EVIDENCE FOR THE GENETIC BASIS AND INHERITANCE OF OCEAN AND RIVER-MATURING ECOTYPES OF PACIFIC LAMPREY (*ENTOSPHENUS TRIDENTATUS*) IN THE KLAMATH RIVER, CALIFORNIA

By

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ABSTRACT

EVIDENCE FOR THE GENETIC BASIS AND INHERITANCE OF OCEAN AND RIVER-MATURING ECOTYPES OF PACIFIC LAMPREY (*ENTOSPHENUS TRIDENTATUS)* IN THE KLAMATH RIVER, CALIFORNIA

Keith A. Parker

Surveys of genetic variation have improved our understanding of the relationship between fitness-related phenotypes and their underlying genetic basis. However, how this information can be used to inform conservation has been unclear in many cases. The objective of this study was to combine next-generation genetic sequencing with traditional ecological knowledge to evaluate imperiled anadromous Pacific lamprey (*Entosphenus tridentatus*) and apply the findings to conservation in the context of resolving Native American traditional food security issues. In the Klamath River of California, a previously identified Pacific lamprey ocean-maturing ecotype was distinguished by a relatively advanced maturity of female fish (e.g., large egg mass) upon freshwater entry compared to a relatively immature river-maturing ecotype. However, relative run-timing and the genetic basis of this ecotypic differentiation was not known. I collected 219 returning adult Pacific lamprey at-entry to the Klamath River over a 12-month period, genotyped them at 308 neutral and adaptive single nucleotide polymorphism (SNP) loci, and recorded morphological traits, including egg mass as an indicator of female sexual maturity. The onset for freshwater migration for the ocean-

maturing ecotype was predominantly the winter whereas the river-maturing ecotype entered during all seasons and a genetic basis of the ecotype diversity was revealed. Genotype-phenotype association mapping identified sixteen SNPs significantly associated to egg mass forming two groups of linked loci and ten other SNPs significantly associated to total length. A duplicate dominant epistasis inheritance model best supported the ocean- and river-maturing ecotypes, accurately predicting ecotype in 83% of the samples. The adaptive genetic variation revealed is useful for conservation planning as it indicates that the river-maturing ecotype carries standing genetic variation capable of producing both ecotypes (e.g., both heterozygous and homozygous individuals), while the ocean-maturing ecotype is almost exclusively homozygous. An ecological application of these molecular findings is that when assessing stream restoration projects, the river-maturing ecotypes could perhaps be prioritized as they contain the genetic diversity capable of producing both ecotypes (i.e., heterozygosity), whereas the ocean-maturing ecotypes do not. I recommend distinguishing the river-maturing and ocean-maturing ecotypes of Pacific lamprey by adopting the names ke'ween (lamprey "eel") and tewol (ocean), respectively, using terms from the Yurok language, in recognition of the importance of Pacific lamprey to Pacific Northwest fishing tribes.

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Wokhlew!

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INTRODUCTION

The ability to survey full genomic variation in natural populations of organisms has paved the way for identification of genomic variation that is associated with fitness-related traits (Narum et al. 2013). This new information offers the potential to focus conservation efforts on the genomic regions that are associated with traits that are likely influenced by selection (Allendorf et al. 2010). While the importance of ecological variation is generally considered in defining conservation units (Waples 2006), how to incorporate the novel insights generated by genotype-phenotype studies into conservation planning is unclear and currently under debate (McMahon et al. 2014; Shafer et al. 2015; Abadia-Cardoso 2016). Herein, I investigate the association of genetic variation with ecotypic differentiation in Pacific lamprey (*Entosphenus tridentatus*) and show how this information could be used to inform conservation efforts. I discuss the results in the context of the ongoing debate regarding the application of adaptive genomic variation in conservation.

The Pacific lamprey is an anadromous species inhabiting coastal rivers and nearshore marine areas from Hokkaido Island, Japan, to California, USA. Range-wide evaluations of neutral genetic structuring using mitochondrial DNA, microsatellites and genome-wide surveys of SNPs indicate Pacific lamprey are nearly panmictic but that limited dispersal at sea precludes full panmixia, resulting in weak but significant isolation by distance over wide-ranging geographic zones (Goodman 2008; Spice et al. 2012; Hess et al. 2012). A single study contrasts with these patterns, where evidence of moderate

temporal genetic differentiation (F_{st} = 0.16 - 0.24 for all pairwise comparisons) was resolved in the Willamette River, Oregon (Clemens et al. 2017). Genome-wide scans have identified 162 SNP loci that are likely associated with adaptive variation in Pacific lamprey (Hess et al. 2013). Linkage analysis shows that these 162 loci are distributed across four linkage groups, termed linkage groups A, B, C, and D (Hess et al. 2013). Loci on linkage group A and B exhibit a strong association with total length and migration distance, but phenotypic associations of the adaptive loci on linkage groups C and D has yet to be elucidated (Hess et al. 2013; Hess et al. 2014).

Beamish et al. (1980) described Pacific lamprey as river-maturing and hypothesized that they spend one-year in freshwater prior to spawning. However, several more recent studies have suggested the existence of multiple ecotypes in Pacific lamprey as indicated by body size and coloration differences, including normal and dwarf (Kostow 2002; Hess et al. 2013), day eels and night eels (Close et al. 2004), normal and praecox (i.e., non-parasitic) (Docker 2009), and river- and ocean maturing (Clemens et al. 2013). However, these studies were conducted in different geographic locations, intercepted individuals at different stages of their river migration (e.g., upstream versus downstream), and used different traits to define the ecotypes (e.g., body size, migration time, maturity, etc.). Thus, it is not clear if these studies are actually using different terminology to refer to the same ecotypes, or if there are unique ecotypes restricted to isolated areas.

In this study, I investigated ocean- and river-maturing ecotypes of Pacific lamprey returning to the Klamath River, California. The primary difference between the ecotypes

was found to be maturity at the onset of freshwater migration, with the female gonadosomatic index of the river-maturing ecotype less than half of the ocean-maturing ecotype (1.2 – 2.8% versus 5.5%) (Clemens et al. 2013). Owing to differences in maturity at entry, it is hypothesized that the ocean-maturing would likely spawn shortly after entering fresh water whereas the river-maturing would spend one year in fresh water prior to spawning (Clemens et al. 2013). However, the study was based upon examination of relatively few females (n=18) and sampling was restricted to collections from April and June (Clemens et al. 2013). Therefore, relative run-timing differentiation between the ecotypes was not identified. Because Klamath River Pacific lamprey have been observed to initiate their anadromous migrations from November to April (Larson and Belchik 1998; Murphey 1959; Petersen 2006; Petersen-Lewis 2009), it is unknown whether the ocean- and river-maturing ecotypes exhibit differences in the season they initiate freshwater migration.

Study Objectives

For this study, adult Pacific lamprey were collected as they initiated their anadromous migration by intercepting individuals as they entered the Klamath River utilizing Native American traditional methods of catch (e.g., eel hook, dip net). Specimens were collected within 0.5 km of the mouth of the Klamath River and collection effort was distributed across a 12-month period, representing the full temporal range of potential river entry times. Fish were genotyped at a panel of 308 SNP loci that were representative of neutral (i.e., not subject to evolution through natural selection) and

adaptive loci in Pacific lamprey (Hess et al. 2013; Hess et al. 2014; Smith et al. 2018). Each individual was also measured for a set of morphological traits, including egg mass for females. These data were used to evaluate the following questions:

(1) Do ocean- and river-maturing ecotypes exhibit differences in the season they initiate freshwater migration?

(2) Is there evidence of temporal genetic population structure between ocean- and river-maturing ecotypes at neutral loci?

(3) Is there evidence for associations between river- and ocean-maturing ecotypes and adaptive genetic loci?

Study Site

The Klamath River Basin originates in Klamath Falls, Oregon and flows southwest before entering the Pacific Ocean at Requa, California, covering an area of 40,720 km^2. The Klamath River Basin supports the highest diversity of lamprey species (n=5) of any single watershed in the world (Thorsteinson et al. 2011; Moyle 2002), with the anadromous Pacific lamprey suggested to have been the river's biomass-dominant fish species historically (Petersen-Lewis 2009). Ecologically, Pacific lamprey are important contributors of marine-derived nutrients and organic matter to the food web of oligotrophic streams (Beamish 1980; Petersen-Lewis 2009) which are far inland from the Pacific Ocean, a primary food source for pinnipeds (Roffe and Mate 1984; Close et al.

1995), and likely a trophic level buffer to some species of migrating salmon as pinnipeds preferentially consume Pacific lamprey (Murphey 1959; Close et al. 2002).

Habitat conditions

Pacific lamprey populations are at risk of extinction due to passage barriers, habitat disturbance and loss (ODFW 2006), and are considered intermediate to intolerant of pollution (Barbour et al. 1999). The lowermost of four dams, Iron Gate, became the terminus for Klamath River migrating fish in 1964, blocking off hundreds of miles of spawning and rearing habitat (Hamilton et al. 2005). In the late summer and fall, the Klamath River is impacted by planktonic bloom forming cyanobacteria resulting in seasonal unsafe levels of microcystins (Gillett et al. 2015). Consequently, high levels of domoic acid (319 ppb) has been detected in Pacific lamprey tissue, along with highly-carcinogenic Polycyclic Aromatic Hydrocarbons (3.3 ppb), above the human health threshold of 2 ppb (Yurok Tribe Environmental Program Final Report to the EPA 2012). Little is known about whether the fish depurate the contaminants once exposure ceases. Without dam removal, deleterious habitat conditions for Pacific lamprey will persist, with only subtle changes due to foreseeable hydrological changes (Close et al. 2010). The four dams are scheduled to undergo a removal project beginning in year 2020 (amended Klamath Hydroelectric Settlement Agreement 2016).

Cultural Significance and Traditional Ecological Knowledge

Culturally, Pacific lamprey are a tribal trust fish species protected under tribal treaty and other rights. The fish continue to provide direct subsistence when other high

lipid foods (e.g., salmon) are unavailable to the Yurok, Hupa, Karuk, and other Native American Tribes of the Klamath River basin (Murphey 1959; Petersen-Lewis 2009). The Klamath River Tribes possess traditional ecological knowledge (TEK) (i.e., evolving knowledge acquired by indigenous peoples over hundreds or thousands of years in direct contact with the environment, specific to a location, and handed down through generations by cultural transmission and spiritual relationships (Barnhardt and Kawagley 2005)) of Pacific lamprey. Tribal harvest methods include the eel hook, eel basket, dip net, trigger net, and hand catch, methods which have been used for hundreds or thousands of years (Petersen-Lewis 2009). Pacific lamprey provide high caloric values (Close et al. 1995) for indigenous people coinciding with the coldest season of the year, ranging 5.92 to 6.34 kcal/g wet mass (Whyte et al. 1993), compared to salmonids with 1.26 to 2.87 kcal/g (Stewart et al. 1983). Over the past fifty years, harvest of Pacific lamprey in the Klamath River has been reduced by several orders of magnitude due to declines in abundance (Petersen-Lewis 2009), impacting Klamath River tribes with adverse health, social, economic, and spiritual effects (Norgaard 2005).

Historically, Pacific lamprey also provided indirect subsistence for Yurok people by facilitating the hunting of pinnipeds (e.g., sea lions) in the Klamath River estuary. (Spott and Kroeber 1942; Warburton and Endert 1966). Until approximately 1890, Yurok tribal people living in the Rekwoi (Requa) village at the mouth of the Klamath River consumed pinnipeds. Pinnipeds followed the freshwater migration of tens of thousands of adult Pacific lamprey ("eel") in late February, March, and early April.

Pacific lamprey would fatigue in fast water during migration and stay close to shore, utilizing eddies to rest, which were created by willows, rocks, or anchored eel baskets. Pinnipeds entering the estuary from the ocean would go directly to the eddies along the bank to consume Pacific lamprey. During Pacific lamprey migration, Yurok men anchored a large ocean-going dugout boat in the willows with a seven-man crew. From shore, a spearman would throw a 15-foot barbed spear made of cedar, blackened with fire, and attached to a long rope into the shoulder area of a pinniped. Once hit, the men would board the dugout, give chase, and attempt to harvest the pinniped for subsistence (Warburton and Endert 1966).

MATERIALS AND METHODS

Pacific Lamprey Collection and Trait Analysis

The methods used this study were reviewed and approved by the Humboldt State University Institutional Animal Care and Use Committee (No. 15/16.F.105-A, May 11, 2016). I collected adult Pacific lamprey at-entry to the Klamath River from the Pacific Ocean (Figure 1.A) (coordinates 41.544, -124.079). Collections were conducted over a 12-month period (June 2016 to May 2017), with efforts to collect during each month, weather dependent. Specimens were obtained via creel survey of the Yurok Tribe subsistence fishery (Figure 1.B), or capture by the author using a traditional Native American eel hook (2-4 ft pole with a 1-2 ft attached wire terminating in a hook) (Figure 1.C) or a long-handled dip net (5-6 ft long handle with a 20-inch bow of fine mesh). All non-creel individuals were euthanized by severing the notochord just posterior, and dorsal, of the eyes, followed by pithing with a metal rod through the brain. A fin clip (2 cm^2) was collected from the dorsal fin of each lamprey and preserved in 95% ethanol until DNA extraction. The following metrics were recorded for each adult Pacific lamprey: day of year, total length (1 mm), body mass (0.1 g), girth just posterior of the rearmost breathing hole (1 mm), interdorsal distance (1 mm) defined as the distance between the posterior most ray insertion of the first dorsal to the insertion of the anterior-most ray on the second dorsal, sex, and egg mass (0.1 g) for females consisting of the total weight of all eggs without the skein. Condition factor (Kn) was calculated as the

ratio between actual body mass and the predicted body mass derived from a length-weight model created from log-transformed lengths and body masses. Gonadosomatic index (GSI) was calculated as the ratio between egg mass and somatic mass. Somatic mass is body mass minus gonad mass.

