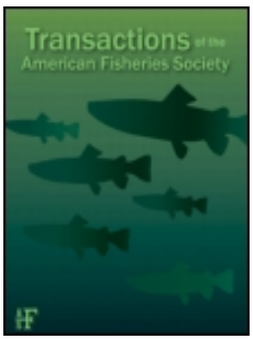

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ARTICLE

Analyses of Genetic Variation in Populations of Oregon Chub, a Threatened Floodplain Minnow in a Highly Altered Environment

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Abstract

The Oregon chub *Oregonichthys crameri* is a small floodplain minnow endemic to the Willamette River basin of western Oregon. Historically the species was widely abundant and probably relied on periodic floods for dispersal and genetic exchange among populations. The species has declined substantially in the past 100 years due to habitat alterations and the introduction of nonnative species and is currently listed as threatened under the U.S. Endangered Species Act. Information on the level of genetic variation within and among populations did not exist when the species was listed or when a recovery plan was being developed. In this study, we used a suite of nine microsatellite loci to characterize genetic variation within and among 16 sampling locations and provide information to help guide future recovery efforts. Even though many locations are presently isolated from one another, we observed relatively high levels of genetic variation within collections. Temporal samples revealed that the levels of genetic variation were stable over time despite fluctuations in abundance. Estimates of effective population size (N_e) for three sampling locations ranged from approximately 120 to 220 and suggest that there is no immediate threat from inbreeding or genetic drift. We observed a significant level of genetic variation among sampling locations (global $F_{ST} = 0.078$) and significant differences in allele frequencies among all sampling locations. Our results suggest that the different locations represent distinct populations of Oregon chub and that greater levels of gene flow occur among populations within the Santiam and Middle Fork Willamette River subbasins. The information presented in this study will help guide Oregon chub recovery efforts, including the refinement of recovery areas and future population introduction efforts, and will help address the challenge of managing populations largely in isolation from one another in order to minimize threats from nonnative species.

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Genetic data have become increasingly important for conserving and managing threatened and endangered species (Allendorf and Luikart 2007). For example, identifying species and populations with reduced genetic diversity is critical for determining extinction risk (Quattro and Vrijenhoek 1989; Saccheri et al. 1998). Genetic data have also been widely used to infer the number of populations or evolutionary groups present (Parker et al. 1999; Currens et al. 2009; Ardren et al. 2011), information that can be useful for defining management and recovery units. More recently, genetic analyses have been used to address increasingly complex conservation issues including the estimation of effective population size (Peterson and Ardren 2009; Small et al. 2009; Osborne et al. 2010), determining levels of migration and gene flow among populations (Howes et al. 2009), and determining the effects that barriers and other landscape features have on populations (Neville et al. 2006; Narum et al. 2008; Boizard et al. 2009). Genetic data have also become important for planning and evaluating population introductions and reintroductions for threatened and endangered species (Parker et al. 1999; Mock et al. 2004; Stephen et al. 2005; Drauch and Rhodes 2007; Drauch et al. 2008).

Genetic data have been important for evaluating the conservation status of many members of the family Cyprinidae (see Table A.1 in the appendix and the references therein). Cyprinid species are found in a number of different geographic regions and habitat types, and populations face many threats to their persistence including habitat fragmentation (Alo and Turner 2005; Skalski et al. 2008; Dehais et al. 2010), alterations in the natural hydrologic regime (Platania 1991; Scheerer 2002), conversion of habitat to agricultural and urban areas (Waite and Carpenter 2000), and introductions of nonnative species that compete with and prey upon native cyprinids (Platania 1991; Simon and Markle 1999; Matthews and Marsh-Matthews 2007). As a result of these threats, many cyprinid species are presently threatened, endangered, or declining in abundance. Studies have documented low levels of genetic variation in many species, low effective population sizes, and reduced gene flow among fragmented populations (Table A.1). Several species and populations have been identified that may face an increased risk of extinction as a result.

