AN ABSTRACT OF THE THESIS OF

Stan J van de Wetering for the degree of Masters of Science in Fisheries Science on May 1, 1998. Title: Aspects of Life History Characteristics and Physiological Processes in Smolting Pacific Lamprey, *Lampetra tridentata*, in a Central Oregon Coast Stream.

Abstract approved:

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Gordon H.Reeves

Rotary screw traps were used to estimate Pacific lamprey smolt yield, outmigration timing, age structure and sex ratio for Tenmile Creek basin, Lane County, Oregon. Traps were fished March to June and August to December 1994 and March to June and October to December 1995. Lamprey smolts caught in the traps were marked and released upstream. Recaptured fish were used to calculate trap efficiencies and daily and weekly estimates of outmigrating smolts. Little movement of smolts occurred before November. Outmigration peaked in late November and was complete both years by 2 December. Ages of smolts were determined using length-frequency analysis and by reading statoliths. Tenmile Creek Pacific lamprey smolts have an extended freshwater residency. Male to female ratios were approximately 1:1 both years.

Larval, metamorphosing and smolting Pacific lamprey were examined for changes in skin guanine concentrations, gill (Na+K)-ATPase activities and plasma thyroid hormone levels during a 14 mo period. Seasonal peaks were observed in gill (Na+K)-ATPase activities and plasma thyroid hormone levels in larvae. Metamorphosing and smolting lamprey showed increases in skin guanine concentrations and gill (Na+K)-ATPase activities along with decreases in plasma thyroid hormone levels. Smolting lamprey challenged with artificial seawater showed no significant changes in the parameters observed.

Aspects of Life History Characteristics and Physiological Processes in Smolting Pacific

Lamprey, *Lampetra tridentata*, in a Central Oregon Coast Stream.

by

Stan J van de Wetering

A THESIS

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APPROVED:

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Dean of Graduate School

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

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Stan J van de Wetering, Author

ACKNOWLEDGEMENTS

CONTRIBUTION OF AUTHORS

Mr. Steven L. Johnson was involved in the data collection and editing of the second chapter. The assays described in chapter three were performed in the laboratory of Dr. Richard Ewing who also assisted in the interpretation of the data and in the writing of the third chapter.

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CHAPTER 1

Introduction

The Pacific lamprey, *Lampetra tridentata,* is distributed in North America from

the Aleutian Islands south along the Pacific coast to Baja California, Mexico and inland

as far as the upper reaches of the Columbia River. In the past two decades, declines in

the returns of adult Pacific lamprey have occurred in rivers throughout Oregon and

Washington. Tribal members from the Columbia River basin have observed reductions

in numbers of returning adults in several Columbia River sub-basins (Close et al. 1995).

Dam counts at Bonneville, The Dalles, McNary, Ice Harbor, and Rock Island appear to

show a pattern of reduction in adult passage numbers during the past 30 yr. Similar

reductions have been noted in Oregon coastal basins. Siletz tribal members have

observed decreased annual harvests from the Siletz River, Oregon (Downey et al. 1993).

Populations in the Umpqua River, Oregon, have also shown declines (Anonymous, North

Umpqua Hydroelectric Project 1994).

Despite its widespread distribution little is known about the life histories of the

Pacific lamprey (Pletcher 1958; Hammond 1979; Richards 1980; Beamish and Levings

1991). No quantitative information is available for Oregon coastal streams. My goal

with the first portion of this study was to provide initial information examining aspects of the life history characteristics of Pacific lamprey in an entire basin on the Oregon coast.

Physiological and behavioral changes underlying the process of parr-smolt transformation have been studied extensively in anadromous salmonids. These include changes in coloration (Staley and Ewing 1992), morphology (Folmar and Dickoff 1980), behavior (McCormick 1994), fat levels (Fessler and Wagner 1969), gill (Na+K)-ATPase (Ewing et al. 1979; Folmar and Dickoff 1979) and seawater tolerance (Varnavsky et al. 1992). In other anadromous species, however, much less information is available. The phylogenetic origins of these changes have not been examined extensively.

One of the most primitive of fishes, the lamprey, incorporates anadromy in its' life history. The freshwater and marine life history stages are bridged by a four to five month metamorphosis during which time morphological and behavioral changes occur similar to those observed in smolting salmonids (van de Wetering Chapter 3 1998). In the second portion of this study I examine the changes in a number of physiological parameters in wild Pacific lamprey which had attained sizes necessary for metamorphosis. In particular, I measured the parameters which have become common for measurement of smolting in salmonids and examined these relative to different life history stages of the Pacific lamprey.

Literature Cited

Anonymous. 1994. North Umpqua Hydroelectric Project.

Beamish, R.J. and Levings, C.D. 1991. Abundance and freshwater migrations of the anadromous parasitic lamprey, *Lampetra tridentata*, in a tributary to the Frazer River, British Columbia. Can. J. Fish. Aquat. Sci. 48:1250-1263.

Close, D.A., Fitzpatrick, M., Li, H., Parker, B., Hatch, D., and James, G. 1995. Status Report of the Pacific lamprey, *Lampetra tridentata*, in the Columbia River Basin. U.S. Dept. Energy, Bonneville Power Admin., Div. Fish and Wildlife, Portland, OR.

Downey, T., Rilatos, D., Sondenaa, A. and Zybach, B. 1993. The Siletz Eels: Oral history interviews with Siletz tribal elders and neighboring residents regarding the decline in Siletz River lamprey populations. Native Americans in Marine Science Program, Oregon State University, Corvallis, Oregon.

Ewing, R.D., Johnson, S.L., Pribble, H.J., and Lichatowich, J.A. 1979. Temperature and photoperiod effects of gill (Na+K)-ATPase activity in chinook salmon, *Oncorhychus tshawytscha*. Can. J. Fish. Aquat. Sci.. Vol. 36, No. 11, pp1347-1353.

Fessler, J.L. and Wagner, H.H. 1969. Some morphological and biochemical changes in steelhead trout during the parr-smolt transformation. J. Fish. Res. Board Can. 26:2823-2934.

Folmar, L.C. and Dickoff, W.W. 1979. Plasma thyroxine and gill (Na+K)-ATPase changes during seawater acclimation of coho salmon, *Oncorhynchus kisutch*. Comp. Biochem. Physiol., vol. 63A. pp. 329-332.

Folmar, L.C. and Dickoff, W.W. 1980. The parr-smolt transformation (smoltification) and seawater adaptation in salmonids. A review of selected literature. Aquaculture 21:1-37.

Hammond, R.J. 1979. Larval biology of the Pacific lamprey, *Entosphenus Tridentatus* (Gairdner), of the Potlatch River, Idaho. Masters Sci. Thesis, Univ. Idaho, Moscow, Idaho.

McCormick, S.D. 1994. Ontogeny and evolution of salinity tolerance in anadromous salmonids: hormones and heterochrony. Estuaries vol. 17 no. 1A pp 26-33.

Pletcher, F.T. 1958. The life history and distribution of lampreys in the Salmon and certain other rivers in British Columbia, Canada. Masters Sci. thesis, Univ. British Columbia, Vancouver, B.C.

Richards, J.E. 1980. Freshwater life history of the anadromous Pacific lamprey, *Lampetra tridentata*. Masters Science thesis, University of Guelph, Guelph, Ont.

Staley, K.B., and Ewing, R.D. 1992. Purine levels in the skin of juvenile coho salmon, *Oncorhynchus kisutch,* during parr-smolt transformation and adaptation to seawater. Comp. Biochem. Physiol. Vol. 101B. No. 3, pp 447-452.

van de Wetering S.J. 1998. Aspects of life history characteristics and physiological processes in smolting Pacific lamprey, *Lampetra tridentata,* in a central coast Oregon stream. Chapter 3 this volume. MS Thesis, Oregon State University, Corvallis, Oregon.

Varnavsky, V.S., Sakamoto, T. and Hirano, T. 1992. Stunting of wild coho salmon, *Oncorhynchus kisutch*, in seawater: Patterns of plasma thyroid hormones, cortisol and growth hormone. Can. J. Fish. Aquat. Sci. 49:458-461.

CHAPTER 2

Aspects of Life History Characteristics of Smolting Pacific Lamprey, *Lampetra tridentata,* in a Central Coast Oregon Stream

Stan J van de Wetering

Abstract

Rotary screw traps were used to estimate Pacific lamprey smolt yield, outmigration timing, age structure and sex ratio for Tenmile Creek basin, Lane County, Oregon. Traps were fished March to June and August to December 1994 and March to June and October to December 1995. Lamprey smolts caught in the traps were marked and released upstream. Recaptured fish were used to calculate trap efficiencies and daily and weekly estimates of outmigrating smolts. Little movement of smolts occurred before November. Outmigration peaked in late November and was complete both years by 2 December. Ages of smolts were determined using length-frequency analysis and by reading statoliths. Tenmile Creek Pacific lamprey smolts have an extended freshwater residency. Male to female ratios were approximately 1:1 both years.

Introduction

The Pacific lamprey, *Lampetra tridentata,* is distributed in North America from the Aleutian Islands south along the Pacific coast to Baja California, Mexico and inland as far as the upper reaches of the Columbia River. After a freshwater residency estimated to average 5 - 6 yr (Beamish and Levings 1991), the lamprey undergoes a metamorphosis before migrating to the ocean as a smolt (macrophthalmia). Ocean residence has been estimated to average 1 - 4 yr (Beamish 1980; Beamish and Levings 1991).

In the past two decades, declines in the returns of adult Pacific lamprey have occurred in rivers throughout Oregon and Washington. Tribal members from the columbia River basin have observed reductions in numbers of returning adults in several

Columbia River sub-basins (Close et al. 1995). Dam counts at Bonneville, The Dalles, McNary, Ice Harbor, and Rock Island appear to show a pattern of reduction in adult passage numbers during the past 30 yr. Similar reductions have been noted in Oregon coastal basins. Siletz tribal members have observed decreased annual harvests from the Siletz River, Oregon (Downey et al. 1993). Populations in the Umpqua River, Oregon, have also shown declines. Data covering the years 1965-1997 show a peak count of 46,785 in 1966, dropping to a low of 7 in 1997 (State of Oregon, Department of Fish and Wildlife 1998).

Little is known about the life histories of the Pacific lamprey despite its widespread distribution (Pletcher 1958; Hammond 1979; Richards 1980; Beamish and Levings 1991). No quantitative information is available for Oregon coastal streams. My goal was to examine aspects of the life history characteristics of Pacific lamprey in an entire basin on the Oregon coast. Specifically, my objectives were to estimate the number and determine the seasonal outmigration timing, age structure and sex ratio of smolting Pacific lamprey in the Tenmile Creek basin.

Study Site

Tenmile Creek is a fourth order stream located on the central Oregon coast (Fig. 2.1). There are approximately 27 km of linear stream miles located in 61 km ^{2} of forested land. Tenmile Creek flows directly into the Pacific ocean. No estuary is present. Porphyritic basalt dominates the lower two thirds of Tenmile Creek basin while the upper

