); differences may also imply metabolic costs relating to warmer or cooler thermal regimes during the return migration.

**Objective 4.** Identify the energetic status of adult Pacific lamprey via energy reserves (total lipid content) from different tissues (muscle, liver, and ovary) to discern sexual dimorphism and reproductive life history types. Comparisons of lipid stores can be used to infer environmentally-induced life history characteristics (Meffe and Snelson 1993), including approximate estimations of fasting as a proxy for time in freshwater.

**Objective 5.** Identify potential links between morphology, and reproductive and energetic status and compare this information with lamprey recently returned from the sea (to be used as a baseline comparison).
Temporal trends in maturation characteristics of adult Pacific lamprey, *Entosphenus tridentatus*, during the freshwater spawning migration

Benjamin J. Clemens, Stan van de Wetering, Stacia A. Sower, and Carl B. Schreck

**Methods**

*Mean daily river discharge and temperature*

Data on river discharge and temperature of the Willamette River at Willamette Falls, Oregon, USA, was acquired from the gauges operated by the Oregon Department of Fish and Wildlife ([http://www.dfw.state.or.us/fish/fish_counts/willamette%20falls.asp](http://www.dfw.state.or.us/fish/fish_counts/willamette%20falls.asp)). These data were graphed to enable comparisons with trends in lamprey maturation and other characteristics.

*Fish collection*

Up to 50 adult Pacific lamprey per month were collected from our target population at Willamette Falls, OR, USA. Willamette Falls is ~204 km upstream from the Pacific Ocean, at river kilometer (RKM) 42 on the Willamette River (Figure 1). Willamette Falls is a 12 m high, horseshoe-shaped, natural falls composed of basaltic rock, flanked by a hydroelectric dam on the west side of the Willamette River. Lamprey were collected weekly to monthly during April–September of 2007 and 2008 and less frequently during 2009 (Figure 2).
During April-June, fish were collected from a fish trap in the fish ladder of the Portland General Electric hydroelectric dam at Willamette Falls. This trap has been used in other studies to capture adult Pacific lamprey migrating upstream to spawn (e.g., see Mesa et al. 2010). During the spring of 2007 and 2008, collections occurred as frequently as opportunities arose to collect lamprey from the fish trap, equating with a weekly to bi-weekly collection frequency. During June – September, when river discharge decreased substantially (compare Figure 2A with Figure 2B), we were able to safely access the base of Willamette Falls, where lamprey congregate, to collect them by hand. We collected fish once per month at the falls. We were able to collect many more fish during monthly collections, totaling 50 fish per collection. During 2009, we collected fewer fish, at about 10-15 per collection once from Willamette Falls during May and once during July.

Adult Pacific lamprey with a known freshwater history (recent migrants entering the estuary from the Pacific Ocean) were collected to use as a baseline comparison for maturation characteristics against fish collected from our target population at Willamette Falls, for which the freshwater history is unknown (Figure 1). The mouth of the Klamath River (RKM 0) provided a unique setting and opportunity to collect these recent migrants (Figure 2). In collaboration with Native American tribes of the Klamath (the Yurok and Karuk tribes) we collected lamprey from their fishing grounds at this location (Figure 2). Adult Pacific lamprey were collected from the Klamath River mouth, once each year, during April 2007, 2008, and 2009, and also once during June 2007. These fish were collected with traditional fishing spears (non-lethal; fishing process described in Peterson
Lewis 2009). The spears were the only effective means of collecting fish in the raging surf, as they entered the Klamath River.

**Measurements / tissue sampling**

A number of measurements and blood and tissue samples were taken from lamprey in the field. The measurements recorded include morphology, reproductive status (gonad histology and reproductive hormones), female fecundity, and lipid reserves. Some of these indices were measured only in certain years. For example, during 2007, we focused on fish morphology, lipid reserves, female fecundity, and gonad histology (no reproductive hormone assays). During 2008, we measured the same indices, and included reproductive hormone assays. During 2009, we focused exclusively on reproductive hormone assays and on a new index, gut morphology. In this way, data from 2007 and 2008 were used to analyze trends in fish morphology and physiology. By contrast, because we focused on reproductive hormone data for 2009, the blood plasma (assayed for 17β-estradiol or E₂—more details below) and brains (lamprey gonadotropin releasing hormone or lGnRH-I, -II, and –III) were sampled more rapidly than during 2008 (within seconds to < 5 minutes of capturing the fish during 2009, compared with several minutes to < 1 h for fish sampled from Willamette Falls and up to a few hours in the field setting of the Klamath River mouth during 2008. This procedural difference allowed us test for possible differences in hormone titres between 2009 and 2008 to test the null hypothesis that there was no difference in reproductive hormones (E₂, lGnRH-I, -II, and –III).
Sampling procedure for collected fish, 2007-2008.—The process for sampling was as follows: The first 10 fish were placed in ice water (< 10 °C) to slow their activity, weighed, and then sampled for blood before being euthanized by decapitation so that we could harvest their brains. The blood and brains were sampled for reproductive hormones (more details below). The carcasses were then thoroughly processed for all indices (morphology, lipids, fecundity, gonad histology). Any fish in excess of the first 10, in many cases up to 40 extra fish, were euthanized by MS-222 (buffered with NaHCO₃) overdose, and processed for fewer indices (morphology and gonad histology).

Sampling procedure for collected fish, 2009.—Approximately 10 fish were collected per month and these animals were sampled for reproductive hormones and gut morphology (more details provided below).

Field measurements / tissue collection / indices.—Fish collected from the trap at Willamette Falls or by fishing spear at the Klamath River mouth were immediately placed in fresh, aerated river water (66 – 132 L aerated bins) until they could be processed. In extenuating circumstances, such as at the mouth of the Klamath River, where fish had to be collected en masse prior to processing, lamprey were held for up to 5 h before they were processed, including taking blood and brain samples for reproductive hormones. At Willamette Falls, this took up to 3 h, but often was within ~1 h. In another study, there was no evidence that this duration of holding affected levels of 17β-estradiol in the blood plasma of immature adult Pacific lamprey (see Appendix B).

During 2007-2008, the fish were immobilized with ice water (~0 °C) prior to taking measurements. During the spring, when ice water did not immobilize the lamprey,
we used 50 mg *L⁻¹ of tricaine methanesulfonate (MS–222) buffered with 125 mg *L⁻¹ of NaHCO₃ to anesthetize the fish.

*Morphology*

2007-2008.—Most of the morphological measurements were done in the field with the use of a digital weight scale and a measuring board. Fish mass (herein, “body weight”) was measured to the nearest 0.1 g; total body length (TL) was measured to the nearest mm, and the internal organs excised. Livers and ovaries were weighed to the nearest 0.1 g. A small subset of the fish did not have accurate weights taken on them (8.9% of all sampled fish), due to the nature of the field setting, and so the body weights for these fish were estimated by sex-specific body weight versus TL regressions. A few of the fish (1.7% of all sampled fish) were missing tails, and so these animals were omitted from body morphology analyses.

Body weight and TL measurements were used to calculate Fulton’s condition factor, or the relative robustness of the fish:

\[(\text{body weight}) \times [(\text{TL}^3) \times 100,000]^{-1}\]

Ovary and body weight measurements were used to calculate the Gonadosomatic Index (GSI):

\[(\text{ovary weight}) \times (\text{body weight} - \text{ovary weight})^{-1}\]
Liver and body weight measurements were used to calculate the Hepatosomatic Index (HSI):

\[(\text{liver weight}) \times (\text{body weight} - \text{liver weight})^{-1}\]

We measured the distance between the two dorsal fins (dorsal fin gap) because this distance is an indicator of sexual maturation (Chapter 2; Clemens et al. 2009). Digital images of each fish were taken with a Pentax Optio W30 camera against a measuring scale (mm denominations) background. The images were used to assess the straight line distance of the dorsal fin gap to the nearest 0.1 mm via a digital software program (VistaMetrix by SkillCrest). The digital scale was ground-truthed against the measurement scale in the image with the fish (Figure 3). We measured the dorsal fin gap as the straight line distance between a super-imposed perpendicular line digitally inserted at the posterior edge of the first dorsal fin (the dorsal fin closest to the head) on the image of the fish and a second superimposed perpendicular line on the image, originating at the anterior base of the second dorsal fin. The positioning of this second perpendicular line on the image was anchored at the point where an orthogonal line inserted directly parallel to, and on top of, the fleshy base of the second dorsal fin in the image (Figure 3).

2009.—As a proxy of the general time since last feeding, we measured characteristics of the gut morphology of the lamprey. A small section of the intestine of the lamprey was excised and placed in 10% buffered formalin. Each sample of gut was processed as a cross-section for histology to a thickness of 7-10 µm, stained with hematoxylin and eosin, and later viewed under a compound light microscope (Leica...
DMLB). Images were taken with a digital camera (SPOT, Diagnostic Instruments, Inc.). The internal gut perimeter was measured to the nearest 0.1 mm, via the SPOT computer software, to trace the inner surface of the gut, including around the villi lining the gut via the software. In a similar fashion, the maximum length of the gut villi was measured as the straight line distance between the surface lining of the gut and the distal tip of individual villi for 4-6 villi, and then averaged.

**Reproductive status**

*Gonad histology.*—Gonad histology was used to determine the maturation stage of lamprey. We collected a small piece of gonad immediately posterior to the posterior tip of the liver as per Sower et al. (1985). The tissue was processed for gonad histology, cut to a section thickness of 7 µm, stained with hematoxylin and eosin, and viewed under a compound light microscope (Leica DMLB). Images were taken with a digital camera (SPOT, Diagnostic Instruments, Inc.). All histology slides were checked several times and cross-checked with each other to insure accurate and consistent staging.

The stage of maturity for oocytes was identified through a modified scale used on sea lamprey, *Petromyzon marinus*, from Lewis and McMillan (1965) and McMillan (2007), and cross-verified with Bolduc and Sower (1992). The long diameter (animal pole to vegetable pole) of four oocytes per slide was also measured with the digital camera software mentioned above and these diameters were used in conjunction with other morphological features to aid in the categorization of the stage of maturity of the oocytes.
The stage of maturity for the testes was identified with the aid of keys for sea lamprey (Fahien and Sower 1990), southern hemisphere lamprey, *Geotria australis* (Potter and Robinson 1991), and salmon, *Salmo* spp. (Dziewulska and Domagala 2003). Because the immature testes showed more complicated and nuanced morphological differences than suggested in the aforementioned papers, and there is a growing literature of complicated testicular cell line iterations in fish and other animals (e.g., see De Rooij and Russell 2000; Leal et al. 2009; Schulz et al. 2010), we opted for identification of more general maturation stages of the testes, akin to Van Eenennaam and Doroshov (1998). This amounted to four stages: 1) pre-meiosis, or an inscrutable proportion of spermatogonia and spermatocytes; 2) beginning meiosis, or the obvious presence of what many authors refer to as secondary spermatocytes (e.g., see Potter and Robinson 1991 and Fahien and Sower 1990); 3) the second meiotic division, resulting in a prevalence of spermatids; and 4) differentiated spermatozoa. Determination of staging was done by averaging the results of staging across four random areas of each testes, similar to Fahien and Sower (1990).

17β-estradiol (*E*₂).—Blood was taken from the fish via cardiac puncture, akin to Sower et al. (1985), using heparinized vacutainers. Plasma was separated by centrifugation and stored at -80 °C until assayed. Estradiol was extracted from 100 µl of plasma as described by Fitzpatrick et al. (1986) and Webb et al. (2002). Extraction efficiency was determined for all samples (69 fish, in duplicate = 138 tubes) by adding a known amount of tritiated E₂ prior to extraction. Average extraction efficiency among all of the tubes was 88.6%, and assay results were corrected for the extraction efficiency of
each sample. Concentrations of E$_2$ in the blood plasma were assayed using radioimmunoassay methods described by Sower and Schreck (1982) and modified by Feist et al. (1990). The lower limit of detection was 1.25 pg/tube (0.25 ng/ml). Any E$_2$ values <0.25 ng/ml were normalized to 0.12 ng/ml, the median between 0 and 0.25 ng/ml. The intra-assay coefficients of variation were <5%, and the inter-assay coefficient of variation was <10%. Preliminary assays showed that serial dilutions of plasma provided E$_2$ values parallel to the standard curve.

*lGnRH-I, -II, and –III.*—Fish were decapitated with a sharp knife immediately behind the last branchiopore, and brains were carefully dissected from the head of the fish via dorsal entry. Brains were promptly placed individually labeled microcentrifuge tubes (1.5 ml), and frozen on dry ice, and stored at -80 °C. The brains were processed and assayed for lamprey gonadotropins (lGnRH) –I, -II, and –III in the laboratory of Dr. Stacia Sower by the process described in Sower et al. (2011).

