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Timing of Population Fragmentation in a Vulnerable Minnow, the Umpqua Chub, and the Role of Nonnative Predators

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ARTICLE

Timing of Population Fragmentation in a Vulnerable Minnow, the Umpqua Chub, and the Role of Nonnative Predators

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Abstract

We examined the distribution of Umpqua Chub *Oregonichthys kalawatseti*, an endemic, vulnerable minnow in western Oregon, and whether six ecological populations (based on distribution patterns) had sufficient genetic cohesion to be considered evolutionary populations. We also evaluated the influence of Holocene geological events and recent nonnative predator introductions on the timing of population formation or fragmentation. Based on data from 10 microsatellite loci, we found evidence for four evolutionary populations of Umpqua Chub. One population, in the Smith River, is isolated by the Umpqua estuary and is more than 100 river kilometers from the other three populations: Elk Creek, Calapooya Creek–Olalla Creek, and Cow Creek–South Umpqua River. Quantile regression was used to examine the timing of genetic divergence among evolutionary populations assuming a genetic isolation-by-distance model. The quantile regression suggested that the genetic differentiation index (F_{ST}) should change by at least 0.0002/km; most fragmentation was recent and with similar timing, but the Smith River isolation event may have been about 2–4 times older. We could not distinguish whether the timing of the Smith River isolation corresponded to the last major tsunami event or the introduction of Striped Bass *Morone saxatilis*, a likely predator. All population fragmentation appears to be relatively recent, with the three upstream populations restricted to third- and fourth-order streams, most likely fragmented by either nonnative Smallmouth Bass *Micropterus dolomieu*, which now dominate sixth-order streams, or in the case of Elk Creek, a dam. The mid-drainage Calapooya–Olalla population was the most genetically diverse and appeared to be a mix of the other populations, which showed a significant isolation-by-distance relationship to this population. We hypothesize that Umpqua Chub populations have formed and fragmented by peripheral isolation from a larger population, the remnant of which is the mid-drainage Calapooya–Olalla population.

The genus *Oregonichthys* is composed of small minnows endemic to western Oregon. One such member, the Oregon Chub *O. crameri*, from the Willamette River is listed as threatened under the U.S. Endangered Species Act (USFWS 2010); another, the Umpqua Chub *O. kalawatseti* from the Umpqua River, is considered a "sensitive–critical species" by the Oregon

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Department of Fish and Wildlife. During the first status survey in 1987, the Umpqua Chub was broadly distributed between Elk Creek and the South Umpqua River near the Umpqua National Forest boundary (Markle et al. 1991). One downstream, isolated, population was discovered in the Smith River, separated by the Umpqua River estuary and 100 river kilometers (rkm) from the nearest population in Elk Creek. However, during a subsequent survey in 1998 (Simon and Markle 1999), distributions upstream of Elk Creek appeared to be fragmented into smaller populations, Umpqua Chub being restricted to lower-order stream sites while many main-stem sites were predominately inhabited by nonnative Smallmouth Bass *Micropterus dolomeiu*.

Events that potentially isolate local populations of Umpqua Chub are temporally variable and encompass Holocene geological episodes to recent anthropogenic activities. For instance, a rising sea level approximately 2,500 years before the present (BP) would have created a salinity barrier (salinity >5‰) separating the Smith River from upstream populations (Nelson 1992; Briggs 1994; Witter et al. 2003). More recent Holocene events include periodic tsunamis with an average recurrence interval of 520 years and the most recent at 330 years BP ($\pm$ 50 years; Briggs 1994).

Potential anthropogenic events include introductions of nonnative predators and dam construction. Striped Bass *Morone saxatilis* were introduced into San Francisco Bay in 1879 and first collected in Coos Bay, Oregon in 1914. By 1945, they were sufficiently abundant such that 8,446 kg were landed from the Umpqua River (Lampman 1946). We assume that if predation pressure isolated the Smith River population, its effects were present by 1930–1950. Smallmouth bass were introduced into Takenitch Lake, 2 km north of the mouth of the Umpqua River, in 1924 or 1925 (Lampman 1946) and were accidentally released into the Umpqua River in 1964 (Simon and Markle 1999). By the 1970s, they were frequently reported in the South Umpqua and main stem. If Smallmouth Bass predation isolated upstream populations, as suggested by Simon and Markle (1999), their effects began prior to the 1987 survey and almost certainly by 1970. We have no way of knowing if their impacts were spatially uniform but assume they were variable over the period 1970–2008. In Elk Creek, the boundary between Smallmouth Bass and Umpqua Chub appeared to be demarcated by Cunningham Dam, which was constructed in 1968. In summary, potential isolation events for the Smith River could have been at approximately 2,500 or more years (Holocene sea level), 330 years (last tsunami), or 70 years (Striped Bass). Potential isolation events for other populations upstream of the Smith River would have been less than 40 years, if caused by Smallmouth Bass predation or in 1968 if attributable to the Cunningham Dam. All populations may simply reflect isolation by distance (IBD).

Understanding the processes of population fragmentation and formation obviously requires the ability to recognize populations, but population definitions are numerous and often vague. Waples and Gaggiotti (2006) have suggested two classes of definitions based on an ecological paradigm and an evolutionary paradigm. Their concept of an ecological population is "a group of individuals of the same species that co-occur in space and time and have an opportunity to interact with each other," while and an evolutionary population is "a group of individuals of the same species living in close enough proximity that any member of the group can potentially mate with any other member." Tagging studies to estimate numbers of migrants are a useful metric for ecological populations (Waples and Gaggiotti 2006) but are currently difficult for small fishes. For practical purposes, an ecological population is often recognized based on discontinuities in distribution. In these cases, a temporal component may be missing if sampling is carried out in a convenient season, such as summer, and spatial gaps between groups may or may not be real if detection is heterogeneous. In contrast, an evolutionary population requires information about gene flow and panmixia (Waples and Gaggiotti 2006).