Figure 1. (A) Aerial view of the collection site at the mouth of the Klamath River, Del Norte County, California, USA. Site was photographed in 2016. (B) Adult Pacific lamprey collected at-entry to the Klamath River during their anadromous migration. (C) Yurok Native American eel hook used for collection of Pacific lamprey. (D) Variation in Pacific lamprey egg mass for individuals collected on same day, 14 April 2017. Egg mass ranged from the smallest of the study at 1.6 g (third from left) to 22.7 g (second from right) (E) Female Pacific lamprey gut cavity prior to egg excision. The individual pictured represents the largest egg mass of the study (25.5 g).

Ecotype designations were based upon identification of 16 SNP loci exhibiting significant association to egg mass in Pacific lamprey with a substantial change in allele frequency corresponding to an egg mass of 12.5 g (ocean-maturing individuals ≥12.5 g egg mass and river-maturing individuals <12.5 g egg mass) (further details in Results). To test for significant differences in run-timing (day-at-entry) between ocean- and river-maturing ecotypes, a Welch two-sample t-test was conducted. The median day-at-entry for both Pacific lamprey ecotypes was identified instead of the mean day-at-entry. The median day-at-entry was less influenced by the four ocean-maturing egg mass outliers in the data.

The coefficient of variation (CV, standard deviation/mean) was calculated for egg mass by month and season to provide a measure of relative variability in a standardized format and compared to other anadromous fish egg mass variability. To test for significant differences in Pacific lamprey egg mass across months, a one-way analysis of variance (ANOVA) was conducted. If significant ANOVA results were observed, post-hoc comparisons using the Tukey's HSD test were used to evaluate pairwise differences.

Multiple linear regression analyses were used to model egg mass as a function of the explanatory variables including day (day 1 = January 1st), total length (TL), interdorsal space (idspace), and ecotype (0 = ocean-maturing, 1 = river-maturing). Weight and girth were eliminated because of strong Pearson correlations with total length, weight (0.82) and girth (0.66). I explored the data graphically and with statistical significance tests for evidence of an interaction between ecotype and day (i.e., strong

differences in regression slopes). Criterion-based model selection (e.g., AIC and BIC comparisons) was conducted with an exhaustive search of all possible variable combinations. Because the distribution of egg mass was log normal, the data were natural log transformed prior to t-tests, ANOVA, and regression. The software package R was used for model fits, statistical analysis, and figure creation (R Core Development Team 2015).

To characterize the relative abundance of adult Pacific lamprey entering the Klamath River during the 12-month study period, the mean catch-per-unit-effort (CPUE) was calculated for each month. Catch-per-unit effort was calculated as the total number of Pacific lamprey captured, using an eel-hook or dipnet, per hour of active fishing. Samples obtained from creel surveys were excluded from the CPUE calculations. During each field collection the same active sampling devices were used consistently (traditional hook or dip net), sampling occurred at the same narrow river passage each trip, and all sampling occurred during daylight hours.

DNA Extraction, PCR, and Genotyping

Tissue samples were transported by the author to the Columbia River Inter-Tribal Fisheries Commission (CRITFC)/University of Idaho/USDA laboratory in Hagerman, Idaho for genetic analysis (ID: Proj184-Etr GT Seq Keith Parker qPCR L-0769). DNA extraction was accomplished using the Chelex 100 method (denaturing protocol). Individual Pacific lamprey tissues were placed in separate wells of a standard

sized PCR plate (n=5) and incubated at room temperature for 30 minutes to evaporate residual ethanol. A total of 5µL ProK was added to each well. A 10% Chelex solution was prepared (2.5g Chelex (Sigman Aldrich C7901) to 25 ml Nuclease-Free H2O) and homogenized. A total of 50µL of the Chelex solution was aliquoted into each well. Pacific lamprey tissue samples were incubated overnight at 55° C in a thermal cycler. The next day each PCR plate was vortexed, centrifuged, and stored at -20° C.

Single nucleotide polymorphism genotypes were generated using the Genotyping-in-Thousands by sequencing (GT-seq) custom amplicon method described in Campbell et al. (2015). The five main steps are: (i) multiplex PCR - forward and reverse primer pairs are tagged with Illumina sequencing primer sites (Read 1 and Read 2) and all targets are amplified in a single multiplex PCR reaction, (ii) index tagging (barcode) - a second PCR step adds a sample specific index sequence (n=96) and a plate specific index sequence (n=96) to each sample allowing up to 9,216 samples to be pooled in a single sequencing lane while maintaining amplicon specificity, (iii) quantification and normalization - the concentrations of tagged amplicons for each sample are measured by qPCR and normalized, (iv) sequencing - SR150 with 2x6 dual index cycles on Illumina NextSeq, and (v) genotype scores identified with a Perl script which takes a list of locus names, allele names, and probe sequences as input (Campbell et al. 2015). The script counts occurrences of each allele, generates a ratio of allele 1 to allele 2, and uses the ratio to generate genotypes for each locus.

All samples were quality control filtered and only those samples that were genotyped at ≥90% of loci were retained, while samples genotyped at <90% loci were discarded. Based upon this filtering, 97.19% of the samples were retained and 2.81% were discarded.

The discovery, selection, and development of a sufficient number of SNP markers to characterize Pacific lamprey population variability was the result of Hess et al. (2013) and Hess et al. (2015). The SNP panel of 308 GT-seq loci were selected to be representative of neutral and adaptive loci across the geographic range of Pacific lamprey, representing a subset of markers developed from the paired end consensus reads from the Hess et al. (2013) RAD-seq dataset. The selection of loci and steps in development began with a group of 457 total SNP loci considered in round 1, which included 120 that had been already designed for TaqMan assays (Hess et al. 2015). A total of 337 SNPs were chosen that had not been designed previously and ensured that all SNP sites were located at base pair position 30 or higher to accommodate the assay primer site in flanking DNA. The following set of guidelines for choosing SNPs for the GT-seq panel were established: (i) pass population genetic quality control filters for a rangewide dataset (Hess et al. 2013), (ii) no duplicate loci, (iii) potential for positioning on a linkage map (i.e., SNPs were polymorphic within the linkage mapping family) (Smith et al. 2018), (iv) high confidence in alignment to the sea lamprey genome, (v) previously designed for TaqMan assays (Hess et al. 2015), (vi) spaced 5cM or greater apart on a linkage group, (vii) putatively neutral and high minor allele frequency (MAF)

for power to perform parentage analyses, and (viii) adaptive SNPs chosen to be equally representative across four groups of statistically linked loci. Loci that appeared to have too many heterozygotes and were likely duplicated regions were removed. There were 401 loci that passed this filter. Although 120 primers were designed from previous work, a consensus sequence was constructed for the rest using paired-end sequence data from Hess et al. (2013) and was successful in developing 266 primer pairs for the loci. A script was used to identify 28 primer interactions which were resolved by dropping 26 primer pairs. This filter resulted in a remaining set of 360 loci (240 new + 120 original primer pairs). Final optimization left 308 markers that worked best in GT-seq genotyping. There are 230 neutral SNPs, 41 adaptive SNPs, and a set of 31 "intermediate" SNPs that did not fit definitions of either putative neutral and putatively adaptive (Hess et al. 2013). Finally, four loci are species diagnostic (Hess et al. 2015), and 2 loci are duplicated. Therefore, there were 302 unique markers available for these association analyses out of the total 308 that were genotyped. These markers include 38 SNPs that were mostly adaptive loci that were categorized into the following four groups of linked loci: A (N=10), B (N=13), C (N=7), and D (N=8, Hess et al. 2013).

Temporal Genetic Structure

The panel of 308 SNPs was filtered as follows to generate a genotype data set suitable for evaluation of temporal genetic structure in Klamath River Pacific lamprey. First, the four species identification loci and all loci missing >=5% of their genotypes were removed. Next, those loci identified as non-neutral using the software

LOSITAN in Hess et al. (2013) were removed. Lastly, linked loci were identified using the software TASSEL (Bradbury et al. 2007), and one locus from each linked pair identified at a significance level of <0.01 was eliminated from the data set. Filtering resulted in a data set consisting of 148 SNP loci that were used to evaluate neutral patterns of genetic structure. Tests for conformance to Hardy–Weinberg proportions and estimates of observed and expected heterozygosity were calculated using the software GENODIVE 2.0b27 (Meirmans and Van Tienderen 2004).

The at-entry collections of the Klamath River Pacific lamprey included samples collected from 2016 and 2017 and samples collected across multiple months in each year allowing the assessment of inter- and intra-annual temporal genetic structure. To evaluate whether Klamath River Pacific lamprey exhibited temporal genetic structure and determine if the ocean- and river-ecotypes were genetically differentiated at neutral loci I analyzed the data using two approaches: (i) Bayesian cluster analysis (Pritchard et al. 2000), and (ii) K-means clustering (Meirmans 2012). Both approaches do not require population hierarchy to be defined a priori and allow assessment as to whether any significant genetic structure is present. However, the two approaches employ different statistical frameworks thereby allowing assessment of consistency across analytical approaches.

The Bayesian clustering algorithm implemented in the software STRUCTURE v 2.3.4 (Pritchard et al. 2000) was used to estimate the number of discrete genetic clusters (K) of individuals in the data and the probability of assignment (q) of each individual to

each cluster. Analyses were run for 200,000 steps (with 100,000 discarded as burn-in) and 20 independent runs were conducted for each value of K. Analyses were run assuming the data consisted of K = 1 … 5 clusters. Summaries of replicate runs were calculated using the software STRUCTURE HARVESTER (Earl and vonHoldt 2012), and the K with highest probability was used as an indicator of the number of genetically distinct groups in the data.

The K-means cluster method implemented in the software GENODIVE was used to sort individuals into an arrangement that maximized variance among groups but minimized within-group diversity. The Sums of Squares from an AMOVA was used to calculate distance matrix between individuals (Meirmans 2012) and simulated annealing from 50,000 steps (repeated 20 times) was used to perform K-means clustering. Analyses were run assuming the data consisted of K=1…5 clusters and selection of optimal K was based on Bayesian Information Criterion (BIC).

Genotype-Phenotype Association Tests

Genotype-phenotype associations were tested using a general linearized model (GLM) and a mixed linearized model (MLM) using the software TASSEL (Trait Analysis by aSSociation, Evolution and Linkage) v. 5.0.8 (Bradbury et al. 2007). GLM reduces the risk of false positives due to population structure by including population membership estimates as covariates in the model. Population structure was estimated based upon principal components analysis (first three PC axes) of 76 neutral SNPs (as

defined in Hess et al. 2014) using the software TASSEL. These 76 neutral SNPs are a subset of the 85 neutral SNPs that were characterized in detail by Hess et al. (2014, 2015) for rangewide collections and were successfully integrated into the GT-seq assay panel. Therefore, these 76 SNPs were expected to be informative to estimate neutral population structure in rangewide populations of Pacific lamprey, including fish from the Klamath River. Genotype-phenotype associations were also investigating using MLM, an approach that controls for false positives that may arise from both population and family structure and is therefore a more stringent association analysis than GLM. The MLM analysis used the population structure as estimated by the principal component analysis (see above) and a kinship matrix ('scaled IBS' method; Endelman and Jannink 2012) which was calculated using TASSEL. Permutation tests (1000) were used to calculate p-values for identification of significant associations of SNPs with traits.

The data set used for association testing consisted of all 308 SNPs genotyped in 92 female adult Pacific lamprey collected at-entry to the Klamath River. Eight traits were used for genotype-phenotype association testing including egg mass, GSI, total length (TL), body mass, girth, interdorsal distance, river entry date (day of year), and condition factor (Kn). Pearson's correlation coefficient and tests of significance were used to examine inter-correlations among the eight traits used in the phenotype-genotype association tests. Tests were conducted using R package Hmisc (R Core Team 2013).

The large number of tests for phenotype-genotype associations (8 traits x 308 loci = 2464 tests) increased the possibility of detecting a significant association by

chance. To account for multiple tests, only those associations with p-values less than the critical value as determined using the false discovery rate procedure described by Benjamini and Yekutieli (2001) were considered significant (critical value = 0.006). The Benjamini and Yekutieli (2001) false discovery rate (FDR) approach has more power to detect significant differences than sequential Bonferroni correction (Rice 1989; Narum 2006).

Inheritance Models for Ocean- and River-Maturing Ecotypes

To test for conformance to an additive genetic variation model, I assessed the relationship between egg mass and the number of river alleles (i.e., O_D, O_B) at the 17 loci exhibiting significant associations to egg mass (see Results). The allele with the highest frequency among the ocean-maturing individuals was used as a reference for designation of the ocean- versus river-maturing alleles. Goodness of fit was evaluated using least-squares linear regression.

Two-by-two contingency tables were constructed to test each of three additional inheritance models. First, individuals were phenotypically classified based upon egg mass, ocean-maturing with egg mass ≥12.5 g and river-maturing with egg mass <12.5 g. Next, individuals were genotypically categorized according to three inheritance models: (i) classical Mendelian inheritance at linkage group D (represented by locus Etr_2878) with river-maturing allele as dominant to ocean-maturing, (ii) classical Mendelian inheritance at linkage group B (represented by locus Etr_2791) with the river-maturing

allele as dominant to ocean-maturing, and (iii) a duplicate dominant epistasis model where ocean-maturing was considered recessive and only produced when genes in linkage groups B (Etr_2791) and D (Etr_2878) were homozygous recessive. Presence of a dominant allele from either linkage group was considered to produce the river-maturing ecotypes. Epistasis refers to genetic interactions in which one gene locus or linkage group masks or modifies the phenotypic effects of another gene locus or linkage group. I compared the assignment accuracy these alternative inheritance models, defined as the number of individuals classified as ocean- and river-maturing by both phenotype and genotype categorizations divided by the total number of individuals examined.

