

Patterns of Intra- and Interspecies Genetic Diversity in Klamath River Basin Suckers

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Abstract.—The Klamath River basin, Oregon, is home to four catostomid species: the Klamath smallscale sucker *Catostomus rimiculus*, the Klamath largescale sucker *Catostomus snyderi*, the shortnose sucker *Chasmistes brevirostris*, and the Lost River sucker *Deltistes luxatus*. A decline in Lost River and shortnose sucker abundances resulted in these species being listed as endangered under the U.S. Endangered Species Act in 1988. The primary aims of this study were to measure interspecific and intraspecific genetic variation in Klamath River basin suckers by examining 15 microsatellite loci. Over 300 suckers were sampled from four subbasins within the Klamath River basin (Lost River, upper Klamath–Sprague rivers, lower Klamath River, and upper Williamson River) and one region outside of the basin (Rogue River). A hierarchical analysis of population subdivision based on groups generated from microsatellite genetic distance (R_{ST}) data was performed by use of analysis of molecular variance. Assignment of individuals to known populations was performed with the program WHICHLOCI. The program STRUCTURE was used to assign individuals probabilistically to homogeneous clusters without population or species identifiers. Klamath smallscale and Lost River suckers were genetically distinct from each other and from shortnose and Klamath largescale suckers. Sympatrically occurring shortnose and Klamath largescale suckers from the Lost and upper Klamath–Sprague River subbasins were genetically indistinguishable from one another, which indicates that interspecific hybridization has occurred between these two species. Managing for the long-term survival and recovery of endangered Klamath River basin suckers should focus on preserving the genetic diversity of all four sucker species. Continued protection of endangered shortnose suckers requires the protection of Klamath largescale suckers, since each of these species may be a crucial contributor to the evolutionary legacy of the other. Maintenance of healthy and abundant sucker spawning and rearing habitat should minimize the chance of losing genetically unique populations while maintaining genetically diverse populations and facilitating the recovery of all Klamath River basin suckers.

The catostomid family of freshwater fishes comprises over 65 species that are widely distributed throughout North America (Miller 1959). Several catostomids, or suckers, are currently listed as threatened or endangered under the U.S. Endangered Species Act and have become the focus of recovery efforts in many western states. The Klamath River basin, Oregon, is home to four catostomid species, including the Klamath smallscale sucker *Catostomus rimiculus*, the Klamath largescale sucker *Catostomus snyderi*, the shortnose sucker *Chasmistes brevirostris*, and the Lost River sucker *Deltistes luxatus*. Shortnose and Lost River suckers were once among the most abundant lake-dwelling fish in the Klamath River basin and supported large native and commercial fisheries and canneries earlier in the last century (Cope 1879; Coots 1965; Andreasen 1975). Large runs of suckers

throughout the 1960s supported a popular sport fishery, although the species' decline became evident toward the end of the decade (Vincent 1968; Golden 1969). Surveys conducted from 1984 to 1987 indicated a major decline in Lost River and shortnose sucker abundances (Bienz and Ziller 1987), resulting in these species being listed as endangered under the Endangered Species Act (USFWS 1988). Factors that have been implicated in the decline of shortnose and Lost River suckers include habitat alteration through river damming, marsh drainage, instream flow diversion, and declining water quality associated with livestock grazing and agricultural activity (Perkins et al. 2000a).

The upper Klamath River basin dates back to the Miocene period and is an ecologically complex system with a long geologic and climatic history (Miller and Smith 1981). Although long-term geologic and climatic changes have largely influenced the evolution of suckers, relatively recent anthropogenic alterations may have also contributed to the genetic patterns observed today. In the early 1900s, an extensive system of dikes,

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canals, and dams was constructed throughout the Klamath River basin to drain the marshes and provide water for agricultural irrigation and hydroelectric power generation. Upper Klamath Lake is one of the largest freshwater lakes in the western USA and is the central feature of the upper Klamath River basin (Figure 1). Upper Klamath Lake's primary sources are the Williamson and Sprague rivers to the north, and the lake's outflow is controlled at the Link River Dam to the south. Much of the water released from the lake is diverted by canal to the Lost River or is passed through hydroelectric facilities before reaching the Klamath River. The continued diversion of waterways and conversion of wetlands has resulted in the loss of adult spawning habitat and lakeshore nursery habitat for larval suckers. Changes in water quality have also adversely affected fish populations; such changes are especially evident in highly eutrophic Upper Klamath Lake. Annual fish kills between 1995 and 1998 attributed to recurrent poor water quality in Upper Klamath Lake resulted in the death of most adult shortnose and Lost River suckers in the lake (Perkins et al. 2000a).