*Female fecundity*

A subsample of the ovary, weighing ~1 g on average, was removed from the middle portion of the ovary and preserved in individually labeled vials of 10% buffered formalin. The eggs were later transferred to 70% ethanol. Individual oocytes were carefully dissected away from the skein via metal dissecting probes with the visual aid of a dissecting microscope. The eggs were carefully spread along the bottom of a large petri dish and the “photocopy method” of fecundity enumeration (see Smith and Marsden 2007) was used to create enlarged photocopies of the eggs, which were then counted. All
samples were counted two times, and the two counts were averaged if they differed by < 10%. If the two counts differed by \( \geq 10\% \), then a third count was done and this third count was averaged with whatever previous count was within < 10\% of the third count. Two female lamprey carcasses were used to get an approximation of potential differences in fecundity across longitudinal sections of the ovary. Accordingly, 3 subsamples of ovary were used to estimate fecundity by the method above. These locations on the ovary were as follows: anterior (same location as for all other fish, described above); middle ovary, and posterior tip of the ovary.

We extrapolated or egg counts to estimate total fecundity via the weight of the entire ovary:

\[
\text{Ovary weight} \times (\text{subsample egg count} \times \text{subsample ovary weight}^{-1}).
\]

This is the method used by Kan (1975).

**Lipid reserves**

Subsamples of trunk muscle (~0.1-0.2 g), ovary tissue (~1-2 g) and whole livers (~2-6 g) were dissected from adult Pacific lamprey for the purpose of estimating the lipid content. The muscle tissue was dissected away from the trunk, from a similar location as used by Beamish et al. (1979; see Figure 3), and the ovary tissue was cut from the middle section of the ovary. All samples were placed in airtight plastic bags on ice and later stored at -20 °C. Tissue samples were thawed and dried, homogenized and then total lipids were extracted from the tissues via a Soxhlet apparatus following the protocol of Anthony et al. (2000). Total lipid mass was estimated as the difference in mass between
the dried, homogenized sample before extraction minus the mass of the same sample after
extraction. Total lipids were expressed as the percentage of the dry mass of the tissues.

Results

Mean daily river discharge and temperature

At Willamette Falls, mean daily river discharge peaked during the winters of 2007-2009
at ~2,200 – 2,500 m^3 * s^{-1}. Relatively high discharge occurred later into the season
during 2008 and 2009, relative to 2007 (Figure 4). Flows waned to a low of ~100 – 200
m^3 * s^{-1} during the summer. It was during the decreasing limbs of this hydrograph and
especially at low water, warm periods (maximum mean daily peak >25 °C) that adult
Pacific lamprey were present and we were able to collect them (Figure 4).

Fish collection

A total of 360 fish were collected at Willamette Falls during 2007-2008. The overall sex
ratio for this total collection was even at 52.3% males, 47.4% females, and 0.2% male
intersex (this intersex fish was omitted from the analyses and is described in Appendix
A). Per month, the sex ratio fluctuated between 34.0 and 63.2% males during 2007, with
weak evidence of a decrease in the percentage of males over the course of the sampling
season, late April – mid September ($r^2 = 0.2741$; % males = -0.1005(numerical sampling
date) +56.778). By contrast, the sex ratio fluctuated between 33.3 and 72.5% males
during 2008, with strong evidence of an increase in the percentage of males over the
course of the sampling season, late April – August ($r^2 = 0.7878$; % males = 0.3612\(\text{numerical sampling date}\) + 33.164). Unlike 2007, no fish were found during September. The skewed sex ratio of 72.5% males : 27.5% females occurred during August. Hundreds of dead lamprey carcasses were found at Willamette Falls during this late summer collection date, when ambient river temperature was 22.6 °C.

A total of 17 fish were collected from Willamette Falls during 2009. The overall sex ratio was biased towards males at 71% males and 29% females. However the total sample number and frequency of collections were much lower than during the previous two years.

Thirty eight fish were collected from the Klamath during 2007-2008, and the overall sex ratio was fairly even at 57.9% males and 42.1% females. Six fish were collected from the Klamath during 2009, and the overall sex ratio was even.

*Morphology*

All morphological measures varied widely over the course of the run during 2007 and 2008. Yet some general temporal trends and differences in migrant status (i.e., fish from Willamette Falls versus those from the Klamath River mouth) were evident for both sexes. Some of the trend data differed annually: 2007 data appeared to show more of a semblance of trends than 2008 data (Figure 5).

*Fish from Willamette Falls, 2007-2008.*—Condition factors, dorsal fin gaps, and total body lengths (TL) varied widely within and throughout collections dates (Figures 5, 6). However there is a weak trend for a peak in condition factors during late April – June
for both sexes, and a few of the dorsal fin gaps (DFGs) were very low at this time, approaching or equaling zero (i.e., the two dorsal fins were touching). There is also a weak trend for decreases in TL throughout the season, with smaller fish being collected during the summer (Figure 5).

Condition factors, DFGs, and TLs overlapped throughout all maturation stages, for both sexes (Figure 7). However there was evidence for a significant increase in condition factor with maturation stage for females (Kruskal-Wallis, 3 df, p = 0.0005; Figure 7A). Similarly there was evidence for a significant increase in condition factor with maturation stage for males (Kruskal-Wallis, 3 df, p = 0.0001; Figure 7B).

There was evidence for a significant decrease in dorsal fin gap with maturation stage for females (Kruskal-Wallis, 3 df, p = 0.0008; Figure 7C). Similarly there was evidence for a significant decrease in dorsal fin gap with maturation stage for males (Kruskal-Wallis, 3 df, p = 0.0001; Figure 7D).

There was evidence for a significant decrease in TL with maturation stage for females (Kruskal-Wallis, 3 df, p = 0.0000; Figure 7E). By contrast, there was no evidence for a significant change in TL with maturation stage for males (Kruskal-Wallis, 3 df, p = 0.3437; Figure 7F).

*Fish from the Klamath River mouth, 2007-2008.*—Recent migrants from the Klamath showed condition factors overlapping those of fish collected from Willamette Falls, and DFGs that were skewed higher than fish from Willamette Falls. Per maturation stage, these recent migrants were also longer than fish from Willamette Falls (Figures 6, 7).
Recent migrants were also significantly heavier than fish from Willamette Falls (MANOVA, p = 0.0001), after controlling for TL and sex. Willamette Falls (WF) males had the greatest proportional increases in body weight per unit increase in TL (body weight = (1.482)*TL – 539.07) followed by WF females (body weight = (1.3188)*TL - 429.28), then Klamath females (body weight = (1.275)*TL – 379.63), and finally, Klamath males (body weight = (1.1739)*TL – 307.15).

Recent migrants showed larger guts with longer, more complex villi than fish from Willamette Falls (Figures 8, 9).

There was no significant difference in body weight for the subset of fish for which physiological samples were taken in relation to the larger dataset (MANOVA, p = 0.8162), after controlling for TL and sex.

Reproductive status

Females.—Fish from Willamette Falls showed four maturation stages: 1) early, 2) mid, and 3) late vitellogenesis; and 4) early maturation (Figure 10). Recent migrants primarily showed the first two of these stages, early and mid vitellogenesis. However, in 2008, 1 recent migrant was in the late vitellogenic stage (Figure 11) and in 2009, 1 of 3 recent migrants was also in the late vitellogenic stage (the other 2 fish were in the early and mid vitellogenic stages; data not shown).

The maturation stages varied within date, over the course of dates, and across years. However, the proportions for each maturation stage were such that there was evidence of maturation during April-May (large proportion of fish were late vitellogenic
or in the early maturation stage). Fish collected between June and September were approximately evenly divided between mid and late vitellogenic fish, with a small percentage of fish being less mature (early vitellogenic; Figure 11). Recent migrants were less mature, being primarily early and mid vitellogenic (with the exception of the late vitellogenic fish noted above). No ovulating or spawned fish were observed.

Oocyte diameter is related to maturation stage, with oocytes increasing in size as yolky vitellogenin is incorporated (Figures 10, 12). Accordingly, oocyte diameter was highest during the spring (Figure 12A), coinciding with maturation timing (Figure 11). And oocyte diameter was lowest in recent migrants (Figure 12A), coinciding with their maturation stages (Figure 11).

Gonadosomatic index (GSI) was highest during the spring, and this also matched the determination of maturation timing observed above (Figure 12B). There was a significant increase in GSI with maturation stage (Kruskal-Wallis, 3 df, p < 0.0000), to what appears to be an exponential degree (Figure 13A). The GSI data for Willamette Falls fish is positively skewed by a few outliers in the ~15-45% range. Most Willamette Falls fish showed GSIs ~ 2-15%. The GSI of recent migrants was comparatively low (< 5%).

Ovary lipids ranged from ~25-65%, with a median around 30-45% (Figure 12C). Whereas ovary lipids trended high during the spring of 2007, ovary lipids appeared relatively low during the following spring of 2008, and the recent migrants showed ovary lipid levels similar to those of fish from Willamette Falls (Figure 12C). There was
evidence for a significant decrease in ovary lipids with maturation stage in females (Kruskal-Wallis, 3 df, p < 0.0000; Figure 13B).

Fecundity was quite variable with no apparent trend during 2007 and a weak upward trend during the spring of 2008 (Figure 12D). There was suggestive, but inconclusive evidence for a decrease in fecundity with maturation stage in females (Kruskal-Wallis, 3 df, p = 0.0903; Figure 13C). Mean monthly values for absolute fecundity ranged from ~100,000-161,000 eggs, with most monthly means ranging between ~114,000 and 140,000 eggs. Mean monthly values for relative fecundities (egg * body weight\(^{-1}\)) ranged between 335 and 460 egg * (g of fish\(^{-1}\)). Recent migrants showed fecundities skewing slightly higher than the median of Willamette Falls fish (monthly mean of ~291,000 estimated total eggs) and one incredibly fecund outlier had an estimated 632,203 eggs (Figure 12D). The relative fecundity of these recent migrants was also higher than that of fish from Willamette Falls at 562 eggs * (g of fish\(^{-1}\)).

There was a 55% decrease in fecundity between the anterior and middle (mean: 3,245 eggs) portions and the posterior (1,447 eggs) portion of the ovary for the female that had a GSI of 13.6%. In contrast, there was a 79% decrease in fecundity between the middle (8,566 eggs) and posterior (1,809 eggs) portions of the ovary in the female that had a GSI of 4.0%.

*Males.*—Fish from Willamette Falls showed four maturation stages: 1) spermatogonia / spermatocytes (SG/SC); 2) spermatocytes (SC), 3) spermatids (SD), and 4) spermatozoa (SZ; Figure 14). Recent migrants from the Klamath River mouth primarily showed the first stage, SG/SC. However in 2007, 1 recent migrant was in the
SC stage (Figure 15) and in 2009, 1 of 3 recent migrants was also in the SC stage (the other 2 fish were in the SG/SC stage; data not shown).

The maturation stages varied within date, over the course of dates, and across years. However, the proportions for each maturation stage were such that there was evidence of maturation during April-June (large proportion of fish had SD and SZ). Fish collected between July and September were mostly SG/SC (Figure 15).

Reproductive hormones (2008 and 2009)

Lamprey gonadotropin releasing hormones (lGnRHs) were incredibly variable over the course of collection dates (Figure 16), and in relation to maturation stage for both sexes (Figure 17).

There was no evidence of a significant increase in lGnRH-I with maturation stage in females (Kruskal-Wallis, 3 df, p = 0.1276; Figure 17A). Similarly, there was no evidence of a significant increase in lGnRH-I with maturation stage in males (Kruskal-Wallis, 3 df, p = 0.5970; Figure 17B).

There was no evidence of a significant increase in lGnRH-III with maturation stage in females (Kruskal-Wallis, 3 df, p = 0.1166; Figure 17C). Similarly, there was no evidence of a significant change in levels of lGnRH-III with maturation stage in males (Kruskal-Wallis, 2 df, p = 0.8427; Figure 17D).

The hormone lGnRH-II peaked during the summer of 2008 in both sexes (Figure 16). There was no evidence of a significant decrease in lGnRH-II with maturation stage in females (Kruskal-Wallis, 3 df, p = 0.7774; Figure 17E). There was no evidence of a
significant increase in lGnRH-II with maturation stage in males (Kruskal-Wallis, 2 df, p = 0.1638; Figure 17F).

Plasma levels of 17β-estradiol (E<sub>2</sub>) were quite variable over collection dates (Figure 18) and maturation stages (Figure 19). There was no evidence of a significant increase in levels of E<sub>2</sub> with maturation stage in females (Kruskal-Wallis, 3 df, p = 0.1114; Figure 19A). However, there was evidence of a significant decrease in E<sub>2</sub> with maturation stage in males (Kruskal-Wallis, 3 df, p = 0.0360; Figure 19B). However, there was a trend for an increase in plasma levels of E<sub>2</sub> with maturation stage for females, and this was evident in the high E<sub>2</sub> levels of 4 outliers (Figures 18A, 19).

There was no significant difference in lGnRH-I, -II, -III, or E<sub>2</sub> with respect to sex (2008-2009 data inclusive; Mann-Whitney U test, p > 0.05). There was no significant difference in these 4 hormones with respect recent migrants vs. Willamette Falls fish (2008-2009 data inclusive; Mann-Whitney U test, p > 0.05).