RESULTS

Pacific Lamprey Collection and Trait Analysis

Pacific lamprey were collected monthly, and sometimes weekly, over a one-year period from June 2016 through May 2017. Adult Pacific lamprey returns were collected during every month except for September and December. A total of 219 adult Pacific lamprey were collected at entry to the Klamath River, including 126 males and 93 females (Table 1; Table S 2). The number of individuals collected per month ranged from 0 to 60.

Table 1. The monthly sample size, total length, body mass, girth, interdorsal distance, and egg mass (females only) for the 219 adult Pacific lamprey collected at-entry to the Klamath River.

	Sample Size			Total Length (mm)			Body Mass (g)			Girth (mm)		
Month	Total	Males	Females	Mean	SD	Range	Mean	SD	Range	Mean	SD	Range
Jun-16	21	11	10	625	29	576-679	413.3	56.1	296-498	123	8	107-133
Jul-16	6	4	2	609	29	564-646	340.6	57.4	277-432	115	6	109-122
Aug-16	6	4	2	612	35	562-655	424.4	51.3	349-481	119	6	111-126
Sep-16	0											
Oct-16	1	1	0	601		601-601	327.5		328-328	104		104-104
Nov-16	1	1	0	588		588-588	440		440-440	126		126-126
Dec-16	0											
Jan-17	5	2	3	629	37	566-659	446	79.1	318-528	123	8	111-132
Feb-17	38	19	19	636	36	565-726	447.3	73.9	313-600	121	8	103-138
Mar-17	60	32	28	618	38	531-697	412.4	92.8	195-636	116	11	82-141
Apr-17	50	35	15	612	40	544-710	399.8	76.4	277-631	116	9	102-139
May-17	31	17	14	616	33	555-673	371.5	71	231-497	111	9	91-128
Total	219	126	93	620	35	531-710	408.7	69.8	195-636	117	8	82-141

	Interdorsal Distance (mm)			Egg Mass (g)		
Month	Mean	SD	Range	Mean	SD	Range
Jun-16	31	7	10-40	5.9	2.2	3.6-10.4
Jul-16	36	6	30-40	4.8	0.3	4.6-5.0
Aug-16	30	6	20-37	12.2	2.5	10.4-13.9
Sep-16						
Oct-16	47		47-47			
Nov-16	25		25-25			
Dec-16						
Jan-17	28	2	26-30	12.3	5.8	7.2-18.6
Feb-17	29	7	15-45	14.8	3.7	7.8-20.6
Mar-17	31	6	18-45	12.4	5.7	5.7-25.5
Apr-17	29	6	18-40	8.4	5.2	1.6-22.7
May-17	30	7	18-41	6.9	4.9	2.9-22.6
Total	30	6	10-47.0	10.5	3.8	1.6-25.5

Significant differences in run-timing (day-at-entry) were found between ocean- and river-maturing ecotypes [$t(83.77) = 4.85$, $p < 0.001$]. The median day of entry was ordinal day 61.5 for ocean-maturing ecotypes and ordinal day 110 for river-maturing ecotypes.

Female Pacific lamprey exhibited substantial variation in egg mass (Figure 1.D) among individuals collected on the same day and in the same month: March (5.7 - 25.5 g), April (1.6 - 22.7 g), and May (2.9 - 22.6 g). Egg mass ranges for January (7.2 - 18.6 g) and February (7.8 - 20.6 g) were less. The CV for April and May had the highest egg mass dispersal with CV's of 0.62 and 0.71, respectively, as compared to January and February CV's of 0.47 and 0.25, respectively. By season, egg mass CV was much higher in the spring as compared to winter and summer, evidenced by winter, spring, and summer CV's of 0.36, 0.60, and 0.21, respectively. No CV was estimated for fall as only one sample was collected per month in the fall.

Egg masses were natural log transformed to meet the assumptions of normality and homogeneity of variance prior to ANOVA analyses. There was a significant effect of month on ln(egg mass) (alpha = 0.05 level), [$F(7, 85) = 8.63$, $p < 0.001$], indicating log of egg mass across months was much larger than the variation of egg mass means within each month and at least one of the group means was significantly different from the others. Post-hoc comparisons using the Tukey's HSD test indicated significant pairwise differences (adjusted $p < 0.05$) with two distinct groups of months: (i) January, February,

and March (significantly higher egg mass means), and (ii) April, May, June, July, and August (significantly lower egg mass means).

Pacific lamprey females were partitioned into two groups based on ecotype categorized by egg mass phenotype-genotype associations (see Results). A multiple linear regression was used to model egg mass as a function of the explanatory variables. Preliminary analyses were performed to check for multicollinearity (weight and girth removed), violations of the assumption of normality, and homogeneity of variance. Evidence of interaction between ecotype and day was not significant ($p = 0.07$). Therefore, pairwise interactions were excluded from the model selection procedure. The best model for egg mass included ordinal day and ecotype as the only predictors (Table 2). A multiple linear regression was calculated to predict Pacific lamprey egg mass based upon ordinal day at-entry and ecotype. Using criterion-based model selection with the lowest AIC and BIC (Table 3), the best model was: ln(egg mass) = 3.026 – 0.003(day) – 0.819(ecotype), [$F(2, 90) = 96.47$, $p < 0.001$, $r^2 = 0.682$], where ecotype was coded as 0 = ocean-maturing and 1 = river-maturing (Figure 2). Based on back-transformed model predictions of egg mass (in grams), Pacific lamprey egg mass declines by 0.3% each day (26% decline in 100 days). River-maturing fish (ecotype=1) have a 56% lower mean egg mass as compared to ocean-maturing Pacific lamprey after controlling for day of year.

Table 2. Explanatory parameter estimates of the final multiple linear regression model.

Parameter	Estimate	SE	t value	Pr(>|t|)
intercept	3.026	0.079	38.291	< 0.001
day	-0.003	0.001	-3.681	< 0.001
ecotype	-0.819	0.077	-10.660	< 0.001

Table 3. Criterion based model selection for predicting ln(egg mass) using Akaike's Information Criteria (AIC), Bayesian Information Criteria (BIC), and parameter information for the top model (1), a competing model (2), and the full model (3).

Model Rank	Model Variables	R^2	Adjusted R^2	BIC	AIC	ΔAIC
1	day+ecotype	0.682	0.675	-93	-209	0
2	day+ecotype+TL	0.682	0.671	-88	-207	2
3	day+ecotype+TL+interdorsal	0.682	0.667	-84	-205	4

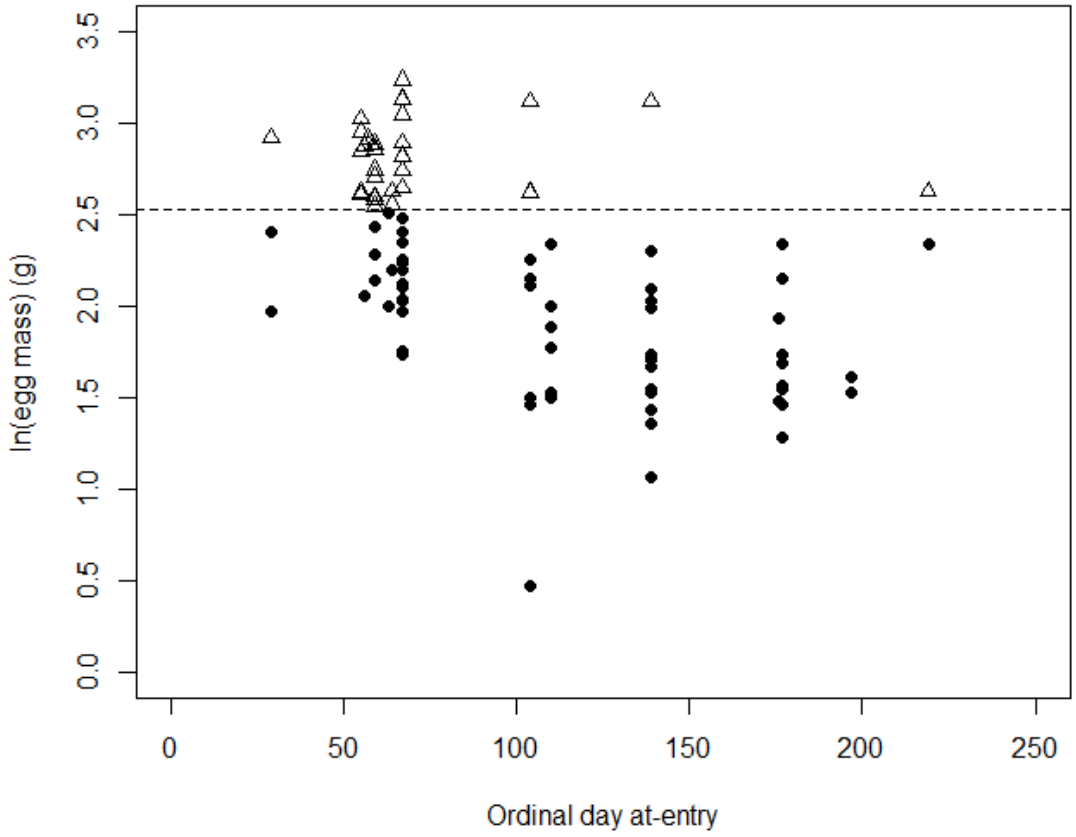

Figure 2. Multiple linear regression fits for the best model (i.e., lowest AIC and BIC) that predicts ln(egg mass) for Pacific lamprey, based on at-entry day and ecotype. River-maturing ecotypes represented with dark circles, and ocean-maturing ecotypes with open triangles. Dashed line represents 12.5 g egg mass break point between ocean- and river-maturing ecotypes. Lamprey egg mass declines by 0.3% each day (26% decline in 100 days).

For estimation of CPUE, a total of 173 Pacific lamprey were captured by dipnet or eel-hook during 142.13 hours of active fishing time (45 trips), resulting in a mean CPUE of 1.2 fish/hour for the study. Fishing effort was applied during all months, and the mean fishing time per month was 11.8 hours (range 3.4 in April to 28.58 in September). The mean CPUE varied throughout the year, with highest catches occurring from February to June (range 1.8 to 7.4 fish per hour) and lower catches from July to January (range 0 to 0.5 fish per hour) (Figure 3).

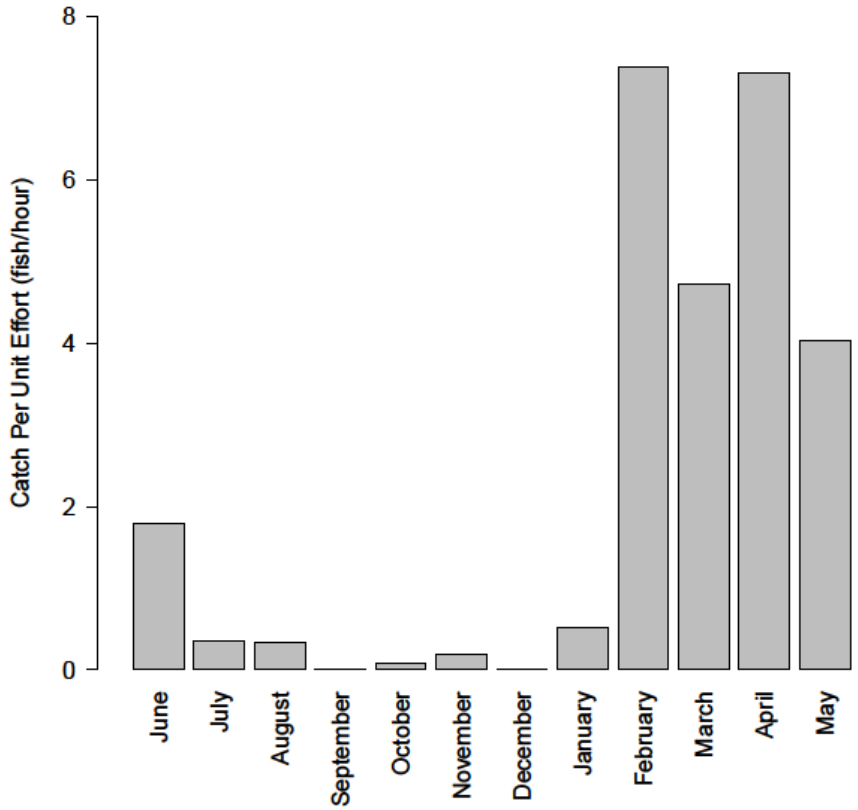

Figure 3. The mean catch-per-unit-effort of Pacific lamprey at-entry to the Klamath River from June 2016 to May 2017. Catch-per-unit effort was calculated as the total

number of Pacific lamprey captured, using an eel-hook or dipnet, per hour of active fishing.

Ln(total length) did not significantly correlate with ln(egg mass) ($r^2 = 0.026$, $p = 0.384$) in the ocean-maturing ecotypes, mature fish hypothesized to be within weeks or months of spawning. For example, one of the smallest fish (EtrKLA17-0061 at 565 mm) had a relatively large egg mass (18 g), and the largest egg mass of the study (EtrKLA17-0136 at 25.5 g) (Figure 1.E) came from a fish with an intermediate body size (615 mm). The largest fish of the study (EtrKLA17-0146 at 710 mm) possessed the smallest egg mass of the study (1.6 g) (Figure 1.D). The total length-egg mass relationship of river-maturing ecotypes was not evaluated as they represented an immature egg maturation stage, hypothesized not to spawn until the following year.

Temporal Genetic Structure

Among the 148 SNPs identified as neutral and therefore suitable for assessment inter- and intra-annual temporal genetic structure, 11 had an expected heterozygosity <0.15, 32 between 0.15 and 0.30, and 105 > 0.35. Tests for conformance to Hardy-Weinberg (Table S 1) expectations revealed significant departures at 39 loci at a $p<0.05$, and nine departures using a Bonferroni corrected p-value for multiple tests of 0.0003 (0.05/148 tests).