Klamath River basin suckers have elongate, muscular bodies and are distinguished by lip size, gill raker

morphology, and preferred habitat. Klamath largescale and Klamath smallscale suckers are river-dwelling species that have large, fleshy lips and widely spaced gill rakers (Miller and Smith 1981; Buettner and Scopettone 1991). Both species are primarily bottom grazers, preferring to feed on invertebrates and algae. By contrast, Lost River and shortnose suckers are primarily lake-dwelling species that have smaller lips and prefer to feed on benthic macroinvertebrates (Markle and Simon 1993; Scopettone et al. 1995). The complex gill raker structure of shortnose suckers suggests that this species is also a filter feeder that targets zooplankton in larger bodies of water (Moyle 1976). In addition, these four species are not completely sympatric (Figure 1). Klamath largescale suckers are restricted to the upper Klamath River basin and primarily occupy the Sprague and Williamson rivers, which are the two main tributaries feeding Upper Klamath Lake. Klamath largescale suckers also occupy the main rivers of the Lost River subbasin. Klamath smallscale suckers are not restricted to the upper Klamath River basin; in addition to occupying the lower Klamath River, they are common in the neighboring Rogue River and Trinity River systems. Lost River and shortnose suckers are restricted to the

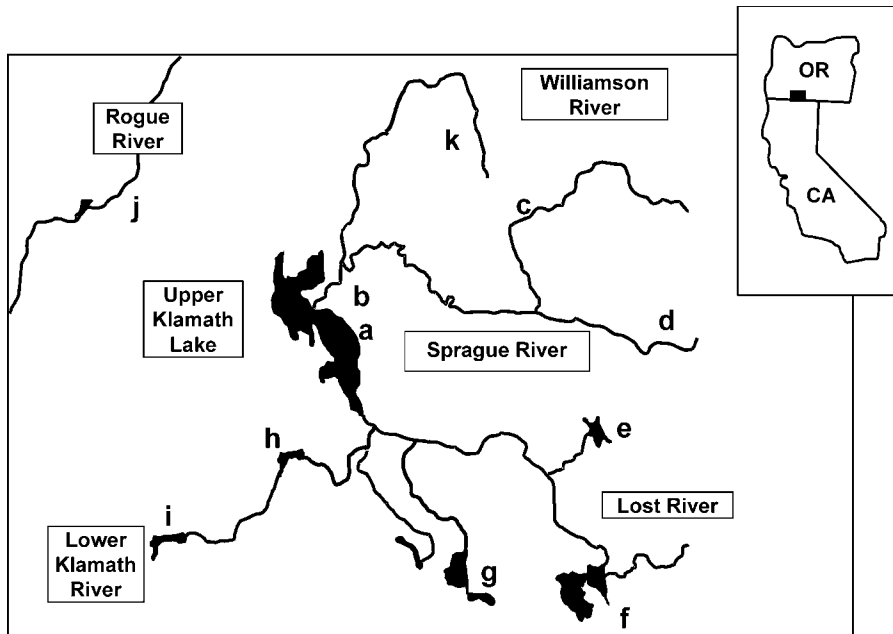


FIGURE 1.—Map of the upper Klamath River basin, Oregon, and sucker sampling sites. Suckers were collected from the Rogue River and from four subbasins: upper Klamath–Sprague rivers (UK), Lost River (LR), lower Klamath River (LK), and upper Williamson River (UW). Sample sites (with their respective subbasins) were (a) Ouxy and Sucker springs of Upper Klamath Lake (UK), (b) the lower Sprague River (UK), (c) Sycan River (UK), (d) upper Sprague River (UK), (e) Gerber Reservoir (LR), (f) Clear Lake (LR), (g) Tule Lake (LR), (h) Topsy Reservoir (LK), (i) Copco Reservoir (LK), (j) Rogue River, and (k) upper Williamson River (UW).

upper Klamath River basin and occur sympatrically in the larger lakes and reservoirs of the Upper Klamath Lake system and in the Lost River subbasins. Shortnose suckers are also common in the reservoirs of the lower Klamath River subbasin. Lake-dwelling Lost River and shortnose suckers make spring spawning runs into adjacent tributaries (Moyle 1976; Scoppettone and Vinyard 1991) and may have temporal and spatial overlap with Klamath largescale suckers in the Sprague River system.

Earlier morphologic and genetic studies have not resolved uncertainties regarding the systematic relationships among the four taxa, and there is little information concerning the basinwide population structure of these species (Miller and Smith 1981; Harris 1991; Harris and Markle 1993). In many cases, morphometric and meristic ambiguities prevent definitive assignment of individuals to a species, suggesting that introgressive hybridization has occurred among Klamath River basin suckers. The primary aims of this study were to (1) measure between-species genetic variation in Klamath River basin suckers collected from four regions of the Klamath River basin and (2) determine whether representatives from each of the four sucker species collected throughout basin make up genetically distinct populations. We present the results of a genetic evaluation based on 15 microsatellite loci, which are discussed in the context of management guidelines recommended by Allendorf et al. (2001), and specific recommendations are targeted toward the recovery of these species.