Oregon

Alsea River

Tenmile Creek

Siuslaw River

Tenmile Creek Watershed

Trap Site
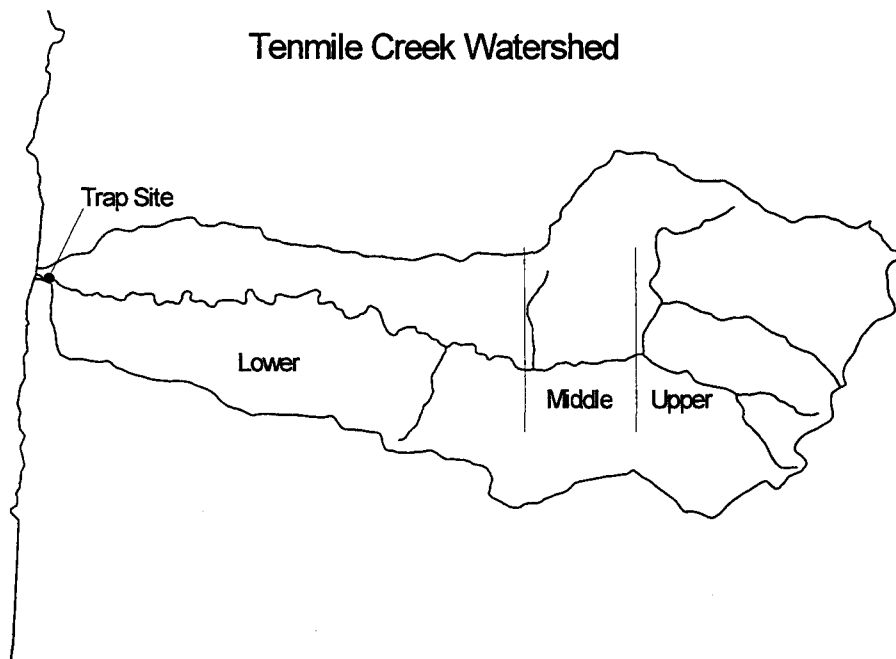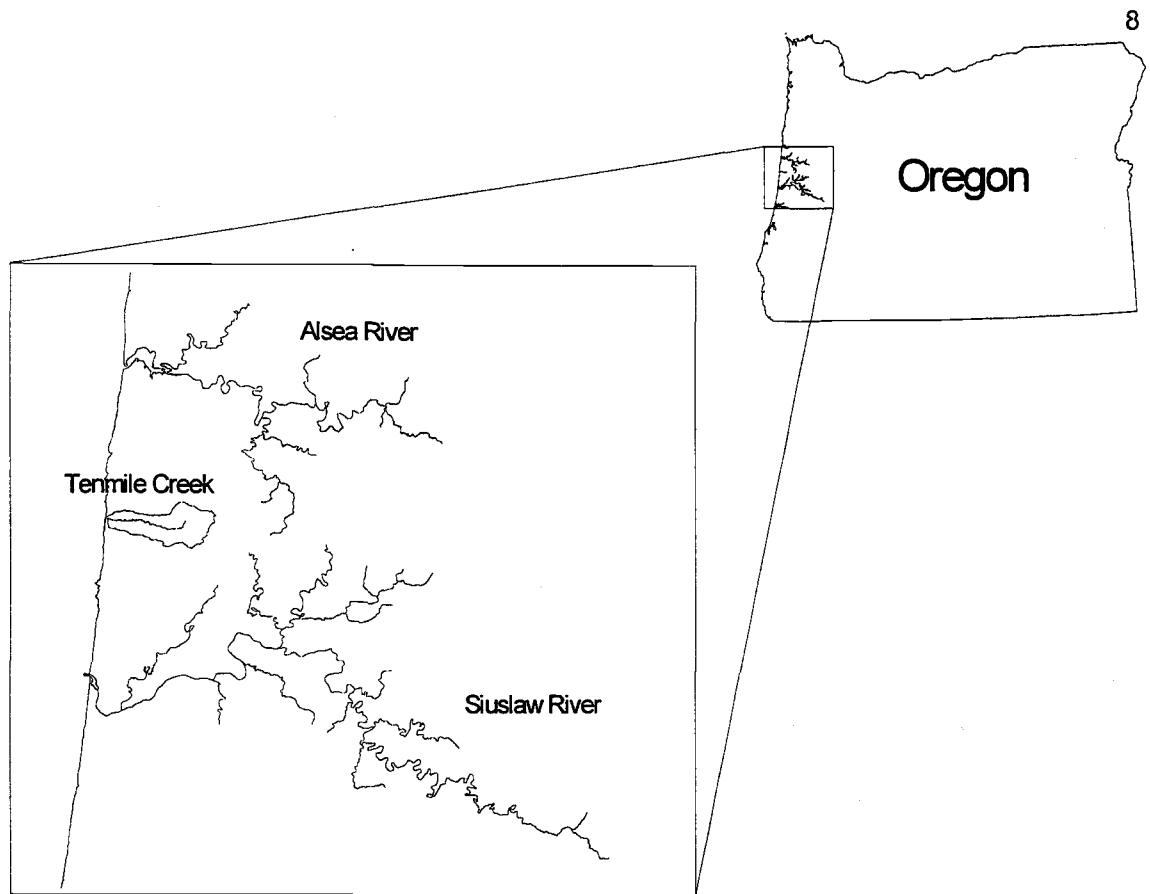
Lower

Middle | Upper

Figure 2.1. Location of Tenmile Creek Study Site

basin is predominantly sandstone. The substrate in the lower and middle portion of Tenmile Creek is composed of 8% sand and silt, 22% gravel, 66% cobble and boulder and 4% bedrock. The upper portion is composed of 5% sand and silt, 23% gravel, 69% cobble and boulder and 3% bedrock. Habitat types in the lower and middle portion of Tenmile Creek are 58% pool, 6% glide and 36% riffle and rapid. The upper portion contains 26% pool, 7% glide and 67% riffle and rapid stream habitat types.

Stream flow is driven by rainfall. Annual rainfall averages 203 - 254 cm. Stream flow estimates range from 35 - 80 $m^3 \cdot sec^{-1}$ for peak winter flows and 2 -5 $m^3 \cdot sec^{-1}$ for low summer flows. Stream temperatures at the mouth reach highs of 18 C° during the summer and lows of 4 C° during the winter. Stream shade averages 45 % in the lower and middle portions and 70 % in the upper portion of the creek. Second and third growth Douglas fir, *Psuedotsuga menziesii*, is the dominant tree species. Sitka spruce, *Picea sitchensis*, is common in the riparian zone of the lower portion of the basin. Red alder, *Alnus rubra*, and big leaf maple, *Acer macrophyllum*, are found throughout all stream reaches. Steelhead trout, *Oncorhynchus mykiss*, cutthroat trout, *O. clarki*, coho salmon, *O. kisutch*, chinook salmon, *O. tshawytsha*, reticulate sculpin, *Cottus perlexus*, riffle sculpin, *C. gulosis*, coastrange sculpin, *C. alueticus*, prickly sculpin, *C. asper*, Pacific giant salamander, *Dicamptodon tenebrosus*, red-legged frog, *Rana aurora*, and tailed frog, *Ascaphus truei* occur in Tenmile Creek.

Materials and Methods

Abundance and Outmigration Timing

A 1.5 m diameter rotary screw trap was fished March to June and August to December during 1994. To increase the total number of fish captured a second larger (2.4 m) trap was also fished during 1995. Traps were fished March to June and October to December during 1995. Traps were operated 24 hr $\cdot$ d^{-1} each year. Both traps had revolving stainless-steel 2 mm mesh cones mounted on aluminum pontoons. The cone entrances were 1.5 m and 2.4 m diameter for the small and large traps respectively, with one-half submerged at anytime. An internal screw rotated the cones. Low summer flows resulted in a rotation of 1 revolution $\cdot$ min^{-1} while peak winter flows resulted in a rotation of 9 revolutions $\cdot$ min^{-1}. Lamprey passing through the cone were collected in a live-box located at the back of the trap. A revolving drum removed small debris from the live-box. Both trap sites were located approximately 400 m upstream from the ocean. In 1995 the traps were separated by 50 m. Each trap fished the head of a pool located below a riffle. The stream was 12 m wide at both trap sites. Each trap fished approximately 10 - 15 % of the stream cross section during all flow conditions.

During mean winter flows the cones were fished in the thalweg at the upper end of each pool approximately 0.1 m above the stream bed. During high flow events the traps were moved downstream 3 - 5 m. While in these positions the traps fished 1 - 2 m outside the thalweg and the cones were 0.2 - 1.0 m above the stream bed. Mean winter flows were estimated at 8 m^3 $\cdot$ s^{-1}. Peak flow was estimated at 35 m^3 $\cdot$ s^{-1} for the 1994 trapping period and 80 m^3 $\cdot$ s^{-1} for the 1995 period.

Fish were usually removed from the traps at dusk each day. During increased flows, fish were removed from the traps more frequently (every 2 hr during daylight and every 1 hr during darkness). Captured Pacific lamprey were counted, anesthetized with buffered tricane methane sulfonate (MS222) and measured to the nearest millimeter. Those lamprey that had not yet begun metamorphosis and were not outmigrating were classified as larvae. Using a modification of Hardisty and Potter's (1982a) methods of classifying stages of metamorphosis, lamprey that were in stages two to seven of metamorphosis and were not outmigrating were classified as pre-smolts. Lamprey considered past stage seven of metamorphosis and found actively migrating downstream were classified as smolts.

Fifty individuals were marked with a caudal fin clip each day. All individuals were marked if there were less than 50 captured. All marked fish were held for 24 hr and released 1 hr after sunset 400 m above the trap site. Recaptured fish were collected daily providing a measure of trap efficiency. All recaptured fish were released below a riffle 100 m downstream of the lower trap site.

Total number of smolts leaving Tenmile Creek was estimated using the following formula:

$$N_{(\text{Week } i)} = C_i / E_i$$

where N_i is the estimated number of smolts outmigrating in Week_i, C_i is the number of unmarked fish captured in Week_i and E_i is the trap efficiency for Week_i.

Trap efficiency was estimated using the following formula:

$$E_{(Week\ i)} = R_i / M_i$$

where R_i is the total number of marked fish recaptured in Week$_i$, and M_i is the total number of marked fish released in Week$_i$. Weekly estimates were summed to provide an estimate for the total migration period. Variance for N_i each weekly period was determined by the bootstrap method developed by Efron and Tibshirani (1986) and summed to obtain a variance for the total migration period.

Smolt yield was also estimated for each day to examine movement relative to changes in stream flow and stream temperature. Daily smolt yield was estimated using the above formula based on the daily trap catch expanded by the appropriate weekly trap efficiency estimate.

Aging

Smolts were collected from the traps during the 1994 and 1995 outmigration period. Each year during the migration period I attempted to collect ten individuals for each 10 mm length range beginning with 101 mm. The first ten fish captured for a given length group were kept for specimens. Collections were filled during the third and fourth week of November each year.

Larvae were used to develop aging criteria to accurately age the smolts. Length-frequency histograms and the statolith, a calcium based structure, were used to define larval ages. A backpack electroshocker was used to collect all larvae. The single largest sample collected during the study period (n=973, 21 August 1995) was used for the length-frequency analysis to increase accuracy in defining larval length at age.

To determine the location of the first statolith annulus and seasonal growth patterns, 75 larvae ranging in length from 12 -138 mm were collected on 21 August 1995, 3 February, 4 April, 31 May, 1 August, 5 October and 2 December 1996. All specimens were stored for 14 d in 50 % ethanol. Statoliths were removed, washed in 50 % ethanol and placed in glycerol on a glass slide. Annuli were most visible immediately after suspension in glycerol. Statoliths left in glycerol more than 10 d degraded making it difficult to discern annuli. A dissecting scope (300X) and transmitted light were used to examine the statoliths for annuli. After reading, statoliths were washed in distilled water and stored dry at room temperature.

Statolith marking techniques were used to validate the statolith aging method. Forty-five larvae were collected from Tenmile Creek on 14 June 1996 with a backpack electroshocker. Larvae were transported to the laboratory, divided into three groups of 15, placed in 95 L aquariums, and provided with a 10 cm layer of mason grade sand for burrowing. Three different temperatures regimes were used. These were a constant 8, 14 and 18 $^{\circ}$ C. A daily cycle of 12 hr of artificial light and 12 hr of darkness was provided. Larvae in each aquarium were fed a mixture of 7 g of bakers yeast diluted in 1 L of water once a week. Larvae were held for 30 d before being injected with oxytetracycline. Larvae received an intraperitoneal injection of 40 µg oxytetracycline $\bullet$ g $^{-1}$ body weight. Larval mortality was monitored during an 80 d period after which time all larvae were measured, killed and statoliths were removed. Statoliths were examined for the presence of oxytetracyline as defined by Medland and Beamish (1991).

Smolt statoliths were aged using the methods developed during the characterization of the larval statoliths. I made three blind readings on each smolt

statolith. I was blind to fish length and specimen number. Eighty percent of the readings for all fish were equal. Of the twenty percent which were not equal, two of the three readings for each statolith were equal. Final age classification was based on the majority reading.

Sex Ratio

Smolts collected for age classification in 1994 and 1995 were also used to determine sex ratios. A lateral cut was made on the ventral surface beginning near the liver and ending at the anus. A dissecting scope (300X) was used to determine the presence of testes or ovaries. A compound microscope (1000X) was used to examine gamete cells to validate ten male and ten female classifications. One hundred four and 66 fish were sexed in 1994 and 1995, respectively.

Results

Abundance and Outmigration Timing

Five hundred ninety-nine smolts were captured and measured in 1994 and 493 in 1995. Smolt lengths ranged from 102 - 171 mm during the two year period. Weekly trap efficiencies ranged from 4 to 17% and 3 to 25% for the 1994 and 1995 migration, respectively. Smolt migration estimates were 6569 and 3592 for 1994 and 1995, respectively (Table 2.1). No outmigration occurred from March to June in either year.

Table 2.1. Estimates and ± 95% confidence intervals (CI) for monthly periods for outmigrating Pacific lamprey smolts in Tenmile Creek, Oregon, during 1994 and 1995.

	1994		1995	
	Number Estimated	± 95% CI	Number Estimated	± 95% CI
Jan. - Feb.	—		—	
Mar. - June	0		0	
July	—		—	
Aug.	2		—	
Sept.	70	87	—	
Oct.	460	768	89	57
Nov. 1 - Dec. 2	6037	1707	3503	1642
Dec. 3 - Dec. 15	0		0	
Dec. 16 - 31	—		—	
All Months	**6569**	**1874**	**3592**	**1643**

The earliest active smolt outmigration observed was on 28 August 1994 and 9 October 1995. All trap captures made before the third week in October were classed as local movement but were included in the annual smolt estimate. This classification was based on the observation that all fish captured prior to the third week in October had not reached stage seven of metamorphosis and were not tolerant to seawater. Greater than 90% of the total outmigration occurred 1 November to 2 December during both years (1994 = 92% and 1995 = 98%).

