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Robust Recolonization of Pacific Lamprey Following Dam Removals

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Abstract
    Removal of two dams in the Elwha River basin, Washington, started one of the largest river restoration projects ever attempted in the Pacific Northwest. These dams had eliminated Pacific Lamprey *Entosphenus tridentatus* populations upstream. After the dam removals, larval production increased in the upper watershed, but the sources and numbers of new adult spawners were unknown. We applied genetic stock identification (GSI), parentage assignment (PA), and sibship assignment (SA) methods to (1) determine the origins of Pacific Lamprey larvae and juveniles, (2) quantify the increase in numbers of successful Elwha River spawners (i.e., effective number of breeders [N_b]) and assess whether the current numbers of spawners have reached levels equivalent to those of neighboring undammed basins, and (3) determine the relative productivity of streams within the Elwha River and how overall productivity originating from this system may be distributed across the broader surrounding region. We utilized a highly accurate set of 263 single-nucleotide polymorphism (SNP) loci to perform PA and SA (>99% accuracy rate) and an additional set of 28 SNPs for GSI. Our results showed that a single stream (Indian Creek) was the source of 41% of larval and juvenile production in the Elwha River. Our N_b estimates for the Elwha River indicated a 12-fold increase in N_b during the 3 years after dam removal, with recent N_b estimates matching those of neighboring Olympic Peninsula basins. These results indicate rapid recolonization potential for this highly dispersive species, and high productivity within the Elwha River suggests that restoring passage to adequate habitat is a highly effective approach for re-establishing populations of Pacific Lamprey in coastal systems.

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Pacific Lamprey *Entosphenus tridentatus* have suffered declines across the species' range due to habitat loss, passage impediments, and even active poisoning. Multifaceted strategies have been employed to re-establish self-sustaining, harvestable abundances of adults, larvae, and juveniles. The greatest level of human intervention may be required in the upper reaches of large river basins. For example, the Columbia River treaty tribes have employed high levels of sustained translocation (i.e., human-mediated transfers of fish to areas upstream from hydropower dams) and developed effective artificial propagation. In smaller coastal systems, the species may need just an opportunity to access appropriate habitat to naturally re-establish because these areas have fewer passage impediments and greater proximity to oceanic sources of Pacific Lamprey spawners. For small coastal rivers with dams that prevent passage of Pacific Lamprey, dam removal could be the only requisite for restoration of robust and self-sustaining Pacific Lamprey abundance via natural recolonization (Reid and Goodman 2020; Moser et al., in press).

Pacific Lamprey in the Elwha River drainage, Washington, present an ideal case study for examining the characteristics of anadromous recolonization of a coastal river basin following dam removal. Removal of two large, obsolete dams started one of the largest river restoration projects ever attempted in the Pacific Northwest. These dams had eliminated Pacific Lamprey populations upstream, but adults rapidly recolonized the upper watershed after dam removal (Moser and Paradis 2017). While natural recolonization was underway, adult lamprey from neighboring watersheds were collected, genetically sampled, radio-tagged, and translocated to the lower Elwha River to document migratory behavior. These collections allowed evaluation of the effectiveness of translocating fish during active natural recolonization efforts.

Although translocation of adult Pacific Lamprey can aid population recovery (Close et al. 2009; Ward et al. 2012), natural recolonization after barrier removal can also occur rapidly (e.g., Hogg et al. 2013; Hess et al. 2015; Pereira et al. 2017; Jolley et al. 2018). Lampreys apparently are not philopatric, and they use pheromonal cues to find watersheds for spawning (reviewed by Moser et al. 2015). The relative rates of population recovery following natural recolonization compared to production resulting from translocated adults likely depend on the system and the sources of lamprey (Reid and Goodman 2020).

Immediately after dam removal was completed in March 2012, Pacific Lamprey were noted upstream from the former Elwha Dam site (Moser and Paradis 2017); however, the exact numbers and sources of effective spawners responsible for this production were unknown. The larvae in one stream (Indian Creek) were observed to increase by 14× in the 5 years following dam removal (from 132 larvae in 2014 to 1,805 larvae in 2016; Moser and Paradis 2017), but it was unknown whether effective spawners increased at the same rate. Given the high fecundity of Pacific Lamprey (Kan 1975), it is possible that the increase in spawners could be much lower than the observed increase in larvae, indicating a less robust recovery.

Second, the relative productivity across streams within the Elwha River basin was unclear because not all streams could be monitored intensively and consistently. One stream, Indian Creek, appears to have supported relatively high increases of juvenile salmonids (i.e., Chinook Salmon *Oncorhynchus tshawytscha*, Coho Salmon *O. kisutch*, and steelhead *O. mykiss*; McHenry et al. 2020) compared to other streams of the Elwha River basin. A similar pattern may occur in Pacific Lamprey recruitment.

Finally, the relative contribution of this new productivity in the Elwha River basin could benefit the entire region given the lack of homing in this species. Understanding the sources and sinks of production is a key question for management of all native anadromous lampreys (Lucas et al. in press). Until recently, the understanding of the population dynamics of anadromous lampreys was rudimentary at best, yet such information is crucial for adaptive management of these imperiled species. With the development of recent genetic methods for Pacific Lamprey like genetic stock identification (GSI), parentage assignment (PA), and sibship assignment (SA), this information has become available.

The three different genetic methods (GSI, PA, and SA) provide ways to address similar objectives (e.g., identify natal origins of individuals), but they differ in precision and sampling requirements. Genetic stock identification identifies individuals to their genetic stock of origin and can be an effective way to perform mixed-stock analysis for management (Shaklee et al. 1999). The precision of GSI depends on the spatial scale at which stock-level differentiation is observed, which can be at small scales within the basin level for some species (e.g., steelhead in the Snake River basin; Nielsen et al. 2009; Campbell et al. 2012) but at larger regional spatial scales for others (e.g., Eulachon *Thaleichthys pacificus*; Candy et al. 2015). Parentage assignment and SA can be used to precisely identify individuals to the spawning site of origin of the sampled parents (PA; Steele et al. 2019) or the natal site of origin of sampled siblings in the baseline (SA; Jones and Wang 2010). In addition, SA can be used to estimate the effective number of breeders (N_b), a potential proxy for adult abundance (Ackerman et al. 2017). Larval and juvenile full-sibling baselines can be effective for estimating the number of effective spawners (breeders) that contributed to each collection of young life stages (e.g., Hess et al. 2015; Whitlock et al. 2017). Here, we define "larvae"

as immature, filter-feeding lamprey without eyes and "juveniles" as fully transformed individuals that have eyes and are ready to feed (Clemens 2019).

We used genetic monitoring of Elwha River Pacific Lamprey to address the following three objectives: (1) determine the origins of Pacific Lamprey larvae and juveniles collected in the Elwha River and thereby attribute production to translocation versus natural recolonization, (2) quantify the increase in N_b in the Elwha River and determine whether the current N_b has reached levels observed in neighboring undammed basins, and (3) determine the relative productivity of streams within the Elwha River basin and how overall productivity originating from the Elwha River is distributed across the broader surrounding region.

We used a highly accurate (>99% accuracy rate) set of 263 single-nucleotide polymorphism (SNP) loci to perform single PA and SA, and we used an additional 28 SNPs to perform GSI. We expected the following answers to questions drawn from our three objectives above. First, we expected that larval and juvenile production in the Elwha River would be primarily sourced from natural recolonization given the relatively modest translocation effort (<50 adults). Second, we anticipated that increases in effective spawners would be as rapid as the 14× increase in larvae during the 5 years after dam removal; however, given the relatively recent recolonization in the Elwha River, we expected N_b to be lower than that in undammed basins. Finally, we anticipated that within the Elwha River, Indian Creek would contribute the most production given its high productivity for salmonids but that the overall contribution of Pacific Lamprey from the Elwha River to the broader surrounding region would be relatively small given its recent founding and small area relative to surrounding sources of Pacific Lamprey.

METHODS

Sample collections.— The collections of Pacific Lamprey included individuals from within the Elwha River basin, neighboring basins of the Olympic Peninsula, ocean-phase individuals along the U.S. West Coast, and adult returns to the Willamette River in the Columbia River basin (Figure 1). In addition, Rangewide reference collections that were previously genotyped were utilized for the GSI baseline (Hess et al. 2013; Figure 1A).

The bulk of the samples comprised larvae, juveniles, and adults collected from the Elwha River ($N = 773$). We refer to groups of these collections from the Elwha River as follows: ELWHA_EF (larval and juvenile Pacific Lamprey that were electrofished), ELWHA_ST (larval and juvenile Pacific Lamprey that were collected via rotary screw trap), and ELWHA_pbt(vol) (volitional adult migrants that were used as candidate parents in the

parentage-based tagging baseline). One sample of adults was translocated into the Elwha River from other basins ($N = 42$; referred to as ELWHA_pbt[trans] in Table 1). All larvae and juveniles were collected via electrofishing or by rotary screw trap; the latter method also recovered some postspawn adults. Adults that were translocated into the Elwha River from neighboring basins (i.e., Deep Creek, East Twin River, and Salt Creek) were always radio-tagged and released on the day of capture.

Pacific Lamprey samples were also collected from outside the Elwha River basin. Electrofished samples from neighboring drainages (Deep Creek, East Twin River, West Twin River, and Lyre River; 2015–2018, $N = 355$) are referred to as "OLY_EF" and were Olympic Peninsula larvae and juveniles. One group of these genetic samples came from electrofished larvae in Salt Creek (sites 9 and 10), which were subsequently translocated in 2018 to the Little River (site 36; Figure 1B), a stream within the Elwha River basin that had no prior lamprey production. Genetic samples were also taken from parasitic, ocean-phase individuals (Ocean; $N = 695$) collected in the 2017 Pacific Hake *Merluccius productus* trawl fishery in the ocean off Washington, Oregon, and northern California (Figure 1A). Samples from miscellaneous adults that were hand collected at Willamette Falls (Columbia River drainage; Figure 1A) in 2014 and 2015 were used as a control group to test assignment methods (MISC_Adult; $N = 1,714$).

