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RESEARCH ARTICLE

Contemporary and historical river connectivity influence population structure in western brook lamprey in the Columbia River Basin

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Abstract

The western brook lamprey *Lampetra richardsoni* (WBL) is a small non-parasitic lamprey that inhabits rivers and streams from southern Alaska to northern California. WBL remain in fresh water throughout their entire life and show limited dispersal. Although adults may migrate short distances upstream to spawn, most movement likely occurs through passive drifting of larvae downstream. Genetic differentiation among populations is thus expected to be high, even within a single basin, but WBL population structure has received little attention. The present study examined population connectivity of WBL from 23 sites throughout the Columbia River Basin and coastal Washington, using eight microsatellite loci and cytochrome b sequence data. Although population structure generally corresponded to contemporary river connectivity, there were some cases where genetic patterns were better explained by historical connections. Microsatellite genetic differentiation among populations separated by < 570 km was moderate to high; F_{ST} values ranged from − 0.0026 to 0.7117 and averaged 0.2929. Tributary distance was the best predictor of F_{ST}, suggesting that most gene flow takes place in tributaries rather than through the mainstem of the Columbia River. As predicted, gene flow occurred primarily in a downstream direction, resulting in lower genetic diversity in upstream sites. WBL populations in these areas may be particularly vulnerable to local extinction. Therefore, whereas anadromous lamprey management efforts are focused on improving passage at mainstem dams, conservation of WBL will require protection of individual watersheds with particular emphasis on headwater areas.

Keywords Population structure · River system · Genetic diversity · Glacial refugia · Gene flow · Non-migratory species

Introduction

Within riverine systems, many species are strongly affected by the unidirectional flow of water. River current is particularly important for population connectivity, especially in systems fragmented by natural or anthropogenic barriers and in species with limited swimming abilities. Many riverine fishes experience passive downstream drift as larvae or adults (e.g., Brown and Armstrong 1985; White and Harvey 2003) and, without compensatory upstream movement, the overall pattern of migration will be biased in a downstream direction. In these situations, upstream populations provide migrants to downstream populations while receiving few migrants themselves, resulting in an overall source-sink metapopulation (e.g., Hänfling and Weetman 2006; Barson et al. 2009). Thus, genetic diversity is generally highest in downstream populations and lowest in those furthest upstream (e.g., Barson et al. 2009; Dehais et al. 2010). Upstream populations, with reduced genetic diversity and little opportunity for "rescue" by migrants from

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downstream populations, may thus be particularly vulnerable to extirpation.

The western brook lamprey *Lampetra richardsoni* (WBL) is a small, freshwater-resident fish found in coastal streams from southern Alaska to northern California (Scott and Crossman 1973). Lamprey larvae of all species are filter-feeders in the sediment of streams and rivers, where they remain burrowed for several years until they undergo metamorphosis. During the larval phase, most movement is expected to occur through passive drifting with the current (reviewed in Dawson et al. 2015). However, unlike parasitic lampreys, which generally migrate out of the stream after metamorphosis to feed on actinopterygian fishes in the ocean or in lakes, brook lampreys do not feed following metamorphosis. Instead, they undergo rapid sexual maturation, and spawn and die in their natal stream within 6–10 months of metamorphosis (reviewed in Docker 2009). Parasitic lampreys, which do not necessarily return to their natal streams to spawn (Waldman et al. 2008; Moser et al. 2015), show minimal genetic differentiation among even widely-separated locations, as has been observed in sea lamprey *Petromyzon marinus* on each side of the Atlantic Ocean (Bryan et al. 2005; Almada et al. 2008; Waldman et al. 2008), Pacific lamprey *Entosphenus tridentatus* in North America (Goodman et al. 2008; Spice et al. 2012; Hess et al. 2013), Arctic lamprey *Lethenteron camtschaticum* in Asia (Yamazaki et al. 2014), and European river lamprey *Lampetra fluviatilis* in northern Europe (Bracken et al. 2015). Furthermore, these large-bodied parasitic lampreys are capable of swimming several hundred kilometers upstream—and surmounting some barriers—during their spawning migrations (Moser et al. 2015), thus providing opportunity for gene flow within as well as among river systems. With their limited dispersal, brook lampreys are expected to show greater population structuring compared to their closely-related parasitic counterparts. This has in fact been shown in European brook lamprey *Lampetra planeri* compared to European river lamprey and in non-parasitic versus parasitic populations of Arctic lamprey (Bracken et al. 2015; Yamazaki et al. 2011b, respectively). Furthermore, since small-bodied brook lamprey adults are capable of upstream migrations of only a few kilometers (Moser et al. 2015), gene flow within a river system may also be limited.

There is a great deal of concern about declining populations of native parasitic lampreys (Maitland et al. 2015; Clemens et al. 2017) but, given their life history differences, conservation strategies developed for anadromous species may not be suitable for brook lampreys. The large parasitic species have long been valued for food and ceremonial purposes, but brook lampreys also play important ecological roles in freshwater systems (Docker et al. 2015). WBL are not considered endangered or threatened (Maitland et al. 2015), although this is partly due to the lack of information regarding population structure and trends, as well as information about specific threats to the species and its habitat (United States Fish and Wildlife Service 2004). The present study thus aims to address some of these knowledge gaps through examination of WBL genetic population structure in the Columbia River Basin (CRB).

The Columbia River system is approximately 2000 km long, with a drainage basin of approximately 668,000 km^2. Many large and small tributaries drain into the mainstem Columbia, and over 200 dams have been constructed within the CRB (Gelfenbaum and Kaminsky 2010). Most of the CRB remained unglaciated during the last glacial maximum (McPhail and Lindsey 1986), and the Columbia River has been suggested as a glacial refugium for a number of fish species, particularly salmonids in the genus *Oncorhynchus* (e.g., Bickham et al. 1995; McCusker et al. 2000; Beacham et al. 2006; Martin et al. 2010). WBL are believed to have persisted in the Columbia River during glaciation (McPhail and Lindsey 1986) and are currently present in the CRB at least as far upstream as the Naches River (Fig. 1).

The objective of the present study was to gain insight into both contemporary and historical population structure in WBL in this large, fragmented riverine system using analysis of microsatellites and mitochondrial DNA (mtDNA). Microsatellite loci, which have a relatively high mutation rate, are suitable for analysis of contemporary ecological questions (Selkoe and Toonen 2006). In contrast, mtDNA retains historical patterns that are more quickly lost in microsatellites, especially with ongoing gene flow among populations (Avise 2000; Heckel et al. 2005). Combining mtDNA and microsatellites can result in particularly informative results (Howes et al. 2006; Flanders et al. 2009; Sala-Bozano et al. 2009), which will help identify appropriate conservation units and suitable management strategies for WBL.

Materials and methods

Study design

We obtained permits from the states of Washington (administered through the Washington Department of Fish and Wildlife) and Oregon (administered through the Oregon Department of Fish and Wildlife) authorizing the capture of and tissue collection from the lamprey used in this study. For microsatellite analysis, approximately 40 WBL were collected from each of 15 sites within the CRB and two sites in coastal Washington (sites 1–17; Table 1; Fig. 1). Sites were grouped into seven regions to facilitate geographic analyses: five provinces (Coastal Washington, Columbia Estuary, Lower Columbia River, Columbia Gorge, and Columbia Plateau) and two sub-basins within the Lower

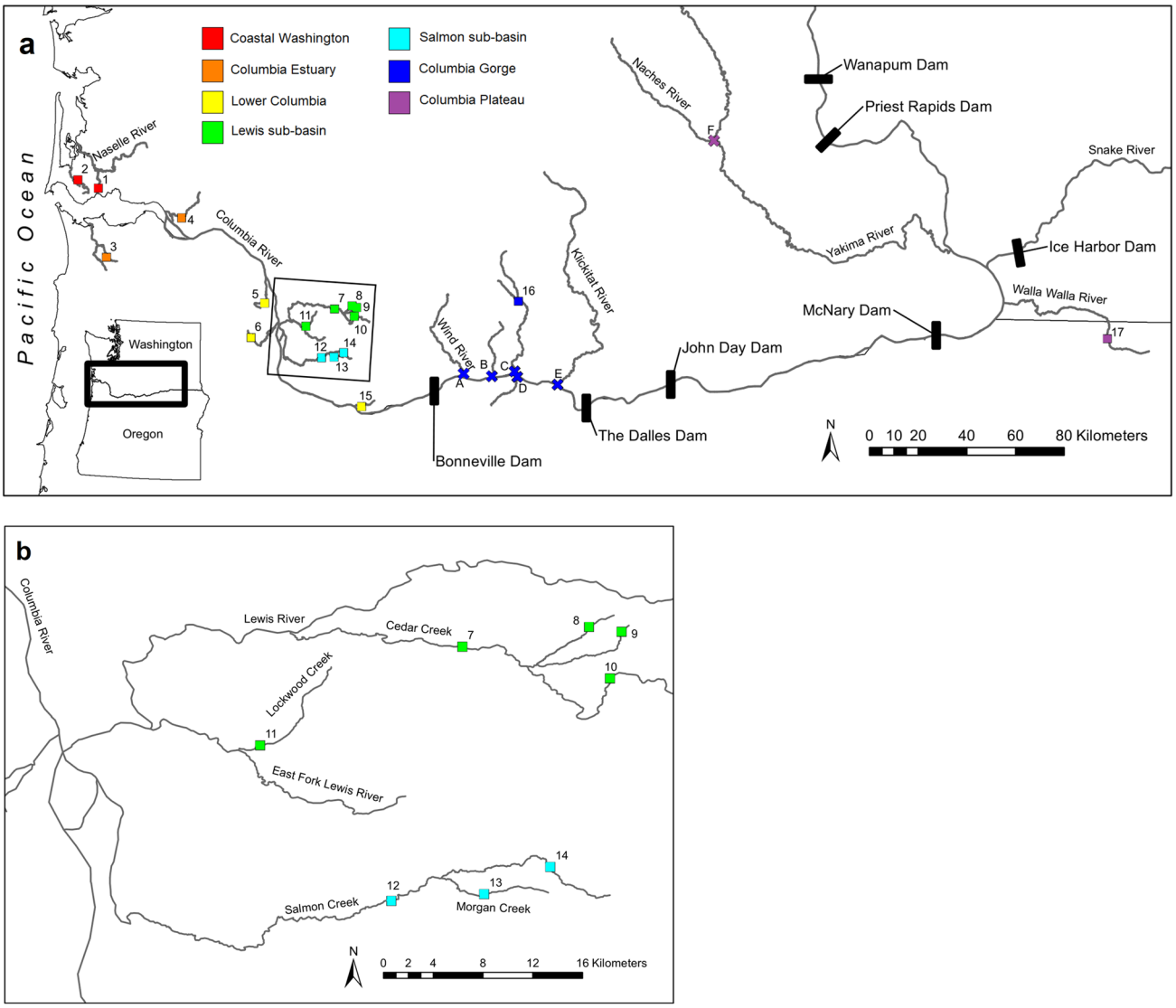

Fig. 1 Map of 23 locations where western brook lamprey were collected. Site labels correspond to those given in Table 1. Squares indicate sites where both microsatellite and mtDNA data were collected; multiplication symbols indicate sites where only mtDNA data were collected. Squares and multiplication symbols are color-coded according to region