The at-entry collections of the Klamath River Pacific lamprey included samples collected from 2016 and 2017 and samples collected across multiple months in each year

allowing the assessment of inter- and intra-annual temporal genetic structure. Also, collections included both ocean- and river-maturing individuals allowing assessment of genetic differentiation between the ecotypes. In the Bayesian cluster analysis using the software STRUCTURE the highest log probability of the data was at K=1 and visual inspection of K>1 revealed that assignments were generally symmetric to all populations, indicative of the absence of ecotypic differentiation and inter- or intra-annual population genetic structure (Table 4). Similarly, K-means clustering using the software GENODIVE indicated the best clustering occurred at K = 1 according to BIC, suggesting there was no significant genetic structure in the data (Table 4).

Table 4. Assessment of neutral genetic structure using Bayesian cluster analysis (number of replicate runs (Reps), mean log probability of the data (Mean LnP(K)), and standard deviation of the log probability of the data (Stdev lnP(K)) and K-means clustering (Bayesian Information Criterion (BIC)) using 148 SNP loci in Klamath River Pacific lamprey. K is the assumed number of clusters.

	Bayesian Cluster			K-means
K	Reps	Mean LnP(K)	Stdev lnP(K)	BIC
1	30	-35047.79	0.3689	2042.18
2	21	-35329.881	181.6567	2043.73
3	20	-35540.04	224.6154	2045.96
4	20	-35669.405	439.0342	2048.52
5	20	-35589.34	410.8487	2051.28

Removal of the 39 loci that significantly departed from Hardy Weinberg expectations, and Bayesian cluster analysis of a data set consisting of 109 SNP loci resolved patterns that were identical to the 148 SNP data set indicative of the absence of

genetic differentiation between ocean- and river-maturing ecotypes and the absence of temporal genetic structure.

Genotype-Phenotype Association Tests

The GLM analyses conducted using TASSEL identified 74 total significant genotype-phenotype associations involving 35 loci and eight traits: egg mass, GSI, TL, weight, girth, interdorsal distance, day, condition factor (Table 5). The results of the genotype-phenotype association tests using MLM were similar but more conservative, identifying 66 total significant genotype-phenotype associations involving 32 loci and seven traits: egg mass, GSI, TL, weight, girth, interdorsal distance, condition factor (Table 5). A total of 61 significant associations were found in common across both GLM and MLM involving 28 loci and seven traits: egg mass, GSI, TL, weight, girth, interdorsal and Kn.

Table 5. P-values for loci exhibiting significant genotype-phenotype associations in the 92 female adult Pacific lamprey collected at-entry to the Klamath River. The data set consisted of 308 SNPs and eight traits (egg mass, gonadosomatic index (GSI), total length (TL), weight, girth, interdorsal distance, river entry date (day), and relative condition factor (Kn)). Significant associations identified only by GLM in red, only in MLM in blue, and both GLM and MLM are bolded in black. Only those associations identified as significant as determined using the false discovery rate procedure described by Benjamini and Yekutieli (2001) are reported (critical value = 0.006). The linkage group identifications follow Smith et al. (2018).

Loci	Egg Mass	GSI	TL	Weight	Girth	Interdorsal Distance	Day	Kn	Linkage group
Etr_1257	1.92E-03		0.005875883						B
Etr_5465	**1.13E-03**								B
Etr_1509	**7.05E-05**	**2.09E-03**	**1.35E-03**	4.48E-03					B
Etr_1613	**2.74E-05**	**3.51E-04**							B
Etr_2151	**6.16E-05**	**9.26E-04**					4.79E-03		B
Etr_2730	**2.74E-05**	**3.51E-04**							B
Etr_2791	**1.39E-05**	**2.93E-04**							B
Etr_4455	**1.39E-05**	**2.93E-04**							B
Etr_1378	**2.45E-04**	**4.83E-04**							D
Etr_1944	**2.18E-04**	**4.13E-04**							D
Etr_2097	**7.30E-04**	**1.47E-03**							D
Etr_211B	**1.12E-04**	**2.22E-04**							D
Etr_2878	**1.12E-04**	**2.22E-04**							D
Etr_4156	**1.12E-04**	**2.22E-04**							D
Etr_464	**2.54E-05**	**4.20E-05**							D
Etr_8649	**2.18E-04**	**4.13E-04**							D

Loci	Egg Mass	GSI	TL	Weight	Girth	Interdorsal Distance	Day	Kn	Linkage group
Etr_3383	**5.25E-05**	**3.37E-05**							NA
Etr_2603			**1.95E-06**	**1.94E-05**	**2.22E-04**	**3.48E-04**			A
Etr_2287			**1.92E-09**	**1.05E-05**	**1.33E-04**				A
Etr_5317			**1.15E-08**	**1.58E-06**	**5.04E-05**				A
Etr_6363			**9.28E-09**	**2.13E-06**	**5.52E-05**				A
Etr_3069			**9.28E-09**	**2.13E-06**	**5.52E-05**				A
Etr_3638			**4.04E-08**	**2.21E-05**	**4.35E-04**				A
Etr_3885			**3.69E-07**	**1.05E-05**	**2.91E-05**				A
Etr_4889			**6.51E-09**	**1.27E-06**	**3.09E-05**				A
Etr_4093			2.46E-03						
Etr_1773			3.04E-03						
Etr_668			1.65E-03	0.005203083					NA
Etr_2776			4.54E-03						C
Etr_5654			**1.77E-03**						NA
Etr_3837		4.30E-03	1.29E-03	7.42E-04					NA
Etr_4414					3.28E-03				NA
Etr_5780								**2.30E-03**	NA
Etr_8681						**7.77E-05**			NA
Etr_2451						**3.88E-04**			NA
Etr_2823						2.50E-03			NA
Etr_4750						3.76E-03			NA

Egg mass at-entry exhibited significant associations with 17 loci in the GLM analysis (Figure 4; Table 5). The mean p-value for the loci showing significant associations in the GLM was 3.00E-04 (range 1.39E-05 to 1.92E-03). The 17 loci found to exhibit significant associations with egg mass were distributed across two linkage groups identified by Smith et al. (2018), including eight loci on linkage group D, eight loci on linkage B (Table 5). One locus, Etr_3383, was of unknown linkage relationship (Table 5). The MLM analysis produced similar results, except that significant associations were detected in 16 loci instead of 17 loci. The mean p-value for the loci showing significant associations in the MLM was 1.33E-03 (range 3.25E-04 to 3.70E-03). Egg mass exhibited a strong correlation with GSI (0.95; Table 5) and therefore GSI exhibited significant associations with 15 of the same loci identified as having significant associations with egg mass (Table 5).

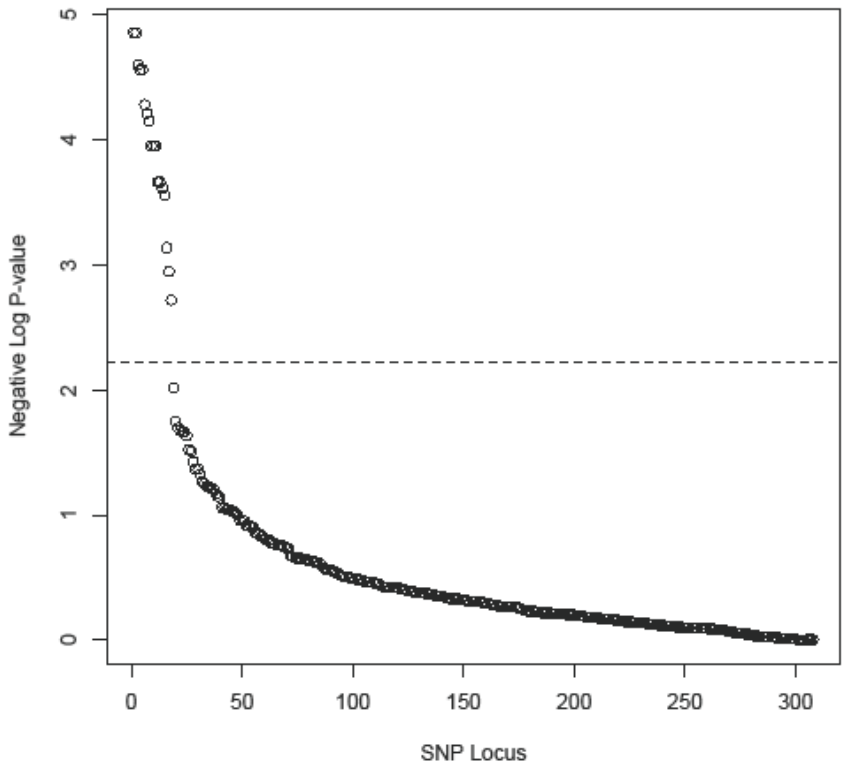

Figure 4. General Linearized Model p-values for associations between egg mass and each of the 308 SNP loci genotyped in 92 adult Pacific lamprey collected at entry to the Klamath River, California. Analyses were conducted using the software TASSEL. P-values are ordered from smallest to largest. The horizontal dotted line indicates the critical value as determined using the false discovery rate procedure described by Benjamini and Yekutieli (2001) (critical value = 0.006).

To visualize the strength of the association between the loci in the B and D linkage groups and eggs mass, a heatmap of each individuals' multilocus genotype was constructed (Figure 5). For standardization, genotypes of each individual were coded as homozygous for small egg mass/river-maturing, homozygous for large egg mass/ocean-maturing, or heterozygous. The allele with the highest frequency among the large egg

mass/ocean-maturing individuals was used as a reference for designation of the ocean-maturing ecotype allele. A distinction occurs in genotypes at both linkage groups A and B at approximately 12.5 g egg mass. The large egg mass individuals (≥ 12.5 g) or ocean-maturing individuals exhibit a higher frequency of the large egg mass allele and are almost exclusively homozygous for the large egg mass allele at both linkage group B and D. In contrast, the small egg mass (< 12.5 g) or river maturing individuals exhibit a higher frequency of the small egg mass allele, but also exhibit high genotypic diversity encompassing both heterozygous and homozygous genotypes at both linkage group B and D.

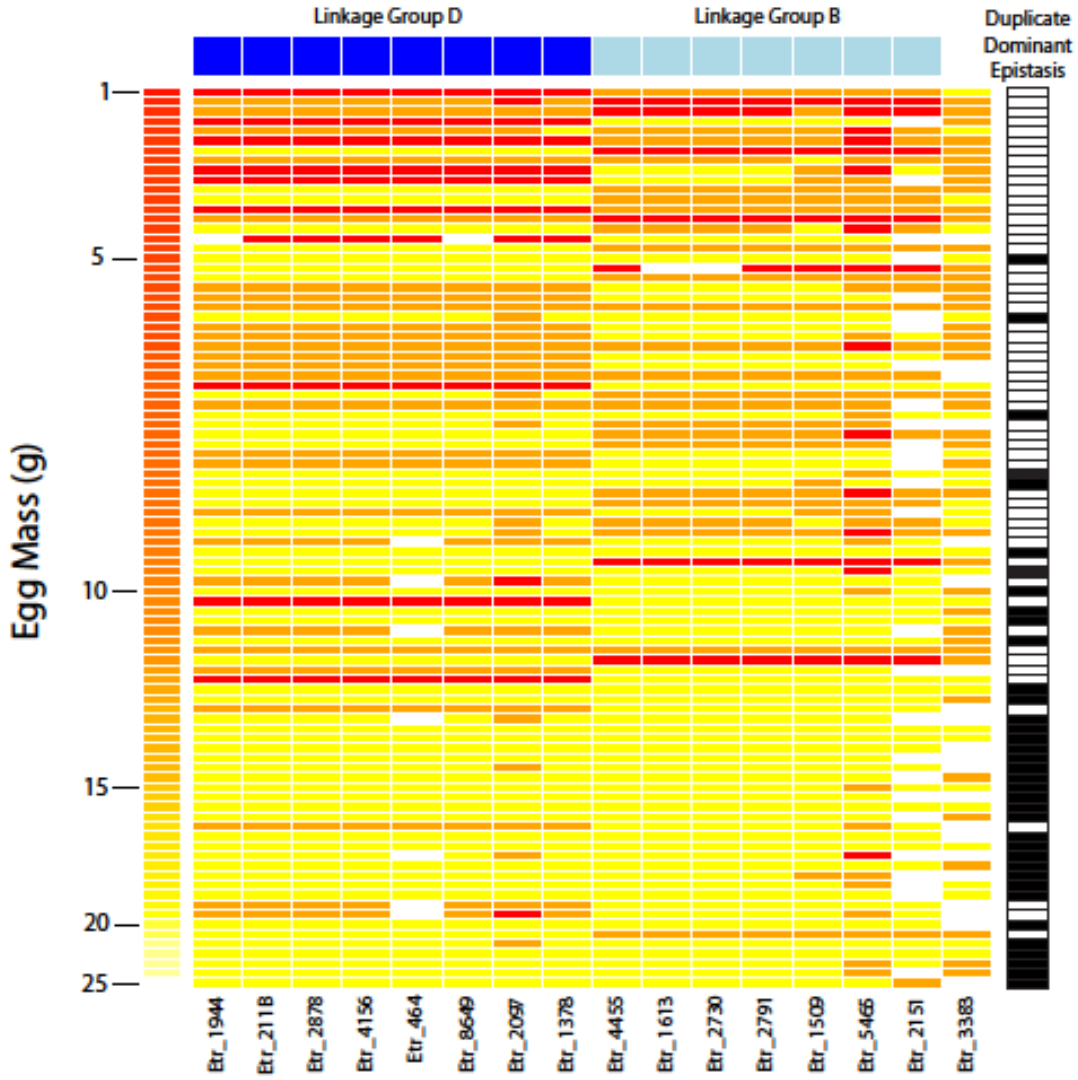

Figure 5. Genotype heatmap for the 16 SNP loci exhibiting significant association to egg mass in 92 adult Pacific lamprey collected at entry to the Klamath River, California. A change in allele frequency was observed corresponding to an egg mass of 12.5 g. Each row indicates an individual multilocus genotype, coded homozygous for small egg/river-maturing (red), homozygous for large egg/ocean-maturing (yellow), and heterozygous (orange). Missing data are coded white. Individuals are ordered top to bottom from small to large egg mass, as indicated by the heat-bar at the left. Loci are grouped by linkage group as indicated by the bar at the top. At right, categorization of individuals into river- or ocean-maturing according to the duplicate dominant epistasis model. Under this model, ocean-maturing is considered recessive and only develops when genes in linkage group

B and D are homozygous recessive. Assignments are based upon one locus from linkage group D (Etr_2878) and one locus from linkage group B (Etr_2791). Shown are the 16 loci that exhibited significant associations in both the GLM and MLM.