Molecular analysis.— We used both ethanol and dry storage of fin tissues followed by extraction and PCR for genotyping-by-sequencing techniques on an Illumina sequencer. Single-nucleotide polymorphism genotypes were generated using the genotyping-in-thousands by sequencing custom amplicon method described by Campbell et al. (2015). The 308 SNP loci in the panel were selected to be representative of neutral and adaptive loci across the geographic range of Pacific Lamprey, as described by Hess et al. (2020). For this study, we used a subset of 295 of these previously published loci. For the most part, this subset was a reduction from the larger number of loci; however, seven loci were reanalyzed to target a different SNP than had been previously genotyped in the same amplicon (*Etr_480-67*, *Etr_930-35*, *Etr_2016-70*, *Etr_3037-68*, *Etr_6318-70*, *Etr_7166-73*, and *Etr_7974-70*). The final 295-SNP panel included 263 SNPs that were used for PA and SA applications (12 of the 263 SNPs are adaptive), 4 SNPs for species determination (Hess et al. 2015), and 28 additional SNPs for characterizing adaptive variation (defined by outlier tests; Hess et al. 2013, 2020).

Statistical analysis.— Using GENEPOP version 4.2, we performed linkage disequilibrium (LD) tests for all locus pairs and characterized minor allele frequency (MAF). For the LD tests, the Rangewide reference collections were pooled into the following four major groups to strike
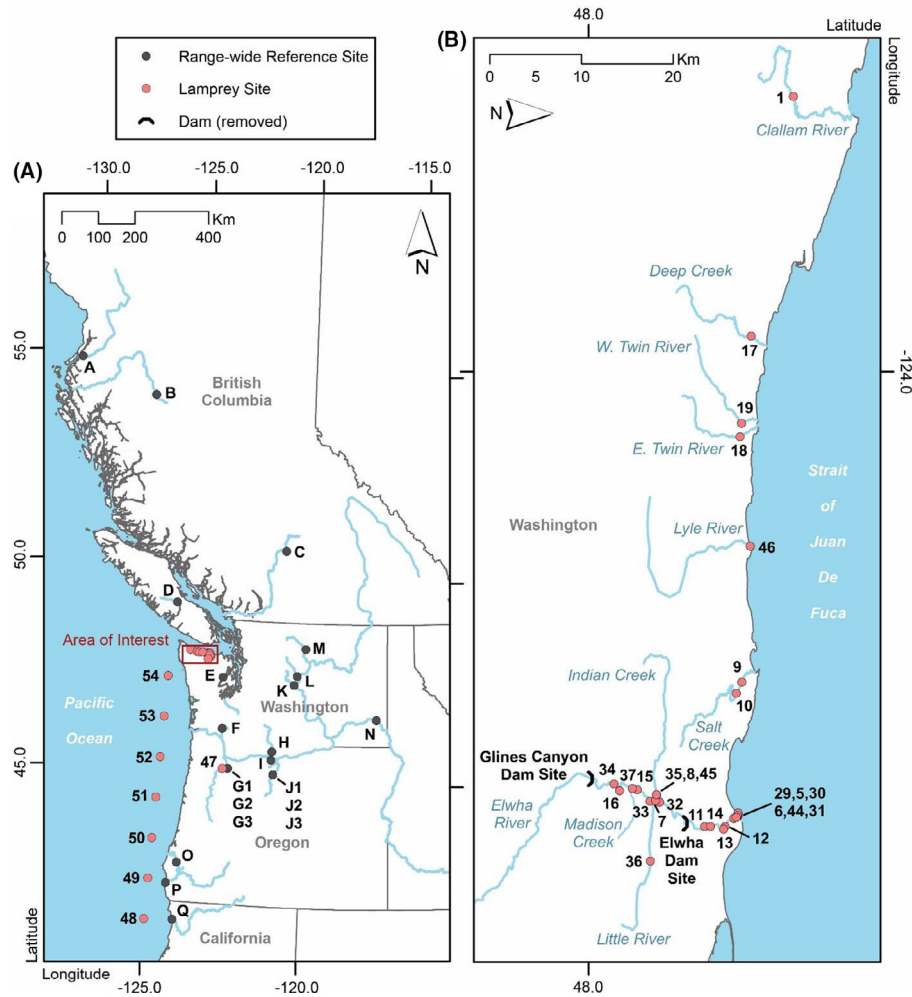
FIGURE 1. (A) Map of the entire study area (with box indicating the area of interest on the Olympic Peninsula), showing Rangewide reference sites (black circles; letter codes defined in Table S1 available in the Supplemental Materials in the online version of this article) and collection sites (red circles; site numbers defined in Table 1) for the Pacific Lamprey samples genotyped in this study. (B) The inset map shows the area of interest, including the Elwha River collections and the additional collections (outside the Elwha River basin) distributed across the Olympic Peninsula that were used for context. Sites where the Glines Canyon and Elwha dams were removed from the Elwha River are indicated on the inset. Note that the inset map north arrow points right.

a balance between maximizing sample sizes per test population and minimizing Wahlund effects (Hess et al. 2013): north (sites A and B), south (sites P and Q), lower Columbia River (sites G2, G3, H, and I), and interior Columbia River (sites J2, J3, K, and L; Figure 1A; Table S1; Hess et al. 2013). We used three software programs to conduct PA with the 263 loci: parent pair assignments using SNPPIT version 1.0 (Anderson 2012) and single parent assignments using COLONY version 2.0.6.5 (Jones and Wang 2010) and SEQUOIA version 2.0.7 (Huisman 2017). These three programs were used in combination for parentage to support results through concordance. The SNPPIT parent pair assignments generally provide the highest confidence but can only be performed when both parents of a candidate offspring are present in the

baseline. When one parent is missing, single parent assignments must be relied upon to identify offspring from a baseline of candidate parents and require greater power to match the accuracy level of SNPPIT. Therefore, we compared any candidate parent assignments in COLONY with results from SNPPIT parent pair assignments and SEQUOIA single parent assignments for concordance. Furthermore, we used new methods to perform simulations in SEQUOIA to measure the accuracy of this approach given the information content (variation) in our markers and data sets. COLONY was also used to reconstruct full-sibling families (Wang 2004) for each study collection and to estimate N_b (Wang 2009) using the 263 SNPs (Table 1).

Individuals were assigned as parent–offspring trios using a likelihood-based method implemented in SNPPIT,

TABLE 1. Locations (river kilometer [rkm]; latitude, longitude), group names (see Methods for group descriptions), number of samples (*N*), and analysis type (Type) for each Pacific Lamprey collection site (Type indicates whether the collection was comprised of larvae and juveniles [L/J], juveniles only [J], or adults only [A] and whether the collections were treated as candidate offspring [Offsp.] or candidate parents [Parent] for the different analyses in this study). Map site numbers correspond to those in Figure 1 (WDFW = Washington Department of Fish and Wildlife).

Map site	Description	rkm	Latitude (°N), longitude (°W)	Pooled group	Collection year(s)	*N*	Type
15	Brannon Creek	0.11	48.048, −123.590	ELWHA_EF	2018	11	L/J Offsp.
16	Campground Creek	0.20	48.030, −123.589	ELWHA_EF	2017	9	L/J Offsp.
29	Elwha River	0.18	48.146, −123.568	ELWHA_EF	2018	2	L/J Offsp.
30	Elwha River	0.38	48.146, −123.564	ELWHA_EF	2018	37	L/J Offsp.
31	Elwha River	0.90	48.142, −123.561	ELWHA_EF	2018	19	L/J Offsp.
32	Elwha River	11.6	48.070, −123.577	ELWHA_EF	2018	1	L/J Offsp.
33	Elwha River	12.7	48.060, −123.578	ELWHA_EF	2018	14	L/J Offsp.
34	Hughes Creek	0.10	48.025, −123.595	ELWHA_EF	2017	8	L/J Offsp.
35	Indian Creek	0.00	48.066, −123.579	ELWHA_EF	2017	18	L/J Offsp.
36	Little River	4.60	48.060, −123.519	ELWHA_EF	2018	38	L/J Offsp.
37	Madison Creek	0.00	48.046, −123.589	ELWHA_EF	2017	51	L/J Offsp.
	Subtotal			ELWHA_EF		208	L/J Offsp.
44	Elwha River	0.67	48.145, −123.562	ELWHA_ST	2016, 2017, 2018	1, 4, 76	L/J Offsp.
45	Indian Creek	0.63	48.067, −123.585	ELWHA_ST	2016, 2017, 2018	10, 11, 455	L/J Offsp.
46	Lyre River mouth	0.00	48.158, −123.828	ELWHA_ST	2018	9	L/J Offsp.
	Subtotal			ELWHA_ST		566	L/J Offsp.
17	Deep Creek	2.00	48.160, −124.035	OLY_EF	2015, 2016, 2017, 2018	47, 20, 74, 27	L/J Offsp.
18	East Twin River	2.20	48.149, −123.936	OLY_EF	2016, 2017, 2018	10, 100, 25	L/J Offsp.
19	West Twin River	1.90	48.158, −123.969	OLY_EF	2017, 2018	32, 11	L/J Offsp.
	Subtotal			OLY_EF		346	L/J Offsp.
48	Eastern Pacific Ocean, U.S. West Coast slope, NLat42SLat41			Ocean	2017	3	J Offsp.
49	Eastern Pacific Ocean, U.S. West Coast slope, NLat43SLat42			Ocean	2017	85	J Offsp.
50	Eastern Pacific Ocean, U.S. West Coast slope, NLat44SLat43			Ocean	2017	216	J Offsp.
51	Eastern Pacific Ocean, U.S. West Coast slope, NLat45SLat44			Ocean	2017	161	J Offsp.
52	Eastern Pacific Ocean, U.S. West Coast slope, NLat46SLat45			Ocean	2017	67	J Offsp.
53	Eastern Pacific Ocean, U.S. West Coast slope, NLat47SLat46			Ocean	2017	130	J Offsp.