Methods

Extensive sampling in 1993 throughout the Klamath River basin and the Rogue River (Figure 1) targeted spawning groups of each of the four species and yielded 309 adult representatives from multiple year-classes (Table 1). All individuals were identified to species morphologically by personnel from the U.S. Bureau of Reclamation and Oregon Department of Fisheries and Wildlife. Attempts were made to sample 30 individuals from each spawning group. Four subbasins were sampled within the Klamath River basin (Lost River [LR], upper Klamath–Sprague rivers [UK], lower Klamath River [LK], and upper Williamson River [UW]). Lost River suckers were collected from Clear and Tule lakes in the LR subbasin ($n = 19$) and from the lower Sprague River and Upper Klamath Lake, including Ouxy and Sucker springs within the UK subbasin ($n = 24$). Shortnose suckers were collected from Gerber Reservoir and Clear Lake in the LR subbasin ($n = 62$), from Copco and Topsy reservoirs in the LK subbasin ($n = 14$), and from the

lower Sprague River and Upper Klamath Lake, including Ouxy and Sucker springs, in the UK subbasin ($n = 31$). Klamath largescale suckers were collected from Gerber Reservoir, Clear Lake, and Tule Lake in the LR subbasin ($n = 37$), from the lower and upper Sprague River and the Sycan River in the UK subbasin ($n = 29$), and from the upper Williamson River in the UW subbasin ($n = 27$). Klamath smallscale suckers were collected from Copco and Topsy reservoirs in the LK subbasin ($n = 23$) and from the Rogue River ($n = 29$).

Genomic DNA was extracted from dried fin samples by use of the tris–NaCl–EDTA–sodium dodecylsulfate–urea procedure (Belfiore and May 2000). All samples were analyzed at 15 microsatellite loci by use of oligonucleotide primers cloned from the Lost River sucker (Tranah 2003). Amplification of target loci was done with 1 unit of *Taq* DNA polymerase (enzyme number 2.7.7.7; IUBMB 1992), 0.4 μM of each primer, 3–10 ng of template DNA, 100 or 175 μM of deoxynucleotide triphosphate, and 1.5–2.5 mM of MgCl_2 in 50 μL of buffer; amplification was conducted in an MJ Research PTC-100 (Waltham, Massachusetts) with a “hot bonnet” lid. We used the following thermal profile: 94°C for 3 min, 35 cycles (94°C for 1 min, 52°C for 30 s, 72°C for 30 s), and 72°C for 5 min. Polymerase chain reaction products were separated on a 5% denaturing acrylamide gel and were visualized with a Molecular Dynamics 595 fluorimager (Sunnyvale, California). The amplification products were fluorescently detected by use of single-primer labeling with fluorescein. Alleles were sized by means of fragment analysis (Molecular Dynamics).

Codominant genotypes were classified and recorded for each microsatellite locus. Allele frequencies per locus and expected heterozygosity (H_E) were calculated with FSTAT 2.9.1 updated from Goudet (1995). Observed heterozygosity (H_O) and deviations from Hardy–Weinberg expectations were calculated with GENEPOP 3.0 (Raymond and Rousset 1995), and significance testing was performed with permutation (Guo and Thompson 1992). Genotypic linkage disequilibrium was measured with GENEPOP 3.0 based on Fisher’s method of analyzing contingency tables (Mehta and Patel 1983).

Pairwise genetic distance was calculated with GENEPOP 3.0, and multilocus estimates of microsatellite genetic distance (R_{ST}) were used (Slatkin 1995). The R_{ST} data were used to generate a tree based on the unweighted pair-group method with arithmetic averages (UPGMA) by use of the NEIGHBOR application in PHYLIP (Felsenstein 1995) and Tree-View 1.6.6 (Page 1996). A hierarchical analysis of

TABLE 1.—Diversity of *Dlu* microsatellite loci among Klamath River basin, Oregon, sucker populations sampled in 1993. Abbreviations and other terms are as follows: *n* = mean number of individuals scored per locus; alleles = number of alleles detected per locus; H_O = observed heterozygosity; H_E = expected heterozygosity; asterisks indicate significant deviation from Hardy–Weinberg equilibrium.