The Oregon chub *Oregonichthys crameri* is a small floodplain minnow endemic to the Willamette River system of western Oregon (Markle et al. 1991). Historically, Oregon chub were widely distributed throughout the Willamette River system (Markle et al. 1991) in off-channel habitats that have minimal or no flow, an abundance of vegetation, and depositional substrate (Pearsons 1989; Scheerer 2002). Oregon chub thrived in an unconstrained Willamette River under a hydrologic regime that featured frequent flood events (Benner and Sedell 1997), which continually created and destroyed off-channel habitats (Lewin 1978; Dykaar and Wigington 2000) and presumably provided a mechanism of dispersal and periodic genetic exchange among populations.

Studies conducted in the 1970s and 1980s found the distribution of Oregon chub to be severely restricted (Bond 1974; Bond and Long 1984; Markle et al. 1991). The primary factors implicated in the species' decline were the loss of habitat due to flood control activities and the introduction of nonnative species. In the past 150 years, the channel length of the Willamette River drainage has been drastically reduced by the construction of 13 major flood control dams, removal of snags for navigation, channelization and revetments, and the drainage of wetlands to increase the land available for agriculture (Sedell and Froggatt 1984; Benner and Sedell 1997). Floods were common before the construction of the dams (1941–1969), averaging 14 floods above bankfull per decade from about 1884 to 1969 (USACOE 1970). A 10-year flood event before dam construction now has a 100-year return interval (Benner and Sedell 1997). As a result, Oregon chub now exist mostly as a series of isolated local populations. Introductions of nonnative fishes in the Willamette River began in the late 1800s (Dimick and Merryfield 1945; Lampman 1946; McIntosh et al. 1989). Nonnative centrarchids and bullhead catfishes (*Ameiurus* spp.) are now common in the Willamette River basin and have been widely implicated in the decline of native fishes (Moyle 1976; Lemly 1985; Rinne and Minckley 1991; Newman 1993; Simon and Markle 1999). Markle et al. (1991) found nonnative fishes were common in historic Oregon chub habitats that no longer contained Oregon chub. Another study by Scheerer (2002) found Oregon chub were absent or in low abundance when nonnative fishes were present in off-channel habitats and described several Oregon chub populations that declined or were extirpated after their habitats were invaded by nonnative fishes following flood events or unauthorized stocking.

Declines in Oregon chub abundance and the species' restricted range led to its listing as endangered under the U.S. Endangered Species Act in 1993 (USFWS 1993). The Oregon Chub Recovery Plan (USFWS 1998) set recovery criteria for downlisting the species to "threatened" status and for delisting of the species. Since 1991, the status of Oregon chub has improved substantially (Scheerer 2007). Recent population surveys have documented a number of newly discovered populations (Scheerer 2007; Bangs et al. 2009). Additionally, a major effort for Oregon chub recovery has focused on introducing Oregon chub into suitable habitats within their historic range, and several new populations have been established since 1988. In 2007, all downlisting criteria were met and the U.S. Fish and Wildlife Service reclassified the species from endangered to threatened status in 2010 (USFWS 2010).

One of the challenges faced by biologists responsible for managing Oregon chub has been evaluating the trade-offs between isolation and connectivity. Many riverine fishes have evolved adaptations to periodic flooding by using off-channel floodplain habitats for reproduction and nursery areas and by using floodwaters to disperse among habitats and colonize new habitats (Zeug et al. 2005; Boizard et al. 2009; Jackson and