TABLE 1. Continued.

Map site	Description	rkm	Latitude (°N), longitude (°W)	Pooled group	Collection year(s)	N	Type
54	Eastern Pacific Ocean, U.S. West Coast slope, NLat48SLat47			Ocean	2017	30	J Offsp.
-				Ocean	2017, 2018	1, 2	J Offsp.
	Subtotal			Ocean		695	J Offsp.
47	Willamette Falls fish ladder	41.1	45.352, −122.618	MISC_Adult	2014, 2015, 2016	865, 580, 269	A Parent
	Subtotal			MISC_Adult		1,714	A Parent
1	Clallam River	9.20	48.201, −124.271	ELWHA_pbt(vol)	2018	1	A Parent
5	Elwha River	0.38	48.147, −123.564	ELWHA_pbt(vol)	2017	1	A Parent
6	Elwha River	0.67	48.145, −123.562	ELWHA_pbt(vol)	2017	1	A Parent
8	Indian Creek	0.63	48.067, −123.585	ELWHA_pbt(vol)	2018, 2017	1, 5	A Parent
	Subtotal			ELWHA_pbt(vol)		9	A Parent
11	Elwha River, pedestrian bridge	5.26	48.114, −123.554	ELWHA_pbt(trans)	2017	7	A Parent
12	Elwha River, sonar	2.47	48.134, −123.554	ELWHA_pbt(trans)	2017	1	A Parent
13	Elwha River, tribal hatchery	2.77	48.132, −123.551	ELWHA_pbt(trans)	2017, 2018	1, 25	A Parent
14	Elwha River, WDFW hatchery	4.63	48.120, −123.554	ELWHA_pbt(trans)	2017	8	A Parent
	Subtotal			ELWHA_pbt(trans)		42	A Parent

with the parameter "–max-par-miss" set to 262 (total SNP $N − 1$). This effectively allows all parents and offspring in the data set to be compared regardless of missing data (missing data were already minimized by excluding individuals that were missing $\geq 10\%$ of their genotypes). We used an estimated SNP genotyping error rate of 1.0% or a per-allele rate of 0.5%. The SNPPIT assesses confidence of PA using a false discovery rate, and we only accepted assignments with a very stringent false discovery rate threshold of less than 0.5% (i.e., we expected the fraction of offspring assigned to incorrect parents to be < 1 in 200). A 0.5% per-allele error rate was chosen as a conservative value for this SNPPIT parameter because it is larger than the observed per-locus error rate of 0.2%.

In COLONY, we analyzed the larval and juvenile collections together as one (i.e., $N = 1,815$ for ELWHA_EF, ELWHA_ST, OLY_EF, and Ocean; Table 1) using the following parameter settings: polygamous mating for males and females without inbreeding, full likelihood, medium length of run, no allele updating, and no sibship priors. These parameter settings were chosen based on previous work that empirically demonstrated the polygamous system of mating and confirmed the accuracy of the sibship reconstruction based on comparisons with known familial relationships (Hess et al. 2015). We also analyzed each collection separately using the same settings to calculate effective population size (N_e) estimates for each collection. For the first analysis that grouped all larval and juvenile collections ($N = 1,815$), we also used the adult collections (i.e., $N = 1,765$ for MISC_Adult and ELWHA_pbt; Table 1) as candidate parents of unknown sex and assumed that the probability that any of these candidates were true parents was 25%. This prior was chosen as our best estimate of the probability that the actual parent of an offspring in the offspring sample is included in the sample of candidate parents, as suggested in the manual. We estimated that we had obtained a sample of candidate parents comprising no more than one-quarter of the fish that spawned.

SingleSEQUOIA (T. A. Delomas; available at https://github.com/delomast/singleSequoia) is an R package (R Development Core Team 2020) that interfaces with IDFGEN (R package available at https://github.com/mackerman44/idfgen) objects to build SEQUOIA (Huisman 2017) inputs for single parent assignment. It also has a function to run simulated analyses in SEQUOIA to estimate expected error rates for single parent assignment given a baseline, marker panel, and number of expected

offspring (see Supplemental Materials available in the online version of this article). The threshold for accepting single parent assignments was set to a value of 0.5 $\log_{10}$ likelihood ratio between a parent–offspring relationship versus unrelated, which was chosen based on prior work demonstrating that this threshold provided an optimal tradeoff between false-positive and false-negative errors. The results from the simulations confirmed that the selected $\log_{10}$ likelihood ratio threshold would yield low error rates for this data set (see Results section).

The GSI analyses required two steps to process the reference collections into GSI baselines: (1) construction of reporting groups and (2) testing of baseline accuracy. Our goal was to construct an optimal set of reporting groups that maximized both geographic and genetic cohesiveness. Ideally, dividing reference collections into reporting groups that represent the smallest geographic regions possible is most informative for GSI if the baseline achieves high accuracy. We used STRUCTURE version 2.3.4 (Pritchard et al. 2000) as a heuristic approach to dividing reference collections into the smallest reporting units for accurate GSI discrimination. We used 291 SNPs (having removed the four species-diagnostic markers) and an initial burn-in of 20,000 iterations followed by 200,000 iterations of the Markov chain–Monte Carlo method to generate posterior probabilities allowing for admixture for each of 40 trials, setting the number of clusters (K) from 1 to 10. To obtain a set of robust individual assignments, a membership coefficient (Q) was calculated for each of K_n clusters, and the Greedy algorithm in CLUMPP (Jakobsson and Rosenberg 2007) was used to find the optimal alignment of clusters across multiple runs using method 2 with 100 repeats. Only the top-10 replicate STRUCTURE trials with the highest mean likelihood of K ($\ln[\Pr(X/K)]$) were averaged using the Greedy algorithm in CLUMPP. The best alignment configuration was computed by 100 configurations that were examined for the highest pairwise similarity (H') to achieve optimal alignment.

We tested GSI baseline accuracy using leave-one-out assignment probabilities estimated with the program GENECLASS2 (Piry et al. 2004), which implemented the Bayesian method of Rannala and Mountain (1997). Following assignment of each individual fish, the estimated probabilities of assignment to each reporting group were averaged for each collection of fish. The following two baselines were tested: (1) Rangewide and (2) Olympic Peninsula. The Rangewide baseline contained 21 reference collections (Hess et al. 2013) divided into five different groups by geographic region. Reporting groups were constructed with guidance from the results of STRUCTURE while attempting to maintain geographic cohesion. The Olympic Peninsula baseline only contained collections within the Elwha River basin (i.e., ELWHA_EF and ELWHA_ST grouped by stream) and its neighboring

tributaries (i.e., OLY_EF grouped by river basin) on the Olympic Peninsula. For leave-one-out tests, a level of 90% correct assignment has been used in fisheries management to indicate that baseline populations have been adequately delineated to assign individuals from fishery mixtures (e.g., Smith et al. 2005; Beacham et al. 2006; Seeb et al. 2007). Neither of the two baselines approached this level of accuracy in correct self-assignments across reporting groups, but the Rangewide baseline performed best.

After establishing the Rangewide GSI baseline, we applied it to our objectives to determine the natal origin of fish based on their assignment probabilities to the reporting groups of the GSI baseline. We performed individual assignments of the following unknown mixtures of fish: (1) ELWHA_EF, (2) ELWHA_ST, (3) OLY_EF, (4) Ocean, and (5) MISC_Adult. Individual assignments to the Rangewide baseline reporting groups were estimated with the Bayesian method implemented in gsi_sim (Anderson et al. 2008), which generated a probability of origin using well-established methods for GSI (Paetkau et al. 1995; Rannala and Mountain 1997; Smouse et al. 1990). The proportion of fish assigned to the reporting group with highest probability ("best estimate" of the reporting group of origin) was calculated for each of the unknown mixtures.

Objective 1: determine the origins of larval and juvenile Pacific Lamprey of the Elwha River basin and attribute production to translocation versus natural recolonization.— We used PA to determine whether larvae and juveniles collected in the Elwha River were the progeny of any of the sampled adults (translocated versus volitional migrants). Candidate offspring (mixtures 1–4 described for GSI above) were assigned to a parent baseline using parent pairs via the program SNPPIT and single parents via the programs COLONY and SEQUOIA. The parent baseline included volitional migrants in the Elwha River and Olympic Peninsula (ELWHA_pbt[vol]; $N = 9$), translocated adults used for telemetry in the Elwha River basin (ELWHA_pbt[trans]; $N = 42$), and a set of negative control adults from the Willamette River (MISC_Adult; $N = 1,714$).