Species	Population	Microsatellite locus (<i>Dlu</i>)														
		476	488	4105	4139	4283	4153	4296	4184	4235	4259	4314	461	4123	466	4183
Shortnose sucker	Lost River (<i>n</i> = 62)															
	Alleles	17	23	22	14	18	15	26	21	18	18	18	13	14	27	
	H_O	0.89	0.90	0.97	0.94	0.89	0.86	0.98	0.95	0.86	0.92	0.77	0.90	0.97	0.88	0.90
	H_E	0.92	0.93	0.95	0.93	0.88	0.88	0.94	0.93	0.92	0.89	0.92*	0.93	0.86	0.89	0.94
	Lower Klamath (<i>n</i> = 14)															
	Alleles	13	17	17	13	12	15	19	14	14	20	15	13	12	10	12
	H_O	0.93	1.00	0.80	1.00	0.80	0.93	1.00	0.93	1.00	1.00	0.80	0.92	1.00	0.83	0.83
	H_E	0.93	0.96	0.96*	0.92	0.92	0.93	0.96	0.94	0.92	0.98	0.95*	0.93	0.92	0.89	0.92
	Upper Klamath–Sprague (<i>n</i> = 31)															
Alleles	18	23	17	12	16	15	21	18	18	20	16	22	11	13	22	
H_O	0.87	0.84	0.84	0.88	0.91	0.91	0.97	0.91	0.91	1.00	0.72	0.88	0.90	0.72	0.85	
H_E	0.94	0.94	0.94	0.92	0.91	0.92	0.95	0.94	0.95	0.95	0.89	0.94	0.92	0.87*	0.97*	
Lost River sucker	Lost River (<i>n</i> = 62)															
	Alleles	17	16	10	11	6	13	16	15	14	10	10	9	12	8	16
	H_O	0.94	0.95	0.89	0.74	0.68	0.95	0.95	0.89	0.89	0.84	0.72	0.88	0.74	0.79	0.78
	H_E	0.93	0.91	0.84	0.89	0.79*	0.90	0.93	0.89	0.92	0.88	0.86	0.88	0.84	0.87	0.94
	Upper Klamath–Sprague (<i>n</i> = 24)															
	Alleles	17	18	18	16	14	17	19	22	18	20	13	16	16	11	22
	H_O	0.96	0.96	0.91	0.96	0.92	0.92	0.96	0.92	0.91	1.00	0.83	0.95	0.92	0.87	0.88
	H_E	0.92	0.97	0.91	0.90	0.87	0.92	0.92	0.96	0.95	0.93	0.92	0.91	0.92	0.81	0.93
	Klamath largescale sucker	Lost River (<i>n</i> = 37)														
Alleles		14	21	21	17	16	15	20	20	14	15	19	17	13	12	25
H_O		0.89	0.89	1.00	0.97	0.74	0.97	0.85	0.87	0.76	0.92	0.83	0.89	0.78	0.91	0.92
H_E		0.89	0.94	0.94	0.94	0.80	0.91	0.94*	0.94	0.91*	0.90	0.93*	0.93	0.86	0.88	0.95
Upper Klamath–Sprague (<i>n</i> = 29)																
Alleles		14	23	16	14	14	14	14	19	18	15	16	16	15	10	18
H_O		0.93	0.93	0.93	0.90	0.71	0.90	0.87	0.90	0.78	0.97	0.80	0.86	0.97	0.87	0.86
H_E		0.95	0.95	0.93	0.89	0.89*	0.92	0.94*	0.92	0.93*	0.93	0.92	0.95	0.88	0.89	0.93*
Upper Williamson (<i>n</i> = 27)																
Alleles		7	16	15	12	8	13	11	10	15	9	15	13	11	6	11
H_O		0.94	0.96	0.89	1.00	0.57	0.96	0.83	0.87	0.93	0.93	0.64	0.79	0.77	0.56	0.52
H_E		0.84	0.92	0.89	0.88	0.74*	0.81	0.82	0.76	0.91	0.85	0.91*	0.87	0.81	0.73*	0.78
Klamath smallscale sucker	Lower Klamath (<i>n</i> = 23)															
	Alleles	16	22	14	12	11	15	25	20	19	18	17	21	14	9	17
	H_O	0.96	1.00	0.92	1.00	0.96	0.87	1.00	0.96	0.96	0.96	0.95	0.95	0.88	0.86	0.91
	H_E	0.93	0.96	0.93	0.90	0.85	0.92	0.97	0.95	0.95	0.93	0.95	0.95	0.92	0.84	0.89
	Rogue River (<i>n</i> = 29)															
	Alleles	16	19	21	14	15	17	19	10	16	14	17	16	10	5	16
	H_O	0.86	0.93	0.90	0.97	0.90	0.90	0.90	0.97	0.87	0.83	1.00	0.83	0.90	0.50	0.36
	H_E	0.92	0.95	0.96	0.92	0.90	0.91	0.93	0.86	0.92	0.92	0.94	0.93	0.84	0.72*	0.87*

population subdivision was performed by use of analysis of molecular variance (AMOVA) based on genetic distance (F_{ST}) from ARLEQUIN 2.000 (Schneider et al. 2000). We assigned populations to groups based on the UPGMA tree generated for R_{ST} data.

Using the critical population method of WHICHLOCI 1.0 (Banks et al. 2003), an individual-based assignment method, we tested the accuracy of assignment for individuals to each population. Assignment accuracy was based on a minimum assignment threshold of 95% and a maximum misassignment

threshold of 5%; no threshold log-likelihood ratio was implemented. The program STRUCTURE (version 2.1) was used to assign individuals probabilistically to homogeneous clusters (*K* clusters) without population or species identifiers (Pritchard et al. 2000). The posterior probability was calculated for each value of *K* by use of the estimated log-likelihood of *K*. All tests for *K* were performed at 100,000 Markov chain Monte Carlo repetitions and a 100,000-run burn-in period without population identifiers and assuming correlated allele frequencies and admixture.

TABLE 2.—Estimates of R_{ST} between all Klamath River basin sucker populations for suckers collected from the upper Klamath–Sprague River subbasin (UK), Lost River subbasin (LR), lower Klamath River subbasin (LK), upper Williamson River subbasin (UW), and the Rogue River (RR) in 1993. Species include shortnose (SN), Klamath largescale (LS), Lost River (LR), and Klamath smallscale (SS) suckers.