Stream temperature in Tenmile Creek begins to decrease in September after mean summer highs during July and August (van de Wetering 1996 unpublished data). Mean winter temperatures in Tenmile Creek occur by November. Because winter stream flows are correlated with rainfall in the Oregon coast range, stream flow and stream temperature normally track with similar increases and decreases. Normal fall rains did not occur until late October and early November 1994. Smolts did show some response to these flow increases with corresponding outmigration pulses (Fig. 2.2). During October 1995 stream flow and stream temperature increased three times. Smolts did not respond to these flow and temperature increases with corresponding outmigration pulses (Fig. 2.2). After the first week in November 1995 smolts responded to increases in stream flow and temperature with corresponding pulses in outmigration. Most movement occurred during the first 3 h after dusk and during the rise and fall of stream flow with less movement during the peak of a given high flow event. Almost no movement occurred between dawn and dusk during high, low and mean flows.
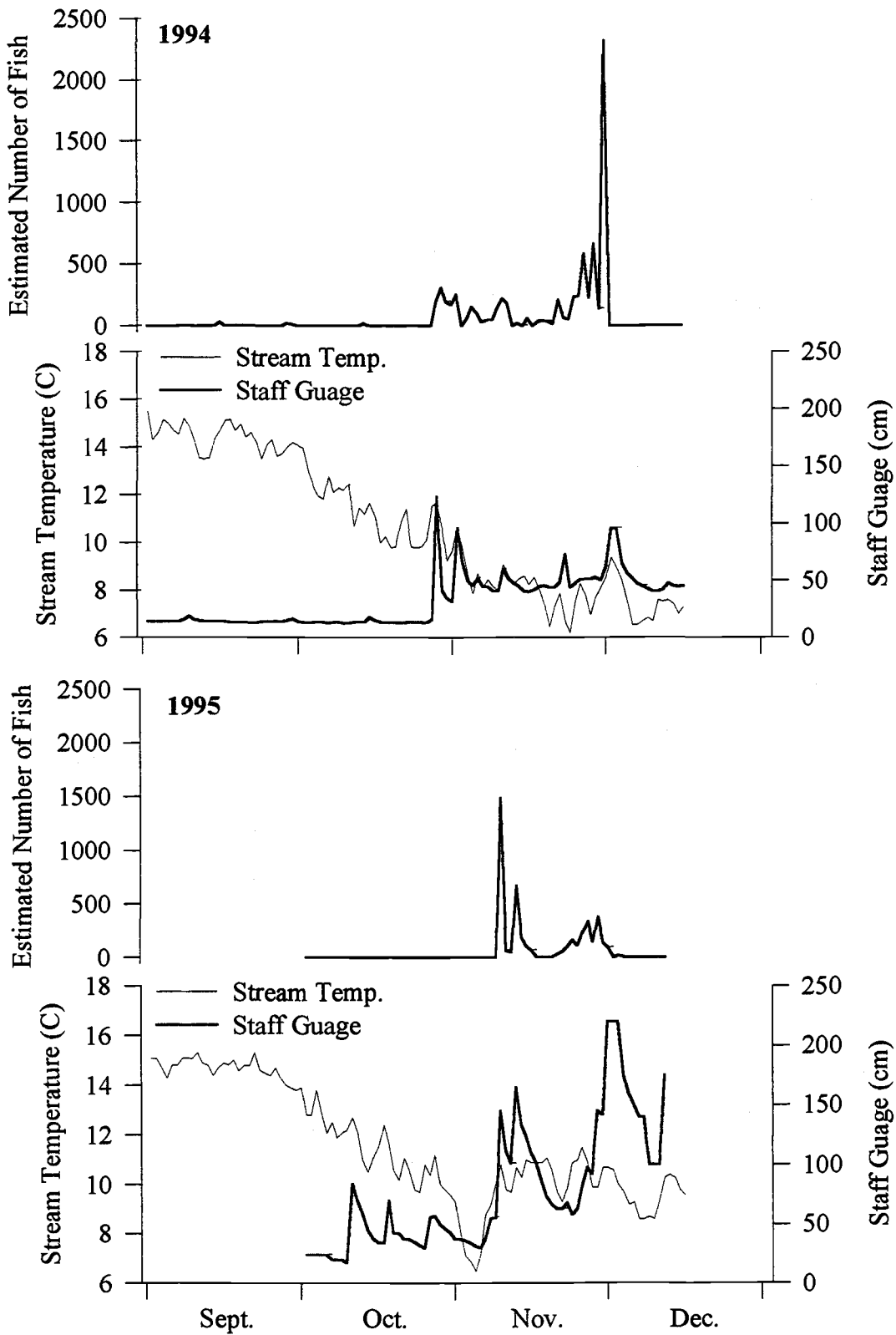
Figure 2.2. Daily estimates of outmigration of Pacific lamprey smolts, stream temperatures and staff guage levels for Tenmile Creek, Oregon, 1994 and 1995.

Aging

Length-frequency histograms and statoliths were used to define age in larvae and smolts. For those larvae sampled, differences were found in mean length, range of length and number of ages estimated using the length-frequency and statolith methods (Fig. 2.3)

Using length-frequency, seven larval age groups were defined by visual interpretation (Fig. 2.3). A graphical interpretation of the larval length-frequency data suggest well defined modes, uniform growth rates and small within group variability. The estimated range of age for the larval population was zero to six years. The smolt length-frequency analysis resulted in a pattern of modes less distinct than that of larvae. Annual increments in growth appeared smaller than that of larvae. Within age group variability appeared similar but less distinct when compared to larvae. Based on the lack of distinct modes, smolt ages were not estimated using length-frequency data.

Based on seasonal sampling, I believe a single statolith annulus was laid down each year during the late fall to early winter. I assigned January first as a birth date for all lamprey. I therefore assumed the 0+ period lasted approximately May - December. Because statoliths collected from the 1994 smolt population were initially stored in glycerol for several weeks prior to aging, defining the annuli for these samples became unreliable. Therefore only results of the age composition for the 1995 smolt population are presented. Using statoliths, five larval and three smolt age groups were defined (Fig. 2.3). The larval data suggest greater mean annual growth rates with more within age group variation, when compared to the length-frequency analysis. The estimated
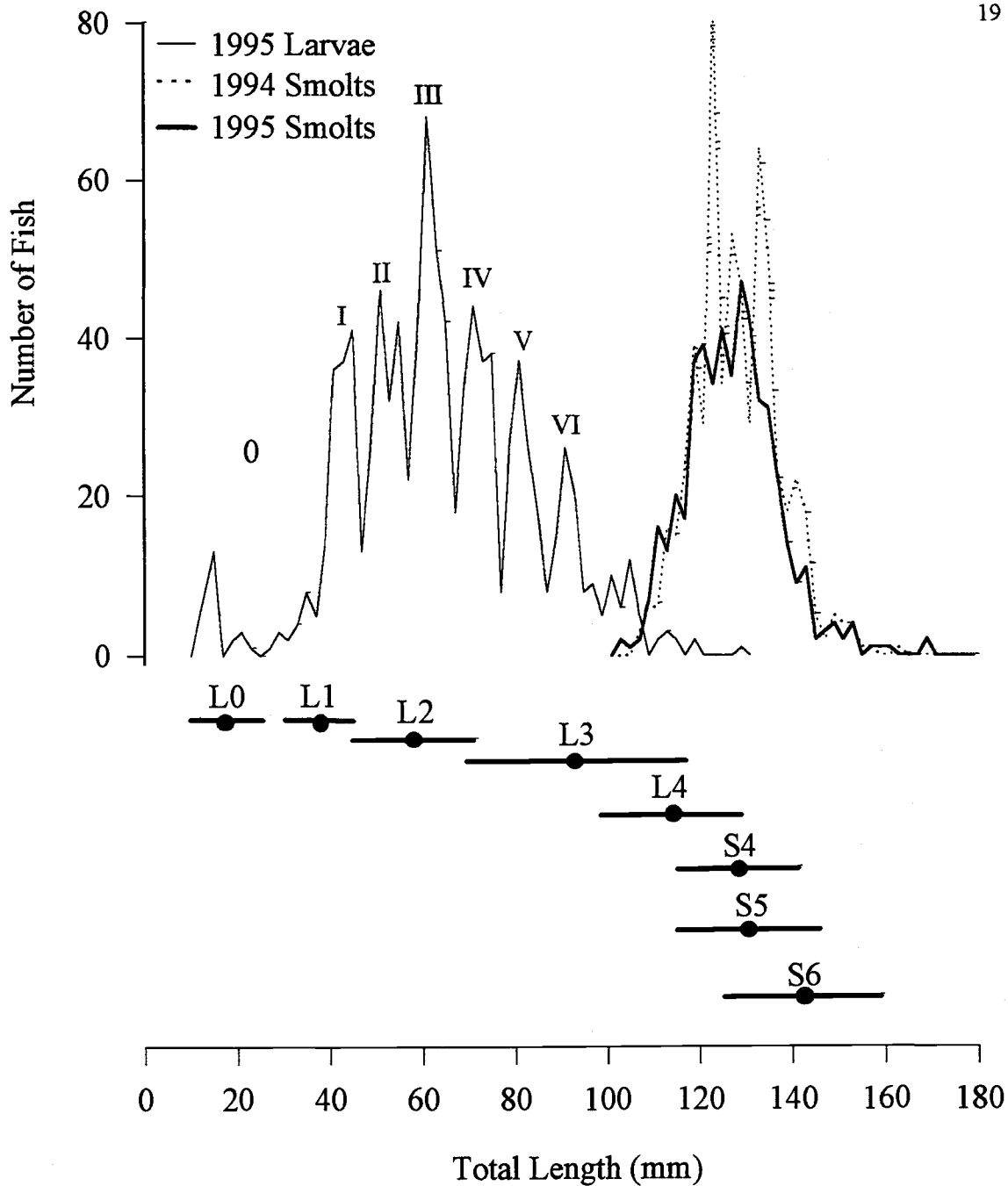
Figure 2.3. Length-frequency distribution of Pacific lamprey larvae collected 21 August 1995 (n=973) and Pacific lamprey smolts collected October to December 1994 (n=599) and 1995 (n=493). Roman numerals correspond to estimated age classes. Horizontal bars are (+/-) 1 SE in length with the mean shown as ● for estimated ages based on statolith readings from larvae collected 21 August 1995 to 2 December 1996 (n=75) and smolts collected in the trap during October to December 1995 (n=60). LO corresponds to an age 0 larva while S4 corresponds to an age 4 smolt.

range of age for larvae was zero to four years. Similar to the length-frequency data, the statolith data suggest reduced growth rates in older age larvae. The three smolt age groups showed smaller distances between mean lengths when compared to the four larval age groups. The within group variation was similar for the larval and smolt age groups. The estimated range of age for smolts was four to six years.

No mortalities occurred and no fish were positive for the presence of oxytetracycline in the statolith, for the 45 larvae which received interperitoneal oxytetracycline injections. Mean increases in lengths were 15, 10, and 13 mm for those larvae held at 8, 14 and 18 °C, respectively.

Sex Ratio

Of those fish sexed, the male to female ratio was approximately 1:1 in 1994 and 1995. Males and females had similar distributions across all length categories in 1994 and 1995 (Fig. 2.4).
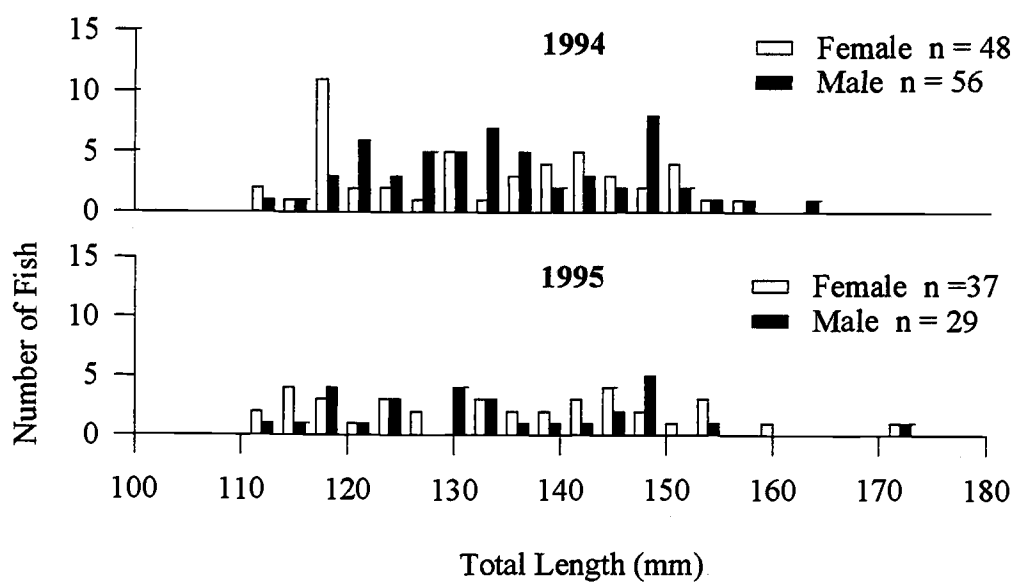
Figure 2.4. Pacific lamprey smolt length distribution for the sex sub-sample collected 1994 and 1995.