Fragmented groups of Umpqua Chub appear to be ecological populations, but given the caveats above in recognizing populations based on single-season spatial discontinuities, we wanted to evaluate whether the nominal ecological populations meet the genetic cohesion criteria of evolutionary populations. Genetic data could also potentially address the timing of historical changes in connectivity between the Umpqua River and Smith River populations. Given concern for the decline in distribution of Umpqua Chub, we saw an opportunity to evaluate whether a larger population had been recently fragmented and, if so, to better understand the history and process of population formation and fragmentation. In 2006–2007, we rapidly surveyed all major tributaries to qualitatively map the distribution of known aggregations or populations, and in 2008 we took individual fin clip and otolith samples from 25 specimens in each of six nominal ecological populations to determine genetic and age structuring (Figure 1). Our objectives were to evaluate the degree of gene flow among the six ecological populations and determine if they were genetically distinct populations. In addition, we tested for a signal of isolation by distance and whether or not the timing of geographical or anthropogenic events contributed to population fragmentation.

METHODS

Surveys in 2006 and 2007.—During the summers of 2006 and 2007, we sampled 141 sites in the Umpqua River basin and noted the presence or absence of all species and performed count of Umpqua Chub and Smallmouth Bass within a stream distance of 50–400 m ($\bar{x}$ = 197 m; Figure 1). Snorkel surveys of 200 m were completed at 113 sites; beach seine hauls (5–11 hauls at 12 sites) and shoreline visual records (16 sites) were used to confirm presence in highly vegetated or otherwise inaccessible sites. Sample bias is unknown and distribution gaps may be real or artifacts of differential sampling efficiency.

Sample collections in 2008.—We collected juvenile and adult individuals from each of six ecological populations: the Smith River, Elk Creek, Calapooya Creek, Olalla Creek, Cow Creek,

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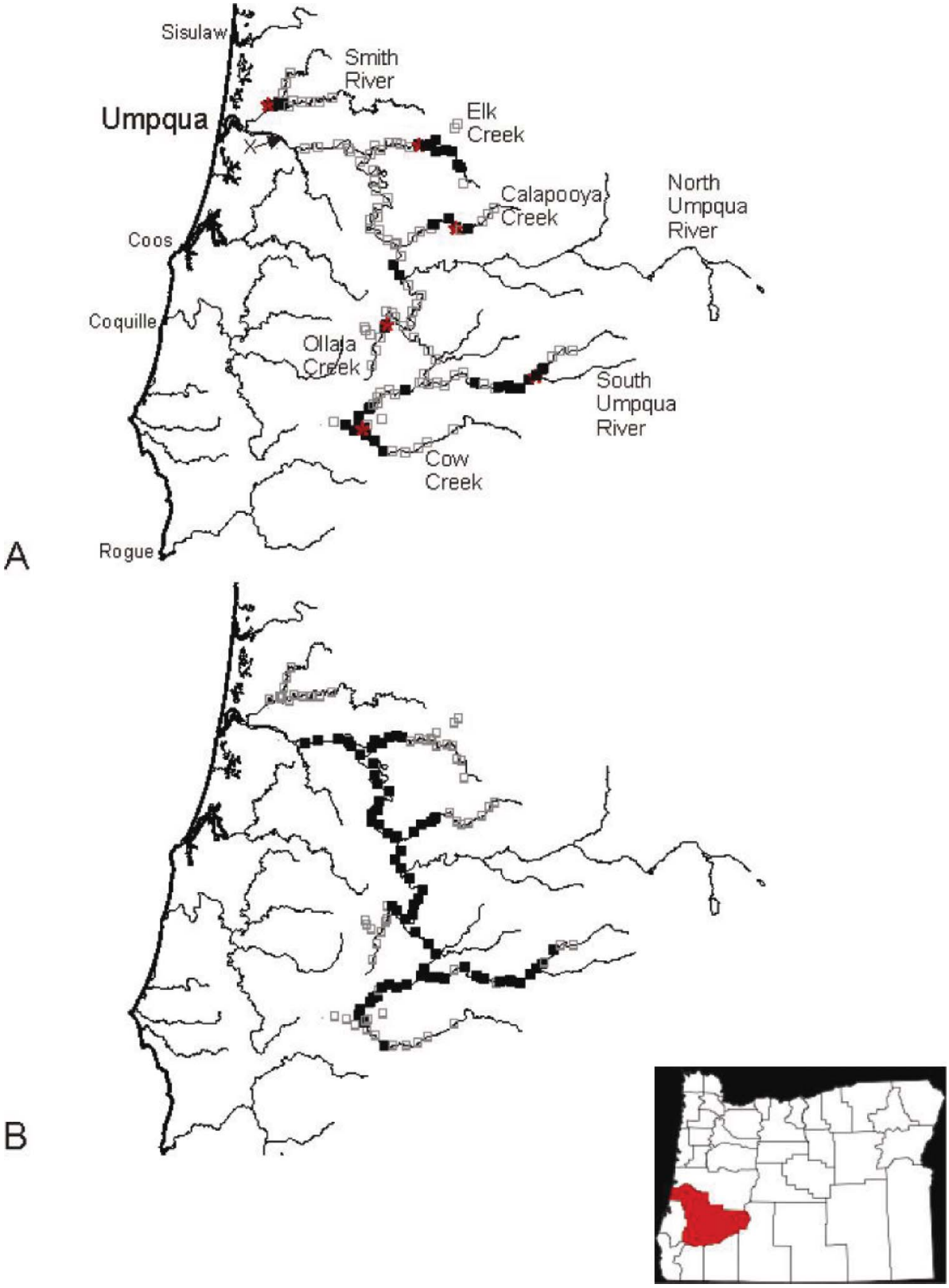

FIGURE 1. Panel **(A)** shows the distribution of Umpqua Chub across Umpaqua River sample sites, where solid squares indicate presence and open squares absence; the stars indicate tissue collection sites in 2008, and the × signs show the known upstream extent of tsunami influence (from Briggs 1994). Panel **(B)** shows the presence or absence of Smallmouth Bass at the same sites. [Figure available online in color.]

and the South Umpqua River using minnow traps and casts nets between 5 September and 2 October 2008. Twenty-five individuals from each population were preserved in 95% ethanol, and otoliths and fin clips were later removed in the laboratory Minimum distance (m) between populations was estimated based on boundaries identified during the 2006–2007 surveys.