For females, there was no significant difference in lGnRH-I, -III, and E<sub>2</sub> with respect to year (Mann-Whitney U test, p > 0.05). However, lGnRH-II was significantly higher during 2008 than during 2009 (Mann-Whitney U test, p = 0.001) (Figure 16E). This difference in lGnRH-II between years may be due in part to the later stage of maturation for the 2008 females (modal gonad stage = “late vitellogenesis”) in comparison to 2009 females (“mid vitellogenesis”). However, the lack of a trend between lGnRH-II and maturation stage (Figure 17E) suggests that maturation stage may have played a minor role in lGnRH-II being significantly higher in 2008 versus 2009.
For males, there was no significant difference in lGnRH-I and E$_2$ with respect to year (Mann-Whitney U test, p > 0.05). However, lGnRH-II was significantly higher during 2008 than during 2009 (Mann-Whitney U test, p = 0.000; Figure 16F) and lGnRH-III was significantly higher during 2009 than during 2008 (Mann-Whitney U test, p = 0.002; Figure 16D). This difference in lGnRH-II and –III cannot be attributed to differences in maturation status, as there were none: spermatocytes were the modal gonad stage, with an identical range of gonad stages, from spermatocytes to spermatozoa for both 2008 and 2009.

**Lipid reserves**

Median values of muscle lipids for both sexes was ~19-45% throughout the sampling periods. Muscle lipids ranged from ~0% to ~45% for females and ~0% to ~60% for males. Muscle lipids peaked during the spring time for both sexes and tapered off during the summer (Figure 20E, F). There was no evidence of a change in muscle lipids with respect to maturation stage for females (Kruskal-Wallis, 3 df, p = 0.2822; Figure 21A). Similarly, there was no evidence of a change in muscle lipids with maturation stage in males (Kruskal-Wallis, 3 df, p = 0.6525; Figure 21B).

Liver lipids for a few females from Willamette Falls were quite low at 8-17% for late vitellogenic and early maturing females (n = 13) during the spring, with most females around ~50-75% (Figure 20C). By contrast, the lowest lipid levels for the livers of males from Willamette Falls was ~45%, with most fish around ~60-70% (Figure 20D).
Liver lipids decreased significantly with maturation stage in females (Kruskal-Wallis, 3 df, p < 0.0000; Figure 21C). Similarly, liver lipids decreased significantly with maturation stage in males (Kruskal-Wallis, 3 df, p = 0.0290; Figure 21D).

There was suggestive statistical evidence of a difference in the HSI of adult Pacific lamprey with regards to fish collected from Willamette Falls versus recent migrants (MANOVA; p = 0.0530) and evidence of a significant difference with respect to sex (MANOVA; p = 0.0001), suggesting different physiological trajectories with regards to migration status and sex. Indeed the HSI for recent migrant females skewed above the median trend line of females from Willamette Falls, whereas for males, the HSI showed an opposite pattern with respect to migration status (Figure 20).

All females (recent migrants collected from the Klamath River mouth plus fish collected from Willamette Falls) showed a significant increase in HSI with maturation stage (Kruskal-Wallis, 3 df, p = 0.0041; Figure 21E). By contrast, there was no evidence of a significant change in HSI for all males (Kruskal-Wallis, 3 df, p = 0.3033; Figure 21F).

**Discussion**

We monitored maturation characteristics of adult Pacific lamprey at Willamette Falls, Oregon, USA, and compared their maturation characteristics with recent migrants. The results suggest a unimodal spawn timing during the spring, with relatively immature fish for both sexes being collected after this period. The immature fish had maturation stages
and phenotypic characteristics similar to recent migrants. Four recent migrants (2 females and 2 males) were surprisingly far along in the maturation process. Below we briefly discuss the availability of fish at Willamette Falls. Next we discuss individual migration characteristics followed by a synthesis of the maturation characteristics in relation to run diversity.

Fish availability at Willamette Falls

Adult Pacific lamprey begin to appear distinctly and abundantly during April – June before tapering off during August – September. They disappear after mid-September (personal observations and Oregon Department of Fish and Wildlife fish counting records, 2005-2008, Sep. 2008). These time periods coincide with when we were able to collect fish.

Individual maturation characteristics

The condition factor of Pacific lamprey collected from Willamette Falls peaked during the spring maturation, at the same time that some of the dorsal fin gaps completely closed. Increases in body length of Pacific lamprey during parasitic feeding at sea are accompanied by a decrease in the fish’s condition factor (Orlov et al. 2008). Conversely shrinkage in body length of Pacific lamprey during fasting, to fuel gonadal maturation (Larsen 1980; Clemens et al. 2009) would explain an increase in condition factor. Our data support these morphological trends with maturation. The condition factors of our recent migrants overlapped the range of values reported for Pacific lamprey captured at
sea: 0.14-0.26 for our Pacific lamprey versus 0.16-0.49 (range of monthly means; Orlov et al. 2008).

In our female Pacific lamprey, none of the lGnRHs showed significant trends with maturation stage, which was surprising, given that the lGnRHs have specific functions in regulating reproductive maturation and function in lampreys (reviewed in Clemens et al. 2010; see also Sower 2003 and Sower et al. 2011). In our Pacific lamprey, lGnRH-III was significantly lower in 2008 versus 2009, which we could not attribute to differences in maturation stage. This may suggest that the longer holding times prior to sampling in 2008 may have decreased lGnRH-III levels in the male Pacific lamprey or that some other unknown factor lowered these levels. The hormone lGnRH-II peaked in our Pacific lamprey during the summer of 2008, and was significantly higher for this year in relation to 2009 in both sexes. Differences in levels of lGnRH-II between years for both sexes could not be attributed to differences in maturation stage. Again this may suggest that the longer holding times prior to sampling in 2008 may have affected both male and female Pacific lamprey or that some other unknown factor increased levels of lGnRH-II in the fish sampled during this year.

In the anadromous sea lamprey, lGnRH-I may influence spawning behavior, and levels of this hormone in females remain low during their final maturation (reviewed in Clemens et al. 2010; see also Sower et al. 2011). In male sea lamprey, lGnRH-I levels varied throughout the season with a final elevation coinciding with final maturation (Sower et al. 2011). The hormone lGnRH-III regulates the final maturation, and it either increases significantly for males or decreases significantly for females during the final
maturation (reviewed in Clemens et al. 2010; see also Sower 2003 and Sower et al. 2011). The function of lGnRH-II is unknown, but in male anadromous sea lamprey, levels of this hormone varied before peaking and then declining prior to spawning. In females, levels of lGnRH-II were relatively high early in the season and then dropped and remained low (Sower et al. 2011).

The function of the lGnRHs in lampreys is still not fully understood, including if there are differences between sea and Pacific lampreys (Clemens et al. 2010). For example, one key difference is that the maturation processes of Pacific lamprey may occur over a more protracted period of time during the prolonged spawning migration of Pacific lamprey (Clemens et al. 2010). Whereas our Pacific lamprey females showed early stages of vitellogenesis, anadromous sea lamprey, collected in freshwater, show only the later stages of vitellogenesis through maturation and spawning (Bolduc and Sower 1992). Similarly, our Pacific lamprey males showed a mix between spermatogonia and spermatocytes, whereas male anadromous sea lamprey in freshwater show only later stages of maturation, from beginning meiosis with spermatocytes onward (Fahien and Sower 1990).

The lamprey brain integrates photoperiod and water temperature stimuli, and relays this information to the hypothalamus of the brain, which controls the release of GnRHs to the pituitary. The pituitary then releases gonadotropin hormone, which targets the gonads to begin steroidogenesis (including the production of E2, which helps regulate reproductive maturation and function in both sexes of lampreys) and gametogenesis (Sower 2003; Bryan et al. 2008).
Plasma levels of E$_2$ in our Pacific lamprey ranged between 0.12 ng/ml and 7.10 ng/ml, overlapping those reported by Mesa et al. (2009) for Pacific lamprey held in artificial streams from fall through late spring. Our male lamprey had a significant decrease in E$_2$ with respect to maturation stage. We found no significant differences in E$_2$ with respect to sex, migration history or year. The lack of a significant difference of E$_2$ between years, with respect to differences in holding time, agrees with our other research, in which we held and then sampled the blood of Pacific lamprey for 0, 1, 3, and 6 h post collection and found no significant differences with respect to holding times (Appendix B).

Our Pacific lamprey had mean monthly fecundities of ~100,000-161,000 eggs. Pacific lamprey were previously reported to have a mean fecundity of 140,312 eggs (range: 98,300 – 238,400 eggs; Kan 1975). The maximum fecundity reported by Kan (1975) is similar to the monthly mean of our recent migrants (~291,000 estimated total eggs).

Our data suggests that the fecundity of the lamprey ovary decreases from the anterior-middle to the posterior sections of the ovary. Hence our fecundity estimates, taken from the middle portion of the ovary, are likely overestimates for most fish. We can only draw conclusions for the lamprey from which we examined longitudinal sections, fish having GSIs of 4.0 and 13.6%. Our data suggests that Pacific lamprey with lower GSI may have a more drastic decrease in fecundity between the anterior-medial and posterior portions of the ovary than more mature fish, so their fecundity is likely to be more strongly overestimated.
The total number of eggs is directly related to adult body size in lampreys. Therefore Pacific lamprey fecundity is intermediate to that of the larger anadromous sea lamprey (mean fecundity of 171,589 to 210,228 eggs) and the relatively small Great Lakes sea lamprey ranging from 34,000 to 110,300 eggs (reviewed in Clemens et al. 2010).

Ovary lipids decreased with maturation, which is counter-intuitive, given that oocytes incorporate vitellogenin (lipids) during maturation. However it might be that the larger, more yolky eggs were packed less densely with more ovarian fluid or spaces between the eggs than smaller, less mature oocytes.

Liver lipids decreased with maturation stage, and liver lipids for a few females from Willamette Falls were quite low at 8-17% during the spring (these fish were in the late vitellogenic to early maturation stages). This is interesting in that the liver supplies lipids to the developing oocytes, which incorporate these lipids in the form of vitellogenin (Youson 1981), so it makes sense that liver lipids would be relatively low in fish that had oocytes in the later stages of maturation. Most of our Pacific lamprey females had ~50-75% lipids in their livers, whereas most males from Willamette Falls were ~60-70%. These lipid contents are very high in comparison with Pacific lamprey that did not feed for extended periods of time, with an overall range of 7 – 26% liver lipids (Whyte et al. 1993). The liver lipid contents of our fish are also very high in comparison with anadromous sea lamprey, which were ≤ 26% (Beamish et al. 1979), and landlocked sea lamprey, which were ≤ 14% (Kott 1971).
Median values of muscle lipids for both sexes was ~19-45% for both sexes throughout the sampling periods. This range is quite high in comparison with anadromous sea lamprey at \( \leq 8\% \) (Beamish et al. 1979), and landlocked sea lamprey at \( \leq 13\% \) (Kott 1971). Males that were recent migrants had higher muscle lipid levels than males from Willamette Falls, which makes sense given that the recent migrants had only recently ceased feeding and began their upstream migration.

*Maturation characteristics: Synthesis*

The goals of this research were to describe the maturation timing, characteristics and diversity of adult Pacific lamprey at Willamette Falls, Oregon, in comparison with recent migrants in the Klamath system. Gonad histology and other characteristics indicate that the final maturation and spawning period was unimodal, occurring late April – early June. This time period agrees with other research on the spawning period of Pacific lamprey (e.g., see Clemens et al. 2010). And this time period preceded the very warm summer temperatures that occur at Willamette Falls (\( > 20^\circ C \)) during July – mid-September. We have unpublished data suggesting that the few mature males that occurred during the end of the spawning period in early July showed evidence of degenerative testes, as did males from other maturation stages during July – September. We also observed a skewing in sex ratios towards a higher percentage of males with collection date, culminating in a population that was 72.5% males during August, the same collection date in which we observed hundreds of dead lamprey carcasses at Willamette Fall, when ambient river temperature was 22.6 \(^\circ C\). This leads us to
hypothesize that there may be a connection between higher water temperatures and relatively fewer females, either because many females left the falls area or died.

Immature fish collected at Willamette Falls during the summer months (late June – September) in many respects resembled recent migrants. This suggests that lamprey collected during the summer at Willamette Falls were relatively recent migrants from the ocean, whereas the mature fish collected during the spring were likely fish that had already resided in the river for a longer period of time. These immature fish might have been able to mature and spawn the following year, after experiencing the warm summer temperatures. Water temperatures have been found to be positively correlated with the reproductive hormones lGnRH-I, -II, and –III and E$_2$ in sea lamprey, *Petromyzon marinus* (Sower et al. 2011). We have also found a positive correlation with water temperature and E$_2$ (Appendix B), and an association with exposure to warm water temperature in the summer and maturation the following spring in adult Pacific lamprey. In the laboratory, 21.8°C (mean) during the summer was associated with sexual maturation in 100% of Pacific lamprey the following spring, whereas only 53% of the lamprey held at 13.6°C matured the following spring (Chapter 2; Clemens et al. 2009).