Objective 2: quantify the increase in numbers of effective spawners in the Elwha River and determine whether current numbers of spawners are equivalent to those in undammed basins.— We used the N_e estimate from COLONY to determine whether N_b increased with time by using two screw trap collections in 2018 from Indian Creek ($N = 447$; within the Elwha River basin) and the mouth of the Elwha River ($N = 69$). These two sites were ideal for this analysis because they had relatively large sample sizes spanning a diversity of fish sizes that represented multiple brood years (BYs). These sites were also ideal because Indian Creek was a site in which larvae were previously quantified to have increased by 14× over recent years and

we could compare whether the trend in N_b was similar to this rate of increase. Furthermore, the mouth of the Elwha River was perfect because it represented the productivity of the entire basin, as all fish had to pass this point before migrating to the ocean, regardless of their natal stream. The effective number of spawners during a single breeding event (i.e., N_b) is approximately equal to the metric N_e when N_b is multiplied by the generation time (Waples 1989). When we separated out each collection of larvae and juveniles based on BY, we were able to estimate N_b; however, when we analyzed collections together as putative mixtures of BYs, our estimate of effective spawners was likely a hybrid between N_b and N_e. For consistency, we distinguish these latter estimates as N_e. Using our size proxies for aging, these collections were estimated to represent at least six BYs (2013–2018). The collection from the Elwha River mouth was split into the following three pairs of consecutive BYs to obtain sample sizes greater than 15 fish/sample: BYs 2013–2014, BYs 2015–2016, and BYs 2017–2018. The collection from Indian Creek was split into four BYs: 2013–2016. We estimated N_b for each BY sample within both sites to examine temporal trends.

Finally, we estimated N_e using collections of larvae and juveniles from undammed tributaries to the Strait of Juan de Fuca, ocean-phase lamprey, and adult returns at Willamette Falls. These estimates provided context for our estimates of N_e among collections within the Elwha River. For these comparisons, only collections with sample sizes greater than 30 were used to provide the most robust estimates. To avoid complexities of differing growth rates among tributaries and older life stages, we did not attempt to split each collection by estimated BY; however, length histograms were generated for each collection to aid interpretation of the results.

Our N_b analyses required information on BY composition of larvae to make accurate comparisons across collections. We used length-based age estimates to determine BYs. Larvae in the study ranged from 31 to 152 mm, while juveniles ranged from 86 to 170 mm, which generally translated to more recent versus older BYs, respectively. However, we also captured larvae and juveniles during a range of dates throughout the year, so we used the following methodology to standardize BY estimates. We delineated age-class intervals based on size by first defining a 1-year age-class informed by two data points of known ages (i.e., from PA). The length range of 30–59 mm was used as a proxy for yearlings, and a linear growth rate of 30 mm/year from year 1 to year 4 was used to delineate the subsequent age-classes: 2 (60–89 mm), 3 (90–119 mm), and 4 (120–150 mm). Juveniles of any length were assumed to be age 5 (the median Pacific Lamprey age at metamorphosis; Dawson et al. 2015). Collection dates were used in conjunction with observed body length to fine-tune the age estimates, and a single birthdate of

May 1 was assumed for consistency when estimating total age. For example, a larva measuring 30 mm collected on May 5, 2018 was assumed to have a birthdate of May 1, 2017 and an estimated age of 1 year and 4 d. For cases in which a larva was captured between October and December of a given year and had an observed length in the lower half of the size range, we assumed that it had experienced the full growing period of that year and we adjusted its birth date accordingly. For example, a larva measuring 30 mm that was collected on October 1, 2018 was assumed to have a birthdate of May 1, 2018 (age = 5 months). In contrast, a larva measuring 59 mm collected on October 1, 2018 was assumed to have a birthdate of May 1, 2017 (age = 1 year and 5 months). These length-based age-class estimates were verified by instances in which we had full-sibship families that were recaptured across years (we examined whether recaptures from a subsequent year fit into the expected range of body lengths for the subsequent age-class).

Objective 3: determine the relative productivity of streams within the Elwha River and its regional contribution.—We used SA to better understand which streams drive productivity in the Elwha River and how this productivity may influence the surrounding region. COLONY results were used to determine the composition of stream of origin for larvae and juveniles exiting the mouth of the Elwha River, in the ocean, and at Willamette Falls. The collections of larvae and juveniles from sites in the Elwha River basin were first ordered from upstream to downstream and by collection year within each site. We reconstructed full-sibling families among all individuals in the data set. For each pair of full siblings, we assumed that the full-sibling family originated nearest to the sibling collected at the most upstream site and was present at least since the earliest collection year of either sibling. For example, a juvenile collected in the screw trap at the Elwha River mouth was categorized as having a natal origin from Indian Creek if it had a full sibling that was collected in Indian Creek. For lamprey collected from the Elwha River basin, we estimated BY using length-based age methodology to further characterize the composition of natal origins in each collection. One natal origin site (Indian Creek) was detected in relatively high abundance ($N > 15$) at the mouth of the Elwha River. To better understand ages at out-migration, we compared the composition of Indian Creek ages (length-based estimates) to that of ages at the river mouth for the same collection year.

RESULTS

Power and Accuracy of Genetic Methods

For PA and SA applications, we omitted all but one locus for each LD pair because parentage requires

independent loci. We identified 138 significant locus pairs in LD (involving 49 unique loci) within the four major pooled groups: North (sites A and B), South (sites P and Q), lower Columbia River (sites G2, G3, H, and I), and interior Columbia River (sites J2, J3, K, and L; Figure 1A; Table S1; Hess et al. 2013). One locus per LD pair was dropped, for a total of 28 dropped and 21 retained, which left a total of 263 independent loci.

The 263 SNP loci were chosen to be highly informative for parentage applications across the species' range; the average MAF across 16 reference populations (Hess et al. 2013) with N greater than 20 varied from 5% to 50%, with a mean average MAF of 31%. The study data set (Table 1) was cleaned to remove individuals with low genotyping success (>10% missing loci) and duplicate individuals (different at ≤ 5 of 263 loci); all loci were genotyped at greater than 70% for this entire data set, with an average genotyping rate of 99%. The MAF for the data set was similar to the Rangewide values (i.e., average MAF across four pooled populations [ELWHA_EF, ELWHA_ST, OLY_EF, and ELWHA_pbt] varied from 2% to 50%, with a mean average MAF of 30%). The Rangewide data set (Hess et al. 2013) was also trimmed in a similar way to reduce failed individuals ($n = 9$; >10% missing loci), and no duplicates were found for 263 SNPs. For the trimmed Rangewide data set, a total of 509 individuals across 21 collections were available for analysis.

The 263 SNPs demonstrated high power for single parent assignments based on the simulations with the singleSEQUOIA function: analysis-wide averages were 0.0% type I errors and 0.1% type II errors across 40 simulations (average accuracy = 99.9%) for the reference collections ($N = 509$ individuals; 21 populations). These values were equivalent to those generated using the two candidate parent pools (i.e., ELWHA_pbt and MISC_Adult).

The two GSI baselines (Rangewide and Olympic Peninsula) were found to have accuracies that were lower than our 90% target based on self-assignment rates in leave-one-out tests. However, the Rangewide GSI baseline performed best and had the greatest potential for utility (Table 2). Weighted average self-assignment rates of 21 collections to five reporting groups of the Rangewide GSI baseline ranged from 54% to 87% (weighted mean = 72%; Table 2). STRUCTURE analyses helped to guide the construction of reporting groups for the Rangewide baseline, as the mean estimates of ln[Pr(X/K)] increased steeply at K-values from 1 to 4 and then plateaued at K-values from 5 to 7. These results indicated that structuring among the reference collections showed support for as many as seven different groups (Figure S1 available in the Supplemental Materials in the online version of this article), and the delineation into five geographic regions was compatible with the pattern of STRUCTURE coefficients of ancestry (Table S2). In contrast with the accuracies of the Rangewide GSI baseline, the Olympic Peninsula self-assignment rates to 14 groups were lower (Table S3) and ranged from 0% to 81% (weighted mean = 31%). STRUCTURE analysis results were not helpful for guiding delineation of groups in the Olympic Peninsula baseline, as the estimates of ln[Pr(X/K)] continued to increase with increasing K-values from 1 to 6 without any apparent correspondence between coefficients of ancestry and geographic regions. Pooling the Olympic Peninsula baseline into two groups increased self-assignment success to 64% and 73% for collections inside and outside the Elwha River basin, respectively (weighted mean = 67%; Table S4).

Objective 1: Natal Origins of Pacific Lamprey in the Elwha River

Our first objective was to use PA, SA, and GSI to determine the origins of Pacific Lamprey larvae and juveniles collected in the Elwha River and thereby attribute production to translocation versus natural recolonization. Results using the PA method supported our expectation that production was primarily driven by natural colonization, as evidenced by a lack of parent assignments to translocation parents. We had expected that natural colonization would be a major source of productivity in the Elwha River given the relatively modest translocation effort (<50 adults) to date and because potential sources of Pacific Lamprey spawners were observed in nearby basins. The other two methods, SA and GSI, were not adequate for addressing this question. Sibship assignment could not be used to identify sources of fish from outside the Elwha River, and GSI was not precise enough to determine the basin of origin for adult colonizers.

There were no parent–offspring trios detected in our data set. This was not surprising given the small number of candidate parents that were genotyped ($N = 51$). However, there were two parents that were assigned as single parents based on COLONY (both adults were volitional migrants collected in the Indian Creek screw trap during 2017) and one of these assignments was concordant with SEQUOIA. Based on our single-parentage power analysis, we would expect only a few type II errors (0.1% of 1,815 candidate offspring; ~2 offspring if all 1,815 candidates were true offspring of parents in the baseline) but would not expect false positives. This expectation fits the small discordance with COLONY (i.e., a putative single false negative observed). Both parents were collected in Indian Creek during the summer (June and July) of 2017, and the two larvae were both collected in October 2018 from the Elwha River screw trap (site 44), which makes their approximate ages around 1 year (1.2 and 1.3 years between collection dates). The larvae measured 40 and 52 mm, respectively, and would have grown 30 and 38 mm in a year (assuming that larvae are 4 mm at hatch; Yamazaki et al. 2003).