Otolith-based aging.—We estimated average generation time from the age range of adults and extrapolation of age at maturity from Oregon Chub (Scheerer and McDonald 2003). Following the methods in Terwilliger et al. (2010), we removed the right lapillus from each fish. Ages were assigned from counts of growth increments that were comprised of a wide translucent and narrow opaque band, and all fish were assigned a nominal birthdate of 1 January. Blind counts of growth marks were made three times over the course of several weeks by one reader and the median age used.

DNA extraction and microsatellite genotyping.—Total genomic DNA was extracted from fin clips following the Glass Fiber Plate DNA Extraction Protocol (Ivanova et al. 2006). Polymerase chain reactions (PCR) were carried out in 5-μl volumes to amplify 11 microsatellite loci via fluorescently labeled primers: *Ocr100*, *Ocr101*, *Ocr103*, *Ocr104*, *Ocr106*, *Ocr110*, *Ocr111*, *Ocr112*, *Ocr113*, *Ocr114*, and *Ocr115* (Ardren et al. 2007). Reaction conditions were initial denaturation at 94°C for 3 min followed by 26 cycles at 94°C for 30 s, then annealing for 30 s at 58°C and 30 s at 72°C, and a final extension at 72°C for 7 min. We electrophoresed PCR products on an Applied Biosystems DNA Analyzer 3730XL and scored these products as length polymorphisms using GENEMAPPER.

Statistical analyses.—We tested populations for conformance to Hardy–Weinberg expectations (HWE) and linkage disequilibrium using the program GENEPOP version 4.0.01 (Raymond and Rousset 1995). We adjusted the initial critical value of 0.05 using sequential Bonferroni corrections (Rice 1989) to account for multiple comparisons made during these tests. To estimate measures of genetic diversity, including mean number of alleles per locus, and observed and expected heterozygosity, we used the program GENETIX (Belkhir et al. 2004).

We calculated pairwise genetic differentiation index (F_{ST}) values (Weir and Cockerham 1984) to estimate the level of genetic variation among each pair, and we used a permutation test with 1,000 iterations to assess the statistical significance of these estimates via GENETIX. To determine the level of genetic variation among populations, we executed exact tests for differences in genic and genotypic frequencies using GENEPOP.

To examine the spatial genetic relationship among the six ecological populations, we constructed a phylogenetic tree using the analysis package PHYLIP version 3.69 (Felsenstein 2005). We estimated chord distances (Cavalli-Sforza and Edwards 1967) between all population pairs in each data set using the program GENDIST and generated a neighbor-joining (NJ) tree using the NEIGHBOR program. To bootstrap the data and estimate statistical support for the topology of this consensus NJ tree, we used the program SEQBOOT. We displayed the trees with TREEVIEW (Page 1996).

Mantel tests for association between geographic and genetic distances among the six populations of Umpqua Chub populations were performed using the software Isolation by Distance Web Service (IBDWS; Jensen et al. 2005). We also used quantile regressions to describe distance as a limiting factor, or constraint, in predicting F_{ST}. Quantile regression uses least absolute deviation regression and is an appropriate methodology for describing limiting factors in ecology (Guo et al. 1998; Cade et al. 1999; Dunham et al. 2002). In this case, we were looking for the relationship that describes the minimum F_{ST} we would expect for any given distance. Because we expected that other unmeasured factors (e.g., time of separation) would also influence F_{ST}, quantile regressions were an appropriate way to describe the data (Cade and Noon 2003). Quantile regressions should be parallel and have the same slope if the predictor variable describes the central tendency of the response and has homogenous variance, but quantile regressions will have different slopes if the predictor constrains the response variable and variance is heterogenous. We used the software program BLOSSOM to calculate nine quantile regressions from 0.10 to 0.90. Because we expected F_{ST} to equal zero when distance is zero, we did not include a constant and forced the regression through the origin. Values of P to test whether slopes differed from zero were calculated using 5,000 permutations with a quantile rank score test having a chi-square distribution. This approach is conservative and reduces type I errors because it is less sensitive to heterogeneous error distributions (Cade and Richards 2005). We used residuals from the lowest significant quantile regression to evaluate whether unmeasured factors were homogenous across ecological populations. As with Mantel tests, our sample size of 15 comparisons is insufficient to justify strong conclusions.

The Bayesian clustering method of Pritchard et al. (2000), as implemented in STRUCTURE version 2.3.2, was used to investigate the most likely number of genetically distinct clusters (K) or populations in the data set. We applied the admixture model that assumes gene flow among populations and allows for correlated allele frequencies across clusters. This admixture model assigns a proportion of each individual's genome to each of the clusters pursuing solutions that maximize HWE and linkage equilibrium within clusters. We performed 20 replicated unsupervised STRUCTURE runs for each K from 1 to 10. All runs had a burn-in of 300,000 iterations followed by 300,000 iterations. The symmetric similarity coefficient (SSC; Jakobsson and Rosenberg 2007) was used to determine the similarity of outcomes among the 20 replicate STRUCTURE runs for each K. Using the LargeKGreedy algorithm of CLUMPP (Jakobsson and Rosenberg 2007) with 1,000 random input sequences, we determined the number of distinct modes among the 20 runs at each K by grouping pairs of runs that had a SSC > 0.9. Graphical displays of STRUCTURE results were generated using the DISTRUCT software (Rosenberg 2004), the

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membership of each individual representing the mean membership over the replicate runs.

Two methods were used to infer the most likely value of K for the data set. Pritchard et al. (2000) showed that the posterior probabilities of K and Bayes' Rule could be used to estimate the most likely value of K. This method simply identifies the K with the highest posterior probability for the data set as the correct value of K. Evanno et al. (2005) suggested that the method of Pritchard et al. (2000) often leads to an overestimation of K and recommended using the second-order rate of change between the K and $K + 1$ clusters (ΔK), as a more effective identifier of the most likely K for the data set. Estimates of ΔK were generated by STRUCTURE HARVESTER (http://taylor0.biology.ucla.edu/ struct_harvest/) using the methods of Evanno et al. (2005).