Total length exhibited significant associations with 16 loci in the GLM analysis. The mean p-value for the loci showing significant associations in the GLM was 1.05E-03 (range 1.92E-09 to 5.88E-03). The 16 loci found to exhibit significant associations with TL were distributed across three linkage groups identified by Smith et al. (2018), including eight loci on linkage group A, one locus on linkage B, one locus on linkage group C. The remaining six loci were of unknown linkage relationship. The MLM analysis produced similar results, except that significant associations with 13 loci instead of 16 loci were detected. The mean p-value for the loci showing significant associations in the MLM was 1.02E-03 (range 1.03E-06 to 5.88E-03). Total length exhibited a moderate correlation with weight (0.82), and girth (0.66) (Table 6) and therefore these traits generally exhibited significant associations with the same loci as TL, including the eight loci occurring on linkage group A (Table 5)

Table 6. Pearson correlation coefficients (below diagonal) and significance tests (above diagonal) for the eight traits used for genotype-phenotype association testing in the 92 female adult Pacific lamprey collected at-entry to the Klamath River.

	Day	Total Length	Body Mass	Girth	Interdorsal Distance	Egg Mass	Gonadosomatic Index	Condition Factor
Day	-	0.8512	0.2549	0.6461	0.2551	0	0	0.0397
Total Length	0.02	-	0	0	0.0015	0.1823	0.341	0.9073
Body Mass	-0.12	0.82	-	0	0.0002	0.1434	0.2239	0
Girth	0.05	0.66	0.87	-	0.0187	0.8869	0.0297	0
Interdorsal Distance	-0.12	0.33	0.38	0.24	-	0.9915	0.3583	0.0789
Egg Mass	-0.49	0.14	0.15	0.02	0	-	0	0.3066
Gonadosomatic Index	-0.47	-0.1	-0.13	-0.23	-0.1	0.95	-	0.716
Condition Factor	-0.21	-0.01	0.55	0.57	0.18	0.11	-0.04	-

To visualize the strength of the association between the loci in the A linkage group and total length, a heatmap of each individuals' multilocus genotype was constructed (Figure 6). An initial visual inspection indicated a change in allele frequency at a total length of 625 mm. Based upon this, genotypes of each individual were coded as homozygous for large size, homozygous for small size, or heterozygous. The allele with the highest frequency among the large individuals (≥625 mm total length) was used as a reference for designation of the "large" allele.

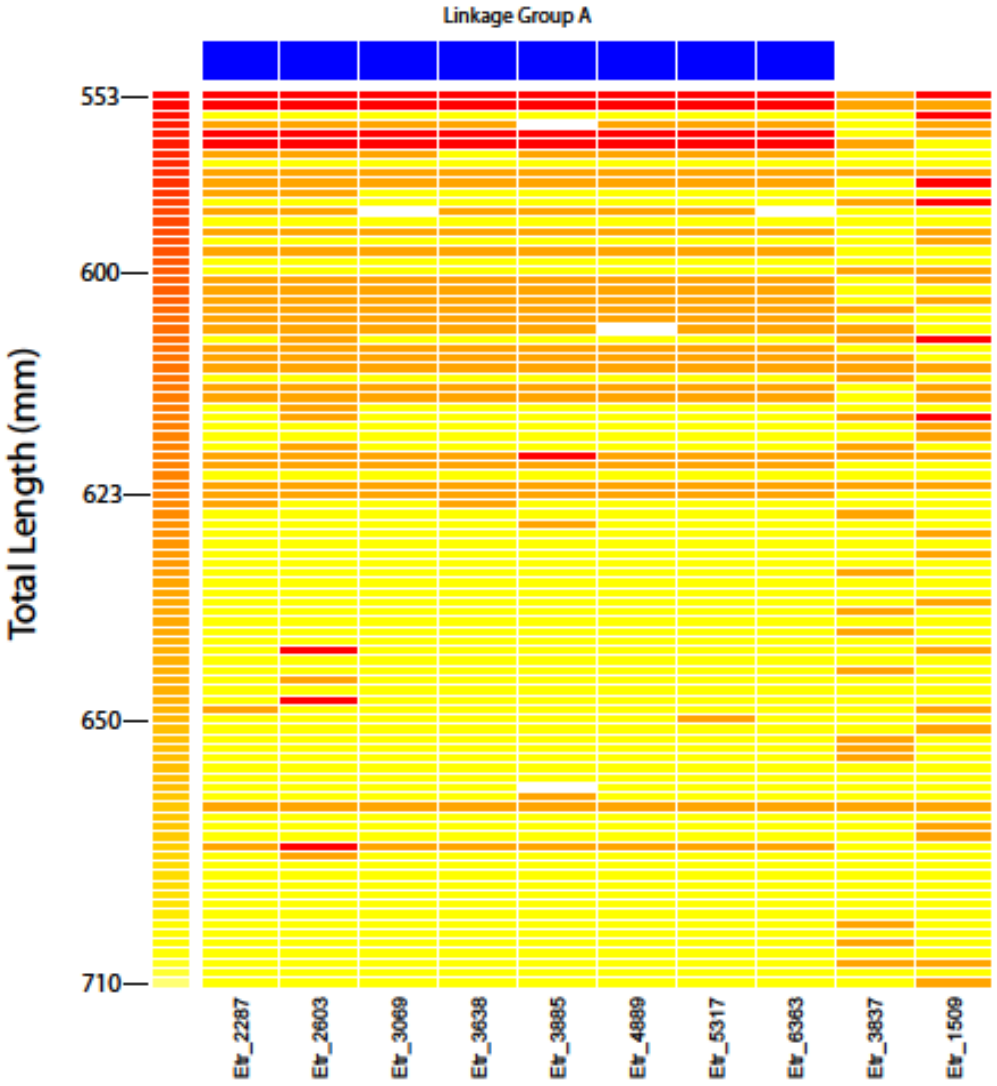

Figure 6. Genotype heatmap for the 10 SNP loci exhibiting significant association to total length in Klamath River Pacific lamprey collected at-entry. A change in allele frequency was observed at a length of 625 mm. Each row indicates an individual multilocus genotype, coded homozygous for shorter length (red), homozygous for larger length (yellow), and heterozygous for shorter length (orange). Missing data are coded white. Individuals are ordered top to bottom from small to large total length, as indicated by the heat-bar at the left. Eight of the ten loci are associated with linkage group A, as indicated by the bar at the top, and two loci are unassigned.

The GLM analysis detected significant relationships between interdorsal distance and four loci, day and one locus, and Kn and one locus (Table 5). The MLM detected significant associations between interdorsal distance and three, and kn and one locus, but failed to produce any associations with day.

Inheritance Model for Ocean- and River-Maturing Ecotypes

The relationship between egg mass and the number of river alleles at the 17 loci associated to egg mass was significant ($p<0.05$), but egg mass associated loci only explained about 39 percent of the variation in egg mass indicating limited support for a model of additive genetic variation. The model assuming classical Mendelian inheritance at linkage group D (Etr_2878) with river-maturing allele as dominant to ocean-maturing resulted in an assignment accuracy of 0.65 (Table 7). Similarly, the model assuming classical Mendelian inheritance at linkage group B (Etr_2791), with river-maturing allele as dominant to ocean-maturing resulted in an assignment accuracy of 0.65 (Table 8). However, when individuals were categorized using a duplicate dominant epistasis model, where ocean-maturing was considered recessive and only produced when genes in linkage groups B (Etr_2791) and D (Etr_2878) were homozygous recessive, resulted in an assignment accuracy of 0.83 (Table 9). Individuals genotypically categorized according to the duplicate dominant epistasis model exhibited significant differences in egg mass [$t(65.64) = 6.90$, $p < 0.001$], providing further support for the inheritance model. The mean egg mass was 14.7 g for ocean-maturing ecotypes and 7.7 g for river-maturing ecotypes categorized using the duplicate dominant epistasis model (Figure 7).

Table 7. Contingency table for 92 adult female Pacific Lamprey collected at-entry to the Klamath River. Classical Mendelian inheritance model with river-maturing allele as dominant (O_D) to ocean-maturing (o_D) at linkage group D (as represented by Etr_2878).

(i) Mendelian Inheritance (Etr_2878, Linkage Group D)

Phenotype	o_Do_D	O_Do_D or O_DO_D
Ocean-maturing	27	4
River-maturing	28	33

Proportion correctly classified: 0.65

Table 8. Contingency table for 92 adult female Pacific Lamprey collected at-entry to the Klamath River. Classical Mendelian inheritance model with the river-maturing allele as dominant (O_B) to ocean-maturing (o_B) at linkage group B (as represented by Etr_2791).

(ii) Mendelian Inheritance (Etr_2791, Linkage Group B)

Phenotype	o_Bo_B	O_Bo_B or O_BO_B
Ocean-maturing	30	1
River-maturing	31	30

Proportion correctly classified: 0.65

Table 9. Contingency table for 92 adult female Pacific Lamprey collected at-entry to the Klamath River. A duplicate dominant epistasis model with ocean-maturing considered recessive and only produced when genes at linkage groups B (represented by Etr_2791) and D (represented by Etr_2878) are both homozygous recessive ($o_Bo_Bo_Do_D$).

(iii) Duplicate Dominant Epistasis (Etr_2791, Linkage Group B and Etr_2878, Linkage Group D)

Phenotype	$o_Bo_Bo_Do_D$	One river allele (O_B or O_D)
Ocean-maturing	26	5
River-maturing	11	50

Proportion correctly classified: 0.83

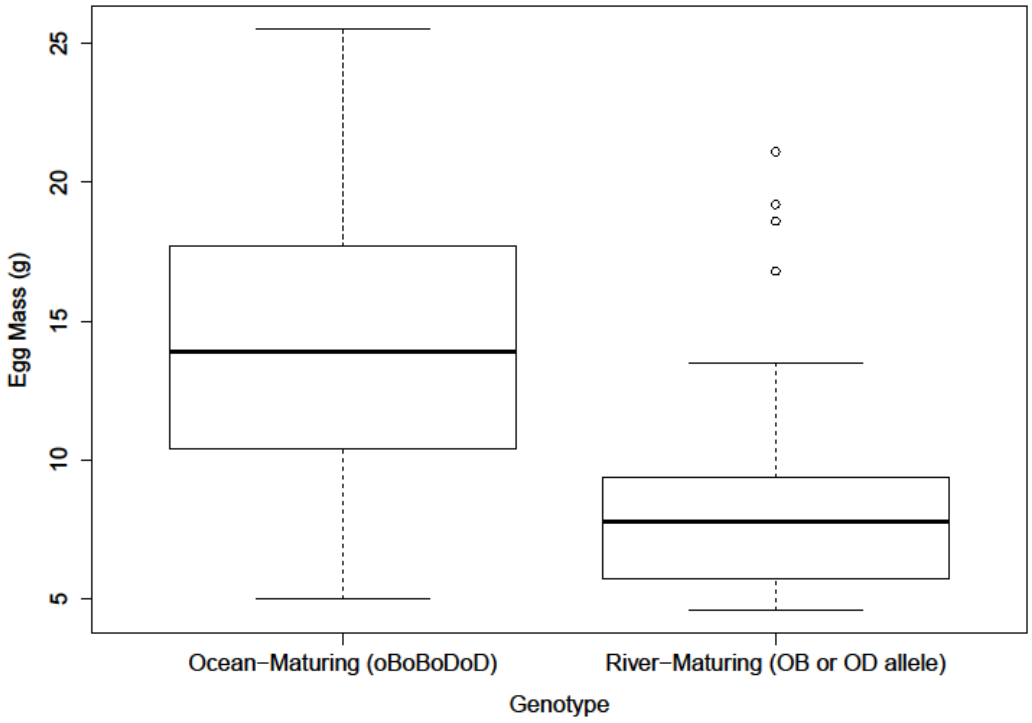

Figure 7. Ocean- and river-maturing Pacific lamprey ecotypes genotypically categorized according to the duplicate dominant epistasis model exhibiting significant differences in egg mass. Presence of a dominant allele (OB, OD) from either linkage group was considered to produce the river-maturing ecotypes.