TABLE 2. Cross validation matrix for the leave-one-out procedure on reference collections in the Pacific Lamprey genetic baseline using 291 single-nucleotide polymorphism loci. Groups are northern British Columbia (NORTBC), Vancouver Island/Puget Sound/lower Columbia River (VIPSLC), Willamette River and Bonneville Reservoir (WILBON), interior Columbia River (INTCOL), and southern U.S. West Coast (SOUTUS). Sample sizes (N) and average self-assignments (Avg. self) are provided for each reference collection. Collections are composed of individuals analyzed by Hess et al. (2013).

Population	Group	N	Collection	NORTBC	VIPSLC	WILBON	INTCOL	SOUTUS	Avg. self (weighted)
A Pop01	NORTBC	17	Nass River	73.7	0.0	9.1	17.1	0.0	63.9
B Pop02	NORTBC	9	Skeena River	45.3	0.0	0.2	54.5	0.0	
C Pop03	VIPSLC	4	Deadman River	0.1	24.6	0.2	75.0	0.0	69.2
D Pop04	VIPSLC	30	Stamp River	0.0	96.7	0.0	0.0	3.3	
E Pop05	VIPSLC	4	Hood River	0.0	100.0	0.0	0.0	0.0	
F Pop06	VIPSLC	30	Toutle River	0.0	43.6	25.9	0.0	30.5	
G1 Pop07	WILBON	30	Willamette River 1995	1.3	6.0	46.5	40.0	6.2	53.9
G2 Pop08	WILBON	29	Willamette River 2011, early	2.0	19.2	56.2	13.9	8.7	
G3 Pop09	WILBON	30	Willamette River 2011, late	3.0	11.8	60.7	16.6	7.9	
H Pop10	WILBON	29	Klickitat River	0.0	9.9	56.3	25.7	7.9	
I Pop11	WILBON	30	Fifteenmile Creek	0.0	0.0	50.1	43.0	6.9	
J1 Pop12	INTCOL	30	Deschutes River 1995	0.0	0.0	1.3	98.7	0.0	86.5
J2 Pop13	INTCOL	30	Deschutes River 2011, early	1.8	0.0	41.9	53.1	3.3	
J3 Pop14	INTCOL	30	Deschutes River 2011, late	0.0	0.0	20.8	75.9	3.2	
K Pop15	INTCOL	30	Wenatchee River	0.0	0.0	4.3	95.7	0.0	
L Pop16	INTCOL	39	Entiat River	0.0	0.0	1.0	99.0	0.0	
M Pop17	INTCOL	5	Methow River	0.0	0.0	0.0	100.0	0.0	
N Pop18	INTCOL	29	Snake River	0.1	0.0	9.1	90.8	0.0	
O Pop19	SOUTUS	9	Coquille River	0.0	3.8	11.3	0.0	84.9	77.4
P Pop20	SOUTUS	30	Rogue River	0.0	12.4	5.7	2.2	79.7	
Q Pop21	SOUTUS	35	Klamath River	0.0	11.4	14.9	0.1	73.6	
Total		509							

Given the limited parent assignments, we could show direct evidence of reproductive success for volitional adults but not for translocated adults. The Rangewide GSI baseline showed that collections of larvae and juveniles from the Elwha River (ELWHA_EF and ELWHA_ST) and those from the surrounding Olympic Peninsula (OLY_EF) were most similar to the reporting group representing the regions of Vancouver Island, Puget Sound, and the lower Columbia River (VIPSLC; Figure 2). This level of geographic resolution did not provide informative results for the current objective. The finer geographic scale of the Olympic Peninsula GSI baseline showed some potential for distinguishing the Elwha River basin versus other river basins but with moderate accuracy according to self-assignments (Table S4). The Little River (site 36) was founded by some translocated larvae from Salt Creek and did have one of the highest rates of assignment to "outside" the Elwha River (68%). However, high "outside" assignment rates were also found in Campground Creek (75%; site 16), a side channel that was washed away the year before the collection was made. The Campground Creek site was located relatively far upstream in the upper basin of the Elwha River and would not be expected to have relatively high rates of influence from the outside compared to lower tributaries, but the recent washout may have influenced the genetic composition.

The sibship simply determined that spawning is likely semelparous, and adults that successfully spawn in one basin do not appear to spawn again in adjacent basins. Hence, neither sibship nor GSI could be used to determine the basin of origin of volitional adults that contributed to Elwha River larval abundance.
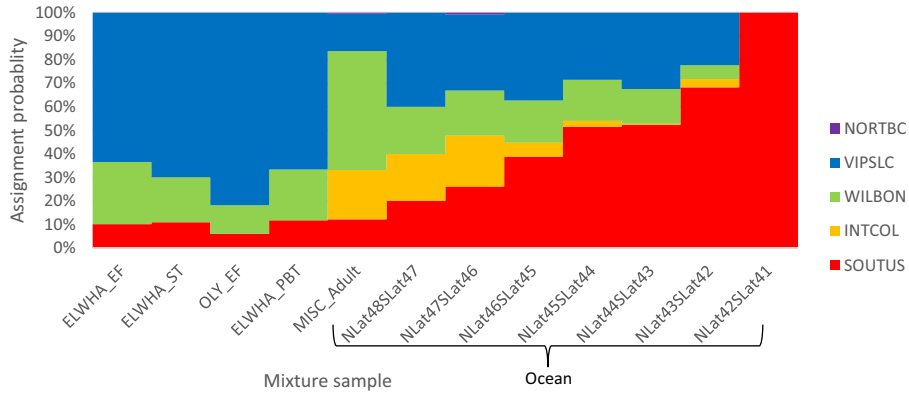
FIGURE 2. Genetic stock composition of Pacific Lamprey samples in this study. Samples include ELWHA_EF, ELWHA_ST, OLY_EF, ELWHA_pbt, MISC_Adult, and Ocean (described in Table 1 and Methods). The colors correspond to proportions of individuals that assigned to the five reporting groups of the Rangewide genetic stock identification baseline: northern British Columbia (NORTBC), Vancouver Island/Puget Sound/lower Columbia River (VIPSLC), Willamette River and Bonneville Reservoir (WILBON), interior Columbia River (INTCOL), and southern U.S. West Coast (SOUTUS; Table 2).

We observed full sibship among fish within the six regional collections that were represented in our data set (Elwha, Lyre, East Twin, and West Twin rivers, Deep Creek, and the Ocean); however, there were no full siblings shared between basins and no full sibship among or within the Ocean collections (Figure 3). Within the Elwha River basin, there was a total of 36 full-sibling families (366 individuals represented the 36 families) that were recaptured across space, time, or both.

We were able to test for positive somatic growth relationships for the 26 full-sibling families that were recaptured across different years (i.e., testing whether individuals from the same family grew larger over time). There were 13 positive and 13 negative growth relationships for these recaptured families, suggesting inconsistent growth between full-sibling recaptures. However, when we examined the five sibship families that contained at least three or more "recaptures" from each of the collection years, we found that four of the five families showed positive growth (Figure S2a). Furthermore, growth appeared to decrease with increasing age when we plotted the estimated age at first capture versus the estimated growth rate (Figure S2b). This relationship may explain why full-sibling families recaptured across different years do not always show positive growth. It may also indicate that length-based age estimates will be less accurate for older ages due to increasing violation of our assumption that growth rate is constant throughout larval life.

Objective 2: Quantify Effective Spawners in the Elwha River

Our second objective was to quantify the numbers of Pacific Lamprey spawners in the Elwha River and determine whether current numbers of spawners have reached

the capacities observed in neighboring undammed basins. Our expectation was for an increase in N_b to occur as rapidly as the increase in larvae observed in Indian Creek during the 5 years after dam removal from the Elwha River. The larvae captured in the screw trap at Indian Creek increased 14-fold (from 132 larvae in 2014 to 1,805 larvae in 2016; Moser and Paradis 2017). Similarly, we estimated that N_b increased 12-fold across the span of at least four BYs (2013–2016) that were represented in our collections of larvae and juveniles from the Indian Creek screw trap in 2018. Furthermore, N_b measured at the mouth of the Elwha River (representative of the overall abundance in the basin) also increased over a 5-year period to values similar to those estimated across neighboring undammed tributaries of the Olympic Peninsula, which indicated that robust recolonization has likely occurred in the Elwha River basin.

The N_e values were generally smallest within the Elwha River (average $N_e = 92$; range = 5–256), larger in other basins of the Olympic Peninsula (average $N_e = 189$; range = 124–264), and larger still in the Ocean collections (average $N_e = 644$; range = 461–755) and Willamette Falls adult collections (average $N_e = 1,374$; range = 843–1,875; Figure 4). The one exception was the electrofished and translocated collection from the Little River (site 36), which had an N_e of 256 (95% CI = 149–805).