Pringle 2010). Suppression of the natural flood regime can result in disruption of spawning behaviors, a loss of access to rearing habitat for juvenile fish, and reduced recruitment (Osmundson and Burnham 1998; Modde et al. 2001; Bunn and Arthington 2002). For fish species such as Oregon chub that presumably rely on floods for dispersal, alteration of the natural hydrologic regime often results in population isolation and loss of gene flow. The effects of population isolation have been well documented and can include reduced genetic variation (Wofford et al. 2005; Neville et al. 2006; Reid et al. 2008; Skalski et al. 2008), loss of population fitness (Morita et al. 2009), and declines of metapopulations (Hanski and Gilpin 1997). However, in certain highly altered ecosystems, reduced hydrologic connectivity can provide greater ecological benefits than increased connectivity (Jackson and Pringle 2010). Fisheries managers are often faced with the dilemma that increasing connectivity to reduce the risk of extinction in fragmented habitats can increase the probability that nonnative fishes will invade and increase the extinction risk (Peterson et al. 2008; Fausch et al. 2009). This dilemma is exemplified by Oregon chub. Scheerer (2002) found that Oregon chub were most abundant in isolated habitats that nonnative species could not access. Ultimately the trade-offs between isolation and connectivity depend on a number of factors, and biologists need to consider a variety of data, including genetic information, when making management decisions in these situations (Peterson et al. 2008; Fausch et al. 2009).

Previously, genetic data important for Oregon chub recovery planning did not exist and decisions such as the delineation of recovery areas and identification of donor stocks for population introductions were made in the absence of this information. Although the status of Oregon chub and our understanding of the species biology has improved since the species was initially listed as endangered, several questions important to the recovery implementation process remain. Our objective in this study was to examine the level of genetic variation in Oregon chub within and among sites to provide information important for recovery implementation. Specifically, we wished to examine the amount of variation within several locations where chub are found and identify any locations with reduced genetic diversity, infer the level of gene flow among locations, and provide information to help clarify the delineation of populations and recovery areas. Furthermore, we wished to examine the effects of isolation due to alterations in the hydrologic regime of the Willamette River.

METHODS

Sample collection.—We collected tissue samples (caudal fin clips) from Oregon chub during annual surveys from April through October from 2004 to 2006 following methods described in Scheerer et al. (2005b). We collected samples from 16 sites where Oregon chub naturally occur (i.e., no introduced populations were sampled) and these sites were distributed throughout the geographic range of Oregon chub (Figure 1; Table 1). For some locations (Geren Island, East Fork [EF] Minnow Creek Pond, Hospital Pond, and Shady Dell Pond), tissue samples were also obtained from the Oregon State University ichthyology collection. These specimens were collected during surveys in 1997 and 1998.

Laboratory analyses.—Total genomic DNA was extracted from fin clips by using a Chelex extraction protocol. A small piece of fin tissue (approximately 1 mm^2) was placed in 190 μL of a 5% Chelex (Chelex 100, Sigma) solution and boiled for 8 min. All individuals were then genotyped at nine microsatellite loci: *Ocr100*, *Ocr103*, *Ocr104*, *Ocr105*, *Ocr106*, *Ocr109*, *Ocr111*, *Ocr113*, and *Ocr114* (Ardren et al. 2007). Polymerase chain reactions (PCR) were carried out in 15 μL volumes that contained 2 μL supernatant from the Chelex extractions and had final concentrations of 1 × PCR buffer (10 mM tris-HCl, 50 mM KCl, 0.1% Triton X-100), 1.5 mM $MgCl_2$, 0.2 mM of each dinucleotide triphosphate, 0.5 μM of forward and reverse primer, and 0.2 units *Taq* DNA polymerase (Promega). Reaction conditions were as follows: initial denaturation at 94°C for 2.5 min, followed by 38 cycles at 94°C for 1 min, 1 min at primer-specific annealing temperature (see Ardren et al. 2007), and 1 min at 72°C, and completed with a final extension at 72°C for 7 min.

Following PCR, we pooled reactions for automated electrophoresis on an Applied Biosystems 3100 genetic analyzer by using the GeneScan-500LIZ size standard (Applied Biosystems). Electropherograms for each individual were analyzed with GENOTYPER version 3.7 NT software (Applied Biosystems). All individuals were double-scored by multiple laboratory personnel. To assess genotyping error rate, 10% of the individuals were re-extracted and regenotyped by a separate laboratory member following the procedures described above.