DISCUSSION

Run-timing

I tested the hypothesis that ocean- and river-maturing Pacific lamprey ecotypes do not display differences in run-timing when they initiate freshwater migration. Significant run-timing differences (*t*-test; $p<0.001$) were observed between ocean- and river-maturing ecotypes. The onset for freshwater migration for the ocean-maturing ecotype was predominantly the winter whereas the river-maturing ecotype entered during all seasons. Egg mass means for February to April were as much as twice the means for May to August. ANOVA and post-hoc tests showed that log of egg mass varied across months with two significantly differing monthly groups: (i) January to March (females with higher egg mass), and (ii) April to August (females with lower egg mass). The group differentiation resulted from both ecotypes being collected concurrently during the group (i) period, exhibiting a large range of egg masses. Whereas other than four ocean-maturing outliers, only the river-maturing ecotype (i.e., small egg mass) was collected during the group (ii) period, extending five months past when the majority of ocean-maturing fish stopped being available for collection. Therefore, significant ecotype run-timing differences between ocean- and river-maturing fish were identified. However, unlike similar ecotypes characterized in a different anadromous species (e.g., premature and mature steelhead (Hess et al. 2016)), the two Pacific lamprey ecotypes do not exhibit the same level of temporal isolation in entry time but instead display overlapping entry

timing. Therefore, freshwater entry date alone does not serve as a good phenotypic proxy of ecotype, as used in spring-run versus fall-run salmon or winter-run versus summer-run steelhead.

Freshwater Migration Strategies

Adult Klamath River Pacific lamprey appear to display at least two spawning migration strategies. Ocean-maturing Pacific lamprey enter freshwater in a sexually mature state during the late-winter and appear to migrate directly to spawning habitat, spawning within weeks or months post river entry. Whereas river-maturing fish appear to migrate either directly to or near spawning locations and hold in-river until the following spring while eggs undergo maturation. With the onset of vitellogenesis, river-maturing holdover fish from the previous year may potentially interbreed with ocean-maturing fish from the current year migration and other river-maturing fish (Figure 8). The diversity in migration behavior of ocean- and river-maturing ecotypes contributes to increased polymorphism, creating a portfolio that may potentially buffer Pacific lamprey against environmental factors that may lead to their decline (Schindler et al. 2010).

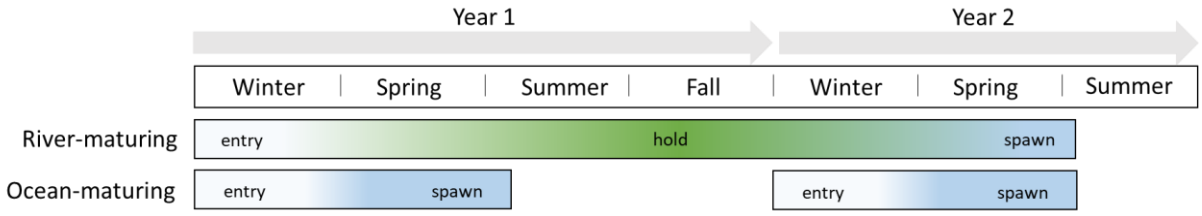

Figure 8. Hypothesized Klamath River Pacific lamprey ocean- and river-maturing freshwater migration strategies.

Trait Analysis

It has been suggested that Pacific lamprey parallel Pacific salmon reproductive strategies (Clemens et al. 2013). Typically, anadromous fish (e.g., salmonids) exhibit a positive body size to egg mass relationship (McGurk 2000; Hendry et al. 2001). In contrast, I observed no significant correlation between body size (total length) and egg mass in ocean-maturing Pacific lamprey, excluding evaluation of the river-maturing ecotype body size-egg mass relationship due to a lack of maturation. The lack of a body size-egg mass correlation is exemplified by one of the smallest fish (565 mm) with a relatively large egg mass (18 g), and the largest egg mass of the study (25.5 g) with an average body size (615 mm). Pacific lamprey body size-egg mass disassociation appears unique as compared to Klamath River Chinook Salmon. Analysis of Fall season "maturing-mature" chinook salmon egg masses collected at-entry to the Klamath River showed a significant body size-egg mass relationship, August to October 2009 (n=142) ($r^2 = 0.286$, $p < 0.001$) and from August to October 2010 (n=104) ($r^2 = 0.236$, $p < 0.001$) (Hearsey and Kinziger 2015), but "maturing-mature" Pacific lamprey ($r^2 = 0.026$, $p = 0.384$) in this study did not have a significant relationship (Figure 9). Pacific lamprey appear to be unique from most fish with regards to the body size-egg mass relationship.

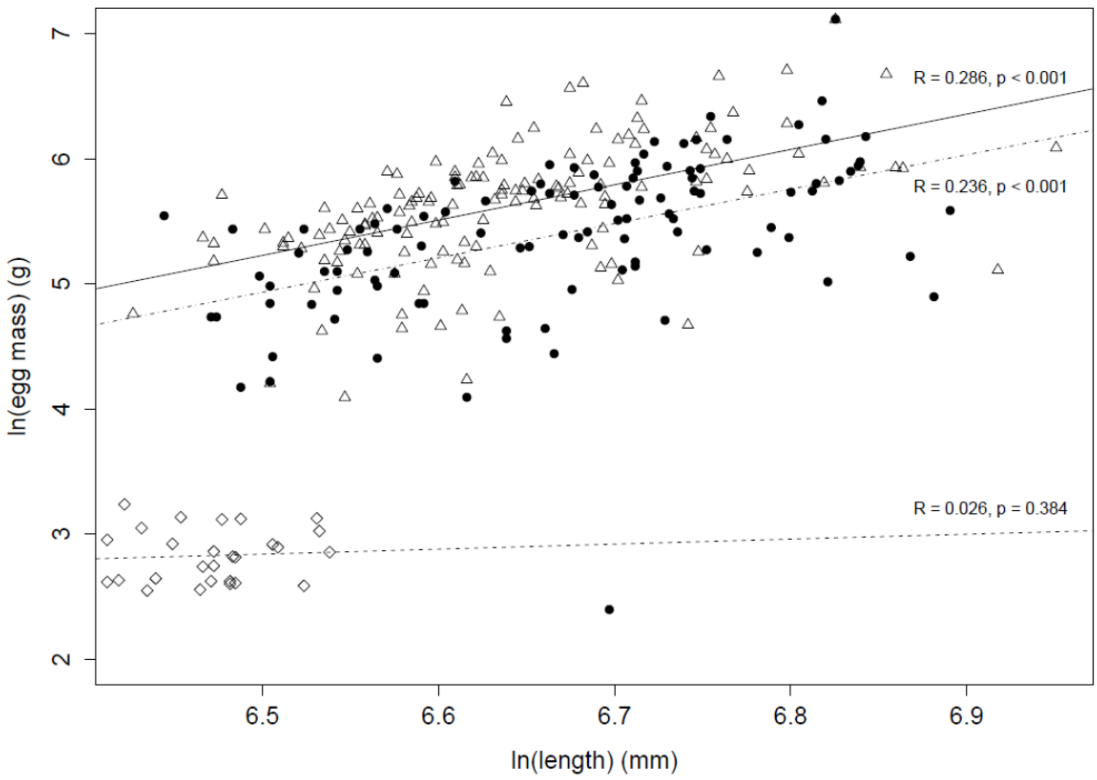

Figure 9. Total length and egg mass relationships for at-entry collections of fall-run Klamath River Chinook Salmon, August to October 2009 (open triangles, solid regression line) showed a significant relationship (r2 = 0.286, p < 0.001), as did August to October 2010 salmon (solid circles, dot-dash regression line) (r^2 = 0.236, p < 0.001), as compared to at-entry Klamath River ocean-maturing Pacific lamprey (open diamonds, dashed regression line) (r^2 = 0.026, p = 0.384) displaying no significant correlation between total length and egg mass.

The Pacific lamprey body size-egg mass disassociation observed may impact run-timing. Quinn et al. (2015) proposed a system of trade-offs to balance the risks and benefits of migration timing in salmonids. Cumulative mortality risk in the ocean increases the longer a fish remains. However, the longer time in the river prior to spawning increases the energetic demands of fasting and so the cumulative mortality risk in the river is lower the shorter the river holding time. Quinn et al. (2015) found that by

leaving the ocean early, salmonids lose growth opportunities and associated reproductive benefits. Specifically, for female salmonids, the reproductive benefits of a later ocean exit is increased total egg mass, positively related to body size. This hypothesis does not appear applicable to Pacific lamprey however. As discussed, if Pacific lamprey stay in the ocean and feed longer, thereby gaining a larger body size, they are not necessarily associated with reproductive benefits such as increased egg mass, like salmonids. Therefore, when Pacific lamprey weigh the benefits and costs of variable freshwater entry timing, the benefit of mortality avoidance in the river likely outweighs the cost of potential egg mass gains from a longer ocean residence time. Pacific lamprey also possess significantly higher lipid reserves than salmonids (Whyte et al. 1993; Stewart et al. 1983), allowing for extended non-feeding time periods in freshwater without interfering with egg maturation. Gonadal growth is based on mobilization of lipid and protein, which has been found in other lamprey species (e.g., anadromous *Lampetra fluviatilis*) to operate in parallel, both being slow before and rapid during sexual maturation (Larsen 1980).

Substantial egg mass variation is observed in female steelhead (winter-run versus summer-run) and salmonids (spring-run versus fall-run chinook), both species exhibiting substantial temporal differences in egg mass segregated by seasonal run times. Initially, Pacific lamprey egg mass variability appeared high for both day and month, but this was later revealed to be caused by the concurrent migration and collection of both ecotypes with distinguishing large and small egg masses. However, statistical analysis showed

Pacific lamprey egg mass variability to be relatively low as compared to Klamath River Chinook Salmon. Analyzing raw data from Hearsey and Kinziger (2015), mean CV for at-entry Chinook Salmon during May to October, 2009 and 2010, were 0.64 and 0.66, respectively, as compared to 0.39 mean CV for lamprey during the 12-month study period. By season, salmon also displayed higher egg mass variability as compared to lamprey with summer differences the most striking (CV=0.70 versus 0.21, respectively).

Annual Variation in Abundance

Estimates of CPUE are important for future management decisions and to assess a fishery condition by comparison. However, CPUE estimates for Pacific lamprey are very limited because large scale harvest typically only occurs in Native American subsistence fisheries, Pacific lamprey are not a priority for fisheries management, and no commercial fishery exists. No CPUE estimates for Klamath River Pacific lamprey were found in the literature. I determined a baseline CPUE estimate for my study period (1.2 fish/hour) with Spring (March to May) having the highest Pacific lamprey mean CPUE estimate (5.4 fish/hour). Eighty-two miles south, the Wiyot Tribe conducts a subsistence fishery for Pacific lamprey on the Eel River. A 2014 creel survey (Stillwater Sciences & USFWS 2016) of at-entry Eel River Pacific lamprey had similar findings to this study: (i) CPUE estimate for January to March 2014 was 0.60 fish/hour, (ii) the beginning of adult Pacific lamprey freshwater migration in both the Klamath River and Eel River is the month of February, and (iii) the mean at-entry total length was nearly identical for Pacific lamprey in both rivers, Eel River (619.4 mm) compared to the Klamath River (619.6

mm). The Eel River may offer an opportunity for a future study to evaluate if the two Pacific lamprey ecotypes I revealed are present there.

Maturity and Interdorsal Distance

Studies have identified a significant correlation between Pacific lamprey interdorsal distance and sexual maturation at collection points far upriver (>100 miles) (Clemens et al. 2009; Hardisty and Potter 1971). The interdorsal space reduces as the fish shrinks during freshwater migration to the point that the two dorsal fins may touch (Clemens et al. 2009). I found no correlation between interdorsal distance and egg mass measurements (r^2=0.00003, p=0.957). It is possible that collection at-entry to the river preempted interdorsal space shrinkage and any corresponding relationship to maturation. When comparing the results from Pacific lamprey studies, it is important to control for the within river collection location (e.g., mouth or upstream), because the freshwater migration distance at collection could affect body size, season of collection, and the likelihood of observing an overwintered or a current year spawner. For example, no evidence for the ocean-maturing ecotype has been found outside the Klamath River (Clemens et al. 2013; Clemens et al. 2016). However, ocean-maturing ecotypes may occur in other rivers besides the Klamath River, but they have not been found at typical collection points far inland (e.g., Willamette Falls (128 RM); Bonneville Dam (145 RM)) possibly due to the onset of vitellogenesis, resulting in spawning prior to potential collection and genotyping. Also, my analysis indicates that ocean-maturing individuals

are more common during the winter, thus winter collection increases the chances of detecting this ecotype.

Neutral Genetic Structure

A goal of this study was to analyze Pacific lamprey SNP loci for evidence of neutral patterns of genetic differentiation between ocean- and river-maturing ecotypes and temporal genetic differentiation (e.g., between months or years). Both Bayesian cluster analysis and K-means clustering analysis failed to resolve evidence for any genetic groups in the data that may have resulted from inter- and intra-annual differences in run-timing or differences between the ocean- and river-maturing ecotypes. The degree that neutral genetic variation can become decoupled with adaptive genetic variation is highly influenced by the level of gene flow maintained across a species range. The gene flow maintained across the Pacific lamprey range is likely quite high as compared to anadromous salmonids (Spice et al. 2012). It is rather surprising that in general, most genetic studies have estimated high levels of neutral gene flow in Pacific lamprey (Goodman et al. 2008; Spice et al. 2012; Hess et al. 2013) despite evidence of strong selective mechanisms acting on adaptive genetic variation (Hess et al. 2013). However, Clemens et al. (2017) suggests that putatively neutral variation based on microsatellite markers, can be divergent within the same river (e.g., Willamette River, Oregon) between years and even between groups of small and large body sizes within the same year. The ways in which neutral and adaptive variation correlate with traits and behaviors of Pacific

lamprey appear to be complex and require further study of a range of temporal and geographical samples to fully understand (Hess et al. 2016).