The trends in N_b for the screw trap collections grouped according to BY were positive for both the Elwha River mouth and Indian Creek (Figure 5). The oldest BY collection (spawn year [SY] 2013–2014) at the mouth of the Elwha River had an N_b value of 14 (95% CI = 7–32), which increased by 11× to an N_b of 160 (95% CI = 70–∞) over a 5-year period (Figure 5). The oldest BY (SY 2013) in Indian Creek had an N_b of 5 (95% CI = 2–20), which
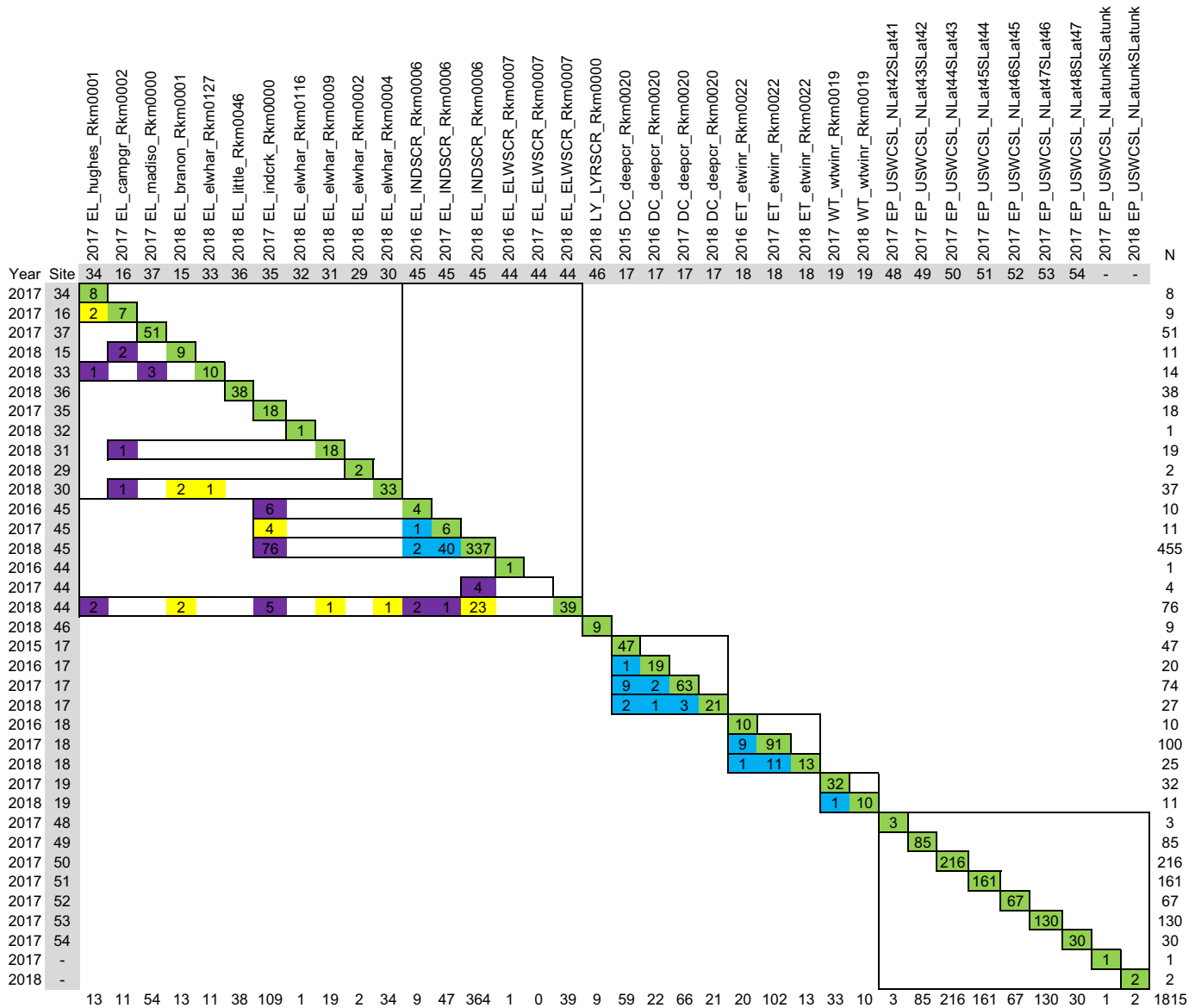
FIGURE 3. Sibship sharing among collections of Pacific Lamprey. Colors indicate the number of full-sibling families that were first captured from their original site (green), first captured in the same year but at a different location (yellow), first captured in a different year but at the same site (blue), or first captured in a different year and at a different site (purple). Site numbers correspond with those in Figure 1 and Table 1 and site names correspond with Table S5.

increased approximately 12× by SY 2015 and stabilized at an N_b of 60 (95% CI = 36–122) through SY 2016 (Figure 5). The N_b values observed for the most recent BYs were not significantly smaller (95% CIs overlapped) than the levels in the undammed basins of the Olympic Peninsula. Moreover, the mean estimate for the most recent BYs of the Elwha River (BY 2017–2018; $N_b = 160$) was in range with the N_e estimates of the undammed basins (Deep

Creek, East Twin River, and West Twin River: N_e range = 124–264). The N_e estimates for the undammed basins were more akin to N_b estimates because the Olympic Peninsula collections were primarily comprised of large juveniles representing a single BY (BY 2012; Figure S3). Therefore, our results demonstrated that the most recent BY N_b estimate for the Elwha River has increased over a 5-year period to levels similar to the N_b for undammed tributaries.
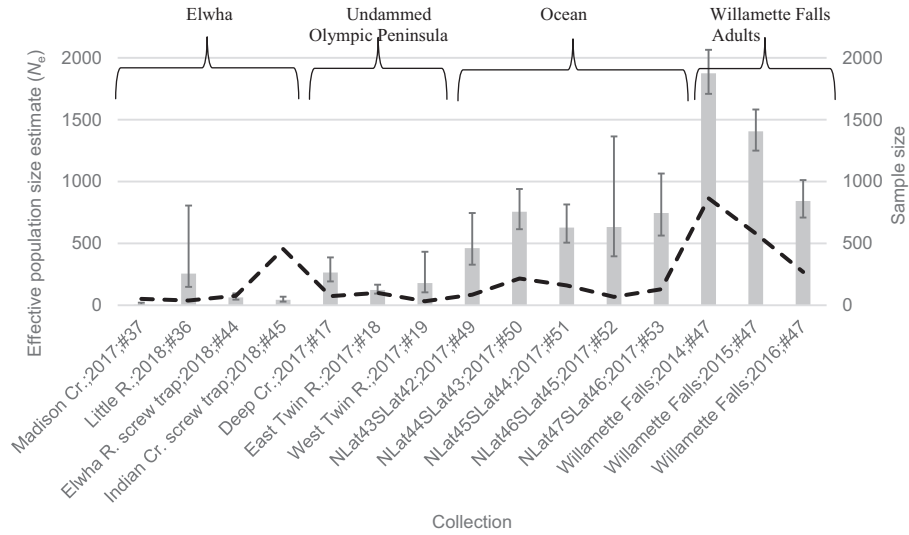
FIGURE 4. Effective population size estimates (N_e; ±95% CI) and sample sizes (dashed line, secondary y-axis) for each Pacific Lamprey collection. Collections are grouped by region and indicate the site name, collection year, and map site number (Table 1; Figure 1) separated by semicolons.
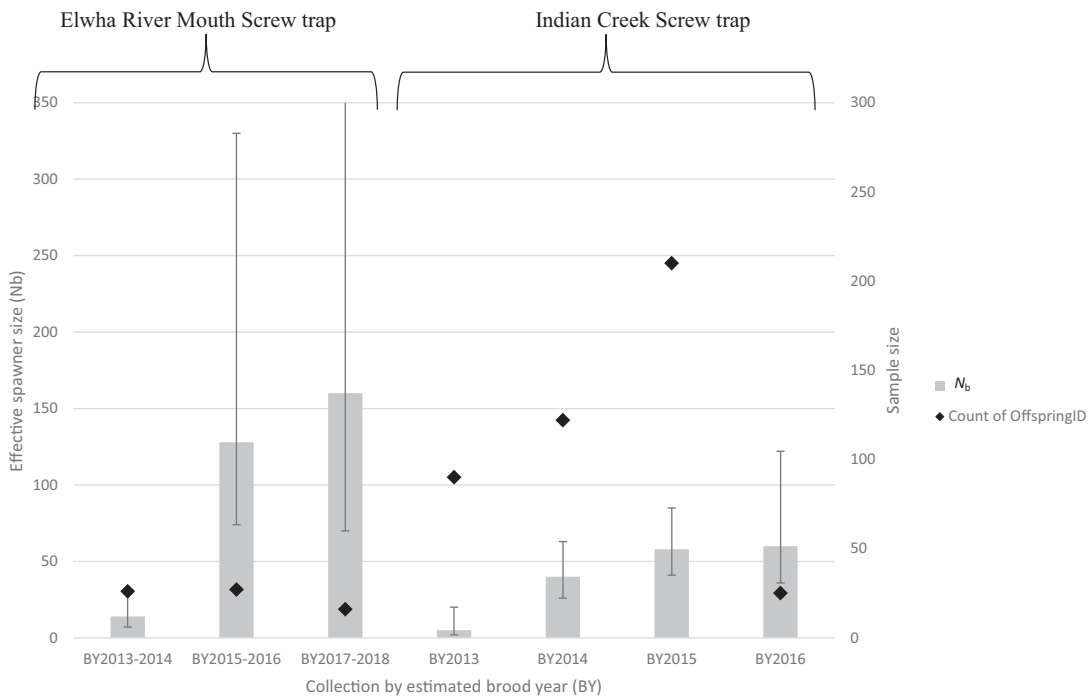

FIGURE 5. Estimates of the effective number of breeders (N_b; ±95% CI) and sample sizes (diamonds, secondary y-axis) for screw trap collections of larval and juvenile Pacific Lamprey in 2018 from the Elwha River basin. Collections are grouped by brood year (BY), as estimated by length-based ages, presented in order from oldest to youngest BY within each site.

Objective 3: Relative Productivity of Elwha River Pacific Lamprey

Our third objective was to determine the relative productivity of streams within the Elwha River and how overall productivity originating from this system is distributed across the broader surrounding region. We expected that within the Elwha River, Indian Creek would be a relatively large source of production given its demonstrated high productivity for salmonids in this basin. Indian Creek, the first tributary upstream from the former Elwha Dam, is an

extremely productive stream based on rapidly increasing juvenile production of several salmonids since dam removal (i.e., Chinook Salmon, Coho Salmon, and steelhead; McHenry et al. 2020). Using SA methods, we found that this stream was also a primary source of Pacific Lamprey larval and juvenile production for the Elwha River basin, as evidenced by the high percentage (i.e., ~41%) of Indian Creek fish detected in the Elwha River mouth screw trap.