Statistical analyses.—For statistical analyses, we grouped individuals according to the 16 sampling locations. Temporal replicate samples from the same location were analyzed as two separate samples (Table 1). We tested each sampling location for conformance to Hardy–Weinberg equilibrium expectations (HWE) by using exact tests implemented in the program GENEPOP version 4.0.7 (Raymond and Rousset 1995). We also used GENEPOP to test each location for evidence of linkage disequilibrium (i.e., nonrandom association between alleles at two loci). We adjusted significance values for HWE and linkage disequilibrium tests for multiple comparisons by using a sequential Bonferroni adjustment (Rice 1989). We estimated measures of genetic diversity, including mean number of alleles per locus, expected heterozygosity, and observed heterozygosity, by using the program GDA (Lewis and Zaykin 2001). Additionally, we used the program HP-Rare version 1.0 (Kalinowski 2005) to estimate allelic richness for each location, based on a minimum sample size of 74 genes (two times the minimum number of individuals sampled). This method provides estimates of allelic richness corrected for differences in sample size.

To test whether levels of genetic diversity were temporally stable, we compared estimates of genetic variation among temporal replicate samples. We used Wilcoxon signed rank tests

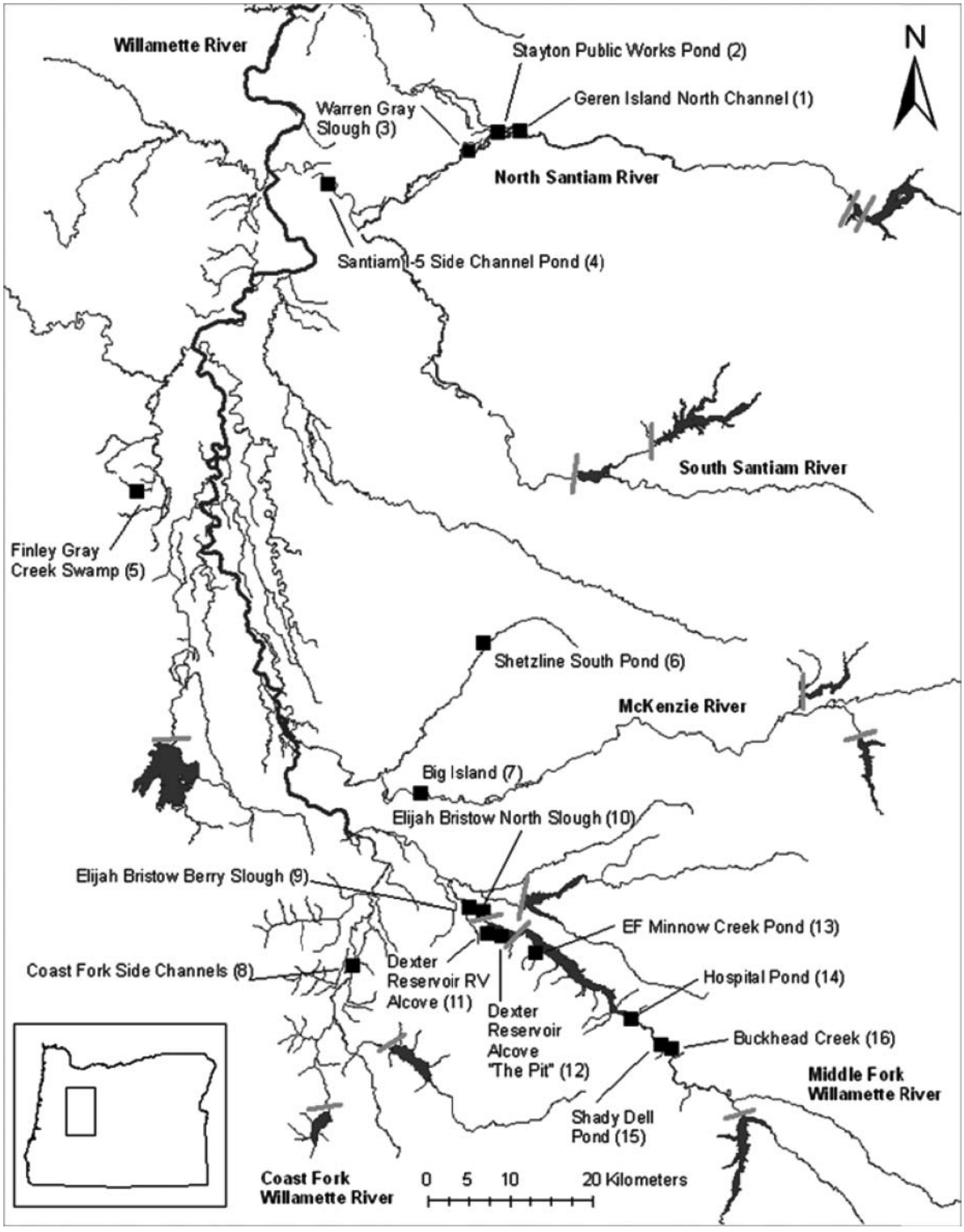

FIGURE 1. Sampling locations within the Willamette River basin, Oregon, where Oregon chub were collected for this study. Numbers in parentheses following site names correspond to numbers in Table 1. Gray bars represent the location of major flood control dams within the basin.