Columbia (Lewis and Salmon sub-basins). Bonneville Dam demarcates the Lower Columbia from the Columbia Gorge provinces, and the Dalles Dam (whose reservoir inundates the former Celilo Falls, a barrier present prior to dam construction) divides the Columbia Gorge and Plateau. Sample sites were chosen to allow examination of gene flow and genetic diversity in several different situations: across saltwater barriers and dams; in different sub-basins separated by the mainstem Columbia; and within individual sub-basins. A subset of individuals from each of sites 1–17 were also analyzed using cytochrome b (see below). Since the Columbia Gorge and Columbia Plateau regions were poorly represented among the original 17 sites, samples were included from six additional sites within these regions (sites A–F; Table 1; Fig. 1) that were collected for other studies (Docker et al. 2016; M.F. Docker, unpublished data). Sample sizes from these additional sites were not large enough to permit microsatellite analysis but allowed cytochrome b analysis to be conducted on a total of 23 sites.

Sample collection

Lamprey were collected using electrofishing (see Harris et al. 2016), briefly anesthetized using tricaine methanesulfonate (MS-222), and preliminary morphological identification was made using the characters in Goodman et al. (2009). A small fin clip was taken for genetic analysis and

Table 1 Collection information for western brook lamprey *Lampetra richardsoni*

Site	River	Region	*N* analyzed using microsatellites	*N* analyzed using cytochrome b	Year	Latitude	Longitude	Life stage(s) collected	Elevation (m)	Distance upstream (km)		
										Mainstem	Tributary	Total
1	S.F. Naselle	Coastal Washington	40	10	2009	46.306	−123.799	Larvae	30	NA	NA	NA
2	Bear	Coastal Washington	40	11	2009	46.33	−123.912	Larvae and adults	7.1	NA	NA	NA
3	Klaskanine	Columbia Estuary	36	10	2010	46.05	−123.723	Larvae, transformers, and adults	113.4	0	27.54	27.54
4	Elochoman	Columbia Estuary	37	9	2010	46.22	−123.345	Larvae	15.9	29.88	21	50.88
5	Merrill	Lower Columbia	39	5	2009	45.925	−122.924	Larvae	139.7	80.87	12.96	93.83
6	N.F. Scappoose	Lower Columbia	41	7	2007	45.795	−122.922	Adults	201	100.82	22.31	123.13
7	Lower Cedar	Lewis sub-basin	40	8	2009	45.924	−122.497	Larvae	136.2	100.82	39.28	140.1
8	N.F. Chelatchie	Lewis sub-basin	40	7	2008	45.935	−122.377	Larvae, transformers, and adults	226.7	100.82	49.83	150.65
9	S.F. Chelatchie	Lewis sub-basin	40	9	2008	45.94	−122.402	Larvae, transformers, and adults	158.7	100.82	52.23	153.05
10	Upper Cedar	Lewis sub-basin	37	7	2008	45.907	−122.382	Larvae, transformers, and adults	276	100.82	55.43	156.25
11	Lockwood	Lewis sub-basin	40	8	2008, 2009	45.853	−122.636	Larvae and transformers	56.2	100.82	16.02	116.84
12	Mainstem Salmon	Salmon sub-basin	40	7	2009	45.742	−122.545	Larvae	64.4	100.82	41.31	142.13
13	Morgan	Salmon sub-basin	38	11	2009	45.747	−122.477	Larvae	99.8	100.82	49.22	150.04
14	Upper Salmon	Salmon sub-basin	40	8	2009	45.764	−122.432	Larvae	128.4	100.82	55.03	155.85
15	Gibbons	Lower Columbia	40	8	2008	45.57	−122.317	Larvae and transformers	8.9	163.58	1.97	165.55
16	Trout Lake	Columbia Gorge	40	5	2008	46.027	−121.575	Larvae, transformers, and adults	600.7	230.85	50	280.85
17	Walla Walla	Columbia Plateau	32	8	2009	45.94	−118.384	Larvae and transformers	309.7	443.21	98	541.21
A	Wind	Columbia Gorge	0	5	2012, 2013	45.714	−121.793	Larvae	24.3	208.33	0.25	208.58
B	Drano Lake	Columbia Gorge	0	2	2013	45.715	−121.638	Larvae	24.1	220.98	0.6	221.58
C	White Salmon	Columbia Gorge	0	2	2013	45.729	−121.522	Larvae	24.1	203.75	0.1	203.85
D	Hood	Columbia Gorge	0	3	2013	45.716	−121.507	Larvae	24.8	232.35	0	232.35
E	Klickitat	Columbia Gorge	0	8	2013	45.696	−121.292	Larvae	24.2	249.85	0	249.85
F	Naches	Columbia Plateau	0	21	2013	46.63	−120.515	Larvae	328	475.51	186.7	662.21

Site numbers correspond to those given in Fig. 1

N.F. North Fork, *S.F.* South Fork

stored in 95% ethanol. Lamprey were released once they had recovered from anesthesia.

Identifications were verified genetically or with adult records since larvae can be difficult to distinguish morphologically. At sites 1–17, larvae from the genus *Entosphenus* were eliminated using the assay described by Goodman et al. (2009), whereby a 433-bp fragment of mitochondrial cytochrome b digested with the enzyme Hae*III* produced fragments of different sizes in *Entosphenus* and *Lampetra*. At sites A–F, the microsatellite assay described by Docker et al. (2016) was used to distinguish *Entosphenus* from *Lampetra*. Within the genus *Lampetra*, the parasitic western river lamprey *L. ayresii* and non-parasitic Pacific brook lamprey *L. pacifica* also occur in the CRB (Kostow 2002; Reid et al. 2011). Mitochondrial DNA sequencing confirmed that no specimens used in the current study were Pacific brook lamprey (Boguski et al. 2012). Western river lamprey and WBL cannot be distinguished genetically (Boguski et al. 2012) or morphologically as larvae (Docker 2009), but they are morphologically distinct at metamorphosis and as adults. Metamorphosing and adult lamprey were collected from 10 of 23 sites in the present study, and all *Lampetra* were morphologically verified to be WBL. At the remaining seven sites, historical records likewise suggest that only non-parasitic *Lampetra* (i.e., WBL) are present.

Calculation of river distances

River distances between populations were determined using ArcGIS (ESRI 2015). For each pair of sites, the total distance between the two sites was separated into three parts: tributary distance from the first site to the Columbia River mainstem; distance in the mainstem; and tributary distance from the mainstem to the second site (Online Resource 1—Table S1). For calculations of individual site distance upstream in the mainstem (see Table 1), the mouth of the Klaskanine River was designated as the furthest downstream point (i.e., 0 km upstream).

DNA extraction, microsatellite amplification, and fragment size determination

DNA was extracted using the Qiagen DNeasy Kit (Qiagen) following the manufacturer's instructions. Eight microsatellite primer pairs developed or optimized for *Lampetra* (Lri-1, Lri-2, Lri-3, Lri-5, Lri-7, Lri-9, Lri-10; Luzier et al. 2010) or *Entosphenus* (Etr-3; Spice et al. 2011) were labeled with Ned and Pet (Applied Biosystems) and 6-Fam and Hex (Sigma Life Science) fluorescent dyes. PCR amplification was performed with a 10 µL reaction volume containing approximately 50 ng genomic DNA, 1 × KAPA Taq Buffer A, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.4 µM of each primer, and 0.2 U Taq DNA Polymerase (KAPA Biosystems). Thermal cycler conditions were an initial denaturation at 94 °C for 2 min; followed by 35 cycles of denaturation at 94 °C for 30 s, annealing at 52 to 58 °C (Luzier et al. 2010; Spice et al. 2011) for 30 s, and extension at 72 °C for 30 s; followed by a final extension at 72 °C for 5 min. Microsatellite fragments were size fractionated using a 3730XL DNA Analyzer (Applied Biosystems) sequencer and allele sizes were determined using GENEMAPPER v. 4.0 (Applied Biosystems).

Cytochrome b amplification and sequencing

A 438-bp fragment of mitochondrial cytochrome b was amplified in 181 individuals (Table 1). Primer sequences were 5′-CACCGTTGTAGAATTCAACTATAAG-3′ (Glu-F; Boguski et al. 2012) and either 5′-GTTAGGGTGGCGTTT GATACTG-3′ (cytb-518-R-*Entosphenus*) or 5′-GTTAAG GTGGCGTTTGATACTG-3′ (cytb-518-R-*Lampetra*). PCR amplification was performed in a 30 µL reaction volume containing approximately 50 ng genomic DNA, 1× GoTaq® Flexi Buffer, 2.5 mM $MgCl_2$, 0.2 mM of each dNTP, 0.4 µM of each primer, and 0.6 U GoTaq® Flexi DNA Polymerase (Invitrogen). PCR amplification conditions were an initial denaturation at 95 °C for 2 min; followed by 10 cycles of denaturation at 95 °C for 1 min, annealing at 60 °C for 1 min, and extension at 72 °C for 1.5 min; followed by 10 cycles of denaturation at 95 °C for 1 min, annealing at 58 °C for 1 min, and extension at 72 °C for 1.5 min; followed by 10 cycles of denaturation at 95 °C for 1 min, annealing at 55 °C for 1 min, and extension at 72 °C for 1.5 min; followed by a final extension at 72 °C for 5 min. PCR products were purified and amplified using the BigDye Terminator v. 3.1 Cycle Sequencing Kit (Applied Biosystems) and sequenced using a 3500 Genetic Analyzer (Applied Biosystems).