Population	Population									
	SN–UK	LS–UK	SN–LK	LS–LR	SN–LR	LS–UW	LR–UK	LR–LR	SS–LK	SS–RR
SN–UK	0.000									
LS–UK	0.005	0.000								
SN–LK	0.009	0.000	0.000							
LS–LR	0.024	0.020	0.021	0.000						
SN–LR	0.037	0.029	0.026	0.004	0.000					
LS–UW	0.059	0.046	0.050	0.099	0.106	0.000				
LR–UK	0.108	0.103	0.065	0.136	0.158	0.115	0.000			
LR–LR	0.133	0.118	0.095	0.142	0.162	0.143	0.008	0.000		
SS–LK	0.177	0.159	0.179	0.236	0.242	0.275	0.309	0.355	0.000	
SS–RR	0.208	0.188	0.241	0.295	0.278	0.274	0.358	0.437	0.142	0.000

Results

Summary data for all microsatellite loci are listed in Table 1. Fifteen to 48 alleles were observed per polymorphic locus (all of which displayed patterns consistent with disomic inheritance), and randomized tests for nearly all loci across populations revealed no evidence of linkage disequilibrium. Additional linkage disequilibrium testing was performed for each of the sympatric “shortnose–largescale” populations from the LR and UK subbasins, and we observed no evidence for linkage disequilibrium in either of these groups.

Average H_o ranged from 0.81 in Klamath largescale suckers from the UW subbasin to 0.94 in Klamath smallscale suckers from the LK subbasin. Most population genotypic distributions conformed to Hardy–Weinberg expectations (Table 1). Deviations from Hardy–Weinberg expectations at *Dlu4314* and *Dlu4183* might suggest the presence of null alleles at these two loci.

Estimates of R_{ST} between all Klamath River basin sucker populations are presented in Table 2, and an unrooted UPGMA tree based on these R_{ST} estimates is presented in Figure 2. Lost River suckers from the LR and UK subbasins were closely related ($R_{ST} = 0.008$) and formed a distinct cluster on the UPGMA tree. Similarly, Klamath smallscale suckers from the LK subbasin and the Rogue River formed a distinct UPGMA cluster; however, the genetic divergence between these two populations was greater than that observed for Lost River suckers ($R_{ST} = 0.142$). Among the shortnose sucker and Klamath largescale sucker populations sampled, clustering was based on sampling location rather than taxonomic status. Shortnose and Klamath largescale suckers from the UK subbasin formed a cluster with shortnose suckers from the LK subbasin. Lost River subbasin shortnose and Klamath largescale suckers clustered independently from the

UK and LK subbasin samples. Klamath largescale suckers from the UW subbasin were the most divergent of all shortnose and Klamath largescale suckers sampled. Within-basin interspecific R_{ST} values for shortnose and Klamath largescale suckers from the LR ($R_{ST} = 0.004$) and UK ($R_{ST} = 0.005$) subbasins were lower than the between-basin intraspecific R_{ST} values for shortnose ($R_{ST} = 0.037$) and Klamath largescale ($R_{ST} = 0.020$) suckers from the LR and UK subbasins.

A hierarchical analysis of population subdivision was performed by assigning populations to groups based on the UPGMA tree generated for R_{ST} data. Populations were assigned to the following five groups: (1) shortnose and Klamath largescale suckers from the UK subbasin and shortnose suckers from the LK

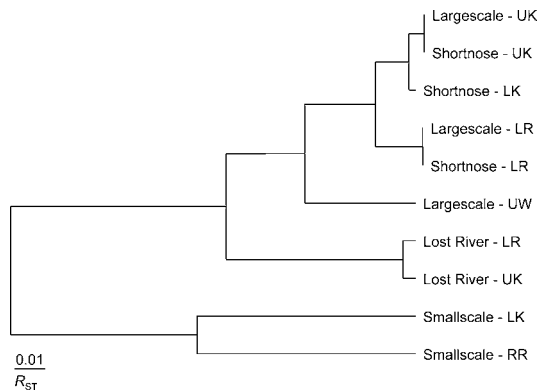


FIGURE 2.—Unrooted tree based on the unweighted pair-group method with arithmetic averages of microsatellite genetic distance (R_{ST}) values for Klamath River basin suckers collected from the Rogue River (RR) and from four subbasins: upper Klamath–Sprague River subbasin (UK), Lost River subbasin (LR), lower Klamath River subbasin (LK), and upper Williamson River (UW). Species are Klamath largescale, shortnose, Lost River, and Klamath smallscale suckers.

TABLE 3.—Assignment of individual Klamath River basin suckers to populations. Suckers were collected from the upper Klamath–Sprague River subbasin (UK), Lost River subbasin (LR), lower Klamath River subbasin (LK), upper Williamson River subbasin (UW), and the Rogue River (RR). Species include shortnose (SN), Klamath largescale (LS), Lost River (LR), and Klamath smallscale (SS) suckers. Assignments were based on minimum 95% assignment and maximum 5% misassignment thresholds; no threshold log-likelihood ratio was implemented. The percentage of individuals correctly assigned to the population or species of origin is indicated.