Genotype-Phenotype Associations

Another goal of this study was to test if phenotype-genotype associations exist between ocean- and river-maturing Pacific lamprey ecotypes and adaptive genetic loci. Both GLM and MLM analysis identified adaptive SNP loci at linkage groups B and D with significant associations to egg mass. Individuals that were homozygous for the ocean-maturing allele at both linkage groups almost always had an egg mass greater than 12.5 g at the onset of their freshwater migration. In contrast individuals that had at least one river-maturing allele in linkage group B or D predominately had an egg mass less than 12.5 g upon river entry (Figure 5). The strong inflection point at 12.5 g egg mass was therefore identified as a reasonable separation point for designating ocean- and river-maturing ecotypes. Also, examination of genotypes at the B and D linkage groups shows strong distinction in the degree of genetic diversity in the ocean- and river-maturing genotypes. The ocean-maturing individuals are almost exclusively homozygous at both linkage groups, but river-maturing individuals exhibit a high degree of polymorphism in their genotypes.

The duplicate dominant epistasis model provided the best fit for explaining the inheritance of the ocean- and river-maturing ecotypes, with an assignment accuracy of 83% (Table 9). Under this model the two linkage groups control the trait and only one

dominant allele in either the B (O_B)or D linkage group (O_D) is necessary to express the river-maturing ecotype, whereas the ocean-maturing ecotype was expressed when genes at both linkage groups were homozygous recessive ($o_Bo_Bo_Do_D$). The model hypothesizes that: (i) a cross between true breeding ocean-maturing (new migrants; $o_Bo_Bo_Do_D$) and river-maturing ($O_BO_BO_DO_D$) individuals would produce river-maturing genotypes only in generation F1, and (ii) intermating of F1 lamprey produces river- and ocean-maturing fish in a 15:1 ratio (assuming 100% penetrance).

Individuals assigned inaccurately with the duplicate dominant inheritance model (16/92) likely resulted from using continuous variation in egg mass (1.6 – 25.5 g) to make a binary diagnosis into ocean- and river-maturing ecotypes. The majority (11/16) of the mis-assigned fish had an ocean-maturing genotype but with a river-maturing phenotype (egg mass < 12.5 g). Most of these individuals had intermediate egg masses ranging from 8.5 – 12.5 g. These individuals may potentially express their ocean-maturing genotype by continuing egg maturation during migration and spawn in their current migratory year. Thus, my field collections may have intercepted individuals too early in gonadal development to allow for perfect diagnosis of the ecotypes. Evidence for the duplicate dominant inheritance model may be improved if individuals could be captured at the defining stage for each ecotype utilizing radiotelemetry tracking.

This study contributes to growing field illustrating the ubiquitous nature of gene interactions in natural systems and the importance of considering epistatic effects when researching the genetic basis of complex traits (Phillips 2008). Epistasis may hinder

efforts to identify the genetic basis of traits owing to the large number of interactions that must be tested and the potential for a genes effects to be obscured by interactions with other loci (Phillips 2008; Yang 2009). Genetic markers may show little effect when considered individually but strong effect when considered in combination with other loci (Cordell 2002). Interestingly, the duplicate dominant epistasis model that I hypothesized to explain inheritance of ocean- and river-maturing ecotypes is a well-known example of epistasis (Miko 2008).

Geography, run-timing, adult body size, and now female maturation (this study) were found to be correlated with adaptive genetic variation in Pacific lamprey (Hess et al. 2013, 2014, 2015). The GT-seq SNP panel used in this analysis was designed to over-represent four groups of linked adaptive loci (Groups A, B, C, and D) which were discovered by Hess et al. (2013). The locus groups B and D, which this study found significantly associated with ocean- and river-maturity traits in females, have not been possible to test in a similar way in other studies. However, association testing conducted with adult females in the Willamette River have at least corroborated a correlation of loci on group D with female Pacific lamprey maturation (Hess et al., unpublished). Further, linkage group A, the only group that was identified as significantly associated with body size (TL) in this study, has also been corroborated in other association studies that have shown a strong association of group A loci with body size traits (Hess et al. 2014, 2015). Aside from maturity and body size, these four adaptive groups of linked loci appear to be associated with other traits and the strength of these associations may be variable across

the species range. Future studies should test whether ocean- and river-maturing Pacific lamprey migration is associated with the same groups of linked loci identified here across multiple populations. It may also be important to integrate genomics, phenomics, and epigenomics, among other tools to evaluate the role of selection as a contributing cause of Pacific lamprey phenotypic diversification.

CONCLUSIONS

The spawning migration differences between ecotypes identified herein has implications for conservation and management. Other studies have found that Pacific lamprey do not exhibit strong natal homing (e.g., local adaptation), nor are they truly panmictic, which has caused difficulty in defining suitable management units (Spice et al. 2012). The river-maturing ecotype identified in this study carries standing genetic variation capable of producing both ecotypes (e.g., both dominant and recessive alleles), while the ocean-maturing ecotype carries a single allele (e.g., recessive only). An ecological application of these molecular findings is that when assessing stream restoration projects for lamprey, the river-maturing ecotypes could perhaps be prioritized as they contain the genetic diversity capable of producing both ecotypes (i.e., heterozygosity), whereas the ocean-maturing ecotypes do not.

Clemens et al. (2016) concluded the relationship between life-history diversity and genetic stock structure of Klamath River Pacific lamprey is not known, and only the Klamath River has shown evidence of an ocean-maturing Pacific lamprey phenotype collected in late vitellogenic stages at-entry (Clemens et al. 2013). Hess et al. (2013) was unable to conclude that Pacific lamprey display multiple run-timing life history strategies in stream habitats, due to a lack of phenotypic information. In this study, I have identified and revealed the genetic basis of ocean- and river-maturing ecotypes. I recommend distinguishing the river-maturing and ocean-maturing ecotypes of Pacific lamprey by adopting the names ke'ween (lamprey "eel") and tewol (ocean), respectively,

using terms from the Yurok language, in recognition of the importance of Pacific lamprey to Pacific Northwest fishing tribes.

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APPENDIX

Table S 1. Locus, test for conformance to Hardy-Weinberg proportions (HWE p-value), observed heterozygosity (Ho), expected heterozygosity (He), and inbreeding coefficient (Gis) at 148 SNP loci used to examine temporal population structure in 216 adult Pacific lamprey collected at-entry to the Klamath River.

Locus	HWE p-value	Ho	Hs	Gis
Etr_1104	0.0072	0.4612	0.4987	0.0752
Etr_1321	0.7921	0.4563	0.4293	-0.0629
Etr_140	0.0199	0.4466	0.4615	0.0323
Etr_2499	0.0018	0.3981	0.4401	0.0955
Etr_3189	0.0003	0.301	0.3255	0.0753
Etr_2226	0.0003	0.3961	0.4519	0.1235
Etr_3601	0.8002	0.4638	0.4455	-0.0411
Etr_4853	0.0011	0.3575	0.3866	0.0754
Etr_172	0.0151	0.4567	0.4902	0.0682
Etr_346	0.0005	0.2788	0.2994	0.0687
Etr_4596	0.0406	0.476	0.4971	0.0425
Etr_518	0.0694	0.4231	0.4233	0.0005
Etr_1569	0.3884	0.5095	0.4738	-0.0754
Etr_1894	0.0001	0.0571	0.0646	0.1157
Etr_231	0.1297	0.4905	0.4979	0.015
Etr_4215	0.1566	0.4667	0.4681	0.003
Etr_4670	0.6403	0.4048	0.3806	-0.0636
Etr_905	0.4513	0.5143	0.4847	-0.061
Etr_1060	0.0039	0.3365	0.3795	0.1134
Etr_1359	0.7975	0.4123	0.41	-0.0057
Etr_1696	0.0001	0.2891	0.4182	0.3088
Etr_2517	0.5877	0.5166	0.5007	-0.0316
Etr_3960	0.0002	0.2844	0.3365	0.1549
Etr_1068	0.0094	0.434	0.4926	0.119
Etr_1667	0.0311	0.3349	0.3588	0.0667
Etr_1762	0.4363	0.5189	0.4967	-0.0447
Etr_190	0.583	0.4292	0.4128	-0.0398
Etr_234	0.1419	0.4292	0.4385	0.0211

Locus	HWE p-value	Ho	Hs	Gis
Etr_4037	0.5928	0.3066	0.2857	-0.0732
Etr_4544	0.711	0.467	0.4624	-0.01
Etr_5654	0.0001	0.3491	0.4837	0.2783
Etr_64	0.5072	0.5047	0.489	-0.0321
Etr_668	0.0574	0.3726	0.3919	0.0492
Etr_1163	0.0471	0.385	0.4176	0.0782
Etr_1238	0.1093	0.4648	0.4926	0.0564
Etr_1341	0.2515	0.3803	0.3817	0.0037
Etr_1349	0.3059	0.4883	0.4906	0.0047
Etr_1556	0.0097	0.2347	0.256	0.083
Etr_1561	0.0281	0.3521	0.3863	0.0886
Etr_1848	0.0001	0.2629	0.3653	0.2803
Etr_225	0.1342	0.3944	0.4097	0.0375
Etr_2272	0.1672	0.3568	0.3651	0.0227
Etr_3292	0.0037	0.2629	0.2999	0.1234
Etr_3330	0.0187	0.4319	0.4862	0.1116
Etr_3885	0.6193	0.3709	0.3626	-0.0229
Etr_5043	0.3306	0.3662	0.3394	-0.079
Etr_810	0.4411	0.4977	0.4788	-0.0395
Etr_1004	0.0001	0.2196	0.3078	0.2865
Etr_181	0.034	0.3972	0.4397	0.0967
Etr_1834	0.0001	0.0047	0.0047	0
Etr_223	0.2744	0.3925	0.3961	0.009
Etr_2409	0.0086	0.2617	0.2988	0.1244
Etr_2642	0.3601	0.5047	0.4853	-0.0399
Etr_2765	0.5242	0.3318	0.319	-0.04
Etr_292	0.6219	0.472	0.4704	-0.0033
Etr_3502	0.445	0.4953	0.478	-0.0363
Etr_4000	0.3672	0.5093	0.4906	-0.0382
Etr_4414	0.5021	0.4065	0.3939	-0.0321
Etr_6076	0.0352	0.4486	0.4985	0.1001
Etr_7142	0.0931	0.4439	0.475	0.0654
Etr_7382	0.2229	0.4112	0.4203	0.0215
Etr_7974	0.0008	0.0561	0.0634	0.1162
Etr_814	0.5087	0.3692	0.3564	-0.0358

Locus	HWE p-value	Ho	Hs	Gis
Etr_824	0.5224	0.4252	0.4145	-0.026
Etr_8281	0.0289	0.3785	0.4222	0.1035
Etr_84	0.0458	0.5654	0.5007	-0.1292
Etr_8960	0.003	0.3925	0.4694	0.1638
Etr_906	0.272	0.4112	0.4164	0.0125
Etr_1007	0.2468	0.4186	0.3948	-0.0602
Etr_1551	0.1221	0.3953	0.4247	0.0692
Etr_1773	0.182	0.2233	0.206	-0.084
Etr_1843	0.0431	0.3814	0.4266	0.1059
Etr_2066	0.2039	0.3302	0.3451	0.043
Etr_2099	0.2052	0.4372	0.4582	0.0458
Etr_212	0.0164	0.4233	0.4888	0.1341
Etr_2414	0.1663	0.4047	0.3746	-0.0801
Etr_2451	0.2269	0.3116	0.3236	0.0369
Etr_2971	0.2342	0.4	0.4153	0.0369
Etr_3038	0.4976	0.4791	0.474	-0.0106
Etr_3128	0.4101	0.4744	0.4772	0.0059
Etr_3234	0.5528	0.2698	0.2669	-0.0108
Etr_3253	0.3004	0.3256	0.3093	-0.0525
Etr_3350	0.0156	0.3256	0.3772	0.1367
Etr_3939	0.037	0.3349	0.3771	0.112
Etr_4093	0.985	0.0186	0.0185	-0.0071
Etr_4173	0.3028	0.3674	0.3502	-0.0493
Etr_4390	0.2055	0.4	0.4191	0.0457
Etr_4716	0.3943	0.3581	0.3603	0.0059
Etr_4750	0.532	0.4512	0.448	-0.007
Etr_4965	0.0787	0.2651	0.2887	0.0818
Etr_5020	0.126	0.4326	0.397	-0.0897
Etr_5193	0.4313	0.4419	0.4318	-0.0234
Etr_5197	0.3946	0.507	0.4938	-0.0267
Etr_5581	0.1248	0.3442	0.315	-0.0925
Etr_5993	0.0515	0.4093	0.4555	0.1014
Etr_766	0.4295	0.5116	0.501	-0.0211
Etr_7918	0.4248	0.4744	0.4634	-0.0238
Etr_9189	0.4751	0.2605	0.2539	-0.0257

Locus	HWE p-value	Ho	Hs	Gis
Etr_963	0.2246	0.3349	0.3477	0.0368
Etr_1187	0.2189	0.4306	0.4042	-0.0653
Etr_1548	0.4743	0.4722	0.4784	0.013
Etr_1589	0.1559	0.3981	0.4325	0.0794
Etr_1684	0.0446	0.2778	0.3197	0.1312
Etr_2016	0.9358	0.037	0.0364	-0.0165
Etr_2068	0.3666	0.2778	0.2658	-0.0449
Etr_2193	0.5235	0.3704	0.3664	-0.0108
Etr_2304	0.3466	0.3843	0.3688	-0.042
Etr_2334	0.3861	0.3009	0.3112	0.0329
Etr_2512	0.5312	0.4722	0.4743	0.0044
Etr_2858	0.1645	0.2315	0.253	0.0852
Etr_3007	0.5765	0.2454	0.2431	-0.0094
Etr_3037	0.5326	0.1065	0.101	-0.0539
Etr_3107	0.2881	0.4074	0.429	0.0503
Etr_3145	0.5491	0.25	0.2464	-0.0146
Etr_3169	0.2966	0.4769	0.4557	-0.0464
Etr_3403	0.5556	0.2315	0.233	0.0066
Etr_4194	0.1602	0.3889	0.4218	0.078
Etr_4288	0.2693	0.3704	0.3516	-0.0535
Etr_4504	0.3634	0.2824	0.2936	0.0383
Etr_4694	0.4642	0.4398	0.447	0.0161
Etr_480	0.9631	0.0278	0.0275	-0.0118
Etr_4845	0.1541	0.1898	0.2088	0.0908
Etr_4859	0.5203	0.5	0.4969	-0.0063
Etr_49	0.2411	0.4213	0.4471	0.0577
Etr_5112	0.224	0.4398	0.4687	0.0617
Etr_5540	0.028	0.2454	0.2157	-0.1376
Etr_5626	0.2101	0.5278	0.4959	-0.0643
Etr_5711	0.8327	0.0602	0.0585	-0.0287
Etr_5762	0.5105	0.4537	0.4572	0.0076
Etr_6179	0.4373	0.3472	0.3386	-0.0254
Etr_6229	0.072	0.5139	0.4637	-0.1084
Etr_6318	0.9983	0.0093	0.0092	-0.0023
Etr_6436	0.0062	0.3565	0.3053	-0.1677