Splitting time (t) and historical effective population size (N_e) were estimated between the Umpqua Chub from the Smith River and Umpqua River using the coalescence method of Hey and Nielsen (2004, 2007) as implemented in the program IMa. The IMa analysis is based on the simple concept that a single ancestral population at time t split into two populations and based on characteristics of microsatellite data, including distribution of allele sizes, the IMa model estimates of the historical N_e of the ancestral population before splitting (N_{eA}), N_e of the Umpqua River after splitting (N_{eU}), N_e of the Smith River after splitting (N_{eS}), and time point (t) at which the Smith and Umpqua populations split. We ran two different IMa models, one allowed for gene flow between populations after splitting while the other did not. The isolation with gene flow model provided estimates of unidirectional migration rates (defined here as individuals/generation) from Smith to Umpqua (m_{SU}) and from Umpqua to Smith (m_{US}). Results for all IMa parameter estimates were converted to biological meaningful units using a microsatellite mutation rate for fishes, $\mu = 5 \times 10^{-4}$ (Estoup and Angers 1998) and a generation time based on age structure data (3–4 years). We ran both models using 25 heated chains parameterized with g1 = 0.8 and g2 = 0.9. Two million MCMC steps were used for a burn-in with 34,897,864 steps needed after the burn-in to reach convergence (i.e., ESS > 42) for the model allowing migration and 15,825,811 steps needed after the burn-in to reach convergence (i.e., ESS > 56) for the model without migration. The following commands were used for the model with migration: -j 1 -qa 75 -q1 50 -q2 50 -m1 100 -m2 100 -t 1 -f g -n 25 -g1 0.8 -g2 0.9 -k 12 -b 2000000 -l 2.0 -s 789 -p 145. Commands without migration were -j 1 -qa 75 -q1 50 -q2 50 -m1 0 -m2 0 -t 1 -f g -n 25 -g1 0.8 -g2 0.9 -k 12 -b 2000000 -l 2.0 -s 999 -p 145.

RESULTS

Distribution of Umpqua Chub and Smallmouth Bass

A majority of the 141 sites surveyed were located in third- through sixth-order streams: first (1), second (5), third (21), fourth (47), fifth (45) and sixth (22). We observed or captured 2,859 Umpqua Chub at 46 sites (Figure 1A). The six populations of Umpqua Chub (the Smith River, Elk Creek, Calapooya Creek, Olalla Creek, Cow Creek, and South Umpqua River) were located in third-order through fifth-order stream sites (Figure 2). Umpqua Chub were found in two (9%) sixth-order streams, both in the Umpqua River between the mouths of Calapooya Creek and North Umpqua River, and in one first-order stream in Elk Creek. The Elk Creek population had the most extensive distribution and highest density. We found age-0 Chub in every population.

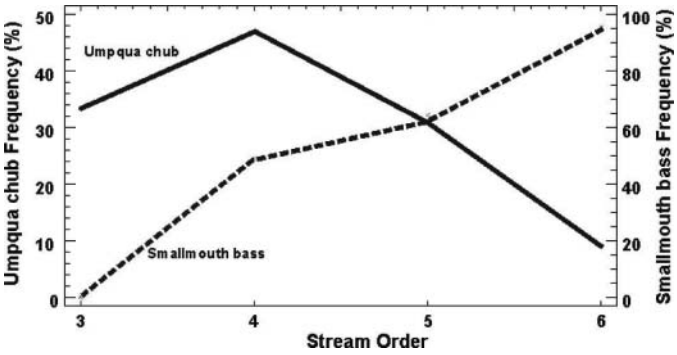

FIGURE 2. Frequency of encounters for Umpqua Chub and Smallmouth Bass by stream order.

We observed or captured an estimated 5,060 Smallmouth Bass at 73 sites, all in fourth-order through sixth-order streams. We detected Smallmouth Bass in all drainages with Umpqua Chub populations except the Smith River (Figure 1B). The frequency of Umpqua Chub and Smallmouth Bass detection was related to stream order. Umpqua Chub were found in over 30% of third-order and fourth-order sites and declined to less than 10% in sixth-order sites (Figure 2). In contrast, Smallmouth Bass were absent from third-order streams but were detected in over 90% of sixth-order streams. Umpqua Chub and Smallmouth Bass appeared sympatric in parts of Cow Creek, South Umpqua River, and Calapooya Creek (Figure 1).

Age Estimates

Otolith sections had well-formed alternating opaque and translucent bands that were relatively easy to count, but the position of the first annulus was slightly difficult to determine. An opaque core was surrounded by an opaque area with growth checks, and we assigned the first annulus to the outer edge of the opaque area surrounding the core. Umpqua Chub from 23 to 65 mm fork length ranged in age from 1 to 7 years (Table 1). Growth in length was relatively rapid until age 2, after which growth slowed and there was greater overlap in length at age. The closely related Oregon Chub have a similar growth trajectory (asymptotic length of 62 mm versus 60 mm) and mature at 40 mm or age 2 (Scheerer and McDonald 2003). Depending on mortality rates, average Umpqua Chub generation time could be expected to be about 3 or 4 years, as also found in Oregon Chub (DeHaan et al. 2012).

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TABLE 1. Age data summary for each ecological population of Umpqua Chub collected in 2008. The sample size was 25 for each population.

Ecological population	Mean age (years)	Age range (years)	Mean fork length (mm)	Fork length range (mm)
Smith River	1.2	0–2	38.4	23–51
Elk Creek	2.3	2–5	51.2	44–60
Calapooya Creek	2.4	2–5	49.2	43–58
Olalla Creek	2.1	2–3	46.8	40–56
Cow Creek	2.6	2–7	51.3	42–65
South Umpqua River	3.1	2–5	53.6	46–59

Genetic Diversity within Populations

We found that the microsatellite locus *Ocr115* did not conform to neutral expectations and therefore excluded it from subsequent analyses. The 10 microsatellites used to characterize the genetic variation within Umpqua Chub populations showed variable levels of polymorphism with the number of alleles per locus ranging from 2 to 28 (mean = 14.4, SD = 9.969). After sequential Bonferroni corrections, we found no evidence for linkage disequilibrium among loci pairs, and all populations conformed to HWE. Estimates of genetic diversity (Table 2)—mean number of alleles per locus (A), expected heterozygosity (H_e), and observed heterozygosity (H_o)—showed that Cow Creek had the lowest mean number of alleles ($A = 6.2$, $H_e = 0.578$, and $H_o = 0.535$). Among the other five populations, Olalla ($A = 9.5$, $H_e = 0.570$, $H_o = 0.590$) and Calapooya ($A = 9.4$, $H_e = 0.567$, $H_o = 0.580$) creeks showed the highest mean number of alleles, while expected and observed heterozygosities were greatest in the South Umpqua River ($A = 8.4$, $H_e = 0.602$ and $H_o = 0.606$).