Finally, we predicted that the contribution of Pacific Lamprey from the Elwha River to the broader surrounding region would be relatively small given its recent founding and small area relative to surrounding sources of Pacific Lamprey. No emigration from the Elwha River could be detected in neighboring streams or in coastal ocean-phase collections using PA and SA methods. While this may indicate the Elwha River's relatively small contribution to panmictic coastal stocks, it may also be an indication of the migratory patterns of Pacific Lamprey (Clemens et al. 2019). Genetic stock identification demonstrated that the genetic composition of ocean-phase lamprey was reflective of latitudinal stock structure. Each of the genetic methods we used provided complementary information that is key to management, and our approach could be useful for application to other highly dispersive species.

We estimated distance traveled for the 32 full-sibling families that were recaptured across space (individual N = 337). Among the electrofishing sites, the largest movements were over 12 km downstream from Brannon, Campground, and Hughes creeks (sites 15, 16, and 34) to sites near the river mouth (sites 30 and 44). The sites with the greatest geographic diversity of natal origins for full-sibling families were near the river mouth, where fish exit to the Strait of Juan de Fuca. Thus, using the sibship results, we were able to trace large portions of the Elwha River productivity based on composition of natal origins of the collections obtained at the river mouth screw trap. In fact, nearly half ($N = 37$; 49%) of all fish in the 2018 collection at the Elwha River mouth screw trap were identified from full-sibling families that were previously sampled upstream from the screw trap (6 unique sites), and most of those fish originated from Indian Creek ($N = 31$, 41%; Figure 6). The remaining 51% of fish in the 2018 Elwha River mouth screw trap collection were unassigned because not all full-sibship families represented in the sample had been previously encountered in the sampling efforts.

Interestingly, the composition of ages (estimated based on length and life stage) at the Indian Creek screw trap in 2018 was weighted toward young ages (i.e., BYs 2013, 2014, and 2015 were 21, 27, and 46%, respectively); however, individuals that were identified as Indian Creek origin at the Elwha River mouth in 2018 were weighted
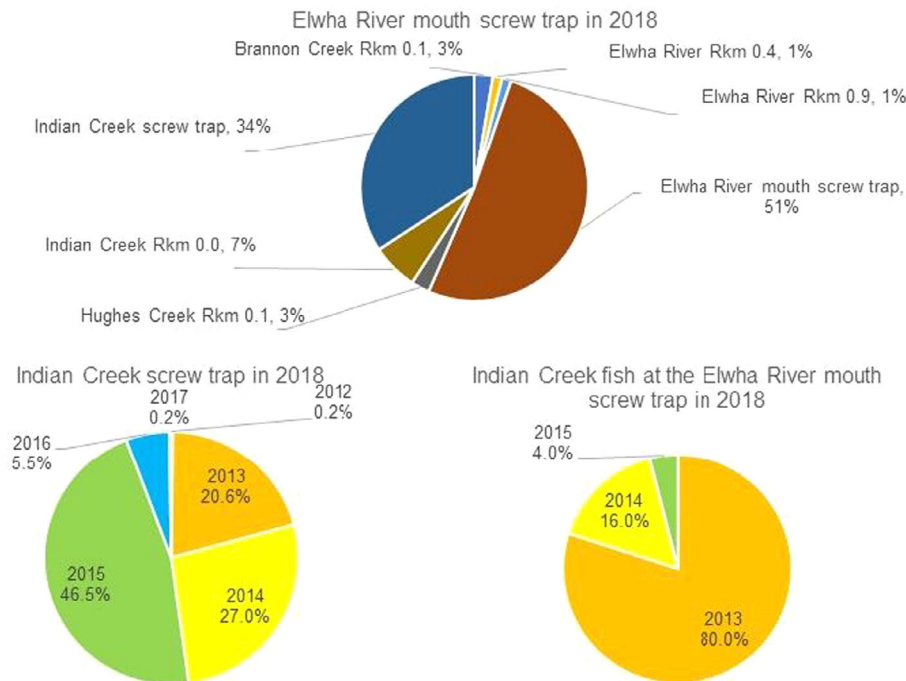

FIGURE 6. Relative percent composition of Pacific Lamprey sibling-derived natal origins (listed by site; rkm = river kilometer) at the Elwha River mouth screw trap in 2018 (top); length-based age composition (brood year) of fish at the Indian Creek screw trap (bottom left); and length-based age composition of Indian Creek fish that were "re-captured" at the Elwha River mouth (bottom right).

oppositely (i.e., 80, 16, and 4% for BYs 2013, 2014, and 2015, respectively; Figure 6). This reverse weighting in composition of ages may indicate either that fish tend to reside in the main-stem Elwha River before emigrating from the river or that there is dramatically different size selection between screw traps (i.e., the Elwha River mouth screw trap selects for larger fish). The evidence points to the former explanation because the overall age composition in 2018 across all streams of origin at the Elwha River mouth trap was relatively even across BYs, similar to the Indian Creek trap (i.e., composition at the Elwha River mouth was 29, 9, 25, 14, 17, and 6% for BYs 2013–2018, respectively). The wide length frequency distribution in the Elwha River screw trap during 2018 (Figure S3) indicates a high degree of age overlap among fish that exit the river, even though there may be a period of residence in the Elwha River main stem.

Sibship was not shared between any collections outside of any basin; therefore, we could not use sibship to make any determinations of Elwha River-origin fish outside of the Elwha River. Instead, we relied on GSI applications for broader regional observations. On average, the individuals in the Elwha River data set across the four pools assigned at a probability of 70% to the most proximate reporting group (i.e., VIPSLC, probability range = 63–82%; Figure 2). The adult collection from Willamette Falls was expected to assign with greatest probability to the Willamette River–Bonneville Reservoir (WILBON) reporting group, and, in fact, 51% assigned to WILBON (Figure 2). The remaining assignment probabilities for the Willamette Falls adult collection were shared among the following three reporting groups (ordered from lowest to greatest probability): Southern U.S. West Coast [SOUTUS], VIPSLC, and interior Columbia River [INTCOL]; assignment probabilities of 12, 16, and 21%, respectively). Relatively high assignment probabilities across multiple reporting groups were similarly observed for the Willamette Falls reference collections (Table 2), which showed leave-one-out assignment probabilities (from least to greatest) of 8% for SOUTUS, 12% for VIPSLC, 23% for INTCOL, and 54% for WILBON.

The assignment probabilities of the unknown ocean-phase mixture showed correlation with geography. For example, the assignment probabilities were inversely proportional with latitude of the ocean-phase collections for SOUTUS and proportional with latitude for INTCOL. The reporting group that included Elwha River and other Olympic Peninsula origins, VIPSLC, showed the highest assignment probability (40%; Figure 2) in the ocean-phase collection (site 54; Figure 1) that was most proximal to these northern Washington sites. A range of modest assignment probabilities to VIPSLC (32–37%) was observed in the ocean-phase collections (sites 50–53; Figure 1) distributed further south to 43.0°N (Figure 2). The

assignment probabilities to VIPSLC decreased to 22% and 0% (Figure 2) for the ocean-phase collections at the most extreme southern sites, 49 and 48 (Figure 1), respectively. These results suggest a relatively broad ocean distribution of VIPSLC (surrogate for Olympic Peninsula origins) from 48.0°N to 43.0°N, with decreasing abundance in the extreme southern end of the ocean-phase survey. This broad distribution contrasts with the more restricted INTCOL reporting group, which was found with an average assignment probability of over 20% (Figure 2) in just the two northernmost ocean-phase collections (sites 53 and 54; Figure 1).

DISCUSSION

Using genetic methods, we were able to demonstrate that adult Pacific Lamprey in the Elwha River were (1) successfully reproducing based on direct evidence from parentage, (2) increasing in abundance through time at rates similar to the observed rate of increase in larval abundance, (3) producing exceptionally high numbers of offspring from one source stream known for its high productivity of salmonids, and (4) likely contributing to heterogeneous mixtures of Pacific Lamprey in the coastal ocean.

Dam removals provide the opportunity for natural recolonization of Pacific Lamprey and other anadromous fishes to previously inaccessible upstream habitat. In the Elwha River drainage, our applications of PA, SA, and GSI provided multiple insights into the process of natural recolonization and into the species' biology in general.

Production by Volitional Migrants versus that of Translocated Adults

When habitat becomes accessible after dam removal or habitat improvements, it is useful to assess the relative merits of taking a passive approach to lamprey recolonization (relying only on volitional migrants) versus actively translocating lamprey to repopulate the newly available habitat (Reid and Goodman 2020). Due to the modest numbers of adults translocated in our study and the limited years available to monitor any translocation production, we could not fully assess the relative merits of these alternative approaches. However, we did document direct evidence of successful spawning by two volitional adults. This suggests that with greater sampling, it would be possible to track the reproductive efforts of both volitional and translocated fish on a larger scale. Compared to volitional migrants, it is sometimes more feasible to sample candidate parents that are translocated into a system (Hess et al. 2015). For example, in the Umatilla River basin, hundreds of adult Pacific Lamprey are genetically sampled and released onto spawning habitats every year (Close et al. 2009). However, our smaller study permitted release of fewer than 50 translocated adults, and none of

these fish was detected as a parent among the collections of larvae that were obtained. Although this was likely due to the small sample, it is also possible that these fish were undetected for other reasons. One possibility is that most of the translocated adults were released in 2018, producing younger, smaller larvae that were difficult to collect. All of the translocated lamprey were radio-tagged, which may have altered their spawning success. If more adults are translocated and sampled, this method could be effective for determining the relative contributions of translocated fish to existing populations.