In comparison with lamprey collected from Willamette Falls, recent migrants generally had greater body sizes, greater dorsal fin gaps, and higher muscle lipids (males) than lamprey collected from Willamette Falls, likely because they had only recently finished feeding (their guts were not atrophied like lamprey from Willamette Falls (Figures 8, 9). In general, recent migrants were also less mature than fish collected from Willamette Falls—with some exceptions.
During 2007-2009 we observed 2 females that were recent migrants in the late vitellogenic maturation phase and 2 males in the spermatocyte phase; all other recent migrants were relatively immature. These data provide modest evidence that some migrant Pacific lamprey will spawn in the same season in which they enter freshwater. It is interesting that Moyle (2002) mentioned “In the Klamath River, there may be at least two distinct runs: a spring run that spawns immediately after the upstream migration and a fall run, which holds over and spawns the following spring”.

Although maturation was unimodal, there was substantial variation in morphology, reproductive hormones, fecundity, and lipid reserves of the Pacific lamprey at Willamette Falls within and across collection dates. Large variations in these parameters were also evident in relation to the stage of gonad maturation. It is unclear to what degree this variation is genetically or environmentally induced. Microsatellite genetic markers, collected from the same fish that we report on for physiological measures, indicate no difference in haplotypes between the early run (most fish are maturing—see figures 11 and 15) and the late run (immature fish) at Willamette Falls (Docker 2011). It is interesting that from a genetic standpoint, there is conflicting evidence for population structure across a very wide geographical scale. This suggests either that population structure does not exist or that there is some form of weak population structuring (reviewed in Clemens et al. 2010). A prima facie consideration of these genetic data suggests a lack of stock structure at Willamette Falls, perhaps akin to the broader finding of little to no stock structure on grander geographical scale. The variability in our biological data from one geographical location (Willamette Falls),
collected over several dates, may support the genetic findings, suggesting a genetic mixing of recent and “old” migrants. Diversity in run cohorts may begin as the fish enter freshwater, as evidenced by the relatively mature female and male lamprey collected from the Klamath River mouth. This suggests some degree of genetic inducement or stock structuring on maturation timing, that it is not solely linked to freshwater temperatures (see Chapter 2; Clemens et al. 2009) or other factors in the freshwater environment. This stock structure or diversity of run cohorts seems to be at least twofold, with the potential for nuanced variations on the second migration/spawning strategy: 1) recent migrants, spawning fish; 2) recent migrants, fish mature and spawn at least 1 or more years later; and old migrants (arrived in freshwater ~ 1+ years prior and will spawn this year. In the laboratory, adult Pacific lamprey can live for up to ~2 years in freshwater without feeding (Whyte et al. 1993) and in our unpublished studies we have had 1 individual live for up to 3 years in freshwater without feeding. Like Pacific lamprey, river lamprey, *Lampetra fluviatilis*, can exhibit a prolonged freshwater residency of several months prior to spawning (Abou-Seedo and Potter 1979; Kelly and King 2001), and run diversity has been noted in this species. This run diversity includes a larger “typical” form and a smaller “praecox” form that was within 20% of the total length of the typical form (Abou-Seedo and Potter 1979). It is interesting that in freshwater, fasting and maturing Pacific lamprey shrink in total body length to around 20% (18-30%) (Clemens et al. 2010), and that river lamprey also shrink to a large extent (Larsen 1980). We therefore wonder whether the morphological diversity in river lamprey is a function of how long the “praecox” forms have been fasting in freshwater,
and if the typical river lamprey forms are recent migrants—possibly akin to “day eels” (praecox) and “night eels” (typical form).

A simultaneous consideration, through multivariate statistics, of several of the characteristics that we have described is needed to objectively identify some of the maturation and migration life history diversity of adult Pacific lamprey (see Chapter 4.2).

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Figure 1. Map of the USA (inset) and Oregon and northern California (expanded view), showing the two locations where adult Pacific lamprey, *Entosphenus tridentatus*, were collected: Willamette Falls, Oregon (Figure 2A, B), and at the Klamath River mouth (interface of the Pacific Ocean and the Klamath River; Figure 2C, D). Additional details are provided in the Methods. Map created by Lance Wyss.
Figure 2. Willamette Falls during spring, with high river discharge (A), and summer, with typical low river discharge (B). The Klamath River mouth from two perspectives: (C) Top down perspective, showing the Klamath River estuary, with the Klamath River to the left of the spit beach in the middle of the image, and the Pacific Ocean with incoming surf on the right. The dashed ellipsis indicates where adult Pacific lamprey were collected; (D) Perspective from the beach (rock outcropping on the right is the perspective from which picture “C” was taken). A Yurok tribal fisherman is shown in the foreground, fishing for adult Pacific lamprey for the study presented in this paper. Photos credits: Suzanne Moellendorf and Ben Clemens (A); Ben Clemens (B, C, and D).
Figure 3. Image of the dorsal fins and tail of an adult Pacific lamprey. The dashed vertical lines encapsulate the dorsal fin gap, the straight line area that we measured (double headed arrow). Also shown is the location from which the trunk musculature was excised (indicated by the rectangle, which is scaled to size) and later measured for lipid content. Photo credit: Tiia Workman and Ben Clemens.
Figure 4. Mean daily flow (top graph) and temperature, by date, at Willamette Falls, Oregon (U.S.A.). In the top graph, the open squares at the base of the graph indicate dates when fish were collected from the trap in fish the ladder of the hydroelectric dam flanking the west side of the falls; the open triangles indicate dates when fish were collected from the base of Willamette Falls. The dark triangles in the bottom graph indicate our temperature measurements at the time that we began our fish collections. Data from the Oregon Department of Fish and Wildlife (http://www.dfw.state.or.us/fish/fish_counts/willamette%20falls.asp).
Figure 5. Scatter plots of morphological measures of adult Pacific lamprey over several collection dates, during 2007-2008 (plots for females in left column, plots for males in the right column). Each symbol = value for an individual fish. Filled squares = values of recent migrants, collected from the Klamath River mouth. All other symbols are data points for fish collected from Willamette Falls. Fulton’s Condition Factor (A and B) and dorsal fin gap (E and F). TL = total body length (E and F). The data are from collections made primarily at Willamette Falls during April 2007-September 2007 and April 2008-August 2008 (no fish were found and so none were collected during September 2008).
Figure 6. Example of size ranges from lamprey collected from the fish ladder trap at Willamette Falls on June 30, 2008. The total body length measures for the largest fish (top) and smallest fish (bottom) are shown; both of these fish were males with spermatogonia and spermatocytes.
Figure 7. Scatter plots of morphological measures of adult Pacific lamprey, collected during 2007-2008, in relation to stages of gonad maturation (plots for females in left column, plots for males in the right column). EVT = early vitellogenesis; MVT = mid vitellogenesis; LVT = late vitellogenesis; EMT = early maturation. SG/SC = spermatogonia/spermatocytes; SC = spermatocytes; SD = spermatids; SZ = spermatozoa. Each symbol = value for an individual fish. Filled triangles = median values for recent migrants, collected from the Klamath River mouth. All other symbols are data points for fish collected from Willamette Falls. The dashed lines indicate general trends from least squares simple regressions for fish from Willamette Falls. TL = total body length (A and B). Fulton’s Condition Factor (C and D) and dorsal fin gap (E and F) are defined in the methods. Asterisks next to the letters indicate trends that were statistically significant when value for all fish (recent migrants plus fish from Willamette Falls) were tested against maturation stage (details in Results section).
Figure 8. Cross-sectional histology (A and B) and gross anatomy (C and D) of guts of adult Pacific lamprey. Scales on the histology images = 1.0 mm; both images are from guts sampled immediately behind the posterior lobe of the liver; the typhosole can be seen in both images. Arrows on the gross anatomy images are indicate the guts in situ. Recent migrant entering the Klamath River mouth from the Pacific Ocean (A and C) and migrant of unknown freshwater history from Willamette Falls, Oregon, U.S.A. (B and D). Note the larger perimeter and diameter of the gut and longer villi from the recent migrant (6 measuring bars are indicated on the histology image) (see also Figure 9).
**Figure 9.** Gut morphology measurements, including gut perimeter (A) and gut villi length (B). Filled triangles = males; open circles = females. The brackets indicate recent migrants entering the Klamath River mouth from the Pacific Ocean during April. All other fish (May and July) were collected from Willamette Falls, Oregon, U.S.A. See also Figure 8.
Figure 10. Images of ovary histology of adult Pacific lamprey. (A) Early vitellogenesis; (B) mid-vitellogenesis; (C) late vitellogenesis; (D) early maturation (pre-ovulation). Scale in all images = 0.1 mm.
Figure 11. Percentage of female Pacific lamprey at each of four stages of maturity, as determined by gonad histology. The top graph is 2007 data and the bottom graph is 2008 data. The left-most column, “Klam” is from recent migrant lamprey, fish that were collected at the interface of the Pacific Ocean and the Klamath River mouth, as they entered the river; the remainder of the fish are from Willamette Falls. Italicized numbers above each month indicate the sample size from which the proportions were determined. Data from 2009 generally agreed with 2007-2008 trends, with 1 fish in the mid vitellogenic stage, and 2 in the late vitellogenic stages during May; and 2 in the late vitellogenic stages during July. And of recent migrants in 2009, 1 fish was in the late vitellogenic stage, 1 was in the early vitellogenic stage, and 1 was in the mid vitellogenic stage (data not shown).
Figure 12. Scatter plots of ovary characteristics for adult Pacific lamprey females, over several collection dates during 2007-2008. Characteristics include oocyte diameter (A), GSI = gonadosomatic index (B); ovary lipids (C), and fecundity = absolute fecundity (D). Each symbol = value for an individual fish. Filled squares = values of recent migrants, collected from the Klamath River mouth. All other symbols are data points for fish collected from Willamette Falls.
Figure 13. Scatter plots of ovary measures of adult Pacific lamprey, collected during 2007-2008, in relation to stages of gonad maturation. EVT = early vitellogenesis; MVT = mid vitellogenesis; LVT = late vitellogenesis; EMT = early maturation. Each symbol = value for an individual fish. Filled triangles = median values for recent migrants collected from the Klamath River mouth. All other symbols are data points for fish collected from Willamette Falls. The dashed lines indicate general trends from least squares simple regressions for fish from Willamette Falls. GSI = gonadosomatic index (A), ovary lipids (B), and fecundity (C). Asterisks next to the letters indicate trends that were statistically significant when value for all fish (recent migrants plus fish from Willamette Falls) were tested against maturation stage (details in Results section).
**Figure 14.** Images of testes histology of adult Pacific lamprey. (A) spermatogonia; (B) spermatocytes; (C) spermatocytes and spermatids (examples of spermatids indicated by arrows); (D) mature spermatozoa. Scale in all images = 20 µm.
**Figure 15.** Percentages of male Pacific lamprey at each of four stages of maturity, as determined by gonad histology. The top graph is from 2007; the bottom graph from 2008. The left-most column, “Klam” is from recent migrant lamprey, fish that were collected at the interface of the Pacific Ocean and the Klamath River mouth, as they entered the river. Italicized numbers above each month indicate the sample size from which the proportions were determined. Data from 2009 generally agreed with 2007-2008 trends, with 2 fish in the SG/SC stage, 1 in the SC stage, and 1 in the SZ stage during May; and 8 in the SG/SC during July. And of recent migrants in 2009, 1 fish was in the SC stage, and 2 were in the SG/SC stage (data not shown).
Figure 16. Scatter plots of lamprey gonadotropin releasing hormones (lGnRHs) for adult Pacific lamprey, collected over several dates during 2008-2009 (plots for females in left column, plots for males in the right column). Each symbol = value for an individual fish. Filled squares = values of recent migrants, collected from the Klamath River mouth. All other symbols are data points for fish collected from Willamette Falls.
Figure 17. Scatter plots of lamprey gonadotropin releasing hormones (lGnRHs) of adult Pacific lamprey, collected during 2008-2009, in relation to stages of gonad maturation (plots for females in left column, plots for males in the right column). EVT = early vitellogenesis; MVT = mid vitellogenesis; LVT = late vitellogenesis; EMT = early maturation. SG/SC = spermatogonia/spermatocytes; SC = spermatocytes; SD = spermatids; SZ = spermatozoa. Each symbol = value for an individual fish. Filled triangles = median values for recent migrants, collected from the Klamath River mouth. All other symbols are data points for fish collected from Willamette Falls during 2008 and 2009. The dashed lines indicate general trends from least squares simple regressions for fish from Willamette Falls. lGnRH-I (A and B), lGnRH-II (C and D), and lGnRH-III (E and F). There was no statistically significant change in any of these measures when all fish (recent migrants plus fish from Willamette Falls) were tested against maturation stage (details in Results section).
Figure 18. Scatter plots of 17β-estradiol from the plasma of adult Pacific lamprey females (A) and males (B), collected over several dates during 2008-2009. Each symbol = value for an individual fish. Filled squares = values of recent migrants collected from the Klamath River mouth. All other symbols are data points for fish collected from Willamette Falls.
Estradiol (ng/ml) vs Gonad stage