Population Genetic Structure

The overall F_{ST} was 0.06 (95% CI = 0.04–0.08). Pairwise estimates of F_{ST} were highly significant ($P \leq 0.01$) and ranged from 0.01 between Olalla Creek and Calapooya Creek to 0.121 between Cow Creek and the Smith River (Table 3). Similarly, exact tests for genic and genotypic differentiation were significant for all population pairwise comparisons ($P < 0.001$).

TABLE 2. Estimates of genetic diversity based on 10 microsatellite loci for Umpqua Chub populations sampled in 2008; diversity variables include the mean number of alleles per locus (A), expected heterozygosity (H_e), and observed heterozygosity (H_o).

Population	A	H_e	H_o
Elk Creek	8.1	0.546	0.572
Calapooya Creek	9.4	0.567	0.580
Olalla Creek	9.5	0.570	0.590
Cow Creek	6.2	0.578	0.535
South Umpqua River	8.4	0.603	0.606
Smith River	6.9	0.564	0.576

The neighbor-joining tree also suggests that the six populations are genetically differentiated (Figure 3). The internal branches, however, are short with weak bootstrap support. Again, the Smith River population, and to a lesser extent the Cow Creek and Elk Creek populations, have the longest branch lengths and appear more divergent.

Mantel tests for the association between genetic and geographic distances indicate a significant isolation-by-distance effect among the six Umpqua Chub populations ($r^2 = 0.325$, $P = 0.0001$; Figure 4). Removing the outlier comparison of Smith

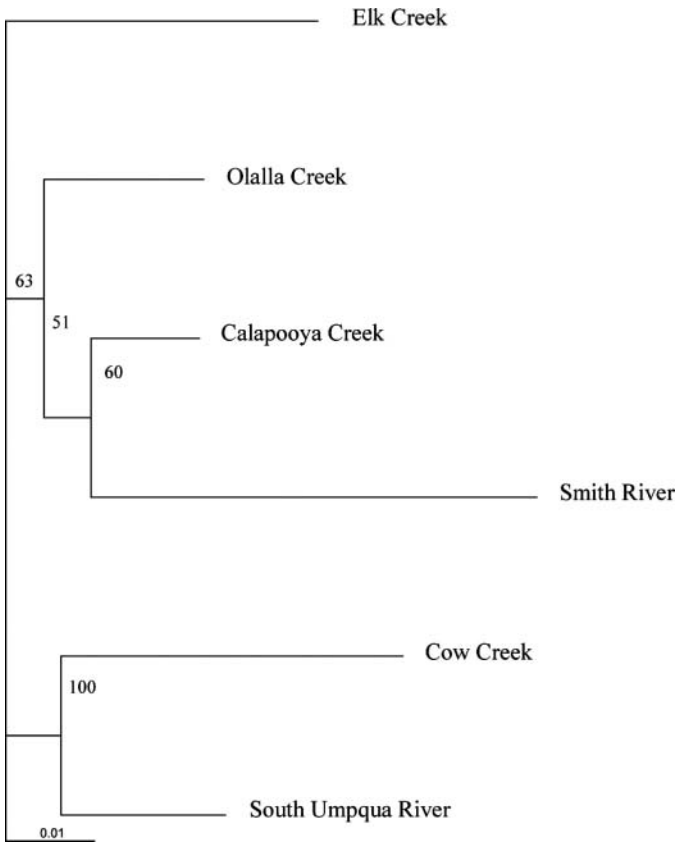

FIGURE 3. Neighbor-joining tree depicting structure among the six Umpqua Chub populations based on Cavalli-Sforza and Edwards' (1967) chord distances. Values are shown only for nodes that received bootstrap support greater than 50%.

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TABLE 3. Pairwise estimates of genetic variation (F_{ST}) among Umpqua Chub populations sampled in 2008 based on 10 microsatellite loci. All values are significant ($P \leq 0.01$).

Population	Calapooya Creek	Olalla Creek	Cow Creek	South Umpqua River	Smith River
Elk Creek	0.042	0.040	0.069	0.051	0.120
Calapooya Creek		0.010	0.053	0.030	0.070
Olalla Creek			0.055	0.028	0.083
Cow Creek				0.040	0.121
South Umpqua River					0.105

and Elk resulted in a twofold increase in the correlation between genetic and geographical distance ($r^2 = 0.617$, $P = 0.0001$). However, excluding the Smith River from the analysis resulted in a nonsignificant isolation-by-distance effect ($r^2 = 0.307$, $P = 0.118$), as did an analysis of the Smith River-only comparisons ($r^2 = 0.001$, $P = 0.95$). A significant isolation-by-distance effect ($r^2 = 0.471$, $P = 0.04$) was seen in all comparisons involving the mid-drainage Calapooya and Olalla groups.