Discussion

The mean number of Pacific lamprey smolts estimated in Tenmile Creek varied between the two study years. The 1994 outmigration was almost twice that of 1995. Beamish and Levings (1991) also reported high variability in annual outmigrant numbers. Whether this variation is common for Pacific lamprey in other cases is unknown. While collecting age samples during the summers of 1994 and 1995, I observed most larvae were distributed in the lower 3 km of the mainstem. Upstream distribution of larvae is limited by the distribution of spawners. Larval lamprey are generally not capable of upstream movement due to limited burst speeds (Hardisty and Potter 1982a). If a spawning population is distributed throughout an entire river system, and larval rearing habitats are being fully utilized, it would follow that a reduction in spawner distribution (no upper watershed spawning) would result in a reduction in habitat available to larvae and therefore a reduction in the number of larvae produced. Variation in annual peak flow event patterns, specifically, flood events, and the presence of degraded habitat, may also result in shifts in larval populations. Pacific larval lamprey appear to shift burrowing habitats during the rise and fall of the stream hydrograph during average winter rain storm events (van de Wetering, 1996 unpublished data). This could be due to preferred feeding currents, preferred depths, or the scouring of burrowing habitat. Large annual peak flow events could greatly affect available burrowing habitats via scouring and deposition of burrowing substrates. Increased shifts in burrowing sites would likely result in a downstream only redistribution of larvae which in turn may result in an overall lower basin-wide larval survival rate.

Limited information is available about the outmigration patterns of Pacific lamprey smolts. Seasonal water temperatures and stream flow patterns may provide the strongest influence on migration timing in Pacific lamprey smolts. Seasonal water temperatures may influence the initiation and duration of the metamorphic process while increased stream flows likely create enhanced migration conditions. My results suggest the seasonal outmigration of Pacific lamprey in Tenmile Creek is a response to the completion of metamorphosis followed by increased stream flow. Ancillary experiments showed most Pacific lamprey smolts were tolerant to full strength seawater, and thus had completed metamorphosis by the first of November (Appendix Table). Studies by Richards (1980) and Richards and Beamish (1981) suggested the Pacific lamprey of the Chemainus River, British Columbia, a small coastal river with temperature and stream flow patterns similar to Tenmile Creek, had a period of metamorphosis from summer to fall, were able to osmoregulate in seawater by fall, and had completed the outmigration by late fall. Beamish and Levings (1991) found outmigration of metamorphosed Pacific lamprey in the Nicola River, a tributary to the Frazier River, British Columbia, extended from the fall (September - November) through the spring (March-May). No observations were made during winter ice-up (December-February). Most (>80%) movement occurred within an eight week period during the spring floods. Flow influenced movement but temperature did not. Hammond (1979) suggested outmigration of Pacific lamprey in the Potlatch River, Idaho, began by mid November and continued into the spring as fish passed through upper Columbia River dams.

It is unclear whether differences in metamorphic development exist between fall and spring outmigrants. Prolonged metamorphic periods have been reported for lamprey

held at reduced temperatures (Youson et al. 1993). Early (fall) migrants in large snowmelt driven river systems may compose the portion of the lamprey population that has completed metamorphosis before annual minimum stream flow and water temperature occurs. Lamprey smolt populations which have completed metamorphosis but do not have flows beneficial for migration may be able to survive a further extended fall/winter period in freshwater without feeding. Although the majority of the Tenmile Creek Pacific lamprey smolt population outmigrates over a short period of time, it appears that these lamprey possess the ability to live in freshwater, post metamorphosis, for a prolonged period of time. An ancillary experiment showed 40 pre-smolts collected in Tenmile Creek and held in a freshwater tank at 10 °C without food from November through March, resulted in an 88% survival rate (van de Wetering 1995 Unpublished data). I believe the variation in seasonal outmigration timing of Pacific lamprey, found between this study, Beamish and Levings (1991), Richards (1980) and Hammond (1979), is due, in part, to: (1) seasonal water temperatures, which affect both the metamorphic process and lamprey activity and (2) flow patterns, which affect migration conditions. An additional area which deserves exploration is the evolutionary influence on outmigration timing created by seasonal prey availability and presence of predators in the estuarine and ocean environments.

Historically, fish biologists have considered the presence of distinct modes in a length-frequency analysis as a validation of well separated component age groups (MacDonald 1987). Manual and computer generated methods exist to form estimates of the shape and area under the curve constructed using length distribution data. Much debate exists as to whether these methods can produce mathematically valid and

biologically meaningful data (MacDonald 1987; Schnute and Fournier 1980). The use of hard parts in aging fish has become common place in much of the fisheries literature. Past research has shown that although hard parts can provide accurate age classification, without an associated validation of the method, through a marking technique, the analysis can be misleading (Summerfelt and Hall 1987). Although the present data raise questions regarding the accuracy of the methods used, they provide a good base from which to begin examining larval Pacific lamprey growth rates. Both the length-frequency and statolith analyses suggest Pacific lamprey exhibit a reduction in growth as they approach the end of the larval period. Beamish and Levings (1991) reported similar results using statolith aging methods while examining Pacific lamprey populations from British Columbia, Canada. Beamish and Levings (1991) reported 84% of a spring migrant sample to be age 5+ and 6+. These ages correspond to 4+ and 5+ during the previous fall. These authors also reported larval length-frequency patterns similar to the present study. Regardless of the aging method used, and therefore the description of larval growth rates, the present study suggests larval lamprey have an extended freshwater residency when compared to anadromous salmonids. The present study also suggests size is of more importance in cueing the smolting process than is age.

The length-frequency and the statolith methods used here require validation to better describe growth patterns in larval Pacific lamprey. A time series sample of length distributions could be used to examine whether the distinct modes observed in this study continue across time or whether they are a product of other processes. These processes could include variation in spawning and emergence timing and or increased or decreased growth rates based on microhabitats utilized. Although other authors (Beamish and

Medland 1988; Medland and Beamish 1991) have described success in marking lamprey statoliths, I believe more work is needed to develop a reproducible method in which researchers can validate annual growth increments in Pacific lamprey larvae from a range of ages.

The sex ratio of other lamprey species has been shown to be related to larval rearing densities. Purvis (1971) reported observing significant increases in the female to male ratio in larval sea lamprey, *Petromyzon marinus*, populations with severely reduced densities. I suspected Pacific lamprey densities were low in Tenmile Creek and that the sex ratio might be skewed. However, sex ratio was similar during the two study years. If the Tenmile Creek larval lamprey densities are low, the population does not appear to be responding by producing a higher proportion of females.

This study and that of Beamish and Levings (1991) suggest Pacific lamprey have an extended freshwater residency prior to smoltification. Much work has been done examining losses in stream habitat complexity and the accompanying deleterious effects on anadromous fish populations in the Pacific Northwest (Bisson et al. 1987). It is logical to assume losses in freshwater habitat complexity result in higher mortalities in those anadromous species with longer freshwater residencies. The present study provides initial data regarding the current status of larval densities in streams of the Oregon coast range. The present study and that of Beamish and Levings (1991) suggest that lamprey populations located in larger inland river systems have very different migration patterns than those in near coastal systems.

Literature Cited

Beamish, R.J. 1980. Adult biology of the river lamprey, *Lampetra ayresi*, and the pacific lamprey, *Lampetra tridentata*, from the Pacific coast of Canada. Can. J. Fish. Aquat. Sci. vol. 37.no. 11 pp 1906-1923.

Beamish, F.W.H. and Medland, T.E. 1988. Age determination for lampreys. Trans. Amer. Fish. Soc. 117:63-71.

Beamish, R.J. and Levings, C.D. 1991. Abundance and freshwater migrations of the anadromous parasitic lamprey, *Lampetra tridentata*, in a tributary to the Frazer River, British Columbia. Can. J. Fish. Aquat. Sci. 48:1250-1263.

Bisson, P.A., Bilby, R.E., Bryant, M.D., Dollof, C.A., Greet, G.B., House, R.A., Murphy, M.L. Koski, K.V., and Sedell, J.R. 1987. Large woody debris in forested streams in the Pacific Northwest: past, present and future. Pages 143-190 *in* E.A. Salo and T.W. Cundy, [ed]. Streamside management: forestry and fishery interactions. Institute of Rorest Resources Contribution 57. University of Washington, College of Forest Resources, Seattle.

Close, D.A., Fitzpatrick, M., Li, M., Parker, B., Hatch, D., and James, G. 1995. Status Report of the Pacific lamprey, *Lampetra tridentata*, in the Columbia River Basin. U.S. Dept. Energy, Bonneville Power Admin., Div. Fish and Wildlife, Portland, OR.

Downey, T., Rilatos, D., Sondenaa, A., and Zybach, B. 1993. The Siletz Eels: Oral history interviews with Siletz tribal elders and neighboring residents regarding the decline in Siletz River lamprey populations. Native Americans in Marine Science Program, Oregon State University, Corvallis, Oregon.

Effron, B., and Tibshirani, R. 1986. Bootstrap methods for standard errors, confidence intervals and other measures of statistical accuracy. Statistical Science 1:54-77.

Hammond, R.J. 1979. Larval biology of the Pacific lamprey, *Entosphenus Tridentatus* (Gairdner), of the Potlatch River, Idaho. Masters Sci. Thesis, Univ. Idaho, Moscow, Idaho.

Hardisty, M.W. and Potter, I.C. 1982a. Stages in metamorphosis, p. 137-164. *In* M.W. Hardisty and I.C. Potter. [ed.] The biology of lampreys. Academic Press, London.

Hardisty M.W. and Potter, I.C. 1982b. The behavior, ecology and growth of larval lampreys, p. 85-120. *In* M.W. Hardisty and I.C. Potter. [ed.] The biology of lampreys. Academic Press, London.

MacDonald, P.D.M. 1987. Analysis of length-frequency distributions. p 371-384 *In* R.C. Summerfelt and G.E. Hall. [ed]. Age and growth of fish. Iowa State University Press, Ames, Iowa.

Medland, T.E., and Beamish, F.W.H. 1991. Lamprey statolith banding in response to temperature, photoperiod, and ontogeny. Trans. Amer. Fish. Soc. 120:255-260.

Anonymous. 1998. State of Oregon, Department of Fish and Wildlife. USA.

Pletcher, F.T. 1958. The life history and distribution of lampreys in the Salmon and certain other rivers in British Columbia, Canada. Masters Sci. thesis, Univ. British Columbia, Vancouver, B.C.

Purvis, H.A. 1971. Variations in growth, age at transformation and sex ratio of sea lampreys reestablished in chemically treated tributaries of the upper Great Lakes. Gt. Lakes Fish. Commn. Tech. Rep. 35:1-36.

Richards, J.E. 1980. Freshwater life history of the anadromous Pacific lamprey, *Lampetra tridentata*. Masters Science thesis, University of Guelph, Guelph, Ont., Canada.

Richards, J.E. and Beamish, F.W.H. 1981. Initiation of feeding and salinity tolerance in the Pacific lamprey, *Lampetra tridentata*. Marine Biology 63:73-77.

Schute, J. and Fournier, D. 1980. A new approach to length-frequency analysis: Growth Structure. Can. J. Fish. Aquat. Sci. 37:1337-1351.

Summerfelt, R.C. and Hall, G.E. 1987. Age and growth of fish. Iowa State University Press, Ames, Iowa.

Youson, J.H., Holmes, J.A., Guchardi, J.A., Seelye, J.G., Beaver, R.E., Gersmehl, J.E., Sower, S.A., and Beamish, F.W.H. 1993. Importance of condition factor and the influence of water temperature and photoperiod on metamorphosis of sea lamprey, *Petromyzon marinus*. Can. J. Fish. Aquat. Sci. 50:248-2456.

CHAPTER 3

Smolting in Pacific Lamprey, *lampetra tridentata*

Stan J van de Wetering

Richard Ewing

Abstract

Larval, metamorphosing and smolting Pacific lamprey were examined for changes in skin guanine concentrations, gill (Na+K)-ATPase specific activities and plasma thyroid hormone levels during a 14 mo period. Seasonal peaks in gill (Na+K)-ATPase specific activities and plasma thyroid hormone levels were observed in larvae. Metamorphosing and smolting lamprey showed increases in skin guanine concentrations and gill (Na+K)-ATPase specific activities along with decreases in plasma thyroid hormone levels. Smolting lamprey challenged with artificial seawater showed no significant changes in the parameters observed.