Locus	HWE p-value	Ho	Hs	Gis
Etr_6440	0.5591	0.287	0.2846	-0.0087
Etr_687	0.5736	0.3981	0.398	-0.0004
Etr_705	0.4187	0.3796	0.3894	0.0252
Etr_7262	0.9352	0.037	0.0364	-0.0165
Etr_7358	0.0368	0.4213	0.485	0.1313
Etr_752	0.516	0.412	0.4082	-0.0093
Etr_785	0.1169	0.4583	0.4198	-0.0919
Etr_7872	0.1642	0.5	0.4649	-0.0754
Etr_8196	0.5236	0.3889	0.385	-0.0102
Etr_832	0.5413	0.4028	0.4042	0.0036
Etr_833	0.2296	0.5	0.4721	-0.0592
Etr_8780	0.4645	0.4306	0.4375	0.0158
Etr_899	0.4225	0.4861	0.4973	0.0225
Etr_930	0.8312	0.0602	0.0585	-0.0287
Etr_972	0.5604	0.3657	0.364	-0.0048

Table S 2. The following metrics were recorded for each adult Pacific lamprey: entry date (month/year), sex, body mass (0.1 g), total length (TL) (1 mm), girth just posterior of the rearmost breathing hole (1 mm), interdorsal distance (ID Space) (1 mm) defined as the distance between the posterior most ray insertion of the first dorsal to the insertion of the anterior-most ray on the second dorsal, egg mass (0.1 g) for females consisting of the total weight of all eggs without the skein, and gonadosomatic index (GSI) defined as the ratio between egg mass and somatic mass, with somatic mass calculated as body mass minus gonad mass.

Entry Date	Sex	Body Mass	TL	Girth	ID Space	Egg Mass	GSI
6/16	M	387.7	612	121	31		
6/16	M	451.8	641	122	26		
6/16	F	458.9	658	125	39	6.9	1.5
6/16	M	449.4	637	127	37		
6/16	M	400.8	626	121	40		
6/16	M	321.7	578	107	36		
6/16	M	295.6	580	110	27		
6/16	F	362.9	627	110	33	4.4	1.2
6/16	M	340.2	576	113	10		

Entry Date	Sex	Body Mass	TL	Girth	ID Space	Egg Mass	GSI
6/16	F	486.6	620	130	25	4.8	1
6/16	F	339.3	578	115	30	4.3	1.3
6/16	F	413.2	622	130	25	4.7	1.2
6/16	F	452.4	679	127	33	8.6	1.9
6/16	M	447.3	626	128	33		
6/16	F	466.5	660	132	35	5.4	1.2
6/16	M	466.3	655	130	40		
6/16	M	397.9	625	125	32		
6/16	F	497.9	640	133	33	10.4	2.1
6/16	F	416	634	124	25	3.6	0.9
6/16	M	398.1	600	127	26		
6/16	F	429	645	128	27	5.7	1.4
7/16	F	385.4	620	122		4.6	1.2
7/16	M	330	615	116			
7/16	F	312.9	621	113		5	1.6
7/16	M	305.4	590	110	40		
7/16	M	432.2	646	122	39		
7/16	M	277.8	564	109	30		
8/16	F	468.1	642	121	37	10.4	2.3
8/16	F	452.9	589	123	35	13.9	3.2
8/16	M	395.6	600	114	31		
8/16	M	349.1	562	111	20		
8/16	M	481.6	655	126	31		
8/16	M	399	624	121	28		
10/16	M	327.5	601	104	47		
11/16	M	440	588	126	25		
1/17	M	483.2	654	125	28		
1/17	F	527.9	659	132	29	11.1	2.2
1/17	M	464.8	636	125	26		
1/17	F	317.5	566	111	30	7.2	2.3
1/17	F	436.4	632	124	29	18.6	4.5
2/17	M	519.9	672	128	35		
2/17	F	522	687	123	37	20.6	4.1
2/17	F	402	609	118	21	19.2	5
2/17	M	313.4	595	103	27		
2/17	F	365.5	596	113	22	17.2	4.9
2/17	F	420	653	110	42	13.8	3.4

Entry Date	Sex	Body Mass	TL	Girth	ID Space	Egg Mass	GSI
2/17	M	414	625	120	29		
2/17	F	461.6	655	122	28	13.6	3
2/17	F	394.4	608	122	36	7.8	2
2/17	F	400.9	580	108	28	17.7	4.6
2/17	M	506.3	664	133	36		
2/17	F	533.9	669	127	27	18.5	3.6
2/17	M	599.6	726	138	36		
2/17	M	495.4	645	133	22		
2/17	F	508.6	647	127	20	15.6	3.2
2/17	M	477.6	677	122	36		
2/17	F	525.3	648	130	45	8.5	1.6
2/17	M	504.1	646	131	37		
2/17	F	400.7	610	122	30	11.4	2.9
2/17	M	540.3	649	133	30		
2/17	F	335.9	565	111	26	18	5.7
2/17	F	403.2	623	116	18	12.8	3.3
2/17	F	330.3	589	105	22	9.8	3.1
2/17	F	546	691	126	36	17.4	3.3
2/17	M	443.4	627	122	30		
2/17	M	429.9	640	120	24		
2/17	M	350.3	577	117	23		
2/17	F	521.7	653	129	26	13.5	2.7
2/17	M	397.3	651	115	33		
2/17	M	390.1	616	114	30		
2/17	F	372	597	114	18	15	4.2
2/17	F	442.8	647	119	30	17.5	4.1
2/17	M	467.5	674	122	25		
2/17	M	443.1	613	122	24		
2/17	M	427.9	618	121	15		
2/17	F	584.4	681	131	39	13.3	2.3
2/17	M	339.3	594	113	28		
2/17	M	468.6	637	131	26		
3/17	M	380.3	610	116	30		
3/17	F	447.6	636	120	20	12.3	2.8
3/17	M	517	652	124	31		
3/17	F	568.9	686	132	41	7.4	1.3
3/17	M	437.8	614	125	18		

Entry Date	Sex	Body Mass	TL	Girth	ID Space	Egg Mass	GSI
3/17	M	368.5	584	112	32		
3/17	F	542.4	672	134	32	9	1.7
3/17	F	523.1	642	128	31	12.9	2.5
3/17	F	425.2	613	120	25	13.9	3.4
3/17	M	441	604	122	34		
3/17	M	532.8	650	129	45		
3/17	F	409.2	622	117	36	5.7	1.4
3/17	F	635.6	665	141	34	9.5	1.5
3/17	F	466.1	643	121	37	15.5	3.4
3/17	M	617.7	672	141	35		
3/17	M	462	630	120	43		
3/17	F	286.1	553	100	26	5.7	2
3/17	M	428.1	648	125	45		
3/17	F	336.5	566	107	28	7.7	2.3
3/17	M	261.2	542	97	31		
3/17	M	195.3	531	82	26		
3/17	M	333.2	630	111	36		
3/17	M	465.5	606	125	35		
3/17	F	460.7	629	117	25	11.1	2.5
3/17	M	411.6	616	112	23		
3/17	M	285.6	566	105	24		
3/17	M	289.7	592	99	31		
3/17	F	364.7	573	117	28	9.4	2.7
3/17	M	321.2	588	111	30		
3/17	F	293.8	572	105	23	8.2	2.9
3/17	F	401.1	686	115	34	22.8	6
3/17	M	299.6	599	104	32		
3/17	M	475.2	655	126	32		
3/17	F	549.7	671	124	25	18.1	3.4
3/17	F	371.5	592	115	34	7.6	2.1
3/17	F	356	612	109	33	12	3.5
3/17	M	313.5	590	103	22		
3/17	F	507.8	626	134	28	14.1	2.9
3/17	M	268.2	553	105	23		
3/17	M	458.4	644	118	37		
3/17	F	453.4	635	122	29	23	5.3
3/17	M	452	643	124	30		

Entry Date	Sex	Body Mass	TL	Girth	ID Space	Egg Mass	GSI
3/17	F	394.7	595	117	34	7.2	1.9
3/17	M	323.8	589	103	40		
3/17	M	431.8	644	118	38		
3/17	M	265.3	564	98	35		
3/17	M	389.2	627	110	31		
3/17	F	468.6	662	117	28	5.8	1.3
3/17	F	557.4	697	132	38	10.5	1.9
3/17	F	478.8	654	123	35	16.8	3.6
3/17	F	468.5	655	124	38	16.7	3.7
3/17	F	445.1	614	117	28	8.4	1.9
3/17	M	404.6	610	116	33		
3/17	M	327.7	565	105	35		
3/17	M	415.5	629	122	31		
3/17	M	344	580	109	22		
3/17	F	452	621	115	22	21.1	4.9
3/17	F	415.9	615	123	35	25.5	6.5
3/17	M	351.3	616	111	27		
3/17	F	394.9	616	112	30	9	2.3
4/17	M	414.9	607	118	22		
4/17	F	467.4	617	130	30	4.5	1
4/17	M	505.1	664	131	20		
4/17	M	382.1	615	116	24		
4/17	M	504.5	672	131	35		
4/17	F	495.4	646	132	36	13.8	2.9
4/17	M	346.6	584	110	34		
4/17	F	631.3	710	139	39	1.6	0.3
4/17	M	377.2	623	114	35		
4/17	F	366.9	600	112	32	8.3	2.3
4/17	M	462.3	640	122	38		
4/17	F	509.4	695	123	39	8.6	1.7
4/17	M	405.2	606	117	34		
4/17	M	463.3	636	114	28		
4/17	M	413.1	639	117	35		
4/17	M	380.6	611	113	30		
4/17	M	400.3	587	117	38		
4/17	F	435.8	622	120	37	4.3	1
4/17	F	366.1	609	104	30	13.7	3.9

Entry Date	Sex	Body Mass	TL	Girth	ID Space	Egg Mass	GSI
4/17	M	391.2	603	111	36		
4/17	F	426.5	657	110	29	22.7	5.6
4/17	M	289	549	108	18		
4/17	M	386.6	610	115	27		
4/17	M	323.4	571	111	34		
4/17	M	277.1	544	106	28		
4/17	M	416.3	605	119	24		
4/17	F	318.8	553	110	19	9.5	3.1
4/17	M	316	567	105	31		
4/17	M	402.3	646	111	36		
4/17	M	337.6	559	116	22		
4/17	M	302.5	560	104	25		
4/17	M	330.9	574	117	40		
4/17	M	446.3	643	118	25		
4/17	M	474.5	629	128	20		
4/17	M	311.3	558	102	19		
4/17	M	420.9	614	122	20		
4/17	M	340.9	592	107	23		
4/17	M	378.4	601	116	37		
4/17	F	370.9	602	117	24	4.6	1.3
4/17	F	569.1	671	133	35	6.6	1.2
4/17	F	375	602	115	30	10.4	2.9
4/17	M	407.4	650	116	36		
4/17	M	278	563	102	24		
4/17	M	484	655	126	35		
4/17	M	323.9	568	107	23		
4/17	F	346.5	576	113	26	5.9	1.7
4/17	F	406.9	623	112	24	7.4	1.9
4/17	M	281.7	560	102	31		
4/17	M	468	649	127	22		
4/17	F	460.9	658	122	25	4.5	1
5/17	F	492.4	640	128	35	5.7	1.2
5/17	F	340.1	586	110	30	2.9	0.9
5/17	M	400	579	118	35		
5/17	F	481.9	653	128	36	5.5	1.2
5/17	F	351.9	602	112	29	7.3	2.1
5/17	F	437.6	630	121	33	4.2	1

Entry Date	Sex	Body Mass	TL	Girth	ID Space	Egg Mass	GSI
5/17	F	398.3	645	113	28	3.9	1
5/17	M	405.6	626	114	28		
5/17	M	353.8	644	110	34		
5/17	F	445	650	124	18	22.6	5.4
5/17	M	327.9	589	104	35		
5/17	M	264.1	571	94	20		
5/17	F	254.2	560	91	39	5.3	2.1
5/17	M	364.6	614	109	36		
5/17	M	295.4	566	110	29		
5/17	F	496.5	653	122	30	4.6	0.9
5/17	M	317.6	590	105	32		
5/17	M	330.6	594	108	35		
5/17	M	313.4	603	104	25		
5/17	F	335	618	111	20	8.1	2.5
5/17	M	368.5	623	108	27		
5/17	M	262.1	571	109	24		
5/17	F	378.9	642	114	20	4.6	1.2
5/17	F	397.4	645	109	22	7.6	2
5/17	M	366	599	113	35		
5/17	F	453.9	645	121	26	4.7	1.1
5/17	M	446.7	656	115	41		
5/17	F	447.4	673	114	41	10	2.3
5/17	M	353.3	628	109	34		
5/17	M	404.4	641	114	36		
5/17	M	230.6	555	93	18		