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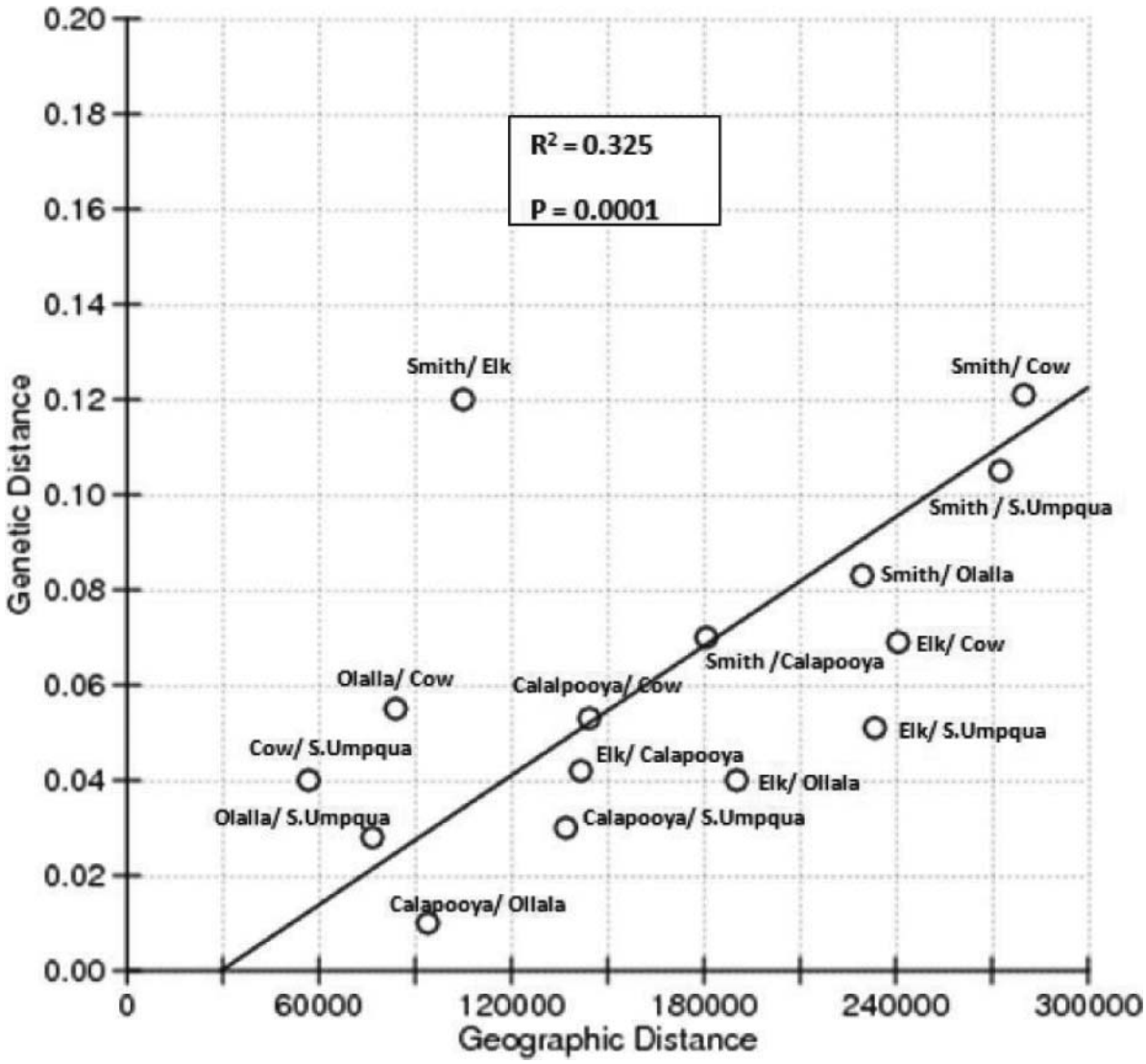

FIGURE 4. Pairwise relationship of genetic distance (as measured by F_{ST}) and geographic distance (m) among the six Umpqua Chub populations as estimated via least-squares regression.

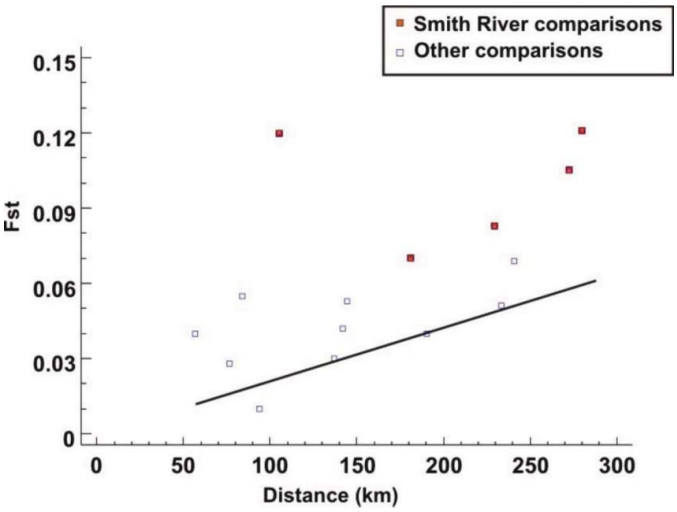

FIGURE 5. Pairwise constraint relationship of F_{ST} and geographic distance based on the 0.1-quantile regression $F_{ST} = 0.0021 \times$ distance (km). The points are the same as those in Figure 4. [Figure available online in color.]

All quantile regressions below 0.8 had slopes significantly different from zero ($P < 0.045$) and slopes were similar (range 0.0002–0.0004). We modeled results using the 0.1 quantile (slope = 0.00021, $P = 0.009$; Figure 5). By comparison, slopes for other low quantiles were 0.00022 for the 0.2 quantile and 0.00029 for the 0.3 quantile. For each ecological population except the Smith River, mean residuals from the 0.1 quantile regression were similar (0.012–0.026) and not significantly different from nonself comparisons ($P > 0.148$; Table 4). The mean residual for the Smith River was 2.1–4.6 times greater than those of the other ecological populations, and these differences were significant (Table 4; Figure 5).

Using STRUCTURE, Bayesian clustering of all individuals resulted in a sharp increase in $\log_e \Pr(X|K)$ from $K = 1$–4 that reached a plateau at $K = 3$ and 4 and then sharply declined (Figure 6A). The ΔK statistic, which identifies the steepest increase in $\log_e \Pr(X|K)$, was clearly highest for $K = 2$ (Figure 6B). Proportional assignment plots of individuals at K of 2, 3, and 4 revealed a hierarchical level of population structure that was closely linked to sampling location (Figure 6C). At $K =$ 2 all individuals from the Smith River formed a distinct genetic Smith River cluster and fish sampled in Cow Creek, Elk Creek and the South Umpqua River formed a second distinct Umpqua River cluster. Fish sampled from Calapooya Creek, and Olalla

TABLE 4. Comparisons of mean F_{ST} residuals from 0.1-quantile regressions for each ecological population (self) and all other comparisons (nonself) and significance of chi-square tests for differences between the self and nonself comparisons (P).