Genetic diversity and population differentiation

A test for null alleles was performed using MICRO-CHECKER v. 2.2.3 (Van Oosterhout et al. 2004), and null allele frequency was calculated in FREENA (Chapuis and Estoup 2007). In order to determine whether null alleles had a substantial effect on the results, pairwise and global F_{ST} values with and without a correction for null alleles were calculated in FREENA and compared. Affected loci were retained for further analysis since the effect of null alleles was small (see "Results") and discarding the loci would have resulted in a loss of power.

Using GENEPOP v. 4.2 (Raymond and Rousset 1995; Rousset 2008), observed and expected heterozygosity (H_O and H_E, respectively) were calculated for each locus and population. As well, each locus was tested for Hardy–Weinberg Equilibrium (HWE), and significance of deviations from HWE was determined after Bonferroni correction.

F_{IS} was calculated using FSTAT v. 2.9.3.2 (Goudet 1995). Allelic richness (AR) and private allelic richness (pAR) were standardized using rarefaction in HP-RARE v. 1.1 (Kalinowski 2005). Effective population size (N_e) was estimated in COLONY v. 2.0.6.1 (Jones and Wang 2010), using a medium run length with random mating and both male and female polygamy permitted (Johnson et al. 2015). A test for recent population bottlenecks was performed in BOTTLENECK v. 1.2.02 (Piry et al. 1999) using the stepwise mutation model and a one-sided Wilcoxon signed rank test for heterozygote excess. To test the hypothesis that populations further upstream or at higher elevations may have lower genetic diversity, one-sided Spearman's rank correlation tests were performed in base R v. 3.4.0 (R Core Team 2016). These tests excluded samples from sites 1 and 2, as these sites were outside the CRB. H_O, AR, pAR, F_{IS}, and N_e were used as dependent variables in separate tests with elevation and total river distance upstream as independent variables. GENEPOP was used to calculate Weir and Cockerham's (1984) F_{ST} for all pairwise comparisons of populations. Significance of F_{ST} values after false discovery rate correction was determined using FSTAT.

Geographic structure and population clustering

Single and multiple regressions of river distance and site elevation versus genetic distance (linearized F_{ST}) were performed using the lm function in base R. Sites 1 and 2 were excluded from this analysis as they are outside the CRB. River distance was separated into three components (tributary distance from the first site to the Columbia River mainstem, distance in the mainstem, and tributary distance from the mainstem to the second site). Models were compared using AIC_C (Burnham and Anderson 2002), which was calculated using the R package AICcmodavg (Mazerolle 2017).

STRUCTURE v. 2.3.4 (Pritchard et al. 2000) was used to analyze population clustering. To compensate for the presence of null alleles, the recessive alleles setting was used. Runs were performed using the admixture model with allele frequencies correlated, with an initial burn-in of 1,000,000 iterations followed by 1,000,000 iterations, and 20 replicates of each K. Preliminary analyses (data not shown) indicated that the most likely number of population clusters was probably less than 10; therefore, K values from 1 to 10 were examined. The most likely number of population clusters was determined using the Evanno et al. (2005) method as implemented in STRUCTURE HARVESTER v. 0.6.94 (Earl and VonHoldt 2012). For this K, results of replicates were combined using CLUMPP v. 1.2.2 (Jakobsson and Rosenberg 2007) and visualized using DISTRUCT v. 1.1 (Rosenberg 2004). To identify additional substructure, the complete dataset was subdivided into the clusters indicated by STRUCTURE, and similar analysis was performed for each cluster. In each of these clusters, K values from 1 to the number of sites were examined.

A neighbor-joining tree was constructed using POPULATIONS v. 1.2.30 (O. Langella, available from http://bioinformatics.org/~tryphon/populations/) with Cavalli-Sforza and Edwards' (1967) chord distance and 500 bootstrap replications on loci. This tree was visualized in TREEVIEW v. 1.6.6 (Page 1996).

Direction of gene flow

To determine the direction of gene flow (i.e., primarily downstream versus bidirectional), spatial models were constructed to compare the proportion of genetic differentiation among sites explained by bidirectional connectivity of sites (distance-based Moran's eigenvector maps; dbMEM) versus only downstream connectivity (asymmetric eigenvector maps; AEM).

dbMEM models were constructed following the methods of Borcard and Legendre (2002), Dray et al. (2006), and Blanchet et al. (2011). Pairwise distances between sites were converted to a minimum spanning tree matrix in R using the package ape (Paradis et al. 2004); the minimum spanning tree matrix was used as the binary edge matrix. Edge weighting formulas were binary (unweighted), distance^{α}, $1-((\text{distance}/\text{distance}_{\max})^{\alpha})$, and $1/(\text{distance}^{\alpha})$; α values were 1 to 3. Adjacent sites (those coded as 1 in the binary matrix) were given the weight corresponding to that edge. The largest weight was identified as the threshold value, and all non-adjacent sites were assigned weights of 4*threshold. The weighted matrix was transformed into eigenvectors through principal coordinates analysis (PCA) in R using ape.

AEM models were constructed following the methods of Blanchet et al. (2008a). The confluence of the Klaskanine River and the mainstem Columbia River was designated as the furthest downstream point. For each site, all river segments connecting it to this point were coded as 1, and all other segments were coded as 0 [see Blanchet et al. (2008a) for a diagram demonstrating how river segments are coded]. The same weighting schemes were used as for the dbMEM models; the weight of each edge was determined by multiplying its binary value by the corresponding weight. This weighted edge matrix was transformed into eigenvectors in R using package AEM (Blanchet et al. 2015).

Redundancy analysis (RDA) was performed to determine how much of the genetic variation was explained by the spatial variation included in each model. This RDA was performed using the R package vegan (Oksanen et al. 2016). The independent variables were the spatial eigenvectors produced by AEM or dbMEM models. The dependent variables were the eigenvectors produced by PCA of the pairwise F_{ST} values (Vangestel et al. 2012; Orsini et al. 2013) using ape. After initial redundancy

analysis, forward selection of spatial eigenvectors was performed following the method of Blanchet et al. (2008b) using the R package packfor (Dray et al. 2016). Eigenvectors were added one by one to the model until one of two stopping criteria was reached: (a) the adjusted r^2 equalled that of the model with all eigenvectors included, or (b) the α value of the next eigenvector being added was ≥ 0.05. After forward selection, redundancy analysis was performed again to determine which model had the highest adjusted r^2 (i.e., the most explanatory power). Finally, variation partitioning was performed for all models, using vegan, to compare the importance of bidirectional connectivity of sites (i.e., dbMEM eigenvectors) versus downstream connectivity (i.e., AEM eigenvectors) in determining genetic differentiation among populations.

Cytochrome b sequence analysis

Sequences were manually verified using CHROMAS LITE v. 2.1.1 (Technelysium; available from http://technelysium.com.au/), and aligned in GENEIOUS v. 5.6.6 (Kearse et al. 2012) using the Geneious Alignment method with default settings. Median-joining networks were constructed using NETWORK v. 4.6.1.1 (Bandelt et al. 1999) with default settings; these networks included samples from the present study and range-wide samples from Boguski et al. (2012).

Results

Genetic diversity and population differentiation

A total of 660 WBL were genotyped at eight microsatellite loci with a missing data rate of 10.44%. Summary statistics for each location are given in Online Resource 1—Table S2. Most loci had low (< 5%) and/or not statistically significant frequency of null alleles; however, null alleles were present at loci Etr-3 and Lri-10 at nine and seven of the 17 sites, respectively. Corrected and uncorrected pairwise and global F_{ST} values were very similar, with corrected values only slightly lower (Table 2; Online Resource 1—Tables S3 and S4). Thus, it appears that the presence of null alleles caused a slight overestimation of population differentiation; this was considered in the interpretation of the results. Locus Lri-9 displayed heterozygote excess in five populations. Homozygote excess was observed in one population each for loci Lri-2, Lri-3, and Lri-7; four populations for Lri-10; and seven populations for Etr-3. Among the 17 sites, average H_O ranged from 0.159 (site 17) to 0.474 (site 4); F_{IS} ranged from −0.328 (site 7) to 0.378 (site 17). N_e ranged from 6 (site 10) to 40 (site 2); AR ranged from 1.683 (site 7) to 3.564 (site 1); pAR ranged from 0.01 (site 13) to 0.33 (site 5). Bottlenecks were not detected in any population (p ranging from 0.6563 to 1 for 17 tests). Distance upstream was negatively correlated with H_O ($\rho = -0.5077$, $p = 0.0334$) and N_e ($\rho = -0.5683$, $p = 0.0170$), and elevation was negatively correlated with H_O ($\rho = -0.5077$, $p = 0.0334$; Online

Table 2 F_{ST} values for pairwise comparisons among populations of western brook lamprey, calculated from eight microsatellite loci

	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17
1	0.0199	0.1823	0.1021	0.1727	0.2381	0.4379	0.3138	0.4032	0.3997	0.2606	0.3098	0.2486	0.2266	0.1941	0.0912	0.3088
2		0.1886	0.1086	0.1430	0.2295	0.4612	0.3216	0.4251	0.4273	0.2534	0.3121	0.2604	0.2307	0.1823	0.1049	0.3239
3			0.0927	0.1470	0.1969	0.4893	0.3594	0.4523	0.4585	0.2033	0.2620	0.2448	0.2075	0.1973	0.2548	0.3884
4				0.1248	0.1427	0.4147	0.2941	0.3767	0.3849	0.1781	0.2055	0.1932	0.1547	0.1523	0.1693	0.4037
5					0.1932	0.4244	0.3033	0.3857	0.3998	0.2495	0.3026	0.2708	0.2275	0.2004	0.1956	0.3573
6						0.5104	0.4008	0.4768	0.4808	0.3264	0.3585	0.3504	0.2998	0.2771	0.2246	0.4070
7							0.2687	-0.0026	0.0081	0.4627	0.5131	0.4811	0.4423	0.4127	0.4663	0.7117
8								0.2407	0.2117	0.2638	0.2972	0.2539	0.2187	0.2056	0.3003	0.5666
9									0.0065	0.4294	0.4774	0.4432	0.4051	0.3776	0.4329	0.6754
10										0.4204	0.4669	0.4268	0.3876	0.3626	0.4213	0.6749
11											0.0334	0.0484	0.0359	0.0324	0.2783	0.4857
12												0.0167	0.0244	0.0832	0.3275	0.5615
13													0.0021	0.0520	0.2585	0.5200
14														0.0261	0.2337	0.4982
15															0.1879	0.4584
16																0.2916

Site numbers correspond to those given in Table 1 and Fig. 1

Light gray indicates values that were significant after false discovery rate correction; mid-gray indicates values that were significant after false discovery rate correction and greater than 0.2500; dark gray indicates values that were significant after false discovery rate correction and greater than 0.5000

Sites 1–2 Coastal Washington; 3–4 Columbia Estuary, 5–6, 15 Lower Columbia; 7–11 Lewis sub-basin; 12–14 Salmon sub-basin; 16 Columbia Gorge; 17 Columbia Plateau

Resource 1—Table S5). Pairwise F_{ST} values ranged from −0.0026 (between sites 7 and 9) to 0.7117 (between sites 7 and 17), with an average of 0.2929 (Table 2). Out of 136 pairwise comparisons, 125 were significant after false discovery rate correction ($p < 0.000327$).