Introduction

Physiological and behavioral changes underlying the process of parr-smolt transformation have been studied extensively in anadromous salmonids. These include changes in coloration (Staley and Ewing 1992), morphology (Folmar and Dickoff 1980), behavior (McCormick 1994), fat levels (Fessler and Wagner 1969), gill (Na+K)-ATPase specific activities (Ewing et al. 1979; Folmar and Dickoff 1979) and seawater tolerance (Varnavsky et al. 1992). In other anadromous fish, however, much less information is available. The phylogenetic origins of these changes have not been examined extensively, although Hoar (1976) has examined some of the aspects in his review.

One of the most primitive of fishes, the lamprey, incorporates anadromy in its life history. The Pacific lamprey, *Lampetra tridentata*, is an anadromous lamprey which undergoes an extended freshwater and marine residence period (Beamish and Levings

1991). Freshwater and marine life history stages are bridged by a four to five month

metamorphosis during which time morphological and behavioral changes occur similar to

those observed in smolting salmonids. The cryptic brown larvae develop a silvery

coating. Condition factor decreases due to metabolism of fats (Hardisty and Potter

1982b), salinity tolerance increases (Richards and Beamish 1981) and an active seaward

migration occurs (Beamish and Levings 1991).

In this study, we examine the changes in a number of physiological parameters in

wild Pacific lamprey which had attained sizes necessary for metamorphosis. In

particular, we measured the parameters which have become common for measurement of

smolting in salmonids and examined these relative to different life history stages of the

Pacific lamprey.

Study Site

Tenmile Creek is a fourth order stream located on the central Oregon coast (Fig.

3.1). There are approximately 27 km of linear stream miles located in 61 km^2 of forested

land. Tenmile Creek flows directly into the Pacific ocean. No estuary is present.

Porphyritic basalt dominates the lower two thirds of Tenmile Creek basin while the upper

basin is predominantly sandstone. The substrate in the lower and middle portion of

Tenmile Creek is composed of 8% sand and silt, 22% gravel, 66% cobble and boulder

and 4% bedrock. The upper portion is composed of 5% sand and silt, 23% gravel, 69%

Oregon

Alsea River

Tenmile Creek

Siuslaw River

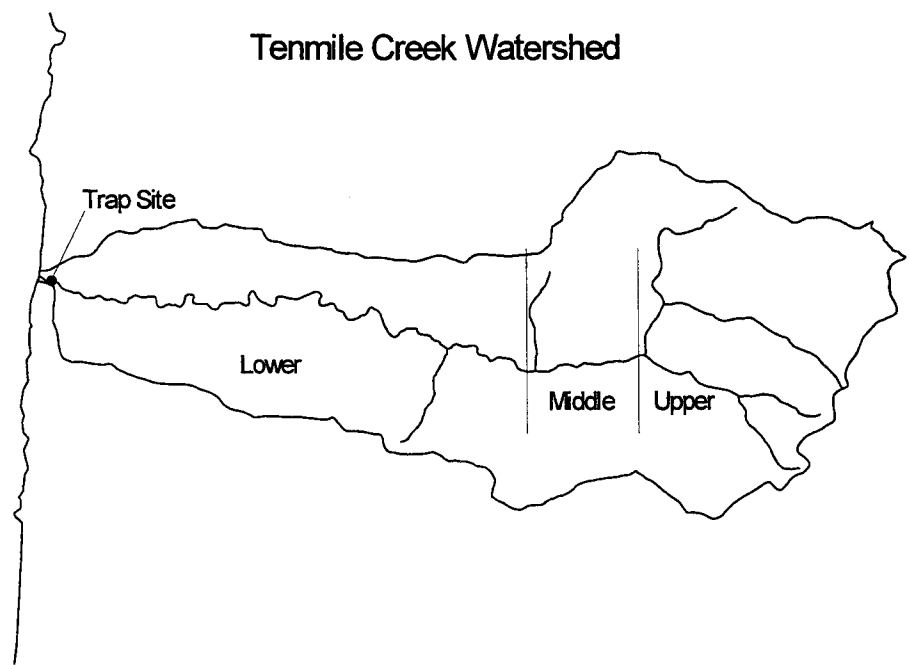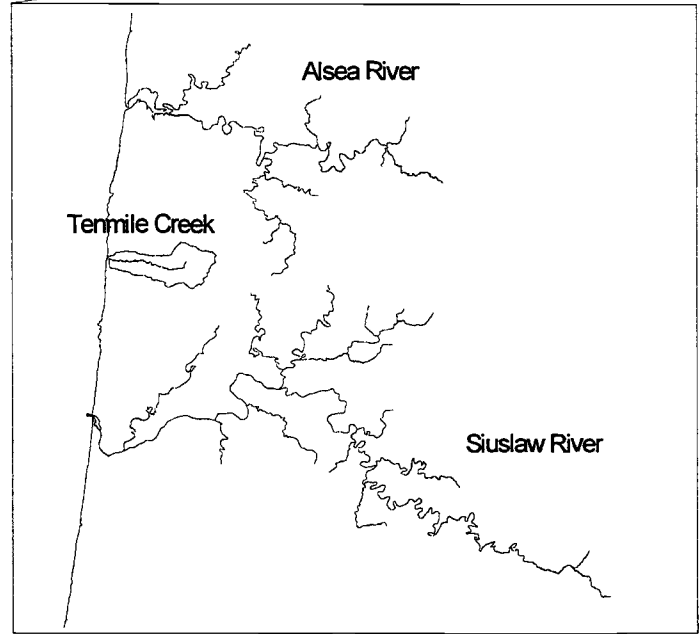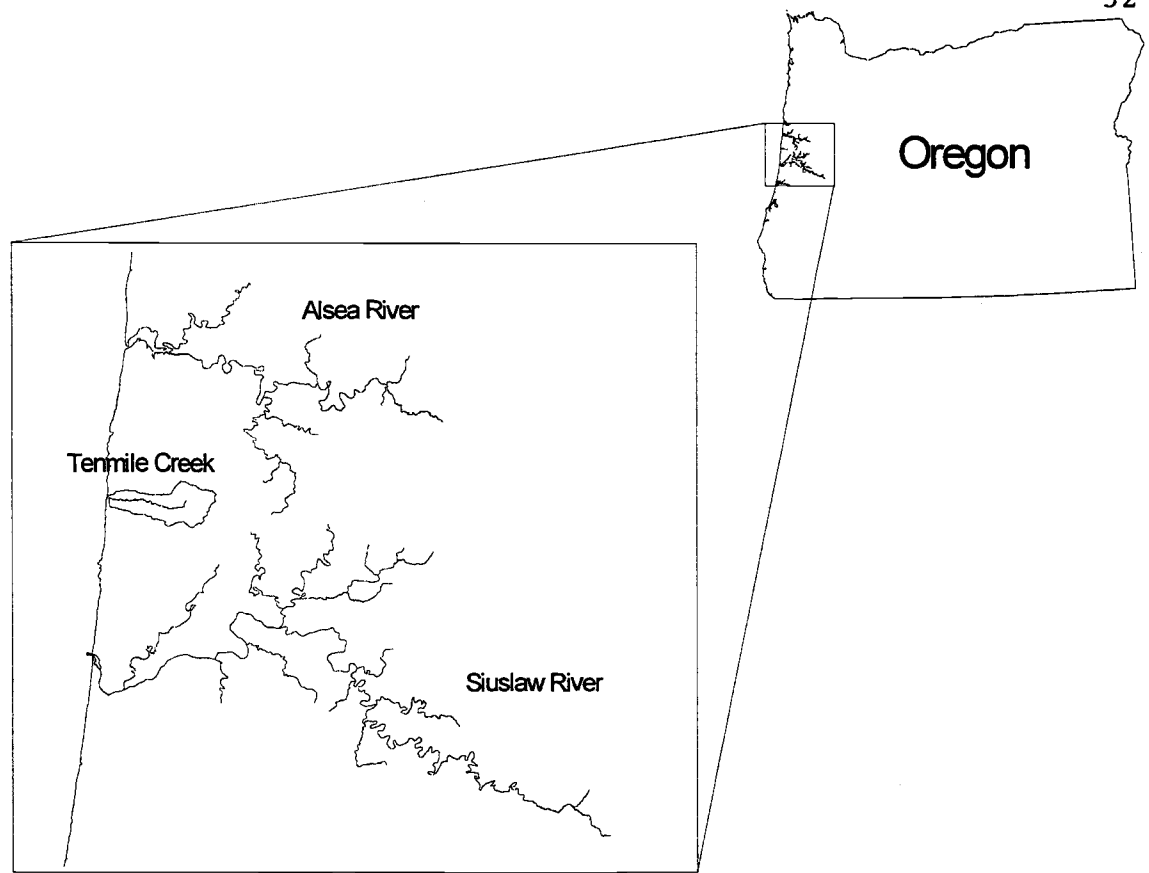## Tenmile Creek Watershed

Trap Site

Lower

Middle | Upper

Figure 3.1. Location of Tenmile Creek Study Site

cobble and boulder and 3% bedrock. Habitat types in the lower and middle portion of Tenmile Creek are 58% pool, 6% glide and 36% riffle and rapid. The upper portion contains 26% pool, 7% glide and 67% riffle and rapid stream habitat types.

Stream flow is driven by rainfall. Annual rainfall averages 203 - 254 cm. Stream flow estimates range from 35 • 80 m^3 • sec^{-1} for peak winter flows and 2 -5 m^3 • sec^{-1} for low summer flows. Stream temperatures at the mouth reach highs of 18 C° during the summer and lows of 4 C° during the winter. Stream shade averages 45 % in the lower and middle portions and 70 % in the upper portion of the creek. Second and third growth Douglas fir, *Psuedotsuga menziesii*, is the dominant tree species. Sitka spruce, *Picea sitchensis,* is common in the riparian zone of the lower portion of the basin. Red alder, *Alnus rubra,* and big leaf maple, *Acer macrophyllum,* are found throughout all stream reaches. Steelhead trout, *Oncorhynchus mykiss,* cutthroat trout, *O. clarki,* coho salmon, *O. kisutch,* chinook salmon, *O. tshawytsha,* reticulate sculpin, *Cottus perlexus,* riffle sculpin, *C. gulosis,* coastrange sculpin, *C. alueticus,* prickly sculpin, *C. asper,* Pacific giant salamander, *Dicamptodon tenebrosus,* red-legged frog, *Rana aurora,* and tailed frog, *Ascaphus truei,* occur in Tenmile Creek.

Materials and Methods

Pacific lamprey in different life stages were collected from Tenmile Creek, during a 14 month period beginning October 1995 and ending November 1996. Hardisty and Potter's (1982a) classification system for larval lampreys was used with new definitions derived from common usage in smolting in salmonids. Larvae were defined as freshwater forms which had not yet begun metamorphosis and were not undergoing

seaward migration. Lamprey that were in stages two to seven of metamorphosis were defined as pre-smolts. Lamprey that were past stage seven and were actively migrating to sea, were considered smolts.

Field Collections

Larvae and pre-smolts were drawn from the substrate with a backpack electroshocker (800 V, 1 Amp.), captured with a net, held in a bucket and sampled for tissues within 1 hr. Smolts were captured in a 1.5 m rotary screw trap from 1700 to 1900 hours during November 1995 and were sampled within 1 hr of capture. All fish were anesthetized with buffered tricane methane sulfonate (MS222) prior to sampling. Stream temperature was monitored every 1 hr during the 14 mo period. Temperature gauges were located in the lower portion of Tenmile Creek.

Laboratory Experiments

To examine the influence of freshwater versus marine habitat on the response of the parameters measured we challenged smolts with artificial seawater. Smolts were collected from a rotary screw trap on 5 December 1995 and transferred in aerated containers to the laboratory. Transfer time was 2 hr. Nine smolts were placed in a 95 L aquarium containing a 10 cm layer of mason's grade sand for burrowing and supplied with artificial seawater (30 ppt) while five were held in freshwater. Water temperature was 10°C in both groups. Both groups were held in complete darkness for 6 d before sampling of tissues.

To examine the effect of electroshocking on tissue parameters, pre-smolts were collected on 5 October 1996 and transferred to the laboratory where they were acclimated for 30 d. Pre-smolts were held in 95 L aquariums containing a 10 cm layer of mason's grade sand supplied with well water at a constant 10°C. Nine of the pre-smolts were drawn from the substrate by electroshock for 3 min with a backpack electroshocker (800 V, 1 Amp.). A control group of nine pre-smolts was captured by running a glass rod through the substrate. Fish were sampled within 1 hr of capture. Fish used in the laboratory experiments were anesthetized with buffered MS222 prior to sampling.

Tissue Parameters

Skin guanine was measured by the enzymatic method of Staley and Ewing (1991). Gill tissue was collected from frozen heads of lampreys by slicing through the buccal cavity and scraping the gill filaments with a razor blade. Gill (Na+K)-ATPase specific activity was measured by the method of Johnson et al. (1977). Anesthetized lampreys were bisected immediately posterior to the heart and blood was collected in heparinized microcapillary tubes. The tubes were blocked with Critoseal and centrifuged for three minutes at room temperature at 6580 x g force in a MSE centrifuge. The tubes were removed and frozen on dry ice for transport to the lab. At the laboratory, the frozen microcapillary tube was snapped at the junction between plasma and red blood cells and plasma was collected in 0.5 ml microcentrifuge tubes. Ten μL samples of plasma were assayed for thyroxine and tri-iodothyronine by the enzyme immunoassay method of Kunst et al. (1988).