Reference population	Population assignment										Correct assignments	
	SN–UK	LS–UK	SN–LK	LS–LR	SN–LR	LS–UW	LR–UK	LR–LR	SS–LK	SS–RR	% population	% species
SN–UK	5	3	15	2	0	0	1	5	0	2	15	61
LS–UK	2	12	12	1	0	0	0	3	1	0	39	42
SN–LK	0	1	12	1	0	0	0	1	0	0	80	80
LS–LR	0	1	9	7	19	0	1	1	0	1	18	21
SN–LR	1	2	12	28	21	0	0	1	0	0	32	52
LS–UW	0	0	0	0	0	30	0	0	0	0	100	100
LR–UK	0	1	7	0	0	0	13	3	0	0	54	67
LR–LR	0	0	0	0	0	0	3	16	0	0	84	100
SS–LK	0	0	7	0	0	0	1	2	12	1	52	57
SS–RR	0	0	0	0	0	1	0	0	0	28	97	97

subbasin; (2) shortnose and Klamath largescale suckers from the LR subbasin; (3) Klamath largescale suckers from the UW subbasin; (4) Lost River suckers from the LR and UK subbasins; and (5) Klamath smallscale suckers from the LK subbasin and the Rogue River. The AMOVA analysis of the genetic diversity found at these 10 loci showed that 97% of the allelic diversity was attributed to individuals within populations. Only 1% of the allelic diversity was found among populations within groups, and 2% was found among groups.

Overall assignment success of individuals to populations ranged from 100% for Klamath largescale suckers from the UW subbasin to 15% for shortnose suckers from UK subbasin (Table 3). In general, shortnose and Klamath largescale suckers from the UK and LR subbasins had poor rates of assignment. Interestingly, shortnose and Klamath largescale suckers from the UK and LR subbasins and Lost River suckers from the UK subbasin were frequently assigned to the LK subbasin population of shortnose suckers. Further investigation into the clustering of shortnose and Klamath largescale suckers in the LR subbasin by use of STRUCTURE did not support the presence of two clusters (Table 4). Predefined shortnose and Klamath largescale suckers in the LR subbasin had equal membership proportions in the two- and three-cluster models (Table 5). The STRUCTURE analysis of shortnose and Klamath largescale suckers in the UK subbasin supported the presence of two clusters (Table 4). In the two-cluster model for the UK subbasin, shortnose suckers had a greater proportion of membership in the second inferred cluster (0.61) than in the first inferred cluster (0.39), whereas Klamath largescale suckers had nearly equal membership in each inferred cluster (0.55 versus 0.46) (Table 5). When considering

shortnose and Klamath largescale suckers from the UK subbasin and shortnose suckers from the LK subbasin, STRUCTURE analysis supported the presence of two clusters (Table 4). In the two-cluster model, shortnose suckers from the UK subbasin again had a greater proportion of membership in the second inferred cluster (0.59) than in the first (0.41), while Klamath largescale suckers from the UK subbasin (first cluster proportion = 0.55; second = 0.45) and shortnose suckers from the LK subbasin (first = 0.45; second = 0.55) subbasin had nearly equal membership in each inferred cluster (Table 5).

Discussion

With the primary aims of measuring between-species genetic variation among Klamath River basin suckers and determining whether species representatives from different geographic locations make up genetically distinct populations, we collected samples throughout the Klamath River basin and used 15 microsatellite loci to conduct a genetic evaluation of these samples. The most divergent populations in our study were the Klamath smallscale suckers collected from the LK subbasin and the Rogue River. Of the four catostomid species that occur in the upper Klamath River basin, Klamath smallscale suckers are the least widely distributed and are only sympatric with shortnose suckers from the lower Klamath River. Additionally, geographic isolation of Klamath smallscale suckers from the Rogue and lower Klamath rivers is probably responsible for the significant genetic divergence found between the two populations in this study. Lost River suckers collected from the LR and UK subbasins were also genetically distinct from the other Klamath River

TABLE 4.—Bayesian clustering results for Klamath River basin shortnose and Klamath largescale suckers collected from the Lost River subbasin, upper Klamath–Sprague River subbasin, and the lower Klamath River subbasin. The letter *K* represents the number of subpopulations; values in bold italics indicate the most likely values of *K*.

Subbasin	<i>K</i>		
	1	2	3
Lost River	-8,059.6	-8,157.9	-9,060.4
Upper Klamath–Sprague	-5,279.3	-5,271.8	-5,348.8
Upper Klamath–Sprague and Lower Klamath	-6,595.4	-6,585.7	-6,633.0

basin suckers; however, divergence between these two populations was limited.