Geographic structure and population clustering

Although all components of river distance were significantly correlated with genetic distance, the best model was the one that included downstream tributary distance and upstream tributary distance as independent variables ($p = 3.14 \times 10^{-12}$, adjusted $r^2 = 0.3934$, $AIC_C = 70.2023$; Online Resource 1—Table S6). The effects of downstream and upstream tributary distances were similar (coefficients of 0.0085 and 0.0071, respectively, with overlapping 95% confidence intervals). This model was not improved by adding site elevations as additional independent variables ($p = 3.91 \times 10^{-11}$, adjusted $r^2 = 0.3975$, $AIC_C = 71.8755$).

STRUCTURE HARVESTER indicated that the most likely number of genetic clusters for WBL was two (mean $\ln P(K) = -8412.7850$; mean $\Delta K = 645.7748$; Fig. 2; Online Resource 1—Table S7). Cluster 1 contained sites 7–15 (the Lewis and Salmon sub-basins and Gibbons Creek from the Lower Columbia). Within cluster 1, two sub-clusters were identified (mean $\ln P(K) = -3066.0850$; mean $\Delta K = 1359.0665$); sub-cluster A contained sites 11–15 (the Salmon sub-basin plus Lockwood and Gibbons creeks), and sub-cluster B contained sites 7–10 (the Lewis sub-basin except for Lockwood Creek). Cluster 2 contained sites 1–6, 16, and 17 (Coastal Washington, the Columbia Estuary, Columbia Gorge, Columbia Plateau, and most of the Lower Columbia) and could be divided into three sub-clusters (mean $\ln P(K) = -4006.1050$; mean $\Delta K = 177.0766$). Sub-cluster C contained Walla Walla River (the Columbia Plateau); sub-cluster D contained sites 3, 4, and 6 (both Columbia Estuary sites and North Fork Scappoose in the Lower Columbia); and sub-cluster E contained sites 1, 2, and 5 (both Coastal Washington sites and Merrill Creek in the Lower Columbia). Trout Lake Creek (site 16) was intermediate between sub-clusters C and E.

A neighbor-joining tree showed generally strong support for geographic grouping of WBL (Fig. 3). Sites 1 and 2 (Coastal Washington) clustered together with 80% bootstrap support. Most of the Lewis sub-basin (sites 7–10) clustered together with 90% bootstrap support. Within the Salmon sub-basin, geographic groupings were not as strong; these sites (12–14) clustered together with 52% bootstrap support. Two sites outside of the Salmon sub-basin (site 11, Lockwood Creek, and site 15, Gibbons Creek) also grouped close to the Salmon sub-basin sites.

Direction of gene flow

One of the dbMEM models (i.e., including bidirectional connectivity of sites) and two of the AEM models (including only downstream connectivity) were rejected completely because no spatial eigenvectors were retained after forward selection. The three best models were those weighted by distance[1], distance[2], and distance[3] (adjusted $r^2 = 0.812$, 0.800, and 0.687, respectively; Online Resource 1—Table S8). The dbMEM models explained little variation (−0.003 to 0.094) on their own; a larger portion of the variation was attributed solely to AEM models (0.183–0.434).

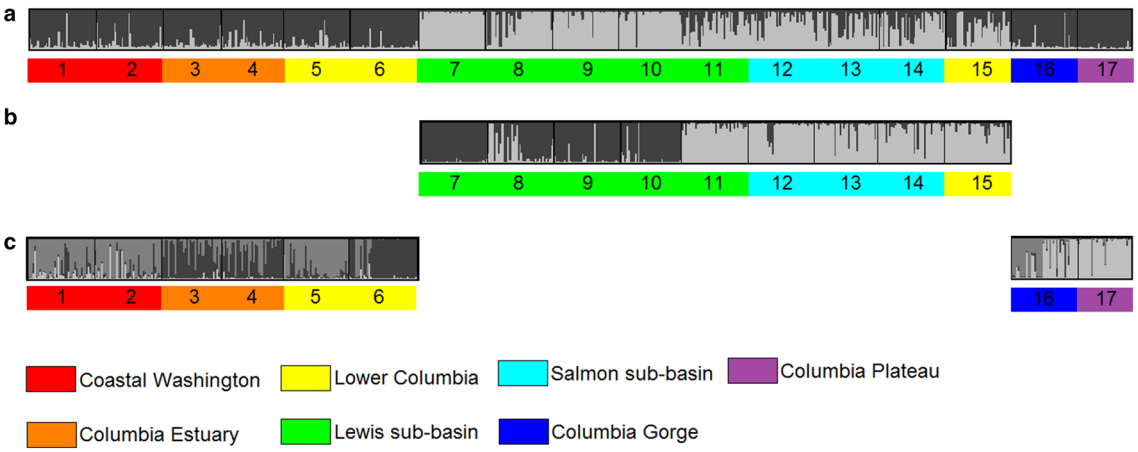

Fig. 2 Bar plot illustrating membership in western brook lamprey population clusters, using data from eight microsatellite loci. Each individual is represented by a single vertical bar broken into colored segments; the height of each color within the bar represents the proportionate genetic assignment of that individual to a population cluster. Numbers beneath the bar plot are color-coded according to region and indicate collection locations corresponding to those given in Table 1 and Fig. 1. **a** Population clusters present in the entire dataset. Cluster 1 = light gray, cluster 2 = dark gray. **b** Sub-clusters present in Cluster (1) Cluster 1a = light gray, cluster 1b = dark gray. **c** Sub-clusters present in Cluster (2) Cluster 2a = light gray, cluster 2b = mid-gray, cluster 2c = dark gray. (Color figure online)

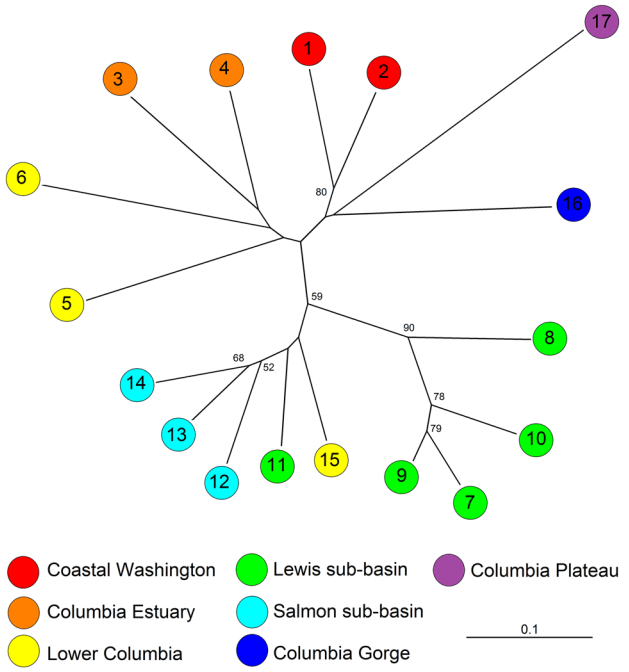

Fig. 3 Neighbor-joining tree showing the relationships among western brook lamprey collected from 17 sites, using data from eight microsatellite loci. Scale bar shows Cavalli-Sforza and Edwards' (1967) chord distance; bootstrap values greater than 50% are shown at nodes. Site numbers correspond to those given in Table 1 and Fig. 1

The largest portion of the variation was shared between AEM and dbMEM models (0.381–0.523).

Cytochrome b analysis

Seven haplotypes were found in the samples analyzed in the present study: three that were shared with other west coast *Lampetra* (H1 to H3) and four that have been reported only in the CRB (H4 to H7; Fig. 4). Coastal haplotypes not present in the CRB were H8 to H10 and H13 to H16; haplotypes H11 and H12 were found in the CRB by Boguski et al. (2012) but not in the present study. Haplotype frequency and distribution are given in Online Resource 1—Tables S9 and S10, along with GenBank accession numbers. Within the CRB, several haplotypes were unique to particular regions: H5 occurred only in the Lewis sub-basin and H7, which was distinct from all other CRB haplotypes by at least five mutations (1.1%), occurred in only one site (site 6, North Fork Scappoose Creek). H3 occurred in the present study only in the Columbia Estuary; however, this haplotype was shared with samples from Coastal Oregon in Boguski et al. (2012). H2, which was found by Boguski et al. (2012) in all six regions from Alaska to Point Conception, was also widely distributed within the CRB, occurring in five