Statistical Analyses

The first analysis examined differences in sample groups at specific points in time during the 14 mo period. Larval parameter values were compared to those of pre-smolts or smolts. Differences between two samples, larval versus pre-smolt or larval versus smolt, for specific sample dates, were analyzed using a Students t test at the 95% level of confidence.

The second analysis examined temporal patterns within three groups. These groups were pre-smolts and smolts 1995, pre-smolts only 1996, and larvae 1995 - 1996. Within group differences in tissue parameters were analyzed by analysis of variance at the 95% level of confidence. To remove non-constant variance between sample dates, data were transformed using natural log prior to statistical analysis. Data presented in graphics are non-transformed data.

A Student's t test, at the 95% level of confidence, was used to examine differences in parameter values from the electroshocking and seawater challenge experiments. Linear regression at the 95% level of confidence was used to examine the correlation between larval plasma T4 levels and stream temperatures.

Results

Skin Guanine Concentrations

Skin guanine concentrations in larvae were significantly ($p < 0.05$, $F_o = 47.76$, d.f.=90) different between times during the 14 mo period (Fig. 3.2.a.). The apparent
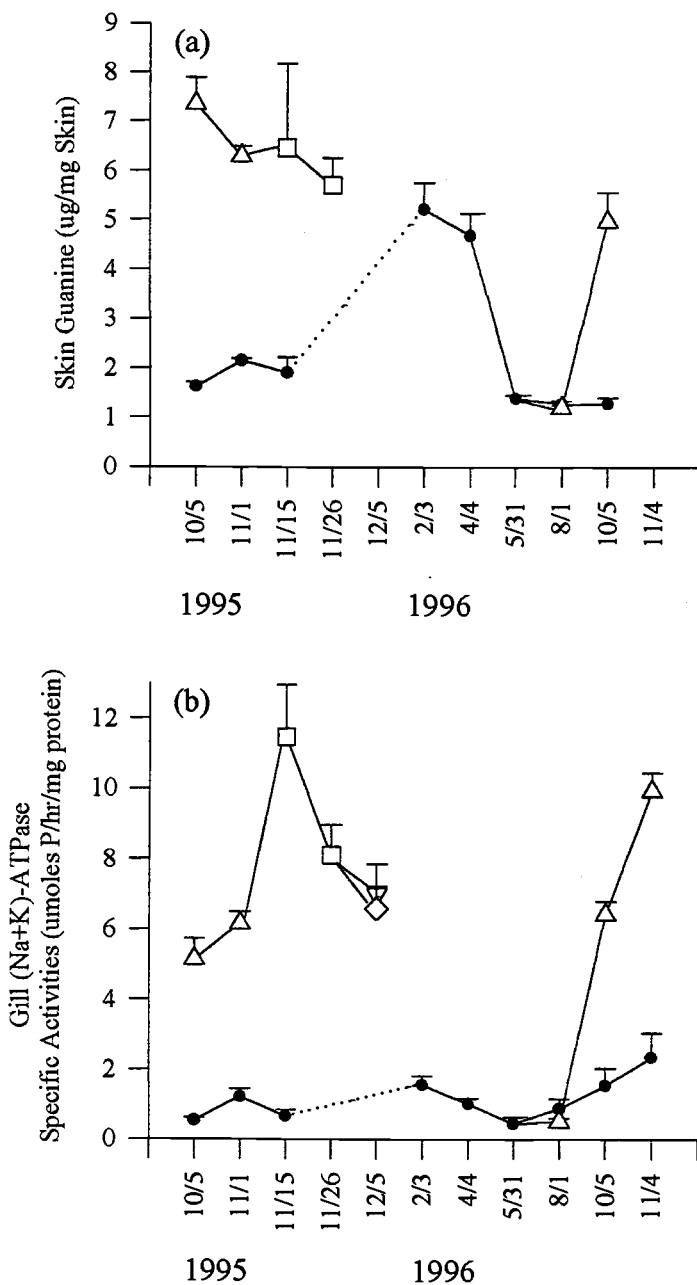
Figure 3.2. Graphs illustrate mean (a) skin guanine (ug guanine · mg^{-1} skin) and (b) gill (Na+K)-ATPase activities (uM · hr^{-1} · mg^{-1} protein), in wild Pacific lamprey larvae (●), pre-smolts (△), migrating smolts (□), seawater challenged smolts (▽), and freshwater challenged smolts (◇) from Tenmile Creek, Oregon, during 1995 and 1996. Vertical bars represent (+/-) 1SE.

increase in larval skin guanine concentrations in μg guanine per mg skin during winter 1996 (sample dates 2 February and 4 April) arose from a thinning of the skin rather than an increase in guanine content. Although the same technique was used for removing the skin, the average weight of the skin dropped by one-third during this time period.

There were also significant ($p < 0.05$, $F_o = 102.5$, d.f.=19) differences in the pre-smolt samples taken during 1996 (Fig. 3.2.a.). This was evident in the change in skin coloration from dull brown (1 August 1996) to silver (5 October 1996) during the metamorphic period. There were no significant ($p > 0.05$) differences in the pre-smolt/smolt samples taken in 1995 (Fig. 3.2.a.).

Guanine content in the pre-smolt/smolt samples was significantly ($p < 0.05$) higher than that of larvae during all sample periods with the exception of 1 August 1996 (Fig. 3.2.a.). Again, these differences reflected an increase in guanine content of the skin of pre-smolts rather than changes in skin thickness.

Gill (Na+K)-ATPase Specific Activities

Pre-smolt/smolt specific activities were significantly ($p < 0.05$) higher than those of larvae measured at the same time with the exception of 1 August 1996 (Fig. 3.2.b.). Gill (Na+K)-ATPase specific activities were less than 2.0 μmoles P/hr/mg protein in larvae. When metamorphosis occurred near 8 August and pre-smolts were first found in the samples, gill (Na+K)-ATPase was not significantly ($p > 0.05$) different between larvae and pre-smolts. In subsequent samples, however, gill (Na+K)-ATPase in pre-smolts was at least three times higher than that of larvae.

Pre-smolt/smolt gill (Na+K)-ATPase activities were significantly (p< 0.05, F_o=9.84, D.F.=46) different during 1995 as were pre-smolts during 1996 (Fig 3.2.b.). These data suggest a peak in gill (Na+K)-ATPase activity occurs during metamorphosis followed by a flattening or decrease during the late smolt and ocean entry periods.

Larval gill (Na+K)-ATPase activities were significantly (p< 0.05, F_o=2.35, D.F.=86) different during the 14 mo period (Fig. 3.2.b.). Although the changes were small, these data suggest the occurrence of a pattern of larval gill (Na+K)-ATPase activities which reach a low in the spring and a peak during the fall to winter seasons.

Plasma Thyroxine

Pre-smolt/smolt plasma T4 concentrations were significantly (p< 0.05) lower than those of larvae measured at the same time (Fig 3.3.a.). The 1996 data show the drop in T4 occurs early in metamorphosis.

Pre-smolt/smolt plasma T4 concentrations were not significantly (p> 0.05) different during 1995 or 1996 (Fig. 3.3.a.). Larval plasma T4 concentrations were significantly (p< 0.05, F_o=3.69, D.F.=89) different during the 14 mo period (Fig. 3.3.a.). These data suggest the occurrence of a fall to winter peak in larval plasma T4 concentrations. Plasma T4 levels did not show a significant (p>0.05) correlation with stream temperatures (r^2 = 0.09, n=8) although the graphical image shows a general pattern of response between the two parameters during the pre-metamorphic period (Fig. 3.3.b.).

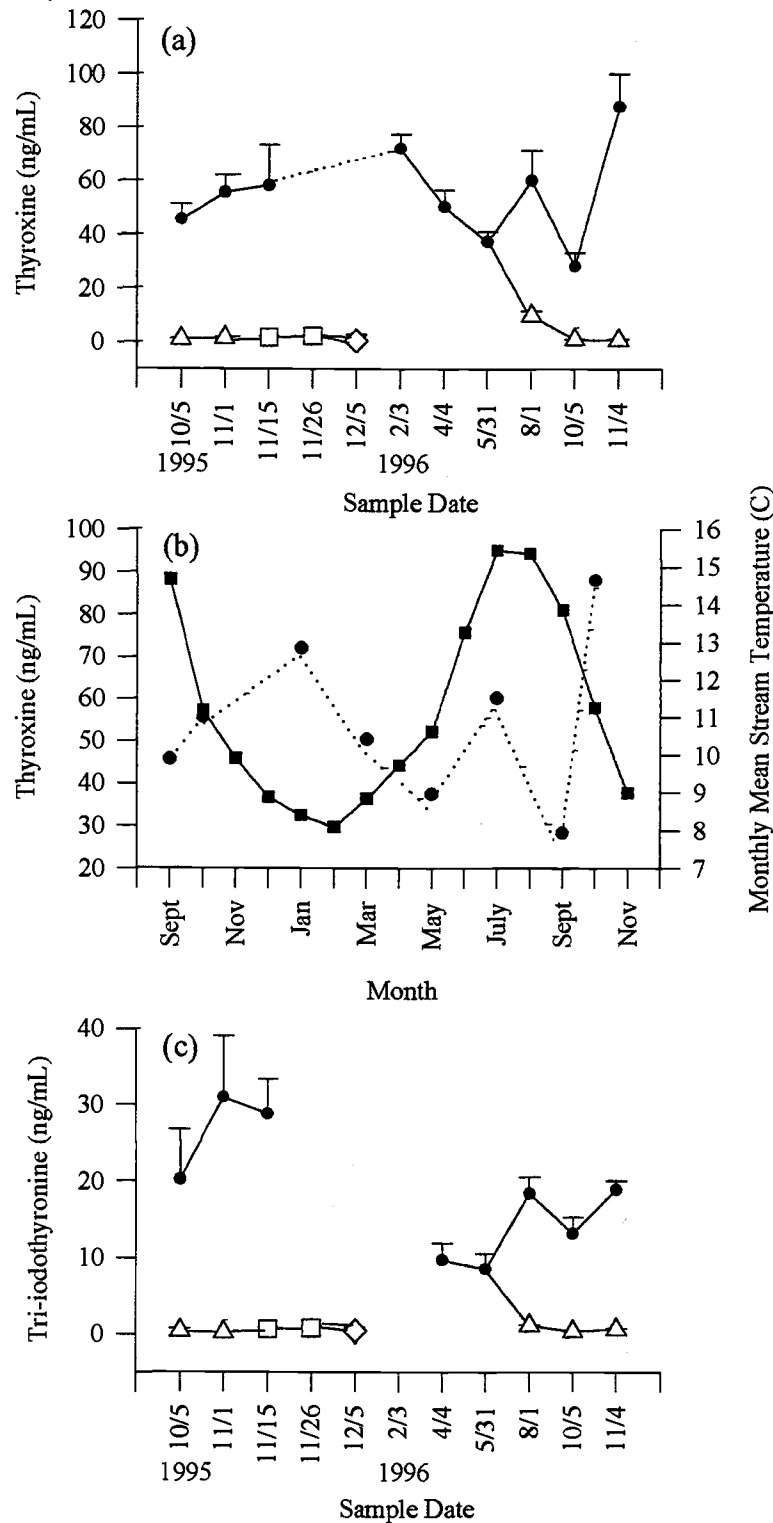Figure 3.3. Graphs illustrate (a) mean plasma thyroxine (ng · mL⁻¹), (b) mean monthly stream temperature and plasma thyroxine and (c) mean plasma tri-iodothyronin (ng · mL⁻¹) in wild Pacific lamprey larvae (●), pre-smolts (△), migrating smolts (□), seawater challenged smolts (▽), and freshwater challenged smolts (◇) from Tenmile Creek, Oregon, during 1995 and 1996. Vertical bars represent (+/-) 1SE.

Plasma Tri-iodothyronine

Pre-smolt/smolt plasma T3 concentrations were significantly ($p < 0.05$) lower than those of larvae measured at the same time (Fig. 3.3.c.). The drop in T3 appears to correspond with that of T4 early in metamorphosis. Pre-smolt/smolt plasma T3 concentrations were not significantly ($p > 0.05$) different during 1995 or 1996. Larval plasma tri-iodothyronine concentrations were significantly ($p < 0.05$, $F_o = 5.79$, D.F.$= 51$) different during the 14 mo period (Fig. 3.3.c.). These data suggest the occurrence of a fall to winter peak in larval plasma T3 concentrations, corresponding to the peak in larval T4 concentrations.