**A**

- Female
- **EVT**
- **MVT**
- **LVT**
- **EMT**

**B**

- Male
- **SG/SC**
- **SC**
- **SD**
- **SZ**

*Note: The diagram illustrates the relationship between estradiol levels and gonad development stages.*
Figure 19. Scatter plots of plasma levels of 17β-estradiol from adult Pacific lamprey, collected during 2008-2009, in relation to stages of gonad maturation (plots for females in left column, “A”; plots for males in the right column, “B”). EVT = early vitellogenesis; MVT = mid vitellogenesis; LVT = late vitellogenesis; EMT = early maturation. SG/SC = spermatogonia/spermatocytes; SC = spermatocytes; SD = spermatids; SZ = spermatozoa. Each symbol = value for an individual fish. Filled triangles = median values for recent migrants, collected from the Klamath River mouth. All other symbols are data points for fish collected from Willamette Falls. The dashed line indicates general trends from least squares simple regressions for fish from Willamette Falls. The asterisk next to the graph for males indicates a statistically significant trend when value for all fish (recent migrants plus fish from Willamette Falls) were tested against maturation stage (details in Results section).
Figure 20. Scatter plots of liver morphology (HIS = hepatosomatic index; A and B) and lipid reserves (liver and muscle; C – F) for adult Pacific lamprey collected during 2007-2008 (plots for females in left column, plots for males in the right column). Each symbol = value for an individual fish. Filled squares = values of recent migrants, collected from the Klamath River mouth. All other symbols are data points for fish collected from Willamette Falls.
Figure 21. Scatter plots of lipid reserves and liver morphology (HSI = hepatosomatic index) of adult Pacific lamprey, collected during 2007-2008, in relation to stages of gonad maturation (plots for females in left column, plots for males in the right column). EVT = early vitellogenesis; MVT = mid vitellogenesis; LVT = late vitellogenesis; EMT = early maturation. SG/SC = spermatogonia/spermatocytes; SC = spermatocytes; SD = spermatids; SZ = spermatozoa. Each symbol = value for an individual fish. Filled triangles = median values for recent migrants, collected from the Klamath River mouth. All other symbols are data points for fish collected from Willamette Falls. The dashed line indicates general trends from least squares simple regressions for fish from Willamette Falls. Muscle lipids (A and B); liver lipids (C and D), and HSI (E and F). Further details can be found in the methods. Asterisks next to the letters indicate statistically significant trends when values for all fish (recent migrants plus fish from Willamette Falls) were tested against maturation stage (details in Results section).
Maturation characteristics and phenotypic diversity of adult Pacific lamprey, *Entosphenus tridentatus*, provide evidence for temporal intermixing of two cohorts or “runs”

Benjamin J. Clemens, Stan van de Wetering, Stacia A. Sower, and Carl B. Schreck

**Methods**

The fish considered here are the same individuals assessed in Chapter 4.1 to ascertain maturation characteristics.

**Statistics**

To determine the maturation and phenotypic diversity of adult Pacific lamprey, two separate cluster analyses were conducted for each sex (1 male intersex from 2007 was omitted and is reported on in Appendix A). The analyses included fish from both the Klamath (recent migrants) and Willamette Falls. The two-step cluster analysis (log-likelihood dissimilarity measure) procedure (SPSS 18.0.0) was used, with gonad stage as the categorical variable to construct preclusters. For males, the continuous variables used to construct the final clusters were Fulton’s condition factor (“K”), the hepatosomatic index (HSI), and dorsal fin gap (DFG; the distance between the two dorsal fins). For females, the same continuous variables were used to construct the final clusters, with the addition of the gonadosomatic index (GSI). In the interest of using several characteristics
to form clusters, fish with missing data for K, HSI, DFG, and GSI were omitted from the analyses. In this way 32% of the 361 lamprey collected from Willamette Falls during 2007-2008, and 53% of the 38 lamprey collected from the Klamath River mouth (recent migrants) during 2007-2008 were omitted from the analyses.

The continuous variables were adjusted for time by regressing each against ordinal date in individual, simple linear regressions, and then adding the residuals from each regression to the mean value for each variable. The data were sorted randomly to minimize any effects of chronological or other types of data ordering.

Pearson Product moment correlations were used to ascertain strength of associations among indices. Indices with an $r \geq 0.5$ were examined further within each cluster for each sex. Specifically, the indices were checked to insure that they met the assumptions of ANOVA, including homogenous variance and normal distributions. Logarithmic transformations were conducted on the data where necessary to improve linearity. The MANOVA models included a dependent variable, an independent variable, and “cluster” as the grouping variable. In this way, the two clusters for a given sex were tested to see if they were significantly different. Indices that had too few data points and obvious outliers did not lend themselves to analyses with linear models (e.g., $\ln$GnRH-I, III, and II, and in some cases, $17\beta$-estradiol or $E_2$). Instead, the Mann-Whitney U tests were used for these particular indices with regards to “location” “sex”, “year” and “cluster”. 
Results

Clusters

Two clusters were identified for females, and 2 for males. For females, the 2 clusters were maturing/mature fish (late vitellogenesis — early maturation gonad stages) and immature fish (early — late vitellogenesis). For males, the 2 clusters were maturing/mature fish (spermatocytes, spermatids, or spermatozoa) and immature fish (spermatogonia/spermatocytes; Table 1). For both sexes, lamprey in the maturing/mature (MM) cluster occurred within the same collection dates as immature fish, indicating that these fish were intermixed in time and space at Willamette Falls. All 8 female lamprey clustered with other immature fish, whereas 1 male lamprey clustered with the maturing/mature fish (it was in the spermatocyte maturation stage), and the other 11 male recent migrants clustered with other immature fish collected at Willamette Falls. The intermixing of the mature/maturing and immature cohorts for both sexes was evident in the overlap of morphological characters (Fulton’s condition factor and dorsal fin gap, Figure 1), and the overlap of the hepatosomatic index (Figure 2), suggesting that morphological characters may hold limited utility in discerning maturation stages. By contrast, GSI appeared to be higher in maturing/mature fish in relation to immature fish (Figure 3). Below we report on the statistical results of reproductive status and character interactions, including morphology, maturation characteristics, and lipid reserves.
Reproductive status

There was no significant difference in each of the reproductive hormones (lGnRH-I, -II, -III, or E2) with respect to maturation status for females (Mann-Whitney U test, p >0.05) and for males (Mann-Whitney U test, p > 0.05). There was no significant difference in lGnRH-I, -II, -III, or E2 with respect to sex (2008-2009 data inclusive; Mann-Whitney U test, p > 0.05) (Table 1; 2009 data shown in Chapter 4.1).

Character interactions

Morphology.—There was no significant difference in body weight between MM and immature female lamprey (MANOVA, p = 0.3786), after controlling for total body length (TL) (Figure 4A). By contrast, there was suggestive, but inconclusive evidence for a greater body weight for immature male lamprey in comparison with MM fish (MANOVA, p = 0.0623), after controlling for TL (Figure 4B; Table 1).

GSI and TL.—There was a significant difference in the GSI between MM and immature females (MANOVA, p < 0.0000): The GSI of MM females showed an exponential increase per unit decrease in TL, whereas immature females showed a weak linear increase (Figure 5A; Table 1).

GSI and liver lipids.—There was a significant difference in GSI between MM and immature female lamprey (MANOVA, p < 0.0000): the GSI of MM females showed a logarithmic increase with logarithmic decreases in liver lipids, whereas the GSI of immature females showed a negligible change in GSI in relation to liver lipids (Figure 5B; Table 1).
Oocyte diameter and GSI.—Oocyte diameter increased with logarithmic increases in GSI for both MM and immature fish, although this relationship was significantly different between MM and immature female lamprey (MANOVA, p < 0.0000; Figure 6A; Table 1).

Oocyte diameter and liver lipids.—Maturing/mature female lamprey showed significantly greater mean oocyte diameters than immature fish (MANOVA, p < 0.0000; Table 1). The mean oocyte diameter of MM fish showed a logarithmic increase per a logarithmic decrease in liver lipids, whereas the mean oocyte diameter of immature females showed little change per unit decrease in liver lipids (Figure 6B; Table 1).

Fulton’s condition factor and liver lipids.—There was a negligible increase in condition factor with decreases in liver lipids for both sexes. There was no significant difference in Fulton’s condition factor between MM and immature female lamprey (MANOVA, p = 0.2144), after controlling for liver lipids (Figure 7A). By contrast, there was a significant difference in Fulton’s condition factor between MM and immature male lamprey (MANOVA, p = 0.0284), after controlling for liver lipids. Liver lipids were lower in MM male lamprey than in immature male lamprey (Figure 7B; Table 1).

Muscle lipids and body weight.—The percentage of lipids in the lateral muscle was lower in smaller females, and there was no significant difference in muscle lipids between MM and immature female lamprey (MANOVA, p = 0.7887; Figure 8A). By contrast, there was a significant difference in muscle lipids between MM and immature male lamprey (MANOVA, p = 0.1182): muscle lipids did not decrease in relation to
decreases in body weight for MM male lamprey, whereas muscle lipids did decrease in relation to decreases in body weight for immature male lamprey (Figure 8B; Table 1).

**Fecundity and body weight.**—There was no significant difference in fecundity between mature and immature female lamprey after controlling for body weight (MANOVA, p = 0.2393). The relationship between fecundity and body weight can be expressed as: fecundity = 329.78*(body weight) + 23,202 (r² = 0.5295).

**Ovary lipids and oocyte diameter.**—Ovary lipids increased exponentially with decreases in oocyte diameter with the function: ovary lipids = 64.566^-0.0872(oocyte diameter) (r² = 0.7268).

**Discussion**

The goal of our research was to use multivariate analyses to discern whether there is evidence for diversity of maturation times and associated characteristics of Pacific lamprey at Willamette Falls, Oregon, USA, and to make a prognosis of such biological diversity. It is not known exactly when Pacific lamprey enter freshwater, and how long they have been in freshwater. Pacific lamprey collected at Willamette Falls, ~205 river kilometers from the Pacific Ocean may have recently entered freshwater and migrated relatively quickly to the falls or they may have been in the freshwater for an extended period of time (months to years?). This is why recent migrant lamprey collected prior to freshwater entry, at the interface of the Pacific Ocean and the Klamath River were essential to use as a baseline comparison.
Our data are suggestive of 2 distinct maturation cohorts across years (2007-2008), comprising a maturing/mature (MM) run and an immature cohort. Each cohort had both sexes, and the MM run included 1 male lamprey that was a recent migrant. This seems to suggest that 1 cohort is comprised of recent migrants that spawn within several weeks of entering freshwater, and 1 cohort is comprised of recent migrants that mature and spawn at least 1 or more years later; and old migrants (arrived in freshwater ~ 1+ years before, and spawn during the current year; see also Chapter 4.1). We hypothesize that the recent migrant that would likely spawn within several weeks of entering freshwater (see also Chapter 4.1) is akin to a winter or “ocean maturing” steelhead (*Oncorynchus mykiss*) that optimizes feeding and growth opportunities in the open ocean for a few years before entering freshwater during the late winter/early spring and spawning relatively low in the river system shortly afterwards (Quinn 2005). Alternatively, ocean maturing lamprey may be similar to coastal cutthroat trout, (*O. clarki clarki*) that feed and grow in the coastal areas of the ocean for a few months before entering freshwater to spawn (Behnke 2002). There could be other less apparent explanations as well. We also hypothesize that the lamprey that would likely spawn within ~ 1 year of entering freshwater are akin to a summer or “stream maturing” steelhead that foregoes feeding and growth opportunities, enters freshwater during the summer – fall, and accesses spawning grounds to spawn at specific temperatures that would promote fitness the following spring.

Early-migrating, stream-maturing Pacific lamprey forego continued feeding and growth opportunities in the ocean to enter freshwater where they will mature and spawn. And there is evidence that, like stream maturing steelhead, the spawning date of Pacific
lamprey must be optimized, as the time-to-hatch and normal development of Pacific lamprey zygotes strongly correlates with ambient water temperatures (e.g., see Meeuwig et al. 2005). It has been inferred that Pacific lamprey enter freshwater early, ~1 year before they spawn (reviewed in Clemens et al. 2010), and adult Pacific lamprey have been held in freshwater for ~2-3 years without food (van de Wetering and Schreck, unpubl. data; Whyte et al. 1993; Clemens et al. 2010). We also have direct evidence of Pacific lamprey maturing in the laboratory, in freshwater, during the March – May after the previous June in which they were collected from Willamette Falls (Chapter 2; Clemens et al. 2009).

We have provided evidence of linear relationships between somatic deterioration and gonadal development indicative of shrinkage in body size to fuel maturation. Accordingly, we agree with Larsen’s hypothesis for lampreys that, “[sexual maturation] may depend on a metabolic signal related to starvation” (1980), at least for stream maturing Pacific lamprey. These relationships include increases in GSI and oocyte diameter in tandem with decreases in TL and liver lipids and concomitant increases in condition factor.

Several of the indices we have used in our cluster analyses overlapped between the 2 maturation cohorts. The level of genetic or environmental inducement on these characters, including body shrinkage and consequent maturation is currently still unknown. However the evidence we present here and in Chapter 4.1 is suggestive of a genetic component to gonadal maturation timing for the ocean maturing fish, whereas an environmental templet (sensu Southwood 1988), including thermal history of the
lamprey, likely has more of an effect on body morphology, bioenergetics, and maturation timing for stream maturing lamprey (e.g., see Chapter 2; Clemens et al. 2009).