Increasing Trends in the Effective Numbers of Spawners Signify a Robust Recolonization and General Use of N_b as a Proxy for Abundance

We found that the value of N_b within collections in the Elwha River basin was positively correlated with BY. This was consistent with our expectation for an increase in N_b over time, given the increased abundances of adults, larvae, and juveniles that were observed immediately after the Elwha Dam was removed (Moser and Paradis 2017). Therefore, results from this study supported the general use of N_b as a proxy for characterizing trends in relative spawner abundance. Furthermore, the use of N_e to estimate effective population size also appeared to reflect expected differences in abundances among the samples we compared—that is, the collections from streams within the Elwha River basin showed relatively low N_e compared to undammed tributaries surveyed in the Olympic Peninsula. This result is consistent with the expectation that streams within a recently recolonized tributary will be low in N_e relative to more established populations. All of the tributary collections showed lower N_e than ocean-phase collections because ocean-phase lamprey would likely represent mixtures of natal origins from multiple tributaries, thereby inflating N_e.

Field observations helped to validate N_b as a proxy for relative spawner abundance. For example, in Madison Creek the first two Pacific Lamprey spawners were observed in 2016. Our sibship reconstruction of electrofished larvae (estimated from BY 2016) in Madison Creek identified just three full-sibship families and five unique parents and estimated an N_b of 5 (95% CI = 2–20), which closely matched the low number of spawners that the field biologists were able to observe. A second example was found in the Indian Creek rotary screw trap, where no lamprey were observed until 2013, when nine adults were captured, and the first larvae were observed in 2014 (Moser and Paradis 2017). The genetic analysis of the juveniles captured (estimated from BY 2013) and sampled in the Indian Creek screw trap during 2018 identified 9 full-sibship families and 16 unique parents and estimated an N_b of 5 (95% CI = 2–20). From these examples, it appeared that the field biologists were able to observe at least half of the total adults that were estimated to have

successfully spawned in the Madison and Indian Creek sites. Given the difficulty observing this cryptic species, this is not a surprising result. The consistent relationship of the field observations and the genetic estimates (genetic estimates of unique numbers of spawners were approximately twice the number observed in the field) helps to validate these results.

Relative Productivity in the Elwha River

Our finding that Indian Creek is a primary source of production for Pacific Lamprey is important because it confirms that stream characteristics that are suitable for salmonid production deliver similar benefits for Pacific Lamprey. This evidence is helpful for habitat improvement projects targeting multiple species but with limited budgets. Our results also provided further evidence of how N_e can act as a useful proxy for abundance. In addition to evidence that Indian Creek was a primary source of Pacific Lamprey abundance for the Elwha River (i.e., a high proportion of Indian Creek siblings was observed at the Elwha River mouth screw trap), we estimated that Indian Creek had relatively high N_e compared to other tributaries. For example, Madison Creek production was not observed at all at the Elwha River mouth and had low N_e.

Distribution of Natal Origins in the Ocean Phase

There are many critical uncertainties concerning the marine phase of Pacific Lamprey, which include characterizing the distribution patterns of the various source stocks (Clemens et al. 2019). Although we could not use PA and SA to precisely estimate the contribution of Elwha River productivity to the broader region, GSI estimation may prove useful for characterizing the marine distributions of source stocks at a broad spatial scale. In our study, the ocean-phase collections showed a decreasing proportion of the GSI reporting group to which Elwha River and Olympic Peninsula Pacific Lamprey were most similar (i.e., VIPSLC) from the northern to southern latitudinal collections (40% to 0%, respectively). Consistent with the low but detectable regional patterns of isolation-by-distance gene flow of Pacific Lamprey as reported by Spice et al. (2012), our results provide evidence of relatively restricted movement compared to a state of panmixia. For example, although the VIPSLC stock is relatively broadly distributed along the coast, it appears to concentrate near natal rivers. In general, Pacific Lamprey stocks appeared as heterogeneous mixtures in the ocean, reflective of the geographic distribution of source stocks. This suggests that anadromous lamprey will occur most frequently in the ocean regions proximate to their natal river. However, our results indicated that marine patterns may depart from this basic assumption because the distribution of the INTCOL reporting group was weighted north in the ocean survey rather than uniformly distributed around

the mouth of the Columbia River. Finally, we found that there were relatively high probabilities of assignment to multiple reporting groups among adult fish in the collections from Willamette Falls. This suggests that Willamette Falls may harbor exceptional diversity of stock origins relative to the other areas that were sampled.

At finer scales within the Olympic Peninsula, GSI may be able to discern Elwha River-origin Pacific Lamprey from those in all other undammed tributaries of the Olympic Peninsula. However, this application is of limited utility because there are few places in which the Elwha River and these other Olympic Peninsula stocks occur as a mixed stock (e.g., Strait of Juan de Fuca). Furthermore, the low degree of self-assignment accuracy for finer-scale discrimination of different Olympic Peninsula tributaries diminishes the potential utility of GSI at fine spatial scales.

Although full-sibling families were detected between years and sites within basins, the occurrence of full siblings did not extend outside tributaries. This result supported the semelparous biology of the species: after adults return to freshwater, they typically lack the ability to repeat-spawn in neighboring tributaries. The high degree of spatial autocorrelation (i.e., lack of full-sibship detections between basins and presence of full siblings shared across time and space within basins) provides support for the accuracy of the SNP panel for pedigree reconstruction given the expectation that relatedness would be spatially autocorrelated. However, the lack of full-sibship sharing between any of the tributary collections and the ocean-phase collections suggests that our sample sizes were insufficient to overcome low detection probabilities, given the scale of mixed natal origins of all the tributaries represented by ocean collections. This low detection probability is one limitation of PA and SA, particularly if the objective is to identify natal origins on a broad spatial scale (e.g., ocean-phase mixed stock).

Use of Size and Life Stage as Proxies for Age-Class in Young Populations of Pacific Lamprey

Accurate aging of larval and juvenile Pacific Lamprey is a difficult challenge for fisheries managers. In bony fishes, otoliths and scales are useful anatomical features with which to derive ages (Campana 2001). Lampreys lack these structures, and statoliths are imperfect substitutes for otoliths (Potts et al. 2015). The use of lengths to derive ages has been implemented in other studies (Beamish and Medland 1988), albeit with increased severity of limitations for older ages (>5 years). Although PA can be an ideal method for aging (Hess et al. 2015), there were not enough parents sampled in this study. Parentage assignment was useful for ground-truthing several size-classes specific to the Elwha River basin, but we realized that there were likely increasing violations of our assumed constant growth rates with increasing age. From our validation testing with sibship

recaptures, our crude length–age relationship served as a useful proxy for age and to consistently separate BYs. Furthermore, similar length–age relationships could be useful in other study populations if they were founded within a short span of years as in the Elwha River (e.g., <5 years). However, systems that have achieved equilibrium for a long period could host enormous overlap in lamprey ages based on the protracted freshwater phase of larval lampreys (e.g., Sea Lamprey *Petromyzon marinus* larvae can persist for 0– 12 + years; Potter 1980), rendering length-based age estimation worthless.

Advancements in Technology

Advancements in technology have enabled implementation of genetic methods (PA, SA, and GSI) to determine natal origins for highly dispersive species. The feasibility of genotyping the high number of markers required by these methods is due to laboratory advancements. For example, use of genotyping-in-thousands by sequencing (Campbell et al. 2015) helped to overcome cost efficiency challenges in this study.

The selection of loci was also key to our success. Making the right choices required striking a balance among multiple objectives for management; SNPs that are ideal for discerning population-level differences (i.e., good GSI candidates) are often relatively poor in informativeness for PA and SA across the species' range (Hess et al. 2015). In this study, the loci selected showed utility for GSI at broad regional scales when using all available SNPs together (291, including all high-F_{ST} [genetic differentiation index] SNPs). We also employed a robust subset of these markers (i.e., 263 independent, high-MAF SNPs) that met the high-informativeness requirements for accurate single parentage analysis and sibship reconstruction. Together, these complementary methods allowed us to achieve our objectives and identify both regional and fine-scale patterns of relatedness for Pacific Lamprey within the Elwha River basin, across the local area in the Olympic Peninsula, and in the coastal ocean.

All of the methods we tested had useful attributes. Management objectives requiring fast turnaround and high precision for natal origin estimation may benefit from a focus on SA baseline sampling. However, when candidate parent sampling challenges can be overcome to achieve high rates of parent sampling, PA methods are advantageous because both age and natal origin can be accurately estimated (Steele et al. 2019). Finally, in cases where sampling resources are more limited and the high level of precision is not needed, GSI is likely the most cost-effective method of determining natal origins.

Conclusion

For a highly dispersive fish species like the Pacific Lamprey, these analyses, methods, and informative molecular

markers can help to illuminate a myriad of management and biological questions that had been stymied by the low population genetic structure of the species. Our results indicate that with more extensive sampling, it would be possible to estimate larval recruitment from various management units—whether they are translocated adult lamprey, artificially propagated larvae, or production from individual drainage basins. Understanding the sources and sinks of lamprey production has been an elusive goal and is critical to recovery of species at risk (Lucas et al. in press). Genetic methods and more extensive sampling could put this goal within reach and shed light on a plethora of unknowns in lamprey biology (e.g., duration of the ocean phase, oceanic distribution and movements, life history variation, etc.).

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SUPPORTING INFORMATION

Additional supplemental material may be found online in the Supporting Information section at the end of the article.