Laboratory Experiments

In laboratory experiments designed to test whether adaptation to a marine habitat would cause changes in the parameters measured, no significant ($p > 0.05$) differences in gill (Na+K)-ATPase specific activities or thyroxine and tri-iodothyronine concentrations were found after 6 d (Table 3.1). In the experiment designed to test the affect of electroshocking on the parameters measured, no significant ($p > 0.05$) differences in gill (Na+K)-ATPase specific activities or T4 and T3 concentrations were found for those fish extracted from the substrate with a backpack electroshocker and those extracted with a glass rod (Table 3.2). This suggests the electroshocking did not affect the parameters of interest and that the values obtained in field samples were not affected by the capture method.

Table 3.1 Gill (NA+K)-ATPase specific activities (μM P $\cdot$ h^{-1} $\cdot$ mg protein $^{-1}$), plasma thyroxine (T4) and plasma tri-iodothyronine (T3) levels (ng $\cdot$ mL^{-1}) for Pacific lamprey smolts held in marine and freshwater. Data are presented as means $\pm$ 1SE. Values in parentheses are numbers of samples.

	Gill (Na+K)-ATPase	T4	T3
Marine	7.00 $\pm$ 2.57 (9)	0.79 $\pm$ .054 (9)	0.24 $\pm$ 0.05 (9)
Freshwater	6.60 $\pm$ 1.42 (5)	0.61 $\pm$ 0.28 (5)	0.52 $\pm$ 0.54 (5)

Table 3.2 Gill (NA+K)-ATPase specific activities (μM P · h^{-1} · mg protein $^{-1}$), plasma thyroxine (T4) and plasma tri-iodothyronine (T3) levels (ng · mL^{-1}), for pre-smolts removed from the substrate with an electroshocker or a glass rod. Data are presented as means ± 1SE. Values in parentheses are numbers of samples.

	Gill (Na+K)-ATPase	T4	T3
Electroshocker	6.86±0.46 (8)	0.67 ± 0.29 (9)	0.74 ± 0.13 (9)
Glass Rod	7.12 ± 0.52 (8)	0.75 ± 0.56 (9)	0.60 ± 0.15 (9)

Discussion

Because the Agnatha are thought to have evolved several hundred million years before the Osteichthyes (Hardisty 1979), similarities between the transformation of anadromous lamprey and parr-smolt transformation in salmonids suggests that the changes observed during the smolting migration are of ancient origin. These changes have probably been retained because of their adaptive significance in shifting from a freshwater to a marine environment. Because the present data were obtained from wild lamprey rather than laboratory-reared fish, the patterns observed probably more closely reflect events occurring during the transformation from larvae to smolt.

Maximum concentrations of skin guanine in Pacific lamprey appear to occur near the end of metamorphosis and before seaward migration. As in smolting salmonids (Staley and Ewing 1992), skin guanine in Pacific lamprey likely prepares smolts for the shift from a freshwater to a marine habitat. We observed some limitations with the methods used. The apparent changes in the thickness of larval lamprey skin observed in the February and April samples altered the resultant concentrations of skin guanine. Because the concentration of guanine is based on the mg of skin in the sample, changes in weight can cause changes in values that reflect skin thickness rather than guanine content. One must account for seasonal changes in skin volume to properly estimate changes in skin guanine content when using the present method for smolting lamprey. The present study suggests Pacific lamprey undergo quantitative and qualitative changes in skin quanine and coloration respectively during the metamorphic period similar to salmonids during parr-smolt transformation.

Gill (Na+K)-ATPase specific activities have been shown to be correlated with the smolting process and seawater survival in salmonids (Folmar and Dickoff 1981). Like salmonids, Pacific lamprey smolts show a distinct increase in gill (Na+K)-ATPase specific activity prior to ocean entry. This increase occurs after the Pacific lamprey has begun metamorphosis. Unlike salmonids, Pacific lamprey show a more dramatic increase in gill (Na+K)-ATPase specific activity and this increase occurs over a shorter period of time. Initial seawater entry does not appear to affect gill (Na+K)-ATPase specific activity levels within six days. Pacific lamprey seaward migration and open ocean entry from Tenmile Creek occurs over a relatively short period of time, most smolts migrate during a two week period and no estuary exists. The gill (Na+K)-ATPase system in these fish may have evolved to allow for a shift to a marine habitat over a more brief period of time when compared to previously studied salmonids. Pacific lamprey smolts located much further inland, which in turn would require a longer post-metamorphic seaward migration, may exhibit a gill (Na+K)-ATPase pattern more similar to that of salmonid smolts with a longer period of migration.

The thyroid data from this study show similarities to that found in other lamprey species. We observed a significant decrease in plasma T4 and T3 during metamorphosis in Pacific lamprey. Leatherland et al. (1990) and Youson et al. (1994) observed decreases in *Petromyzon marinus* plasma T4 and T3 during metamorphosis. We observed no significant recovery during post-metamorphic seaward migration or seawater entry. Lintop and Youson (1983) did not observe a significant recovery of plasma T4 and T3 levels during the post-metamorphic period in *Petromyzon marinus*.

The present data also suggest thyroxine may play a role in the metabolic requirements of pre-metamorphic lamprey via stream temperature. Lintop and Youson (1983) suggest an inverse relation between water temperature and thyroid hormone levels exists in *Petromyzon marinus* larvae. Although the present data do not show a significant correlation between plasma T4 levels in pre-metamorphic larvae and winter and spring temperatures, there exists a general pattern of increasing plasma T4 during decreasing winter temperatures followed by decreasing T4 during increasing spring temperatures. Thyroxine has been associated with metabolic rates in homeothermic vertebrates but its' affect on basal metabolic rates in poikilotherms is less clear (McNabb 1992). Growing evidence suggests thyroid hormones in poikliotherms may play a role in growth and energy partitioning prior to metamorphosis. Lipogenesis has been associated with thyroid activity in homeothermic vertebrates. Freake et al. (1989) showed increased thyroid hormone levels were correlated with increased lipogenesis in the liver of Sprague-Dawley rats. Lipogenesis has also been associated with the pre-metamorphic process in the sea lamprey, *Petromyzon marinus*. Youson et al. (1993) reported pre-metamorphic sea lamprey show an increase in lipogenesis and a reduction in growth during the winter prior to metamorphosis. Reduced growth in pre-metamorphic teleosts has been correlated with thyroid hormone activity. Miwa and Inui (1987) and Inui and Miwa (1985) suggest that thyroid hormones both inhibit growth prior to metamorphosis and stimulate the metamorphic process in the flounder, *(Paralichthys olivaceus)*.

The present study supports the suggestion of Leatherland et al. (1990) for the role of thyroid hormones in the control of growth and energy partitioning prior to the metamorphic process rather than in control of the developmental processes in

anadromous lamprey. Lamprey provide an opportunity to examine the role of thyroid hormones in the energy partitioning processes leading up to seaward migration in anadromous fishes.

In summary, we have shown changes in skin guanine concentrations, gill (Na+K)-ATPase specific activities and thyroid hormone patterns in Pacific lamprey during the period prior to the seaward migration of smolts, that are similar to those during parr-smolt transformation of salmonids. Salmonids and lamprey share many similarities in life histories and geographic range. Lamprey offer an evolutionary older system in which to study the physiological changes involved in the smolting process. We suggest the study of Pacific lamprey provides a means to define basic mechanisms involved in the physiological processes which enable anadromous salmonids and lamprey to enter the smoltification process and to shift habitats (freshwater to seawater) and food sources at the completion of this process.

Literature Cited

Beamish, R.J. and Levings, C.D. 1991. Abundance and freshwater migrations of the anadromous parasitic lamprey, *Lampetra tridentata*, in a tributary to the Frazer River, British Columbia. Can. J. Fish. Aquat. Sci. 48:1250-1263.

Ewing, R.D., Johnson, S.L., Pribble, H.J., and Lichatowich, J.A. 1979. Temperature and photoperiod effects of gill (Na+K)-ATPase activity in chinook salmon, *Oncorhynchus tshawytscha*. Can. J. Fish. Aquat. Sci.. Vol. 36, No. 11, pp1347-1353.

Fessler, J.L. and Wagner, H.H. 1969. Some morphological and biochemical changes in steelhead trout during the parr-smolt transformation. J. Fish. Res. Board Can. 26:2823-2934.

Folmar, L.C. and Dickoff, W.W. 1979. Plasma thyroxine and gill (Na+K)-ATPase changes during seawater acclimation of coho salmon, *Oncorhynchus kisutch*. Comp. Biochem. Physiol., vol. 63A. pp. 329-332.

Folmar, L.C. and Dickoff, W.W. 1980. The parr-smolt transformation (smoltification) and seawater adaptation in salmonids. A review of selected literature. Aquaculture 21:1-37.

Folmar, L.C. and Dickoff, W.W. 1981. Evaluation of some physiological parameters as predictive indices of smoltification. Aquaculture 23:309-324.

Freake, H.C., Schwartz, H.J. and Oppenheimer, J.H. 1989. The regulation of lipogenesis by thyroid hormone and its contribution to thermogenesis. Endocrinology. 125(6): 2868-2874.

Hardisty, M.W. 1979. Biology of the cyclostomes. Chapman and Hall Ltd.

Hardisty, M.W. and Potter, I.C. 1982a. Stages in metamorphosis, p. 137-164. *In* M.W. Hardisty and I.C. Potter. [ed.] The biology of lampreys. Academic Press, London.

Hardisty M.W. and Potter, I.C. 1982b. The behavior, ecology and growth of larval lampreys, p. 85-120. *In* M.W. Hardisty and I.C. Potter. [ed.] The biology of lampreys. Academic Press, London.

Hoar, W.S. 1976. Smolt transformation: evolution, behavior and physiology. J. Fish. Res. Board Can., 33:1234-1252.

Inui, Y. and Miwa, S. 1985. Thyroid hormone induces metamorphosis of flounder larvae. Gen. Comp. Endo. 60:450-454.

Johnson, S.L., Ewing, R.D. and Lichatowich, J.A. 1977. Characterization of gill-(Na+K)-activated adenosine triphosphate from chinook salmon, *Oncorhynchus tsawytscha*. J. Exper. Zool., 199:345-354.

Kunst, A., Burk, H., Schaurer, S., Haug, H., Lehmann, P. and Volker, E. 1988. One-step enzyme immunoassay for free thyroxin. Clinical Chemistry 34/9 1830-1833.

Leatherland, J.F., Hilliard, R.W., Macey, D.J. and Potter, I.C. 1990. Changes in serum thyroxine and tri-iodothyronine concentrations during metamorphosis of the southern hemisphere lamprey, *Geotria australis*, and the effect of propylthiouracil, tri-iodothyronine and environmental temperature on serum thyroid hormone concentrations of ammocoetes. Fish Physiol. Bioch. 8(2):167-177.

Lintop, S.P. and Youson, J.H. 1983. Concentration of tri-iodothyronine in the sera of the sea lamprey, *Petromyzon marinus*, and the brook lamprey, *Lampetra lamottenii*, at various phases of the life cycle. Gen. Comp. Endo. 49:187-194.

McCormick, S.D. 1994. Ontogeny and evolution of salinity tolerance in anadromous salmonids: hormones and heterochrony. Estuaries vol. 17 no. 1A pp 26-33.

McNabb, F.M. 1992. Control of the thyroid gland function, p. 49-73. *In* M.E. Hadley [ed.] Thyroid Hormones. Prentice Hall.

Miwa, S. and Inui, Y. 1987. Effects of various doses of thyroxine and tri-iodothyronine on the metamorphosis of flounder, *Paralichthys olivaceus*. Gen. Comp. Endo. 67:356-363.

Richards, J.E. and Beamish, F.W.H. 1981. Initiation of feeding and salinity tolerance in the Pacific lamprey, *Lampetra tridentata*. Marine Biology 63:73-77.

Staley, K.B., and Ewing, R.D. 1992. Purine levels in the skin of juvenile coho salmon, *Oncorhynchus kisutch*, during parr-smolt transformation and adaptation to seawater. Comp. Biochem. Physiol. Vol. 101B. No. 3, pp 447-452.

van de Wetering S.J. 1998. Aspects of life history characteristics and physiological processes in smolting Pacific lamprey, *Lampetra tridentata*, in a central coast Oregon stream. Chapter 1 this volume. MS Thesis, Oregon State University, Corvallis, Oregon.

Varnavsky, V.S., Sakamoto, T. and Hirano, T. 1992. Stunting of wild coho salmon, *Oncorhynchus kisutch*, in seawater: Patterns of plasma thyroid hormones, cortisol and growth hormone. Can. J. Fish. Aquat. Sci. 49:458-461.

Youson, J.H., Holmes, J.A. Guchardi, J.A., Seelye, J.G., Beaver, R.E., Gersmehl, J.E., Sower, S.A. and Beamish, F.W.H. 1993. Importance of condition factor and the influence of water temperature and photoperiod on metamorphosis of sea lamprey, *Petromyzon marinus*. Can. J. Fish. Aquat. Sci. 50:248-2456.

Youson, J.H., Plisetskaya, E.M. and Leatherland, J.F. 1994. Concentrations of insulin and thyroid hormones in the serum of landlocked sea lampreys, *Petromyzon marinus*, of three larval year classes, in larvae exposed to two temperature regimes and in individuals during and after metamorphosis. Gen. Comp. Endo. 94:294-304.