By contrast, shortnose and Klamath largescale suckers collected from the UK subbasin were indistinguishable, as were shortnose and Klamath largescale suckers collected from the LR subbasin. Within-species comparisons among shortnose and Klamath largescale suckers from the UK and LR subbasins revealed that each of these species is genetically distinct between these regions. Historically, the Lost River formed an extensive marshland that was linked to Upper Klamath Lake through periodic flooding, presumably allowing a free exchange of fish between these systems. The Lost River has since lost its historic legacy as a marshland through the construction of the Link River Dam to the south of Upper Klamath Lake. Water now flows from Upper Klamath Lake to the Lost River through a highly controlled canal, allowing limited one-way movement of shortnose, Klamath largescale, and Lost River suckers (Gutermouth et al. 2000). In addition, STRUCTURE analysis supported the presence of two population clusters among shortnose and Klamath largescale suckers more strongly in the UK subbasin than in the LR subbasin.

Among Klamath largescale and shortnose suckers, the Klamath largescale sucker population sample from the UW subbasin was the most divergent (and the only population in this study with a 100% correct assignment rate). Klamath largescale suckers from the upper Williamson River may have been isolated from Upper Klamath Lake by geographic distance and by intervening unfavorable habitat. The Williamson River has its headwaters in the northern part of the Klamath River basin, where it flows north and west before entering Klamath Marsh, filling shallow ponds, sloughs, and channels. The water collects to the south of the marsh, forming the lower Williamson River before joining the Sprague River and flowing into Upper Klamath Lake. This complex geography may be responsible for isolating upper Williamson River Klamath largescale suckers for a relatively long period of time and thus minimizing their contact with suckers from the southern basin.

Relatively poor rates of population assignment were found among Lost River, shortnose, and Klamath largescale suckers collected from the UK subbasin and Klamath smallscale suckers from the LK subbasin. Interestingly, many of the incorrectly assigned individuals were assigned as shortnose suckers from the LK subbasin. Like the Lost River, the lower Klamath River begins at the Link River Dam, but water flow into the lower Klamath River is not as highly regulated as the flow to the Lost River. Frequent passage of shortnose and Klamath largescale suckers from Upper Klamath Lake into the lower Klamath River has been documented (Peck 2000) and may account for the lack of genetic differentiation between shortnose suckers from Upper Klamath Lake and the lower Klamath River.

To explain the lack of genetic differentiation between sympatric shortnose and Klamath largescale suckers within the UK and LR subbasins, we propose

TABLE 5.—Proportional membership of each predefined population of Klamath River basin shortnose and Klamath largescale suckers in each inferred cluster (see Table 4). Abbreviations are as follows: UK = upper Klamath–Sprague rivers and LK = lower Klamath River; *n* is the number of individuals sampled.

Species	Inferred cluster			<i>n</i>
	1	2	3	
Lost River				
Largescale	0.50	0.50		39
Shortnose	0.50	0.50		65
Largescale	0.33	0.33	0.33	39
Shortnose	0.33	0.33	0.33	65
Upper Klamath–Sprague				
Largescale	0.55	0.46		31
Shortnose	0.39	0.61		33
Largescale	0.29	0.30	0.41	31
Shortnose	0.37	0.37	0.26	33
Upper Klamath–Sprague and Lower Klamath				
Largescale (UK)	0.55	0.45		31
Shortnose (UK)	0.41	0.59		33
Shortnose (LK)	0.45	0.55		15
Largescale (UK)	0.24	0.44	0.32	31
Shortnose (UK)	0.33	0.26	0.41	33
Shortnose (LK)	0.42	0.25	0.33	15

two possible scenarios. Our first scenario depicts Klamath largescale and shortnose suckers as ecophenotypes, or life history variants of the same species that show some subdivision with geography. Within this setting, Klamath largescale suckers represent a river form and shortnose suckers represent a lake form of the same species that coexist in both habitats. However, consistent morphological and ecological differences between Klamath largescale and shortnose suckers support the existence of two entities. In addition to differences in lip and gill raker morphology, Klamath largescale and shortnose sucker larvae and adults exhibit distinct ecological differences. While larval shortnose suckers proceed to lakeshore nursery habitat soon after hatching, larval Klamath largescale suckers remain in the stream environment, where they continue to reside as adults (Simon et al. 2000). As adults, shortnose suckers remain in large bodies of water and only return to the smaller tributaries to spawn. Differences in the timing of spawning runs have also been observed for shortnose and Klamath largescale suckers. Spring runs of Klamath largescale suckers have been observed from mid-March to early April, while shortnose suckers make spawning runs from mid-April to early May (USBR 2001). Moreover, the existence of allopatric populations of Klamath largescale and shortnose suckers in the upper Williamson and lower Klamath rivers, respectively, argues against these being ecophenotypes of the same species.