Acknowledgments

The same individuals and organizations acknowledged in Chapter 4.1 are acknowledged here.

Literature cited


Table 1. Means, standard errors of the mean (SE), and sample size of each of two clusters for each sex. The clusters grouped into immature and maturing/mature fish.

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Relative fecundity (eggs/g body wt) 414 23 10 397 13 30

Absolute fecundity (eggs) 148,240 10,770 10 127,178 6,099 30

G.S.I. (% body wt) 3.9 0.1 67 10.5 1 59

Oocyte diameter (mm) 0.4 0.0 67 0.7 0 59

Egg lipids 44.5 1.4 12 34.3 1 30

Muscle lipids 29.1 3.1 12 26.6 1.6 29

Liver lipids 58.3 1.5 26 33.8 3.6 32

H.S.I. (% body wt) 1.3 0.0 67 1.3 0.0 59

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Figure 1. Fulton’s condition factor for female (A) and male (B) and dorsal fin gap for female (C) and male (D) Pacific lamprey. Open circles = maturing/mature fish from Willamette Falls, filled triangles = immature lamprey from Willamette Falls, open triangles = immature recent migrants collected from the Klamath River mouth, and asterisks = mature recent migrants (n = 1 male).
Figure 2. Hepatosomatic index (HSI) for female (A) and male (B) Pacific lamprey.

Open circles = maturing/mature fish from Willamette Falls, filled triangles = immature lamprey from Willamette Falls, open triangles = immature recent migrants collected from the Klamath River mouth, and asterisks = mature recent migrants (n = 1 male).
Figure 3. Gonadosomatic index (GSI) for female Pacific lamprey. Open circles = maturing/mature lamprey from Willamette Falls, filled triangles = immature lamprey from Willamette Falls, and open triangles = immature recent migrants collected from the Klamath River mouth.
Figure 4. Body weight versus total body length (TL) for female (A) and male (B) Pacific lamprey collected from Willamette Falls and from the Klamath River mouth (recent migrants). Open circles = maturing/mature fish and filled triangles = immature fish. The lines are least squares simple linear regressions. Maturing/mature males had a significantly different relationship than immature fish, which had greater proportional increases in body weight per unit of increase in TL (further details in text).
A

$y = 378.02e^{-0.007x}$

$R^2 = 0.2109$

$y = -0.0148x + 12.738$

$R^2 = 0.4329$

B

$y = 131.36x^{0.782}$

$R^2 = 0.7778$

$y = 0.0662x^{0.9684}$

$R^2 = 0.1594$
Figure 5. Gonadosomatic indices (GSI) versus total body length (TL) (A) and liver lipids (B) for female Pacific lamprey collected from Willamette Falls and from the Klamath River mouth (recent migrants). Open circles = maturing/mature fish and filled triangles = immature fish. The lines are least squares simple linear regressions. Maturing/mature females had a significantly different relationship between GSI and TL, and also between GSI and liver lipids than immature fish (further details in text).
**A**

- Mean oocyte diameter (mm) vs. GSI (%)
- Relationship: $y = 0.158\ln(x) + 0.236$ with $R^2 = 0.427$

**B**

- Liver lipids (% dry weight) vs. GSI (%)
- Relationship: $y = 1.4248x^{0.21}$ with $R^2 = 0.7047$
Figure 6. Mean oocyte diameter versus gonadosomatic indices (GSI) (A) and liver lipids (B) for female Pacific lamprey collected from Willamette Falls and from the Klamath River mouth (recent migrants). Open circles = maturing/mature fish and filled triangles = immature fish. The lines are least squares simple linear regressions. Maturing/mature females had a significantly different relationship between oocyte diameter and GSI, and also between oocyte diameter and liver lipids than immature fish (further details in text).
A

\[ y = -0.014 \ln(x) + 0.2259 \]
\[ R^2 = 0.2045 \]

B

\[ y = -0.0019x + 0.199 \]
\[ R^2 = 0.1716 \]

\[ y = -0.0016x + 0.2651 \]
\[ R^2 = 0.2337 \]
Figure 7. Fulton’s condition factor versus liver lipids in females (A) and males (B) for Pacific lamprey collected from Willamette Falls and from the estuary (recent migrants). Open circles = maturing/mature fish and filled triangles = immature fish. The lines are least squares simple linear regressions. Maturing/mature females had a significantly different relationship between GSI and TL, and also between GSI and liver lipids than immature fish (further details in text).
A

\[ y = 0.0594x + 7.1902 \]
\[ R^2 = 0.2619 \]

B

\[ y = 0.1475x - 16.474 \]
\[ R^2 = 0.5634 \]
Figure 8. Muscle lipids versus total body weight in female (A) and male (B) Pacific lamprey collected from Willamette Falls and from the estuary (recent migrants). Open circles = maturing/mature fish and filled triangles = immature fish. The lines are least squares simple linear regressions. Maturing/mature males had a significantly different relationship between muscle lipids and body weight than immature fish (further details in text).
General Conclusions

*You cannot see the whole pattern of life, the whole movement of life, if you merely take one part of it and are tremendously concerned about that particular part.* (Jiddu Krishnamurti)

The primary goal of this research was to describe the maturation and migration characteristics and timing of adult Pacific lamprey, *Entosphenus tridentatus*, at Willamette Falls, Oregon, USA. A second goal was to discern whether there is evidence for diversity of maturation and migration times and associated characteristics of Pacific lamprey, and to make a prognosis of such biological diversity.

I used a variety of approaches to achieve these goals through three complementary studies, including a manipulative laboratory study on the effects of temperatures on maturation of lamprey; a field study in which I tracked the presence and movement of radio-tagged lamprey in the Willamette River Basin; and a field monitoring study in which I measured the maturation characteristics of lamprey over time at Willamette Falls and in comparison with recent migrants. Below I will briefly summarize the results of these three studies. I will then define apparent run diversity of Pacific lamprey. Next I will attempt to synthesize this information into a plausible scenario for the freshwater biology and ecology of adult Pacific lamprey before concluding with an estimation of the life history strategy employed by Pacific lamprey.
In Chapter 2 I presented results on manipulative, controlled research that I conducted on adult Pacific lamprey at the Fish Performance and Genetics Laboratory at Oregon State University. The goals of this study were: 1) to examine the effects of relatively warm, summer temperatures (>20 °C) on the maturation characteristics of Pacific lamprey over time, and 2) to ascertain whether there was evidence for a cause-and-effect relationship between body size and maturation timing. Because high temperatures increase metabolism and activity in lampreys (Johansen et al. 1973; Lewis 1980) as in other fishes, we predicted that shrinkage in body size during freshwater residency would be accentuated at temperatures >20 °C experienced during the summer. We also predicted that if summer temperatures led to significant decreases in body size of Pacific lamprey, then a higher percentage of fish would mature early in comparison with lamprey held at 13.6 °C, which occurs in the upper tributaries of the Willamette River Basin. Our results supported our predictions. Pacific lamprey matured during the spring, 8-10 months after temperature exposure. The warm water group (> 20 °C; and several in the cool water group) matured within the spring spawning period of fish in the wild. Maturation and mortality were associated with a previously existing small body size in cool water fish and also to significant proportional decreases in body size in warm water fish. This suggests that a minimum, threshold body size exists at which maturation must occur or sufficient energy reserves will not be available for reproduction.

In Chapter 3 I presented tracking data from radio-tagged lamprey, including location, timing and minimum rates of movement in the Willamette Basin above Willamette Falls. I tested four predictions. I predicted that adult Pacific lamprey would:
1) display a normal distribution with respect to the distance migrated; 2) cease their migrations during the summer, when temperatures > 20 °C; 3) show a positive correlation between upstream migration distance and body size; and 4) show a correlation between migration distance and date of passage of Willamette Falls. The data supported the hypothesis that adult Pacific lamprey would distribute evenly throughout the basin. However the data did not support the hypotheses that all fish would stop migrating during the summer or that there would be a positive correlation between the maximum distance migrated versus total body length, or a correlation between the maximum distance migrated and the date fish passed Willamette Falls. Fish migrated primarily during the spring – early summer before stopping during the rest of the summer, when river temperature peaked at ≥ 20°C. However, at least three fish continued to migrate upstream after September. Behavior ranged from relatively slow migration, followed by holding; to rapid migration, followed by slow migration further up in the basin. The lack of correlation between body size and migration distance in our lamprey was likely a result of a size-biased assortment of fish to tag: tag size precluded tagging fish across all ranges in body size. Our data suggests that fish that migrated earlier in the year did not migrate a different distance than those that migrated later.

In Chapter 4 I presented information on the maturation characteristics of adult Pacific lamprey that I collected from Willamette Falls, over time. I compared these characteristics with those of recent migrants collected from the interface of the Klamath River mouth and the Pacific Ocean. The two goals of this research were to: 1) provide a description of the trends of maturation characteristics and timing of lamprey over the
course of the run, and 2) characterize any potential diversity that might be exclusive of obvious spawn timing. I measured morphology, reproductive status, female fecundity, and lipid levels of the lamprey. The results suggest a unimodal spawn timing between April and June, at water temperatures < 20 °C. Between July and mid-September, water temperatures at Willamette Falls peak at > 25 °C. This summer period coincides with evidence of relatively immature fish for both sexes (no early maturation stages for females and no obvious meiotic stages in males). The few mature males that occurred during the end of the spawning period in early July showed evidence of degenerative testes, as did males from other maturation stages during July – September (B. Clemens, T. Peterson, and C. Schreck, unpubl. data). We also observed a skewing in sex ratios towards a higher percentage of males with collection date, culminating in a 72.5% males : 27.5% females during August of 2008, the same collection date in which we observed hundreds of dead lamprey carcasses at Willamette Fall, an obvious fish kill when ambient river temperature was 22.6 °C.

Immature adult fish collected at Willamette Falls during the summer months (late June – September) in many respects resembled recent migrants with regards to maturation stages and phenotypic characteristics. This suggests that lamprey collected during the summer at Willamette Falls were relatively recent migrants from the ocean, whereas the mature fish collected during the spring were likely fish that had already resided in the river for a longer period of time. These immature fish might have been able to mature and spawn the following year, after experiencing the warm summer temperatures. Water temperatures have been found to be positively correlated with the
reproductive hormones lGnRH-I, -II, and –III and E2 in sea lamprey, *Petromyzon marinus* (Sower et al. 2011). We have also found a positive correlation with water temperature and E2 (Appendix B), and an association with exposure to warm water temperature in the summer and maturation the following spring in adult Pacific lamprey. In the laboratory, 21.8°C (mean) during the summer was associated with sexual maturation in 100% of Pacific lamprey the following spring, whereas only 53% of the lamprey held at 13.6°C matured the following spring (Chapter 2; Clemens et al. 2009).

*Run diversity*

The data suggest a life history more complex than alternating cohorts (one years spawners versus next years spawners). For example, some evidence from recent migrants is suggestive of spawning in the same year the fish enter freshwater. This information, along with the large variability within and across collection dates is suggestive of different maturation and migration life histories (different “runs”) that overlap somewhat in time. Cluster analyses indicated 2 distinct maturation cohorts across years, comprising a maturing/mature (MM) run and an immature cohort. For females, the MM cohort was comprised of late vitellogenic and early maturation stages; for males, this cohort was comprised of spermatocytes, spermatids, and spermatozoa stages. For females, the immature cohort was comprised of early to mid vitellogenic females, and for males this cohort was comprised of spermatogonia/spermatocytes. Each cohort had both sexes, and the MM run included 1 male lamprey that was a recent migrant. This seems to suggest that 1 cohort is comprised of recent migrants that spawn within several weeks of entering
freshwater, and 1 cohort is comprised of recent migrants that mature and spawn at least 1 or more years later; and old migrants (arrived in freshwater ~ 1+ years prior, and spawn during the current year). I hypothesize that the recent migrant that would likely spawn within several weeks of entering freshwater is akin to a winter or “ocean maturing” steelhead (*Oncorynchus mykiss*) that optimizes feeding and growth opportunities in the open ocean for a few years before entering freshwater during the late winter/early spring and spawning relatively low in the river system shortly afterwards (Quinn 2005). Alternatively, these lamprey may be similar to coastal cutthroat trout, *O. clarki clarki*, that feed and grow in the coastal areas of the ocean for a few months before entering freshwater to spawn (Behnke 2002). There could be other less apparent explanations as well. I also hypothesize that the lamprey that would likely spawn within ~ 1 year of entering freshwater is akin to a summer or “stream maturing” steelhead that foregoes feeding and growth opportunities, enters freshwater during the summer – fall, and accesses spawning grounds to spawn at specific temperatures that would promote fitness the following spring.

In Chapter 4 I tested the hypothesis that there is a genetic component to gonadal maturation timing for ocean maturing Pacific lamprey, whereas an environmental inducement, including the severity and frequency of warm temperature exposures en route to spawning areas, likely has more of an effect on reproductive hormone levels (see above), shrinkage in body size and subsequent gonad maturation for stream maturing lamprey (e.g., see Chapter 2; Clemens et al. 2009).
Freshwater biology and ecology: the environmental temple

The available information from my research and others suggests that summer temperatures regulate migration behavior and maturation timing of Pacific lamprey in the Willamette Basin, and these temperatures have also been associated with testicular damage and mortality.