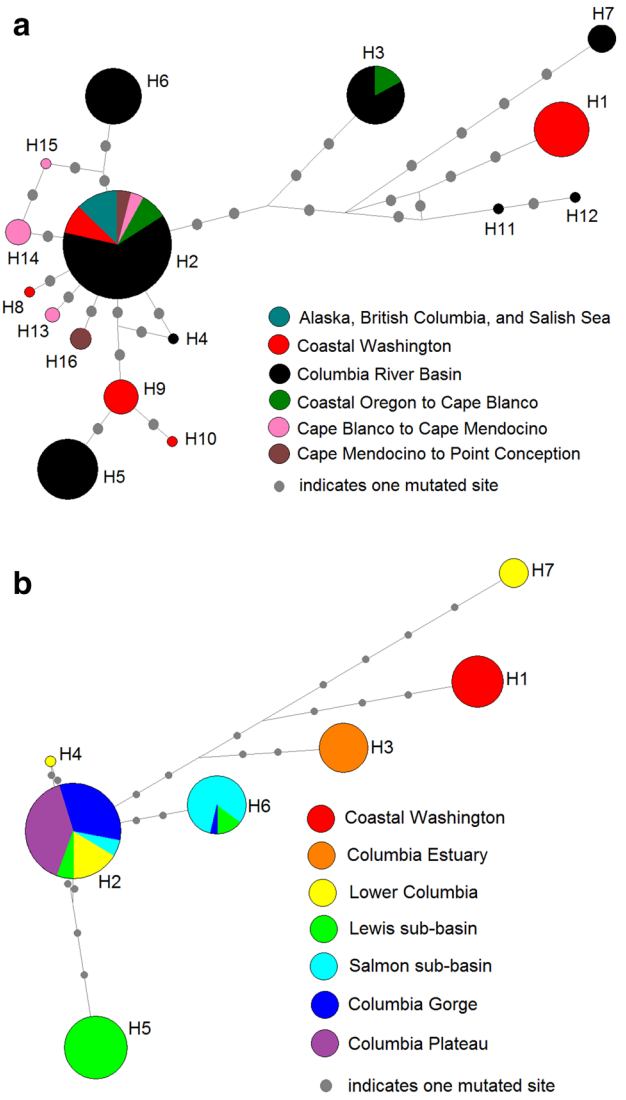

Fig. 4 Median-joining networks constructed from 438 base pairs of cytochrome b sequence. Labels H1 through H16 indicate haplotypes. **a** Network including *Lampetra* samples from the entire west coast of North America, from Boguski et al. (2012) and the present study. Color-coding indicates regions used by Boguski et al. (2012). Haplotype frequency is given in Online Resource 1—Table S9. **b** Network including only western brook lamprey samples from the present study. Color-coding indicates regions given in Table 1. Haplotype H5 was found in sites 7–10; H2 and H6 were found in site 11; H7 was found only in site 6. Haplotype frequency is given in Online Resource 1—Table S10. (Color figure online)

of the seven regions examined in the present study (i.e., all except Coastal Washington and the Columbia Estuary). Virtually all (53/54) of the WBL surveyed from the upstream-most CRB sites (i.e., sites 16–17 and A–F in the Columbia Gorge and Columbia Plateau) possessed H2; only one individual from Trout Lake Creek had a haplotype other than H2.

Discussion

In the Columbia River Basin, clear geographic structuring and moderate to high genetic differentiation were evident among populations of western brook lamprey. This contrasts dramatically with the low levels of genetic differentiation seen among anadromous lampreys from even widely-disparate locations (e.g., Spice et al. 2012; Yamazaki et al. 2014). A trend of isolation by distance (IBD) was present, indicating that some gene flow among geographically proximate populations occurs, but most of this gene flow appears to take place in tributaries rather than in or through the mainstem Columbia River. Spatial models indicated that gene flow occurs primarily in a downstream direction, and genetic diversity decreased from downstream to upstream. Microsatellite genetic structure usually corresponded to mtDNA genetic structure and contemporary river connectivity, but the few discrepancies noted may help provide insight into historical processes within the CRB.

Contemporary connectivity among WBL populations

This study is novel in that it examined population structure of a freshwater-resident, non-parasitic lamprey in a single large river basin and showed moderate to high genetic differentiation among populations. Most studies of lamprey population structure to date have focused on anadromous parasitic species and found low (although sometimes significant) genetic differentiation among locations (e.g., Almada et al. 2008; Spice et al. 2012; Hess et al. 2013; Yamazaki et al. 2014). F_{ST} values for WBL populations separated by < 570 km ranged from -0.0026 to 0.7117, with an average of 0.2929; these values are more than an order of magnitude greater than those for anadromous Pacific lamprey separated by up to 2600 km (maximum 0.0584, average 0.0165; Spice et al. 2012).

Most population genetic studies conducted on brook lampreys used only mtDNA markers (Mateus et al. 2011; Boguski et al. 2012), compared sites between river basins separated by saltwater barriers (Rougemont et al. 2016, 2017), or included relatively few sites within a single basin. Nevertheless, these studies also suggest that brook lamprey populations tend to be genetically distinct over small to moderate spatial scales. In France, in tributaries of the Garonne River separated by less than 100 km, genetic differentiation between two European brook lamprey populations was moderate and significant ($F_{ST} = 0.210$; Rougemont et al. 2015). Among four European brook lamprey populations separated by 108–222 km in the Humber River drainage in the U.K., F_{ST} values were likewise moderate and significant (0.103–0.191; Bracken et al. 2015). Lack of genetic differentiation among populations of brook lampreys has been reported only over very small spatial scales ($F_{ST} = -0.020$ to 0.005 among three populations of *Lethenteron* sp. N separated by approximately 10 km; Yamazaki et al. 2011a) or in recently established populations ($F_{ST} = 0.037$ between two non-parasitic populations of Arctic lamprey separated by approximately 40 km but apparently founded by the anadromous parasitic form within the last century; Yamazaki et al. 2011b).

Even in comparison to other freshwater fish species with limited dispersal, WBL have relatively strong population structure. The comparatively high F_{ST} values seen in the present study may be partially due to the large size of the CRB; however, moderate differentiation was sometimes present even between spatially proximate sites. For example, F_{ST} was 0.2687 between lower Cedar Creek and North Fork Chelatchie Creek (a tributary to Cedar Creek) which are separated by only 10.6 km. In contrast, in Rocky Mountain sculpin *Cottus* sp., pairwise F_{ST} values ≤ 0.02 were observed among sites separated by 0.2–11 km within the same river and dispersal in this species appears very limited; 50% of tagged individuals moved ≤ 10 m over a 5-month period (Ruppert et al. 2017). In the more mobile but still non-migratory European chub *Squalius cephalus*, which is able to cover distances of up to 15 km to spawn, pairwise F_{ST} values among 11 sites within a 219 km stretch of the mainstem Durance River ranged from 0 to 0.055 with a global F_{ST} of 0.015 (Dehais et al. 2010). Population structure observed in guppy *Poecilia reticulata* was more similar to that observed in the present study (Barson et al. 2009). In five pairs of upstream and downstream sites separated by less than 10 km, pairwise F_{ST} values calculated from eight microsatellite loci were significant and ranged from 0.013 to 0.927. Upstream and downstream sites, however, were also separated by changes in elevation, which would presumably be a barrier to upstream gene flow for fish without strong swimming abilities. In the present study, genetic distance was much more strongly affected by river distance than by elevation; however, elevation was negatively correlated with observed heterozygosity. Future research should examine the effects of elevation changes on gene flow in WBL.

The presence of significant IBD shows that some gene flow among WBL populations occurs (Hutchison and Templeton 1999); however, tributary rather than total distance was the best predictor of F_{ST}, indicating that most gene flow occurs within tributaries rather than in or through the mainstem of the Columbia River. Although evidence suggests that WBL larvae drift downstream into the mainstem fairly often (Harris and Jolley 2017), and *Lampetra* sp. larvae that may be WBL have been detected in large rivers such as the Willamette River (Jolley et al. 2012), the results

of the present study suggest that larvae that drift into the mainstem Columbia rarely succeed in re-entering tributaries and spawning. Our finding that genetic distance was better predicted by AEM models (including only downstream connectivity) than dbMEM models (bidirectional connectivity) also indicates that there is very little upstream gene flow.

In river systems, asymmetric gene flow is expected to result in reduced genetic diversity in upstream populations (Morrissey and de Kerckhove 2009). This should be particularly evident in brook lampreys since compensatory upstream movement for spawning is limited relative to the largely passive downstream movement experienced during the prolonged larval period. Other studies of freshwater fishes with limited migration have found that both allelic richness and heterozygosity increase from upstream to downstream (Hänfling and Weetman 2006; Dehais et al. 2010). The same pattern has been observed in other sedentary riverine organisms, such as mussels (e.g., Mock et al. 2010, 2013). In WBL in the CRB, heterozygosity (but not allelic richness) was lower in upstream and high elevation sites. Lack of correlation between allelic richness and upstream distance may be due to a confounding effect related to historical patterns of post-glacial colonization (see below).

Headwater areas are expected to have particularly low genetic diversity due to the lack of incoming gene flow from upstream; however, they may also have a high frequency of private alleles (Morrissey and de Kerckhove 2009). In the present study, relatively low genetic diversity was observed at upstream sites within the Lewis and Salmon sub-basins. The frequency of private microsatellite alleles was not correlated with upstream distance, but unique or mostly unique mtDNA haplotypes (H5 and H6) were observed in both the Lewis and Salmon sub-basins. Relatively high N_e (39) was found at lower Cedar Creek (furthest downstream in the Lewis sub-basin). Very low N_e was found in the remaining sites, with the lowest N_e (6) at upper Cedar Creek (furthest upstream in the Lewis sub-basin). Headwater areas tend to have higher stream gradients and less fine sediment than areas lower in a watershed, potentially giving them a lower capacity to support larval lamprey (see Dawson et al. 2015). Given the low effective population sizes and unique haplotypes observed in headwater sites, conservation of WBL in headwater areas may be particularly important. Unlike large-bodied parasitic lampreys that are capable of swimming up to several hundred kilometers upstream, brook lampreys will be more limited in their recolonization abilities if extirpated. Anadromous Pacific and sea lampreys have successfully colonized or recolonized rivers within years of the removal of dams or natural barriers (e.g., Farlinger and Beamish 1984; Hogg et al. 2013; Jolley et al. 2018). Where barriers persist, translocation from downstream sites is being used to introduce Pacific lamprey into upstream sub-basins (Ward et al. 2012). However, given the moderate to strong genetic differentiation observed in WBL in the CRB, translocation in this species could severely disrupt population structure and local adaptation. In vulnerable headwater regions, therefore, conservation efforts should focus on habitat conservation and restoration (see Maitland et al. 2015).

Historical connectivity among WBL populations in the CRB

One of the major questions raised by the present study is whether the population structure observed in WBL is due to patterns of colonization or present connectivity. The answer is likely that the effects of both can be seen within the CRB. In other relatively sedentary freshwater fish species, changes in drainage systems have resulted in phylogeographic patterns that reflect past rather than present river connectivity (Waters et al. 2001; Adamson et al. 2012; Wu et al. 2016). In mottled sculpin *Cottus bairdii*, for example, microsatellite population structure was found to be more reflective of historical patterns of colonization than present patterns of connectivity (Homola et al. 2016). Similarly, the presence of an unexpected haplotype in European river and/or brook lampreys in one tributary of the Tagus River was attributed to geological events which previously isolated this tributary (Mateus et al. 2011), and microsatellite data likewise show that lampreys on the Iberian Peninsula have been isolated for long periods (Mateus et al. 2016). The present study found two major genetic groups (the Lewis and Salmon sub-basins, all other sites), with additional sub-structuring apparent within each larger group. This sub-structuring roughly corresponds to contemporary drainages. It should be noted that sampling focused heavily on the Lewis and Salmon sub-basins, which may have made it easier to detect genetic groupings in these areas. There were several cases where the genetic groupings did not correspond exactly to present geography, and these discrepancies may provide information about the history and biogeography of the CRB.