CHAPTER 4

Summary

Chapter 2

This study suggests Pacific lamprey have an extended freshwater residency prior to smoltification. In the Pacific Northwest numerous research projects have examined losses in stream habitat complexity and the accompanying deleterious effects on anadromous fish populations (Bisson et al. 1987). It is logical to assume losses in freshwater habitat complexity result in higher mortalities in those anadromous species with longer freshwater residencies. This study provides initial data regarding the current status of larval densities in streams of the Oregon coast range. Comparing the present study to that of Beamish and Levings (1991), suggests that lamprey populations located in larger inland river systems have very different migration patterns that those in near coastal systems.

Chapter 3

Because the Agnatha are thought to have evolved several hundred million years before the Osteichthyes (Hardisty 1979), the similarities between the transformation of anadromous lamprey and parr-smolt transformation in salmonids suggests that the changes observed during anadromous migration are of ancient origin. These changes have probably been retained because of their adaptive significance in shifting from a

freshwater to a marine environment. Because the present data were obtained from wild lamprey rather than laboratory-reared fish, the patterns observed probably more closely reflect events occurring during the transformation from larvae to smolt. I have shown changes in skin guanine concentrations, gill (Na+K)-ATPase specific activities and thyroid hormone patterns in Pacific lamprey during the period prior to the seaward migration of smolts, that are similar to those during parr-smolt transformation of salmonids. Salmonids and lamprey share many similarities in life histories and geographic range. Lamprey offer an evolutionary older system in which to study the physiological changes involved in the smolting process. I suggest the study of Pacific lamprey provides a means to define basic mechanisms involved in the physiological processes which enable anadromous salmonids and lamprey to enter the smoltification process and to shift habitats (freshwater to seawater) and food sources at the completion of this process.

Literature Cited

Beamish, R.J. and Levings,C.D. 1991. Abundance and freshwater migrations of the anadromous parasitic lamprey, *Lampetra tridentata*, in a tributary to the Frazer River, British Columbia. Can. J. Fish. Aquat. Sci. 48:1250-1263.

Bisson, P.A., R.E. Bilby, R.E., Bryant, M.D., Dollof, C.A., Greet, G.B., House, R.A., Murphy, M.L., Koski, K.V., and Sedell, J.R. 1987. Large woody debris in forested streams in the Pacific Northwest: past, present and future. Pages 143-190 *in* E.A. Salo and T.W. Cundy, [ed]. Streamside management: forestry and fishery interactions. Institute of Rorest Resources Contribution 57. University of Washington, College of Forest Resources, Seattle.

Hardisty, M.W. 1979. Biology of the cyclostomes. Chapman and Hall Ltd.

BIBLIOGRAPHY

Anonymous. 1994. North Umpqua Hydroelectric Project.

Anonymous. 1998. State of Oregon, Department of Fish and Wildlife. USA.

Beamish, F.W.H. and Medland, T.E. 1988. Age determination for lampreys. Trans. Amer. Fish. Soc. 117:63-71.

Beamish, R.J. 1980. Adult biology of the river lamprey, *Lampetra ayresi*, and the Pacific lamprey, Lampetra tridentata, from the Pacific coast of Canada. Can. J. Fish. Aquat. Sci. vol. 37 no. 11 pp 1906-1923.

Beamish, R.J. and Levings, C.D. 1991. Abundance and freshwater migrations of the anadromous parasitic lamprey, *Lampetra tridentata*, in a tributary to the Frazer River, British Columbia. Can. J. Fish. Aquat. Sci. 48:1250-1263.

Bisson, P.A., Bilby, R.E., Bryant, M.D., Dollof, C.A., Greet, G.B., House, R.A., Murphy, M.L., Koski, K.V., and Sedell, J.R. 1987. Large woody debris in forested streams in the Pacific Northwest: past, present and future. Pages 143-190 *in* E.A. Salo and T.W. Cundy, [ed]. Streamside management: forestry and fishery interactions. Institute of Rorest Resources Contribution 57. University of Washington, College of Forest Resources, Seattle.British Columbia, Vancouver, B.C.

Close, D.A., Fitzpatrick, M., Li, H., Parker, B., Hatch, D., and James, G. 1995. Status Report of the Pacific lamprey, *Lampetra tridentata*, in the Columbia River Basin. U.S. Dept. Energy, Bonneville Power Admin., Div. Fish and Wildlife, Portland, OR.

Downey, T., Rilatos, D., Sondenaa, A. and Zybach, B. 1993. The Siletz Eels: Oral history interviews with Siletz tribal elders and neighboring residents regarding the decline in Siletz River lamprey populations. Native Americans in Marine Science Program, Oregon State University, Corvallis, Oregon.

Effron, B., and Tibshirani, R. 1986. Bootstrap methods for standard errors, confidence intervals and other measures of statistical accuracy. Statistical Science 1:54-77.

Ewing, R.D., Johnson, S.L., Pribble, H.J., and Lichatowich, J.A. 1979. Temperature and photoperiod effects of gill (Na+K)-ATPase activity in chinook salmon, *Oncorhychus tshawytscha*. Can. J. Fish. Aquat. Sci.. Vol. 36, No. 11, pp1347-1353.

Fessler, J.L. and Wagner, H.H. 1969. Some morphological and biochemical changes in steelhead trout during the parr-smolt transformation. J. Fish. Res. Board Can. 26:2823-2934.

Folmar, L.C. and Dickoff, W.W. 1979. Plasma thyroxine and gill (Na+K)-ATPase changes during seawater acclimation of coho salmon, *Oncorhynchus kisutch*. Comp. Biochem. Physiol., vol. 63A. pp. 329-332.

Folmar, L.C. and Dickoff, W.W. 1980. The parr-smolt transformation (smoltification) and seawater adaptation in salmonids. A review of selected literature. Aquaculture 21:1-37.

Folmar, L.C. and Dickoff, W.W. 1981. Evaluation of some physiological parameters as predictive indices of smoltification. Aquaculture 23:309-324.

Freake, H.C., Schwartz, H.J. and Oppenheimer, J.H. 1989. The regulation of lipogenesis by thyroid hormone and its contribution to thermogenesis. Endocrinology.

Hammond, R.J. 1979. Larval biology of the Pacific lamprey, *Entosphenus Tridentatus* (Gairdner), of the Potlatch River, Idaho. Masters Sci. Thesis, Univ. Idaho, Moscow, Idaho.

Hardisty, M.W. 1979. Biology of the cyclostomes. Chapman and Hall Ltd.

Hardisty, M.W. and Potter, I.C. 1982a. Stages in metamorphosis, p. 137-164. *In* M.W. Hardisty and I.C. Potter. [ed.] The biology of lampreys. Academic Press, London.

Hardisty M.W. and Potter, I.C. 1982b. The behavior, ecology and growth of larval lampreys, p. 85-120. *In* M.W. Hardisty and I.C. Potter. [ed.] The biology of lampreys. Academic Press, London.

Hoar, W.S. 1976. Smolt transformation: evolution, behavior and physiology. J. Fish. Res. Board Can., 33:1234-1252.

Inui, Y. and Miwa, S. 1985. Thyroid hormone induces metamorphosis of flounder larvae. Gen. Comp. Endo. 60:450-454.

Johnson, S.L., Ewing, R.D. and Lichatowich, J.A. 1977. Characterization of gill-(Na+K)-activated adenosine triphosphate from chinook salmon, *Oncorhynchus tsawytscha*. J. Exper. Zool., 199:345-354.

Kunst, A., Burk, H., Schaurer, S., Haug, H., Lehmann, P., and Volker, E. 1988. One-step enzyme immunoassay for free thyroxin. Clinical Chemistry 34/9 1830-1833.

Leatherland, J.F., Hilliard, R.W., Macey, D.J. and Potter, I.C. 1990. Changes in serum thyroxine and tri-iodothyronine concentrations during metamorphosis of the southern hemisphere lamprey, *Geotria australis*, and the effect of propylthiouracil, tri-iodothyronine and environmental temperature on serum

Lintop, S.P. and Youson, J.H. 1983. Concentration of tri-iodothyronine in the sera of the sea lamprey, *Petromyzon marinus*, and the brook lamprey, *Lampetra lamottenii*, at various phases of the life cycle. Gen. Comp. Endo. 49:187-194.

MacDonald, P.D.M. 1987. Analysis of length-frequency distributions. p 371-384 *In*

McCormick, S.D. 1994. Ontogeny and evolution of salinity tolerance in anadromous salmonids: hormones and heterochrony. Estuaries vol. 17 no. 1A pp 26-33.

McNabb, F.M. 1992. Control of the thyroid gland function, p. 49-73. *In* M.E. Hadley [ed.] Thyroid Hormones. Prentice Hall.

Medland, T.E., and Beamish, F.W.H. 1991. Lamprey statolith banding in response to temperature, photoperiod, and ontogeny. Trans. Amer. Fish. Soc. 120:255-260.

Miwa, S. and Inui, Y. 1987. Effects of various doses of thyroxine and tri-iodothyronine on the metamorphosis of flounder, *Paralichthys olivaceus*. Gen. Comp. Endo. 67:356-363.

Pletcher, F.T. 1958. The life history and distribution of lampreys in the Salmon and certain other rivers in British Columbia, Canada. Masters Sci. thesis, Univ. British Columbia, Vancouver, B.C.

Purvis, H.A. 1971. Variations in growth, age at transformation and sex ratio of sea lampreys reestablished in chemically treated tributaries of the upper Great Lakes. Gt. Lakes Fish. Commn. Tech. Rep. 35:1-36.
R.C. Summerfelt and G.E. Hall. [ed]. Age and growth of fish. Iowa State

Richards, J.E. 1980. Freshwater life history of the anadromous Pacific lamprey, *Lampetra tridentata*. Masters Science thesis, University of Guelph, Ont., Canada.

Richards, J.E. and Beamish, F.W.H. 1981. Initiation of feeding and salinity tolerance in the Pacific lamprey, *Lampetra tridentata*. Marine Biology 63:73-77.

Schute, J. and Fournier, D. 1980. A new approach to length-frequency analysis: Growth Structure. Can. J. Fish. Aquat. Sci. 37:1337-1351.

Staley, K.B., and Ewing, R.D. 1992. Purine levels in the skin of juvenile coho salmon, *Oncorhynchus kisutch*, during parr-smolt transformation and adaptation to seawater. Comp. Biochem. Physiol. Vol. 101B. No. 3, pp 447-452.

Summerfelt, R.C. and Hall, G.E. 1987. Age and growth of fish. Iowa State University Press, Ames, Iowa.thyroid hormone concentrations of ammocoetes. Fish Physiol. Bioch. 8(2):167-University Press, Ames, Iowa.

van de Wetering S.J. 1998. Aspects of life history characteristics and physiological processes in smolting Pacific lamprey, *Lampetra tridentata*, in a central coast Oregon stream. Chapter 1 this volume. MS Thesis, Oregon State University, Corvallis, Oregon.

Varnavsky, V.S., Sakamoto, T. and Hirano, T. 1992. Stunting of wild coho salmon, *Oncorhynchus kisutch*, in seawater: Patterns of plasma thyroid hormones, cortisol and growth hormone. Can. J. Fish. Aquat. Sci. 49:458-461.

Youson, J.H., Holmes, J.A. Guchardi, J.A., Seelye, J.G., Beaver, R.E., Gersmehl, J.E., Sower, S.A. and Beamish, F.W.H. 1993. Importance of condition factor and the influence of water temperature and photoperiod on metamorphosis of sea lamprey, *Petromyzon marinus*. Can. J. Fish. Aquat. Sci. 50:248-2456.

Youson, J.H., Plisetskaya, E.M. and Leatherland, J.F. 1994. Concentrations of insulin and thyroid hormones in the serum of landlocked sea lampreys, *Petromyzon marinus*, of three larval year classes, in larvae exposed to two temperature regimes and in individuals during and after metamorphosis. Gen. Comp. Endo. 94:294-304.

APPENDIX TABLE

Appendix Table. Seawater challenges for Pacific lamprey pre-smolts and smolts collected in Tenmile Creek 1994 and 1995. Classification, salinity, percent survival and challenge period (in days) are shown in columns. Sample size is shown in parentheses.

Date	Pre-smolt/Smolt	Salinity (ppt)	% Survival	Challenge Time (d)
9/2/94	pre-smolt (14)	29	0	1
9/9/94	pre-smolt (10)	29	0	1
9/16/94	pre-smolt (8)	29	0	1
9/22/94	pre-smolt (14)	30	0	1
10/15/94	pre-smolt (10)	29	0	1
10/29/94	**smolt (6)**	29	**100**	**10**
9/28/95	pre-smolt (5)	30	0	1
10/14/95	pre-smolt (6)	29	0	1
11/2/95	pre-smolt (11)	29	**40**	**5**
12/5/95	**smolt (9)**	29	**100**	7