Given that most migrating lamprey pass Willamette Falls during the warm summer period (≥ 20 °C; Mesa et al. 2010) and then cease further migration during this time (Chapter 3; Clemens et al. 2011), the association I have found in relation to warm summer temperatures and 100% maturation in Pacific lamprey the following spring may suggest that the thermal histories of these lamprey could uncouple spawn timing with optimal habitat characteristics in the upper watershed for spawning, embryonic development and larval emergence, rearing and growth (Chapter 2; Clemens et al. 2009). We also have found an increased prevalence of gonad damage in Pacific lamprey, coincident with warm summer temperatures (B. Clemens, T. Peterson, and C. Schreck, unpubl. data), and a large die-off of hundreds of lamprey at Willamette Falls during the low discharge, > 20 °C period of August 2008. At this time there were proportionally more males : females ~71% : 29%. Based on the foregoing, I hypothesize that summer temperatures can act as a strong selection factor against stream maturing Pacific lamprey; conversely this scenario may select for ocean maturing Pacific lamprey.

Pacific lamprey life history strategy
Evolution has favored late maturity in Pacific lamprey (~ 8 – 13 years; Chapter 1), semelparity, and incredibly high fecundity. This fecundity is ~ 13-150X greater than the fecundity of steelhead (fecundities ~ 2,000 – 12,000; Bulkley 1967; Quinn et al. 2011), the fish of which we have compared with Pacific lamprey run diversity. This late maturity and high fecundity resembles a “periodic” life history strategy, somewhat akin to sturgeons (Acipenseriformes), although they are repeat spawners), and unlike sharks (Carcharhiniformes) and guppies (Cyprinodontiformes), which reside at the opposite ends of the life history spectrum. Periodic strategists produce massive quantities of small offspring to capitalize on infrequent opportunities for favorable reproduction in environments with large spatial or seasonal variation (Winemiller and Rose 1992). It is unknown how the intersex fish (Appendix A) might fit into this life history strategy, or if intersex is a maladaptive trait.

*From there to here, from here to there, funny things are everywhere.*

(Theodor Seuss Geisel)

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Appendix A.

Incidence of male intersex in adult Pacific lamprey, *Entosphenus tridentatus*, with a brief discussion of intersex and hermaphroditism in lampreys

Benjamin J. Clemens, Stacia A. Sower, and Carl B. Schreck

Abstract.—We report the incidence of male intersex in adult Pacific lamprey, *Entosphenus tridentatus*, collected from Willamette Falls, Oregon, USA. Although “hermaphrodites” have been suggested in other adult lampreys, this is the first detailed description and discussion of this phenomenon. A total of 0.5% of our adult Pacific lamprey from Willamette Falls (2 out of 431 adults) were intersex, with female germ cells in the testes. This phenomenon was identifiable only by histological examination. The testes of the intersex males were immature, being in the beginning stages of meiosis. One intersex male possessed primary growth/perinucleolar stage eggs loosely interspersed throughout the testes, and the other possessed at least 6 mid-vitellogenic oocytes (0.6 mm, mean long diameter) separate from, and adjacent to, the testes. Because pre-metamorphic lamprey can possess both female and male gonial cells, it seems likely that intersex is a remnant larval trait, and that these fish failed to fully develop to either sex during metamorphosis. Another, perhaps not mutually exclusive hypothesis, is that hermaphroditism is the ancestral trait common to the two paraphyletic groups of cyclostomes, the hagfishes (Myxiniformes), and the lampreys.
(Petromyzontiformes), and that intersex in lampreys is a non-functional, possibly muted form of hermaphroditism.

**Introduction**

Hermaphroditism, either sequential or synchronous, exists in ~5 – 6% of all animal species (Grober and Rodgers 2007) and ~2% of all extant teleost fishes, across 20 families (Avise and Mank 2009). Grober and Rodgers (2007) noted: “*Ancestral fish lineages (e.g., lamprey, sharks/rays, gars, paddlefish, sturgeon, lungfish…) are remarkably devoid of hermaphroditic species, whereas the more recently diverged percomorph fishes (advanced teleosts) boast a large variety of hermaphroditic forms, suggesting that hermaphroditism is on the rise, not in decline…”*. “Ancestral” or primitive fish such as the hagfishes (Myxiniformes), can indeed be hermaphroditic or sexually dimorphic (Powell et al. 2004 and references therein), and so hermaphroditism is not absent in this ancient, basal vertebrate. There have also been obscure reports on “hermaphroditism” in lampreys (Petromyzontiformes) (Breder and Rosen 1966; Holcik and Delic 2000), a Cyclostome like the hagfishes (Janvier 2008). Here, we report on and describe the occurrence of male intersex in adult Pacific lamprey, *Entosphenus tridentatus*. We discuss the intersex condition in larval and adult lampreys, in relation to hermaphroditism. We define intersex as the co-occurrence of cells of both sexes in the gonad, with one sex cell type predominating in a species that is otherwise gonochoristic. We define hermaphroditism as the existence of approximately equal masses of each sex
cell within the gonad, either simultaneously (e.g., synchronous hermaphroditism) or at different times (e.g., sequential hermaphroditism; Hecker et al. 2006). We conclude by proposing a hypothesis on the potential taxonomic relationship between intersex and hermaphroditism in the cyclostomes (Myxiniformes and Petromyzontiformes).

**Methods**

Lamprey were collected from Willamette Falls during April – September of 2007 and 2008. During April-June, fish were collected from a fish trap in the fish ladder of the Portland General Electric hydroelectric dam that flanks the 12 m high basaltic falls. During June – September, fish were collected by hand from the base of the falls, where they congregate prior to going upstream or back downstream to spawn. Several different morphological and physiological measurements were taken on the fish as part of another study (see Chapter 4). We collected a small piece of gonad immediately posterior to the posterior tip of the liver. The tissue was processed for gonad histology and stained with hematoxylin and eosin, and viewed under a compound light microscope (Leica DMLB). Images were taken with a digital camera (SPOT, Diagnostic Instruments, Inc.).

The stage of maturity for oocytes was identified through methods used by Lewis and McMillan (1965) and McMillan (2007). The stage of maturity for the testes was based on descriptions in Potter and Robinson (1991) and Dziewulska and Domagala (2003). Because the immature testes showed more complicated and nuanced morphological differences than suggested in those references, and there is a growing
literature indicating complicated cell line iterations in fish and other animals (e.g., see De Rooij and Russell 2000; Leal et al. 2009; Schulz et al. 2010), we identified the maturation stages of the testes following Van Eenennaam and Doroshov (1998).

**Results**

A total of 0.5% of all adult lamprey (2 out of 431) collected from the Willamette River at Willamette Falls, OR, USA, were intersex fish possessing primarily testicular tissue. This includes 1 out of 210 fish collected from Willamette Falls during 2007 (Figure 1) and 1 out 217 fish collected from the same location during 2008 (Figure 2).

The intersex fish were collected late in the year (during September of 2007 and August of 2008), long after the spawning season. Our gross identification of the sex of both hermaphroditic adult Pacific lamprey indicated that they were male (the eggs were only evident from histological examination of gonad tissue).

The intersex fish were immature and although they possessed mostly spermatogonia / early stage spermatocytes, the few oocytes that were apparent were in vastly different stages of maturation between the two fish. The intersex fish from 2007 possessed at least 6 mid-vitellogenic oocytes (0.6 mm, mean long diameter) separate from, and adjacent to, the testes (Figure 1), whereas the intersex fish from 2008 possessed primary growth / perinucleolar stage eggs interspersed throughout the testes (Figure 2). This latter fish also showed signs of testicular atresia, indicated by eosin-stained voids where other gonial cells used to be (Figure 2).
Discussion

Other intriguing, albeit unclear, and in at least some cases unsubstantiated reports of “hermaphroditism” in lampreys exist in the literature. Recently brook lamprey, *Eudontomyzon mariae*, was reported to have 6% of collected “adults” (2 out of 33 fish) as hermaphrodites (Holcik and Delic 2000). However, it is not clear that the “adult” fish had metamorphosed, as the descriptions in the paper are vague, and the reports of 2 fish having eyes covered by skin suggests that at least some of the fish had not fully metamorphosed. Larval lamprey are sexually labile and indeterminate prior to metamorphosis (Wicks et al. 1998; Barker and Beamish 2000; Beamish and Barker 2002).

Some older papers from the early 1800s – early 1900s provide further tantalizing reports of “hermaphroditism” in various other lamprey species (reviewed in Breder and Rosen 1966), yet the interpretation of these claims is problematic for several reasons. For example, the anatomical descriptions and observations of early observations are neither clear, nor scientific, nor accurate (e.g., Home 1815). It was later noted, “*That Sir Edward Home was mistaken in supposing the Lamprey to be a hermaphrodite animal has long been well known*” (Ewart 1876). Others have made extraordinary claims that have not been independently verified, including the hypothesis of parthenogenesis in lampreys, based on the supposed observation of egg development to the morula stage without fertilization (Gage 1928—cited in Breder and Rosen 1966). Still others reported on
hermaphroditism in pre-metamorphic and metamorphosizing fish (e.g., Okkelberg 1921—cited in Breder and Rosen 1966), which seems reasonable because of the aforementioned lability and indeterminacy prior to metamorphosis.

We refer to our fish as “male intersex” because of the predominance of testicular tissue compared with the few oocytes, and because lampreys are gonochoristic, not hermaphroditic. It seems highly unlikely that the adult male intersex fish that we discovered would be able to self-fertilize (i.e., the fish were very likely not functional hermaphrodites) or that they would be able to spawn viable eggs. We do not know whether these fish would be able to fertilize eggs from females. One of the two male intersex Pacific lamprey showed signs of testicular atresia, like the “normal” male lamprey collected at the same location (Willamette Falls) and during the same time period (late summer).

Because pre-metamorphic lamprey can possess both female and male gonial cells (Wicks et al. 1998; Barker and Beamish 2000; Beamish and Barker 2002), we hypothesize that the presence of male intersex fish is a remnant larval trait, and that these intersex fish failed to fully commit development to either sex during metamorphosis. Another, perhaps not mutually exclusive hypothesis, is that hermaphroditism may be the ancestral trait common to the paraphyletic cyclostomes (Dawkins et al. 2004; Powell et al. 2004; Janvier 2008). That hermaphroditism (we suggest the “intersex” is more accurate and appropriate) has been reported, albeit not clearly or thoroughly from other lampreys, could suggest an infrequent tendency of this trait to occur across species of lampreys, which lends additional support to the ancestral trait hypothesis. Intersex in
adult lampreys might be more prevalent than indicated by the literature, and it could be more widely reported if more researchers examined the gonad histology from adult lamprey. If occurrence is as low as the 0.5% we report in this paper, a minimum, random sample of at least 200 random lampreys would be required to observe an intersex individual.

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**Figure 1.** Gonad histology of a male intersex adult Pacific lamprey, *Entosphenus tridentatus*, collected from Willamette Falls, OR, USA during September of 2007.  

**A.** Immature testes towards the top left of the image (circled), and the mid-vitellogenic oocytes towards the bottom right (indicated by arrow); 25X focus, scale = 1 mm.  

**B.** Testes from “A” with spermatogonia and or spermatocytes; 100X focus, scale = 20 micrometers (0.02 mm).  

**C.** Mid-vitellogenic oocytes from “A”; 50X focus, scale = 0.1 mm.
Figure 2. Gonad histology of a male intersex adult Pacific lamprey, *Entosphenus tridentatus*, collected from Willamette Falls, OR, USA during August of 2008. Immature testes (spermatogonia and/or spermatocytes), and oocytes in the primary growth/perinucleolar stage (oocytes indicated by arrows); 200X magnification, scale = 100 micrometers.
Appendix B.

Warm temperature, but not holding and transportation, raises blood plasma levels of the reproductive hormone, 17β-estradiol, in immature adult Pacific lamprey, *Entosphenus tridentatus*

Benjamin J. Clemens, and Carl B. Schreck

Abstract

In some instances it may be useful to collect lamprey and keep them alive until their blood can be sampled in more favorable conditions than in the field. However, we do not know if capturing and holding sexually immature adult Pacific lamprey may induce stress and therefore affect plasma levels of the reproductive hormone, 17β-estradiol (E$_2$). We determined the effects of temperature, holding and transporting on E$_2$ in the blood plasma of immature Pacific lamprey. We predicted that: 1) holding adult Pacific lamprey for up to 1 month at ~14.5°C would result in significantly low levels of E$_2$ compared to fish sampled immediately at 21.5°C during the summer; 2) levels of E$_2$ would not significantly differ with respect to collecting, holding and transporting lamprey over a few hours during the summer; 3) sex would not be associated with significant differences in levels of E$_2$ in these immature fish. The results were consistent with our predictions: 1) fish held for 29 d in captivity at ~14.5°C had significantly lower levels of E$_2$ (0.17±0.02SE ng/ml; N = 16) than fish sampled the following day at 21.5°C (0.62 ±0.12 ng/ml; N = 8); 2) there was no significant difference in levels of E$_2$ (N = 37) with respect to holding times of 0 to 6 h at 21.5°C (range: 0.12 – 3.13 ng/ml); 3) there was no significant difference in levels of E$_2$ with respect to sex in lamprey held in captivity for
29 d at ~14.5°C or lamprey held for up to 6 h. We conclude that warm temperatures, but not holding and transportation, are associated with relatively high plasma levels of E₂ in sexually immature Pacific lamprey.