Coastal Washington, the Columbia Estuary, and the Lower Columbia were generally grouped together by microsatellite analyses (except for site 15; see below); however, some degree of historical isolation among and within these sites was suggested by the mtDNA analysis. Haplotype 7, for example, was found only in North Fork Scappoose Creek, which drains into Scappoose Bay and then into the Multnomah Channel (a distributary of the Willamette River) near its confluence with the Columbia River. Haplotype H7 was the most distinctive haplotype identified in the present study, differing by at least 1.1% from any other WBL examined to date, including those from nearby (12 km linear distance, 55 km river distance) Merrill Creek. Most of the Willamette drainage samples examined by Boguski et al. (2012) were Pacific brook lamprey, but the WBL in North

Fork Scappoose Creek differed from Pacific brook lamprey by 3.7% in cytochrome b sequence and were clearly not Pacific brook lamprey. The remaining two Willamette drainage samples analyzed by Boguski et al. (2012) contained haplotypes H11 and H12, which are distinct from both H7 from North Fork Scappoose (1.1–1.4%) and H2 (0.9–1.1%) which is common in other parts of the CRB and along the west coast. During the last glaciation, the Missoula Floods resulted in periodic flooding of the Willamette Valley (Waitt 1985). Perhaps WBL colonized different parts of the Willamette Valley during these floods and were subsequently isolated, resulting in the development of distinctive haplotypes, before drainage patterns shifted again to allow the present river connectivity. The distinctiveness of haplotypes H7, H11, and H12 (and restriction of Pacific brook lamprey to this drainage) indicates that the Willamette drainage warrants further study.

One surprising result was the low genetic differentiation (F_{ST} = 0.0199) between the two Coastal Washington sites. Although all individuals from these two rivers had the Coastal Washington-specific haplotype H1, suggesting colonization by a common lineage, patterns of colonization alone cannot explain the low genetic differentiation observed with microsatellite data. Other pairs or groups of sites sharing region-specific haplotypes had much higher pairwise F_{ST} values (e.g., 0.0927 between the two Columbia Estuary sites; up to 0.2687 among sites 7 to 10, the tributaries of the mainstem Lewis River). Anadromous western river lamprey might mediate contemporary gene flow between coastal WBL populations. Western river lamprey and western brook lamprey form a species pair, and the two cannot be genetically differentiated using cytochrome b sequence (Docker 2009; Boguski et al. 2012). Although size differences between non-parasitic WBL and parasitic western river lamprey generally prevent successful hybridization, some interbreeding has been observed in the laboratory (Beamish and Neville 1992). As well, WBL populations may occasionally give rise to western river lamprey (see Jolley et al. 2016); however, western river lamprey have not been directly recorded from either Bear River or South Fork Naselle River. Alternatively or additionally, non-parasitic lampreys may have some ability to osmoregulate in salt water (see Dawson et al. 2015), perhaps permitting contemporary gene flow mediated by WBL. The Bear and South Fork Naselle rivers drain into the Willapa Bay estuary, and *Lampetra* sp. larvae have been detected in estuarine areas in Ellsworth Creek, a tributary of the Naselle River (Silver 2015). With only two Coastal Washington sites included in the present study, it is difficult to properly assess the possibility or magnitude of gene flow through salt water or estuaries. Future studies should examine population structure of WBL in a larger number of coastal rivers and directly estimate rates of migration between populations.

The Lewis and Salmon sub-basins appeared to contain WBL from at least two different lineages, which did not directly correspond to contemporary drainages. Most Lewis sub-basin sites surveyed (sites 7–10, i.e., excluding Lockwood Creek) drain into the mainstem of the Lewis River. These four sites grouped together in the microsatellite analyses, and all individuals sequenced had the Lewis-specific haplotype H5. However, Lockwood Creek, which drains into the East Fork Lewis River, grouped with the Salmon sub-basin in both microsatellite and mtDNA analyses. The haplotype distribution of Lockwood Creek was more similar to the Salmon sub-basin (a mix of H2 and H6) than to the rest of the Lewis sub-basin (exclusively the Lewis-specific haplotype H5). Tributaries of Salmon Creek are within a few kilometers of the East Fork Lewis River, and a post-glacial headwater capture seems plausible. Headwater capture has been documented as a mode of dispersal and gene flow in many other freshwater fish species (e.g., Waters et al. 2001; Burridge et al. 2006; Adamson et al. 2012), and it may be particularly important in non-migratory species such as WBL. As well, the lower elevation of the Salmon sub-basin may make contemporary connectivity with Lockwood Creek possible. The rest of the Lewis sub-basin appears to have been colonized by a different lineage. Cytochrome b sequence of WBL from the tributaries of the mainstem Lewis River differed by only 1–2 substitutions (0.23–0.46%) from those sampled by Boguski et al. (2012) in northwestern Washington, including those from one tributary of the Chehalis River. The Chehalis River and surrounding areas were a refugium—separate from the Columbia River refugium—for other freshwater fish species during the most recent glaciation (McPhail and Lindsey 1986), and it seems likely that the mainstem Lewis River and its tributaries were colonized from this refugium. Relatively recent colonization and successive founder events may contribute to the low microsatellite genetic diversity found in this area. It is notable that, although all individuals from North Fork Chelatchie Creek had the Lewis-specific haplotype H5, North Fork Chelatchie Creek was somewhat distinct from the rest of the Lewis sub-basin (F_{ST} = 0.212–0.269). As well, STRUCTURE analysis placed about one-third of individuals from this site in the Salmon sub-cluster rather than the Lewis sub-cluster. The reasons for this are unclear, and further study of population structure in this area is recommended.

Gibbons Creek, the uppermost site in the Lower Columbia, grouped with the Salmon sub-basin in microsatellite analyses; this is surprising because the river distance between these sites is about 110 km. Pairwise F_{ST} values between Gibbons Creek and the Salmon sub-basin were only 0.026–0.083, suggesting some degree of historical connectivity. In contrast, F_{ST} values between Gibbons Creek and the other two Lower Columbia sites outside of the Lewis and Salmon sub-basins (Merrill and North Fork Scappoose creeks, sites 5 and 6) were 0.200

and 0.277, respectively, despite their closer proximity (83 and 22 km). Thus, although Gibbons Creek extends only a few kilometers upstream from the Columbia mainstem, it seems to defy simple categorization as a Lower Columbia River population, and the genetic similarity between WBL in Gibbons Creek and those in the Salmon sub-basin requires further study. Since Gibbons Creek is separated from the Salmon sub-basin by the Washougal River, historical headwater capture is unlikely, although a major flooding event (such as the Missoula Floods) could have provided a temporary connection between these drainages. If individuals from Gibbons Creek or the mainstem Columbia were swept into the Salmon sub-basin, this would account for the presence of the H2 haplotype alongside the H6 haplotype common in the Salmon sub-basin. Further study of this region (including any WBL present in the Washougal River drainage) is necessary to ascertain the nature of the connection between Gibbons Creek and the Salmon sub-basin.

In general, the Columbia Gorge and Plateau regions displayed low haplotypic diversity; of the 54 individuals sequenced from these regions, 53 had the common haplotype H2. This suggests WBL may have persisted primarily in the Lower Columbia during glaciation, and upstream regions were later colonized from downstream and subjected to multiple founder events. However, both microsatellite and mtDNA diversity were higher than expected in Trout Lake Creek, a tributary of the White Salmon River. Four of the five individuals sequenced from Trout Lake Creek had the common haplotype H2; however, one individual had haplotype H6, which otherwise occurs only in the Salmon sub-basin and Lockwood Creek. Both headwater capture and contemporary connectivity between Trout Lake Creek and Salmon Creek seem unlikely, as river distance between these sites is approximately 230 km and linear distance is approximately 80 km. This site may have been colonized by more than one group of WBL, but analysis of other sites in this region will be needed to better elucidate colonization patterns. Trout Lake Creek is also of particular interest because it is upstream of several waterfalls and the Condit Dam site. Condit Dam was in place for over 100 years and, along with the waterfalls, was a clear barrier to upstream passage of fish (see Jolley et al. 2018). In the present study, WBL samples were collected prior to the decommissioning of the dam in 2011. Additional study of the White Salmon River and its tributaries would therefore also be of interest to determine whether decommissioning the dam has caused any changes in population connectivity or genetic diversity.

Conclusions

Although considerable research and management efforts are being directed at anadromous Pacific lamprey (e.g., Wang and Schaller 2015; Reid and Goodman 2015; Clemens et al. 2017), WBL have received little research attention, and a lack of knowledge about this species inhibits conservation efforts. Most programs designed to monitor anadromous lamprey abundance (e.g., during upstream migration) are not suitable for detecting brook lampreys. Even in the CRB, where lamprey abundance is closely monitored, most of the available data are for Pacific lamprey (Kostow 2002). The present study aimed to rectify some of these information deficits through examination of historical and contemporary WBL population structure in the CRB. Future studies using other markers (e.g., single nucleotide polymorphisms) and more extensive sampling outside of the Lewis and Salmon sub-basins may provide additional insight into WBL population structure in this large river basin. However, even conservative interpretation of the current results shows significant geographic structuring and limited gene flow among populations. Gene flow appeared to occur primarily in a downstream direction, resulting in a decrease in genetic diversity in upstream sites and suggesting that WBL populations in these areas may be particularly vulnerable to local extinction. Furthermore, most gene flow appeared to take place in tributaries rather than through the mainstem Columbia River. Therefore, whereas Pacific lamprey management efforts are focusing on improving passage at mainstem dams (Moser et al. 2015; Clemens et al. 2017), WBL will likely benefit more from conservation of populations in individual watersheds, particularly headwater areas. Compared to Pacific lamprey, WBL will be more far limited in their ability to recolonize headwater areas if extirpated and, given the moderate to strong genetic differentiation observed over even relatively short distances, translocation within the CRB could severely disrupt population structure and local adaptation. Conservation efforts, therefore, should focus on habitat protection and restoration.

The present study also demonstrated that conservation decisions should be informed by both historical and contemporary patterns of river connectivity. Although population structure often corresponded to contemporary river connectivity, there were some cases in the study where historical connectivity provided a better explanation for the observed patterns of genetic diversity. Due to their limited dispersal, non-migratory freshwater fish species such as WBL may be ideal for examining biogeography and patterns of colonization. Additional analysis of WBL populations in the broader Cascade Range area and the Willamette drainage may shed light on the biogeography of the CRB as a whole.