Ecological population	Self	Nonself	P
Smith River	0.055	0.012	0.001
Elk Creek	0.026	0.027	0.965
Calapooya Creek	0.012	0.034	0.148
Olalla Creek	0.015	0.032	0.263
Cow Creek	0.017	0.032	0.500
South Umpqua River	0.018	0.031	0.427

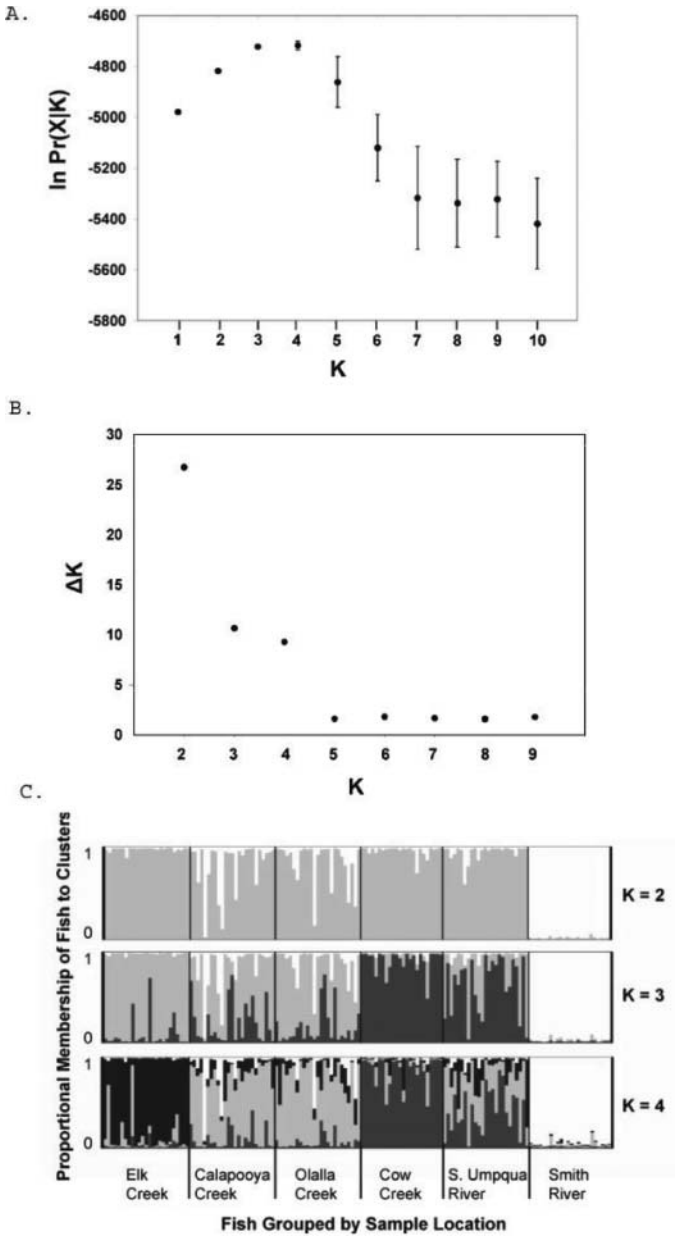

FIGURE 6. Summary of Bayesian clustering results of Umpqua Chub sampled from six locations in the Smith and Umpqua River basins based on a STRUCTURE analysis. Panel **(A)** shows the estimated probabilities of the number of genetic clusters ($K = 1$ to 10; 20 replicates/K value) based on data at 10 microsatellite loci. Panel **(B)** shows the results of the ΔK analysis used to determine the optimum number of genetically distinct Umpqua Chub clusters represented in this data set. Panel **(C)** shows the proportional membership of Umpqua Chub individuals in genetic clusters assuming $K = 2$, 3, and 4. Each vertical bar in panel (C) corresponds to an individual, and each genetic cluster is represented by a different shading. Individuals are grouped by sample location. The proportional membership of an individual in each genetic cluster can be gauged by the y-axis and shading of the bar.

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Creek largely grouped with the Umpqua River cluster; however, a few fish had high membership probabilities to the Smith River cluster, and some fish were an admixture of the two clusters. Weak substructuring in the Umpqua River cluster was observed at $K = 3$ and $K = 4$. At $K = 3$, fish sampled from Elk Creek and Cow Creek formed distinct clusters, while fish sampled from the other three populations in the Umpqua River generally had mixed membership. The main distinction between the $K = 3$ and $K = 4$ plot is weak evidence for a fourth cluster composed primarily of fish sampled from Calapooya Creek and Olalla Creek.

Splitting time (t) between Umpqua Chub from the Smith River and Umpqua River based on the IMa model was estimated to be 196 years before present (90% credible interval; 90% CI = 44–340) assuming no migration between rivers and years before present (90% CI = 20–396) allowing for migration between rivers. Estimates of historical demographic parameters for the IMa model with migration were N_{eA} of 14,035 (90% CI = 9,394–18,544), N_{eU} of 474 (90% CI = 63–913), N_{eS} of 77 (90% CI = 13–238), m_{SU} of 0.009 (90% CI = <0.0000–0.0234), and m_{US} of 0.0200 (90% CI = <0.0000–0.0419). Estimates of demographic parameters for the IMa model without migration were N_{eA} of 12,262 (90% CI = 8381–16144), N_{eU} of 682 (90% CI = 238–1138), and N_{eS} of 164 (90% CI = 38–338).

DISCUSSION

Despite recent declines in distribution and apparent isolation of Umpqua Chub populations, estimates of genetic diversity remained relatively high among all sampled populations. Our observed estimates are similar to those reported for the closely related Oregon Chub, a species whose recently fragmented populations do not appear threatened by the effects of low genetic diversity (DeHaan et al. 2012). Furthermore, estimates of genetic diversity for both Oregon and Umpqua Chub were greater than or equivalent to those observed in several other cyprinid species, many of which are listed as threatened or endangered (Parker et al. 1999; Mesquita et al. 2005; Sousa et al. 2008).