**Introduction**

Sexually immature Pacific lamprey cease their parasitic lifestyle in the ocean, return to freshwater during the spring (April – June), and begin their initial upstream migration during the summer (July – September) before overwintering during October – March. They mature, spawn, and die during April — July (reviewed in Clemens et al. 2010). Pacific lamprey returning from the Pacific Ocean to the Willamette River via the Columbia River migrate 204 km before encountering 12 m high Willamette Falls comprised of basaltic bedrock and boulders, flanked by a hydroelectric dam and paper mill (with a fish ladder) on the west side of the river. The lamprey congregate at the falls before attempting to ascend and continue their migration to spawning areas upstream or towards unknown destinations downstream (Mesa et al. 2010).

Knowledge of the details of sexual maturation for Pacific lamprey is relatively low (Clemens et al. 2010) and this information will be useful in improving understanding of lamprey biology (Chapter 4) and potentially conservation practices (e.g., translocation of these fish; see Close et al. 2009; Ward et al. 2010).

Efforts to collect lamprey and sample their blood in a timely manner in the field are challenging, particularly when balancing the demands of acquiring other measurements and tissues from the same fish (BJC, pers. obs.; Chapter 4). In some
instances it may be useful to collect lamprey and keep them alive until their blood can be sampled on stable ground, rather than in non-pristine field conditions in the sun, wind, and water spray, and inclement weather. We do not know if capturing Pacific lamprey and then holding them may induce stress and therefore affect plasma levels of the reproductive hormone, 17β-estradiol (E₂), that we are trying to estimate. The present study tests the effects of temperature, holding and transporting on E₂ in the blood plasma several months before the animals would be expected to mature and spawn.

There is ample evidence that E₂ is a major hormone regulating reproductive maturation and function in males and female lampreys (e.g., see Sower 2003; Bryan et al. 2008; Mesa et al. 2010). In Pacific lamprey, levels of plasma E₂ were 0.5 – 2 ng/ml in both sexes during fall – early spring, before peaking during mid spring at 2 – 4 ng/ml and decreasing during late spring. Levels of E₂ were often higher in males than females, particularly during peak maturation in the spring (Mesa et al. 2010).

We tested three predictions. Specifically, we predicted that: 1) holding adult Pacific lamprey for up to 1 month at ~14.5°C, would result in low levels of E₂ in the blood plasma in comparison with fish sampled immediately at 21.5°C during the summer; 2) levels of E₂ in the blood plasma would not significantly differ with respect to collecting, holding and transporting lamprey over the course of a few hours during the summer; 3) sex would not be associated with significant differences in levels of E₂ in these sexually immature fish.

**Methods**
**Sampling**

*Captive-held fish (29 d post collection).*—Adult Pacific lamprey were collected by hand from Willamette Falls, Oregon on July 22, 2008, and transported within 2.5 h to the Oregon State University Fish Performance and Genetics Laboratory where they were stocked into three outdoor 1,365 L tanks with cobble and gravel substrate and shade screen covers. Ambient water temperature at Willamette Falls upon collection was ~21.5°C. Fish were transported in a cooler (66 L) of aerated river water chilled to 6.5°C with ice to minimize stress. The mean, daily temperature of the holding tanks was ~14.5°C (± 1.5°C). Pathogen-free water was supplied to each tank (4.9 L/min), and the water was circulated between the tanks to insure all fish experienced the same pheromonal milieu.

On August 20, 2008, 29 d post collection, the fish (N = 16) were collected from the tanks and immediately placed into ~ 60 L of ice water (<1°C) to immobilize them. Blood was subsequently drawn from each fish via cardiac puncture, as per Sower et al. (1985), with heparinized vacutainers. The tanks were sampled sequentially, and so blood was drawn between 08:51 (first fish) and 10:28 h (last fish). The blood was immediately placed on ice and then centrifuged to separate plasma. Plasma was stored at -80°C prior to analysis. Body length was measured to the nearest mm, and fish were euthanized by severing the head with a knife. Gonad tissue was taken immediately posterior to the liver of euthanized fish. This tissue was prepared for histology, as per Sower et al. (1985). Gonad histology was used to supplement hormonal measures of reproductive readiness, as in Bolduc and Sower (1992).
Fish sampled from the wild, up to 6 h post collection.—During the next day, on August 21, 2008, blood was collected from 38 fish immediately upon collection (time zero) at Willamette Falls, Oregon, and at 1, 3, and 6 h post collection in the field. Ambient river temperature at Willamette Falls during sampling was 21.5°C. Fish collected and sampled immediately (time zero fish) were collected at 09:55—09:57 h, and were placed in ~ 60 L of ice water (<1°C) at 10:00 h to immobilize them. Blood from these fish was sampled between 10:03 and 10:22 h. Additional fish were collected between 11:15 and 11:20 h and held in approximately equal numbers for either 1, 3, or 6 h in 66 L coolers prior to sampling their blood. Blood was drawn from the last fish at 17:31 h. All coolers were maintained within 0.3°C of ambient river temperatures by adding ice. The ice water used for immobilizing the fish prior to drawing blood was consistently <1°C. Body length, sexing, and sampling of gonads for histology occurred after euthanizing the fish, as described above. Plasma was obtained and stored as described above.

Radioimmunoassays

I extracted 17β-estradiol (E₂) from 100 µl of plasma as described by Fitzpatrick et al. (1986) and Webb et al. (2002). Extraction efficiency was determined for all samples (46 fish, in duplicate = 92 tubes) by adding a known amount of tritiated estradiol prior to extraction. Average extraction efficiency among all of the tubes was 89%, and assay results were corrected for the extraction efficiency of each sample. Concentrations of E₂
in the blood plasma were assayed using the methodology described by Sower and Schreck (1982) and modified by Feist et al. (1990). The lower limit of detection was 1.25 pg/tube (0.25 ng/ml). Any E₂ values <0.25 ng/ml were normalized to 0.12 ng/ml, the median between 0 and 0.25 ng/ml. The intra-assay coefficients of variation were <5%, and the inter-assay coefficient of variation was <10%. Preliminary assays showed that serial dilutions of plasma provided E₂ values parallel to the standard curve.

Statistics

A two-sample t-test was conducted on levels of E₂ in the blood plasma of fish held in captivity versus fish sampled immediately from the wild (time 0 h). This particular test was also conducted on levels of E₂ with respect to sex for fish held in captivity. For fish sampled from the wild, up to 6 h post collection, a two-way ANOVA (MANOVA) was conducted, with levels of E₂ being the dependent variable and sex and sampling time category (0, 1, 3, and 6 h post-collection) being the blocking variables. To meet the assumptions of ANOVA, including normal distribution and homogenous variance, the E₂ data were log-transformed.

Results

The results (summarized in Table 1 and Figure 1) were consistent with our predictions. Fish held for 29 d in captivity at ~14.5°C had significantly lower levels of E₂
(0.17±0.02SE ng/ml; N = 16) than fish sampled the following day at 21.5°C (0.62 ±0.12 ng/ml; N = 8) (two-sample t-test, 22 df, P < 0.0001). There was no significant difference in levels of E₂ (N = 37) with respect to holding times of 0 h (immediate sampling) to 6 h at an ambient temperature of 21-22°C (range: 0.12—3.13 ng/ml; MANOVA, P = 0.9287). There was no significant difference in levels of E₂ with respect to sex in lamprey held in captivity for 29 d at ~14.5°C (two-sample t-test, 14 df, P = 0.2171) or lamprey held for up to 6 h (MANOVA, P = 0.5647).

All females were sexually immature, but their eggs were all vitellogenic, and ranged between 0.4 and 0.7 mm, with an average long diameter of 0.6 mm. Based on this size and stage of the oocytes, it is estimated that these fish could have been ready to spawn during the next spring, ~8-9 months away. All males were sexually immature, with their testes comprised of spermatogonia and spermatocytes. One intersex individual, collected on August 21, 2008, was omitted from the analyses. I report on this fish elsewhere (see Appendix A.).

**Discussion**

Our results were consistent with our predictions, suggesting that warm temperatures, but not holding and transportation, may raise plasma levels of E₂ in sexually immature Pacific lamprey. The corollary is that cooler temperatures may lower or prevent the rise of E₂. Increases in E₂ and gonadotropic-releasing hormones are associated with changes in photoperiod and warming river temperatures in sea lamprey, *Petromyzon marinus*. 
(Sower 2003; Sower et al. 2011). In the laboratory, 21.8°C (mean) during the summer was significantly associated with sexual maturation in 100% of Pacific lamprey the following spring, whereas only 53% of the lamprey held at 13.6°C matured the following spring (Clemens et al. 2009). This line of evidence leads us to hypothesize that warm summer temperatures (see Figure 4 in Chapter 4) are associated with high levels of E₂ (present study) and expedited maturation timing in Pacific lamprey. Conversely, levels of E₂ may be reversible in summer with lowering of water temperature.

The plasma E₂ levels for Pacific lamprey that we report, 0.12-3.13 ng/ml during late summer, overlap the lower range of mean E₂ values for Pacific lamprey reported by Mesa et al. (2010). They collected fish in the summer and held them in artificial streams, at seasonally varying temperatures of 5-14°C. During the fall through spring, they found mean E₂ in their fish ranged between 0.5-4.0 ng/ml.

We hypothesize that sexual maturation of Pacific lamprey in the Willamette Basin may be triggered by warm summer temperatures (>20°C; see Clemens et al. 2009), and the high levels of E₂ in the blood plasma that we have reported. We also hypothesize that these physiological conditions may occur when the upstream migration of adult Pacific lamprey slows or ceases (Chapter 3; Clemens et al. 2011). If these hypotheses are correct, then the trend for warm summer temperatures (>20°C) in the Willamette Basin may uncouple the timing of sexual maturation with potentially optimum spawning locations in the upper, cooler stretches of the Willamette Basin (Clemens et al. 2009).

With the recently described stress hormone in lampreys — 11-deoxycortisol— (Close et al. 2010) the possibility of estimating stress levels now exists. In addition it
will be informative to study the cross talk between stress and maturation of Pacific lamprey, in relation to thermal exposures.

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Yakama, and Warm Springs Tribes, May 18 2008.  68 pp.  Available:  


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Table 1. Origin, number, and duration of holding and descriptive statistics of adult Pacific lamprey sampled for the present study. Temperature at Willamette Falls was 21.5°C during collection of the lamprey that were subsequently held at the Fish Performance and Genetics Laboratory (FPGL) for 29 d at ~14.5°C prior to sampling on August 20, 2008. Temperature at Willamette Falls during collection of the fish held and sampled over time (0, 1, 3, and 6 h) on August 21, 2008 was ~21.5°C. Numbers in parentheses following the means are standard errors of the mean. All values less than the lowest standard (< 0.25 ng/ml) were corrected by changing these values to the median of 0 and 0.25 ng/ml, 0.12 ng/ml.

<table>
<thead>
<tr>
<th>Origin</th>
<th>N</th>
<th>Males (N) : Females (N)</th>
<th>Holding duration</th>
<th>Mean level of 17β-Estradiol (ng/ml)</th>
<th>mean TL</th>
</tr>
</thead>
<tbody>
<tr>
<td>FPGL</td>
<td>16</td>
<td>5 : 11</td>
<td>29 d</td>
<td>0.17 (±0.02)</td>
<td>560 (±8)</td>
</tr>
<tr>
<td>Willamette Falls</td>
<td>8</td>
<td>6 : 2</td>
<td>0 h</td>
<td>0.62 (±0.12)</td>
<td>564 (±15)</td>
</tr>
<tr>
<td></td>
<td>9</td>
<td>5 : 4</td>
<td>1 h</td>
<td>0.67 (±0.32)</td>
<td>594 (±20)</td>
</tr>
<tr>
<td></td>
<td>8</td>
<td>6 : 2</td>
<td>3 h</td>
<td>0.48 (±0.05)</td>
<td>580 (±22)</td>
</tr>
<tr>
<td></td>
<td>12</td>
<td>4 : 8</td>
<td>6 h</td>
<td>0.51 (±0.10)</td>
<td>566 (±9)</td>
</tr>
</tbody>
</table>

aFish were sampled immediately.
Figure 1. Plasma levels of estradiol in Pacific lamprey from Willamette Falls, Oregon. The values shown are from two different groups of fish: 1) collected at ~22 °C and held in captivity for 29 d at a mean temperature of ~14.5 °C (FPGL) prior to sampling on August 20, 2008, and 2) collected at ~22 °C and sampled 0, 1, 3, and 6 h post collection on August 21, 2008.