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References

Adamson EAS, Hurwood DA, Mather PB (2012) Insights into historical drainage evolution based on the phylogeography of the chevron snakehead fish (*Channa striata*) in the Mekong Basin. Freshw Biol 57:2211–2229

Almada VC, Pereira AM, Robalo JI, Fonseca JP, Levy A, Maia C, Valente A (2008) Mitochondrial DNA fails to reveal genetic structure in sea-lampreys along European shores. Mol Phylogenet Evol 46:391–396

Avise JC (2000) Phylogeography: the history and formation of species. Harvard University Press, Cambridge

Bandelt HJ, Forster P, Röhl A (1999) Median-joining networks for inferring intraspecific phylogenies. Mol Biol Evol 16:37–48

Barson NJ, Cable J, Van Oosterhout C (2009) Population genetic analysis of microsatellite variation of guppies (*Poecilia reticulata*) in Trinidad and Tobago: evidence for a dynamic source-sink metapopulation structure, founder events and population bottlenecks. J Evol Biol 22:485–497

Beacham TD, McIntosh B, MacConnachie C, Miller KM, Withler RE, Varnavskaya N (2006) Pacific Rim population structure of sockeye salmon as determined from microsatellite analysis. Trans Am Fish Soc 135:174–187

Beamish RJ, Neville CM (1992) The importance of size as an isolating mechanism in lampreys. Copeia 1992:191–196

Bickham JW, Wood CC, Patton JC (1995) Biogeographic implications of cytochrome b sequences and allozymes in sockeye (*Oncorhynchus nerka*). J Hered 86:140–144

Blanchet FG, Legendre P, Borcard D (2008a) Modelling directional spatial processes in ecological data. Ecol Model 215:325–336

Blanchet FG, Legendre P, Borcard D (2008b) Forward selection of spatial explanatory variables. Ecology 89:2623–2632

Blanchet FG, Legendre P, Maranger R, Monti D, Pepin P (2011) Modelling the effect of directional spatial ecological processes at different scales. Oecologia 166:357–368

Blanchet FG, Legendre P, Gauthier O (2015) AEM: tools to construct asymmetric eigenvector maps (AEM) spatial variables. R package version 0.6/r127. https://R-Forge.R-project.org/projects/sedar/

Boguski DA, Reid SB, Goodman DH, Docker MF (2012) Genetic diversity, endemism and phylogeny of lampreys within the genus *Lampetra sensu stricto* (Petromyzontiformes: Petromyzontidae) in western North America. J Fish Biol 81:1891–1914

Borcard D, Legendre P (2002) All-scale spatial analysis of ecological data by means of principal coordinates of neighbour matrices. Ecol Model 153:51–68

Bracken FSA, Hoelzel AR, Hume JB, Lucas MC (2015) Contrasting population genetic structure among freshwater-resident and anadromous lampreys: the role of demographic history, differential dispersal, and anthropogenic barriers to movement. Mol Ecol 24:1188–1204

Brown AV, Armstrong ML (1985) Propensity to drift downstream among various species of fish. J Freshw Ecol 3:3–17

Bryan MB, Zalinski D, Filcek KB, Libants S, Li W, Scribner KT (2005) Patterns of invasion and colonization of the sea lamprey (*Petromyzon marinus*) in North America as revealed by microsatellite genotypes. Mol Ecol 14:3757–3773

Burnham KP, Anderson DR (2002) Model selection and multimodel inference: a practical information-theoretic approach. Springer, New York

Burridge CP, Craw D, Waters JM (2006) River capture, range expansion, and cladogenesis: the genetic signature of freshwater vicariance. Evolution 60:1038–1049

Cavalli-Sforza LL, Edwards AWF (1967) Phylogenetic analysis: models and estimation procedures. Am J Hum Genet 19:233–257

Chapuis MP, Estoup A (2007) Microsatellite null alleles and estimation of population differentiation. Mol Biol Evol 24:621–631

Clemens BJ, Beamish RJ, Coates KC et al (2017) Conservation challenges and research needs for Pacific Lamprey in the Columbia River Basin. Fisheries 42:269–280

Dawson HA, Quintella BR, Almeida PR, Treble AJ, Jolley JC (2015) The ecology of larval and metamorphosing lampreys. In: Docker MF (ed) Lampreys: biology, conservation and control, vol 1. Springer, Dordrecht, pp 75–138

Dehais CR, Eudeline R, Berrebi P, Argillier C (2010) Microgeographic genetic isolation in chub (Cyprinidae: *Squalius cephalus*) population of the Durance River: estimating fragmentation by dams. Ecol Freshw Fish 19:267–278

Docker MF (2009) A review of the evolution of nonparasitism in lampreys and an update of the paired species concept. In: Brown LR, Chase SD, Moyle PB, Beamish RJ, Mesa MG (eds) Biology, management, and conservation of lampreys in North America. American Fisheries Society, Symposium 72, Bethesda, Maryland, pp 71–114

Docker MF, Hume JB, Clemens BJ (2015) Introduction: a surfeit of lampreys. In: Docker MF (ed) Lampreys: biology, conservation and control, vol 1. Springer, Dordrecht, pp 1–34

Docker MF, Silver GS, Jolley JC, Spice EK (2016) Simple genetic assay distinguishes lamprey genera *Entosphenus* and *Lampetra*: comparison with existing genetic and morphological identification methods. N Am J Fish Manag 36:780–787

Dray S, Legendre P, Peres-Neto PR (2006) Spatial modelling: a comprehensive framework for principal coordinate analysis of neighbour matrices (PCNM). Ecol Model 196:483–493

Dray S, Legendre P, Blanchet G (2016) packfor: forward Selection with permutation (Canoco p. 46). R package version 0.0-8/r136. https://R-Forge.R-project.org/projects/sedar/

Earl DA, VonHoldt BM (2012) STRUCTURE HARVESTER: a website and program for visualizing STRUCTURE output and implementing the Evanno method. Conserv Genet Resour 4:359–361

ESRI (2015) ArcGIS desktop: release 10.3. Environmental Systems Research Institute, Redlands

Evanno G, Regnaut S, Goudet J (2005) Detecting the number of clusters of individuals using the software STRUCTURE: a simulation study. Mol Ecol 14:2611–2620

Farlinger SP, Beamish RJ (1984) Recent colonization of a major salmon-producing lake in British Columbia by Pacific lamprey (*Lampetra tridentata*). Can J Fish Aquat Sci 41:278–285

Flanders J, Jones G, Benda P, Dietz C, Zhang S, Li G, Sharifi M, Rossiter SJ (2009) Phylogeography of the greater horseshoe bat, *Rhinolophus ferrumequinum*: contrasting results from mitochondrial and microsatellite data. Mol Ecol 18:306–318

Gelfenbaum G, Kaminsky GM (2010) Large-scale coastal change in the Columbia River littoral cell: an overview. Mar Geol 273:1–10

Goodman DH, Reid SB, Docker MF, Haas GR, Kinziger AP (2008) Mitochondrial DNA evidence for high levels of gene flow among populations of a widely distributed anadromous lamprey *Entosphenus tridentatus* (Petromyzontidae). J Fish Biol 72:400–417

Goodman DH, Kinziger AP, Reid SB, Docker MF (2009) Morphological diagnosis of *Entosphenus* and *Lampetra* ammocoetes (Petromyzontidae) in Washington, Oregon, and California. In: Brown LR, Chase SD, Moyle PB, Beamish RJ, Mesa MG (eds) Biology, management, and conservation of lampreys in