Efficient methods have been developed to translate patterns in neutral genetic markers (i.e., microsatellites) into inferences about demography, gene flow, effective population size (*Ne*), metapopulation structure, and phylogeography to obtain information about the current (and past status) of threatened populations (Excoffier and Heckel 2006). Consequently, considerable effort has been devoted to delineating units of conservation within species that are distinct enough to warrant separate management; these include ESUs, distinct population segments, and management units (Allendorf et al. 2010). We utilized several methods to determine the number of evolutionary populations represented in our sample collection. Pairwise F_{ST} estimates, exact tests for genic and genotypic differentiation, and Cavalli-Sforza and Edwards (1967) chord distances confirm that the six populations are genetically distinct. Alternatively, STRUCTURE analysis suggested four evolutionary populations. For $K = 4$, there is an upstream population composed of Cow Creek and South Umpqua, a diverse mid-drainage mixed population from Calapooya Creek and Olalla Creek, a downstream population at Elk Creek, and another downstream population at the Smith River.

Our analyses identify hierarchical structure with four evolutionary populations and peripheral isolation as the mechanism of fragmentation. The most downstream group is the Smith River, which is separated from the next closest group, Elk Creek, by the Umpqua River estuary, a dam on Elk Creek, and 100 rkm that harbors introduced Striped Bass and Smallmouth Bass. The 0.1-quantile regression suggested that F_{ST} should change by at least 0.0002/km. Significant quantile slopes differed by a factor of two, suggesting some heterogeneity in the error term. For each ecological population, other than the Smith River, mean residuals from the 0.1-quantile regression were similar (0.12–0.26) and not significantly different from nonself comparisons, suggesting relative homogeneity in our error term for those groups. Residuals for the Smith River were 2.1–4.6 times greater than other comparisons and significantly different from nonself comparisons, suggesting that the heterogeneity in error is associated with the Smith River. The Smith River was also the first group separated in the STRUCTURE analysis, and pairwise estimates of F_{ST} were highest for the Smith River (mean = 0.100, range = 0.070–0.121) and relatively uniform for distances ranging from 105 to 280 km (CV = 22.7%). Excluding the very low Calapooya and Ollala creeks pairwise comparison, estimates of F_{ST} outside the Smith River were still less than half of the Smith River comparisons (mean = 0.045, range 0.028–0.069) and relatively uniform for distances ranging from 57 to 241 km (CV = 26.2–32.2%). Even if the latter is restricted to distances >105 km, the mean F_{ST} is still only 0.048, less than half the Smith River mean. Interestingly, the highest F_{ST} pairwise comparisons with the Smith River were with the closest group, Elk Creek, and the most distant group, Cow Creek. If most of the error term in our IBD analyses is due to time of separation, it suggests that the Smith River separated first and the other populations separated at about the same time. If the residuals from the quantile regression scale linearly with time, then the age of the Smith River separation is 2.1–4.6 times greater than the separation of other populations. The mean of the Smith River IMa model with migration (188 years) puts the other separations at about 41–89 years. These estimates coincide with the timing of nonnative predator introductions. However, the confidence interval on the IMa model with migration is broad, 20–396 years, so that the data seem most robust for eliminating Holocene sea level rise as an isolating mechanism.

The other four ecological populations upstream of the mouth of Elk Creek have no physical barriers but appear to have been separated by introduced Smallmouth Bass, which occupy mid-order and high-order streams and effectively isolate Umpqua Chub in low-order streams. Some mid-order and high-order sites were occupied by Umpqua Chub as recently as 1987

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(Markle et al. 1991). It's interesting to note that the mixed Calapooya and Ollala creeks population also included individuals with high probabilities of membership to the Smith River population and had the highest and second highest mean number of alleles and the lowest pairwise F_{ST}. This diverse mixed population in the mid-Umpqua drainage could either be the source from which all other groups could be derived or a population to which all other groups contribute. We find the latter hypothesis less likely, especially because upstream movement from the Smith River is both low (m_{SU}, 0.009) and half as likely as movement in the opposite direction (m_{US}, 0.0200). Instead, we hypothesize that Umpqua Chub populations formed by peripheral isolation from a larger population, the remnant of which is the mid-drainage Calapooya–Olalla population. If a mid-drainage group is the source for peripheral isolation of populations downstream in the Smith River and Elk Creek and upstream in Cow Creek and South Umpqua River, we would not expect an isolation-by-distance model to fit all of our data well, but we would expect and did find a significant relationship restricted to comparisons with Calapooya and Olalla creek groups. For non-Calapooya–Olalla comparisons, we would expect isolation by time. Unfortunately, we can only estimate relative temporal splitting, which shows the Smith River population splitting first and the Elk Creek and Cow Creek and South Umpqua subpopulations splitting more recently from the mid-drainage group.

Umpqua Chub is currently listed as a sensitive–critical species by the Oregon Department of Fish and Wildlife, but there is no conservation plan. The listing is due in part to apparent declines and fragmentation of Umpqua Chub documented during two cursory surveys 11 years apart (Simon and Markle 1999). The observed shift in distribution patterns was attributed to displacement of Umpqua Chub by nonnative Smallmouth Bass (Simon and Markle 1999). Here, we provide a more in depth examination based on our analyses of genetic isolation by distance and time. Correcting for distance, our results suggest that the Smith River population has been isolated for about four times as long (about 200 years) as the recent separation of the three other evolutionary populations. Furthermore, one of the three populations, Elk Creek, may have been isolated by a dam, such that only the fragmentation of Calapooya Creek–Olalla Creek from Cow Creek–South Umpqua River appears attributable to Smallmouth Bass.

Although levels of genetic diversity observed in Umpqua Chub are relatively high, continued disruption of natural connectivity by nonnative predators will probably lead to an increase in genetic drift and a reduction in genetic diversity in small, isolated populations. The tributary-based populations show reduced levels of genetic variation and may face a greater risk of extinction as a result (Rieman and Allendorf 2001). Thus, the Umpqua River system provides an excellent opportunity for understanding the progressive impact on the distribution, abundance and genetic diversity of fragmented populations. Developing a conservation plan that includes periodic monitoring of Umpqua Chub distributed throughout the river system would seem prudent.

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