Our second scenario involves a significant degree of introgression between sympatric populations of Klamath largescale and shortnose suckers. Intraspecific genetic distance measures between LR and UK populations of Klamath largescale ($R_{ST} = 0.020$) and shortnose ($R_{ST} = 0.037$) suckers exceed interspecific distance measures for Klamath largescale and shortnose suckers from the LR ($R_{ST} = 0.004$) and UK ($R_{ST} = 0.005$) subbasins. The lack of genetic differentiation observed between sympatric populations of Klamath largescale and shortnose suckers is probably not due to low statistical power, since the microsatellite markers used in this study sufficiently resolved genetic differences between these species from different subbasins (including the UW and LK subbasins). This evidence lends support to the hypothesis that sympatric populations of Klamath largescale and shortnose suckers within the UK and LR subbasins have undergone introgression. The lack of linkage disequilibrium within combined "shortnose-largescale" populations from the UK and LR subbasins suggests that the potential introgression between these species is not a recent phenomenon and may be the result of longer-term interspecific hybridization.

Allendorf et al. (2001) recently published conserva-

tion guidelines for hybridizing species. They defined six categories of hybridization and developed conservation guidelines based on these specific categorizations. The primary distinction made by the authors is between anthropogenic and natural hybridization. The consequences of anthropogenic hybridization can range from hybridization without introgression to complete admixture of the participating taxa. Anthropogenic hybridization is often caused by species translocations or introductions (Allendorf and Leary 1988). Hybridization is considered natural when it occurs as a natural part of the evolutionary legacy of the taxa involved. The outcomes of natural hybridization include the formation of a hybrid taxon or a distinct hybrid zone and can result in ongoing natural introgression. Allendorf et al. (2001) described natural introgression as hybridization resulting in one or both of the species resembling the other genetically more than they resemble conspecifics while the two species maintain phenotypic and ecological differences. Shortnose and Klamath largescale suckers in the LR and UK subbasins seem to fit this definition, as intraspecific genetic differentiation between the LR and UK subbasin populations was greater than interspecific genetic differences for each of the sympatric populations. The mechanism for this phenomenon was proposed by Clarke et al. (1998) as occasional hybridization that leads to molecular leakage and the "convergence of neutral and mutually advantageous genes" in the two species. Since this type of introgression may be considered a part of the evolutionary process, Allendorf et al. (2001) recommend protection of the taxa that result from such hybridization. In the case of shortnose and Klamath largescale suckers, continued protection of shortnose suckers currently protected by the Endangered Species Act necessarily includes the protection of Klamath largescale suckers (not listed under the act), since each of these species may be a crucial contributor to the evolutionary legacy of the other.

Distinguishing between natural and anthropogenic causes of hybridization can be difficult, and in the case of Klamath River basin suckers we have no historical samples that would allow us to determine rates of introgression prior to human alteration of the basin. Catostomids are well known for the ability to hybridize in nature (Hubbs et al. 1943), and it has been estimated that 1 out of every 100 West Coast suckers is an interspecific hybrid (Hubbs 1955). Hybridization may be facilitated in Klamath River basin suckers because they are highly fecund and long-lived (Miller and Smith 1981), have an allotetraploid genetic heritage (Uyeno and Smith 1972; Ferris and Whitt 1980), and have temporal and spatial overlap during spawning

runs (Perkins et al. 2000b). Among the four species analyzed in this study, levels of genetic divergence among all four taxa are well within the range that is usually found between intraspecific populations. This may indicate that the four species in the Klamath River basin have naturally been exchanging genes for a relatively long period of time while maintaining distinct morphological and behavioral differences.

The loss of several spawning stocks has been documented for Barkley Spring and Harriman Springs and for Odessa, Fourmile, Crooked, Fort, and Seven-mile creeks (Andreasen 1975; Perkins et al. 2000a; USFWS 2001). Large sucker die-offs related to poor water quality were documented from Upper Klamath Lake in the mid-1990s and contributed to an estimated 80–90% reduction in the adult population size of both shortnose and Lost River suckers (Perkins et al. 2000a). It is unknown how the loss of several spawning stocks and the large die-offs in Upper Klamath Lake have affected patterns of genetic diversity in Klamath River basin suckers. Historically, suckers in the upper Klamath River basin adapted to many ecological shifts, but the rate of habitat change has dramatically increased since anthropogenic alterations began early in the last century, and interspecific hybridization is frequently documented in habitat that has been modified naturally or anthropogenically (e.g., Campton 1987; Arnold 1997). To most effectively facilitate the long-term survival and recovery of endangered Klamath River basin suckers in a rapidly changing ecosystem, management priorities should focus on preserving the genetic diversity of all four sucker species. Results of this study suggest that in addition to preserving maximum amounts of genetic diversity of endangered Lost River and shortnose suckers, particular attention should be paid to Klamath largescale suckers. In addition to the possible contribution of Klamath largescale suckers to the evolutionary legacy of the endangered shortnose sucker, the upper Williamson River population of Klamath largescale suckers may be among the most genetically unique populations of Klamath largescale or shortnose sucker in the upper Klamath River basin. Management practices that focus on preserving the maximum amount of genetic diversity in all four Klamath River basin sucker species must be especially mindful of all genetically unique populations, such as the upper Williamson River Klamath largescale suckers. Priority should be placed on maintaining healthy and abundant sucker spawning and rearing habitat, which should minimize the chance of losing genetically unique populations and should maintain large, genetically diverse populations and facilitate the recovery of all Klamath River basin suckers.

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