North America. American Fisheries Society, Symposium 72, Bethesda, Maryland, pp 223–232
Goudet J (1995) FSTAT (version 1.2): a computer program to calculate F-statistics. J Hered 86:485–486
Hänfling B, Weetman D (2006) Concordant genetic estimators of migration reveal anthropogenically enhanced source-sink population structure in the river sculpin, *Cottus gobio*. Genetics 173:1487–1501
Harris JE, Jolley JC (2017) Estimation of occupancy, density, and abundance of larval lampreys in tributary river mouths upstream of dams on the Columbia River, Washington and Oregon. Can J Fish Aquat Sci 74:843–852
Harris JE, Jolley JC, Silver GS, Yuen H, Whitesel TA (2016) An experimental evaluation of electrofishing catchability and catch depletion abundance estimates of larval lampreys in a wadeable stream: use of a hierarchical approach. Trans Am Fish Soc 145:1006–1017
Heckel G, Burri R, Fink S, Desmet JFF, Excoffier L (2005) Genetic structure and colonization processes in European populations of the common vole, *Microtus arvalis*. Evolution 59:2231–2242
Hess JE, Campbell NR, Close DA, Docker MF, Narum SR (2013) Population genomics of Pacific lamprey: adaptive variation in a highly dispersive species. Mol Ecol 22:2898–2916
Hogg R, Coghlan SM, Zydlewski J (2013) Anadromous Sea Lampreys recolonize a Maine coastal river tributary after dam removal. Trans Am Fish Soc 142:1381–1394
Homola JJ, Ruetz CR, Kohler SL, Thum RA (2016) Complex postglacial recolonization inferred from population genetic structure of mottled sculpin *Cottus bairdii* in tributaries of eastern Lake Michigan, U.S.A. J Fish Biol 89:2234–2250
Howes BJ, Lindsay B, Lougheed SC (2006) Range-wide phylogeography of a temperate lizard, the five-lined skink (*Eumeces fasciatus*). Mol Phylogenet Evol 40:183–194
Hutchison DW, Templeton AR (1999) Correlation of pairwise genetic and geographic distance measures: inferring the relative influences of gene flow and drift on the distribution of genetic variability. Evolution 53:1898–1914
Jakobsson M, Rosenberg NA (2007) CLUMPP: a cluster matching and permutation program for dealing with label switching and multimodality in analysis of population structure. Bioinformatics 23:1801–1806
Johnson NS, Buchinger TJ, Li W (2015) Reproductive ecology of lampreys. In: Docker MF (ed) Lampreys: biology, conservation and control. Springer, Dordrecht, pp 265–303
Jolley JC, Silver GS, Whitesel TA (2012) Occupancy and detection of larval Pacific lampreys and *Lampetra* spp. in a large river: the lower Willamette River. Trans Am Fish Soc 141:305–312
Jolley JC, Kovalchuk G, Docker MF (2016) River lamprey *Lampetra ayresii* outmigrant upstream of the John Day Dam in the mid-Columbia river. Northwest Nat 97:48–52
Jolley JC, Silver GS, Harris JE, Whitesel TA (2018) Pacific lamprey recolonization of a Pacific Northwest river following dam removal. River Res Appl 34:44–51
Jones OR, Wang J (2010) COLONY: a program for parentage and sibship inference from multilocus genotype data. Mol Ecol Resour 10:551–555
Kalinowski ST (2005) HP-RARE 1.0: a computer program for performing rarefaction on measures of allelic richness. Mol Ecol Notes 5:187–189
Kearse M, Moir R, Wilson A, Stones-Havas S, Cheung M, Sturrock S, Buxton S, Cooper A, Markowitz S, Duran C, Thierer T, Ashton B, Meintjes P, Drummond A (2012) Geneious basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics 28:1647–1649
Kostow K (2002) Oregon lampreys: natural history, status, and management issues. Oregon Department of Fish and Wildlife, Portland
Luzier CW, Docker MF, Whitesel TA (2010) Characterization of ten microsatellite loci for western brook lamprey *Lampetra richardsoni*. Conserv Genet Resour 2:71–74
Maitland PS, Renaud CB, Quintella BR, Close DA, Docker MF (2015) Conservation of native lampreys. In: Docker MF (ed) Lampreys: biology, conservation, and control, vol 1. Springer, Dordrecht, pp 375–427
Martin KE, Steele CA, Brunelli JP, Thorgaard GH (2010) Mitochondrial variation and biogeographic history of Chinook salmon. Trans Am Fish Soc 139:792–802
Mateus CS, Almeida PR, Quintella BR, Alves MJ (2011) MtDNA markers reveal the existence of allopatric evolutionary lineages in the threatened lampreys *Lampetra fluviatilis* (L.) and *Lampetra planeri* (Bloch) in the Iberian glacial refugium. Conserv Genet 12:1061–1074
Mateus CS, Almeida PR, Mesquita N, Quintella BR, Alves MJ (2016) European lampreys: new insights on postglacial colonization, gene flow and speciation. PLoS ONE 11:e0148107
Mazerolle MJ (2017) AICcmodavg: model selection and multimodel inference based on (Q)AIC(c). R package version 2.1-1. https://cran.r-project.org/package=AICcmodavg
McCusker MR, Parkinson E, Taylor EB (2000) Mitochondrial DNA variation in rainbow trout (*Oncorhynchus mykiss*) across its native range: testing biogeographical hypotheses and their relevance to conservation. Mol Ecol 9:2089–2108
McPhail JD, Lindsey CC (1986) Zoogeography of the freshwater fishes of Cascadia (the Columbia system and rivers north to the Stikine). In: Hocutt CH, Wiley EO (eds) The zoogeography of North American freshwater fishes. Wiley, New York, pp 615–637
Mock KE, Brim Box JC, Chong JP, Howard JK, Nez DA, Wolf D, Gardner RS (2010) Genetic structuring in the freshwater mussel *Anodonta* corresponds with major hydrologic basins in the western United States. Mol Ecol 19:569–591
Mock KE, Brim Box JC, Chong JP, Furnish J, Howard JK (2013) Comparison of population genetic patterns in two widespread freshwater mussels with contrasting life histories in western North America. Mol Ecol 22:6060–6073
Morrissey MB, de Kerckhove DT (2009) The maintenance of genetic variation due to asymmetric gene flow in dendritic metapopulations. Am Nat 174:875–889
Moser ML, Almeida PR, Kemp PS, Sorensen PW (2015) Lamprey spawning migration. In: Docker MF (ed) Lampreys: biology, conservation, and control, vol 1. Springer, Dordrecht, pp 215–263
Oksanen J, Blanchet FJ, Kindt R, Legendre P, Minchin PR, O'Hara RB, Simpson GL, Solymos P, Stevens MHH, Wagner H (2016) vegan: community ecology package. R package version 2.3-5/r2997. https://R-Forge.R-project.org/projects/vegan/
Orsini L, Mergeay J, Vanoverbeke J, De Meester L (2013) The role of selection in driving landscape genomic structure of the waterflea *Daphnia magna*. Mol Ecol 22:583–601
Page R (1996) TreeView: an application to display phylogenetic trees on personal computers. Comput Appl Biosci 12:357–358
Paradis E, Claude J, Strimmer K (2004) APE: analyses of phylogenetics and evolution in R language. Bioinformatics 20:289–290
Piry S, Luikart G, Cornuet JM (1999) BOTTLENECK: a computer program for detecting recent reductions in the effective population size using allele frequency data. J Hered 90:502–503
Pritchard JK, Stephens M, Donnelly P (2000) Inference of population structure using multilocus genotype data. Genetics 155:945–959
R Core Team (2016) R: a language and environment for statistical computing. R Foundation for Statistical Computing, Vienna, Austria. https://www.R-project.org/

Raymond M, Rousset F (1995) GENEPOP (Version 1.2): population genetics software for exact tests and ecumenism. J Hered 86:248–249

Reid SB, Goodman DH (2015) Detectability of Pacific lamprey occupancy in western drainages: implications for distribution surveys. Trans Am Fish Soc 144:315–322

Reid SB, Boguski DA, Goodman DH, Docker MF (2011) Validity of *Lampetra pacifica* (Petromyzontiformes: Petromyzontidae), a brook lamprey described from the lower Columbia River Basin. Zootaxa 3091:42–50

Rosenberg NA (2004) DISTRUCT: a program for the graphical display of population structure. Mol Ecol Notes 4:137–138

Rougemont Q, Gaigher A, Lasne E, Côte J, Coke M, Besnard AL, Launey S, Evanno G (2015) Low reproductive isolation and highly variable levels of gene flow reveal limited progress towards speciation between European river and brook lampreys. J Evol Biol 28:2248–2263

Rougemont Q, Roux C, Neuenschwander S, Goudet J, Launey S, Evanno G (2016) Reconstructing the demographic history of divergence between European river and brook lampreys using approximate Bayesian computations. PeerJ 4:e1910

Rougemont Q, Gagnaire PA, Perrier C, Genthon C, Besnard AL, Launey S, Evanno G (2017) Inferring the demographic history underlying parallel genomic divergence among pairs of parasitic and non-parasitic lamprey ecotypes. Mol Ecol 26:142–162

Rousset F (2008) GENEPOP'007: a complete re-implementation of the genepop software for Windows and Linux. Mol Ecol Resour 8:103–106

Ruppert JLW, James PMA, Taylor EB, Rudolfsen T, Veillard M, Davis CS, Watkinson D, Poesch MS (2017) Riverscape genetic structure of a threatened and dispersal limited freshwater species, the Rocky Mountain Sculpin (*Cottus* sp.). Conserv Genet 18:925–937

Sala-Bozano M, Ketmaier V, Mariani S (2009) Contrasting signals from multiple markers illuminate population connectivity in a marine fish. Mol Ecol 18:4811–4826

Scott WB, Crossman EJ (1973) Freshwater fishes of Canada. Fisheries Research Board of Canada, Ottawa

Selkoe KA, Toonen RJ (2006) Microsatellites for ecologists: a practical guide to using and evaluating microsatellite markers. Ecol Lett 9:615–629

Silver GS (2015) Investigations of larval Pacific lamprey *Entosphenus tridentatus* osmotic stress tolerance and occurrence in a tidally-influenced estuarine stream. M.S. thesis, Portland State University, Portland, Oregon

Spice EK, Whitesel TA, McFarlane CT, Docker MF (2011) Characterization of 12 microsatellite loci for the Pacific lamprey, *Entosphenus tridentatus* (Petromyzontidae), and cross-amplification in five other lamprey species. Genet Mol Res 10:3246–3250

Spice EK, Goodman DH, Reid SB, Docker MF (2012) Neither philopatric nor panmictic: microsatellite and mtDNA evidence suggests lack of natal homing but limits to dispersal in Pacific lamprey. Mol Ecol 21:2916–2930

United States Fish and Wildlife Service (2004) Endangered and threatened wildlife and plants; 90-day finding on a petition to list three species of lampreys as threatened or endangered. Fed Reg 69:77158–77167

Van Oosterhout C, Hutchinson WF, Wills DPM, Shipley P (2004) Micro-checker: software for identifying and correcting genotyping errors in microsatellite data. Mol Ecol Notes 4:535–538

Vangestel C, Mergeay J, Dawson DA, Callens T, Vandomme V, Lens L (2012) Genetic diversity and population structure in contemporary house sparrow populations along an urbanization gradient. Heredity 109:163–172

Waitt RB (1985) Case for periodic, colossal jökulhlaups from Pleistocene glacial Lake Missoula. Geol Soc Am Bull 96:1271–1286

Waldman J, Grunwald C, Wirgin I (2008) Sea lamprey *Petromyzon marinus*: an exception to the rule of homing in anadromous fishes. Biol Lett 4:659–662

Wang C, Schaller H (2015) Conserving Pacific lamprey through collaborative efforts. Fisheries 40:72–79

Ward DL, Clemens BJ, Clugston D et al (2012) Translocating adult Pacific lamprey within the Columbia River basin: state of the science. Fisheries 37:351–361

Waters JM, Craw D, Youngson JH, Wallis GP (2001) Genes meet geology: fish phylogeographic pattern reflects ancient, rather than modern, drainage connections. Evolution 55:1844–1851

Weir B, Cockerham C (1984) Estimating F-statistics for the analysis of population structure. Evolution 38:1358–1370

White JL, Harvey BC (2003) Basin-scale patterns in the drift of embryonic and larval fishes and lamprey ammocoetes in two coastal rivers. Environ Biol Fish 67:369–378

Wu TH, Tsang LM, Chen IS, Chu KH (2016) Multilocus approach reveals cryptic lineages in the goby *Rhinogobius duospilus* in Hong Kong streams: role of paleodrainage systems in shaping marked population differentiation in a city. Mol Phylogenet Evol 104:112–122

Yamazaki Y, Yamano A, Oura K (2011a) Recent microscale disturbance of gene flow in threatened fluvial lamprey, *Lethenteron* sp. N, living in a paddy water system. Conserv Genet 12:1373–1377

Yamazaki Y, Yokoyama R, Nagai T, Goto A (2011b) Formation of a fluvial non-parasitic population of *Lethenteron camtschaticum* as the first step in petromyzontid speciation. J Fish Biol 79:2043–2059

Yamazaki Y, Yokoyama R, Nagai T, Goto A (2014) Population structure and gene flow among anadromous Arctic lamprey (*Lethenteron camtschaticum*) populations deduced from polymorphic microsatellite loci. Environ Biol Fish 97:43–52