TABLE 1. Sampling location identification (ID) numbers, sample locations, sample years, sample sizes, and estimates of genetic diversity based on nine microsatellite loci for the Oregon chub collections sampled for this study. Abbreviations are as follows: CF = Coast Fork, MF = Middle Fork, EF = East Fork, n = the number of individuals analyzed, A = the mean number of alleles per locus, A_R = allelic richness, H_e = expected heterozygosity, and H_o = observed heterozygosity.

Sample location ID	Subbasin	Sample location name	Year(s) sampled	n	A	A_R	H_e	H_o
1	Santiam	Geren Island North Channel	2005	50	8.778	8.392	0.779	0.802
1A	Santiam	Geren Island North Channel	1997	47	11.222	10.547	0.783	0.757
2	Santiam	Stayton Public Works Pond	2005	43	10.222	10.001	0.794	0.817
3	Santiam	Warren Gray Slough	2004	49	10.889	10.374	0.799	0.770
4	Santiam	Santiam I-5 Channel Pond	2004, 2005	46	12.111	11.573	0.796	0.802
5	Middle Willamette	Finley NWR Gray Creek Swamp	2004, 2005	40	6.778	6.741	0.687	0.660
6	McKenzie	Shetzline South Pond	2004, 2005	45	3.444	3.381	0.558	0.523
7	McKenzie	Big Island	2004	48	8.333	7.960	0.753	0.757
8	CF Willamette	CF Willamette Side Channel	2004, 2005, 2006	44	8.667	8.497	0.751	0.750
9	MF Willamette	Elijah Bristow Berry Slough	2004, 2005	47	12.222	11.677	0.777	0.790
10	MF Willamette	Elijah Bristow North Slough	2004, 2005	44	11.333	11.142	0.789	0.770
11	MF Willamette	Dexter Reservoir RV Alcove	2004	46	11.222	10.808	0.763	0.752
12	MF Willamette	Dexter Reservoir Alcove - The Pit	2005	47	11.111	10.661	0.798	0.828
13	MF Willamette	EF Minnow Creek Pond	2004	45	11.222	10.985	0.804	0.804
13A	MF Willamette	EF Minnow Creek Pond	1997	48	11.667	11.327	0.793	0.796
14	MF Willamette	Hospital Pond	2005	47	11.333	10.990	0.801	0.788
14A	MF Willamette	Hospital Pond	1998	47	12.111	11.523	0.806	0.832
15	MF Willamette	Shady Dell Pond	2004, 2005	80	11.333	10.147	0.779	0.783
15A	MF Willamette	Shady Dell Pond	1998	48	10.889	10.363	0.788	0.777
16	MF Willamette	Buckhead Creek	2004, 2005	45	9.889	9.547	0.758	0.753

to determine whether there was a significant difference in allelic richness and expected heterozygosity among the temporal samples from EF Minnow Creek Pond, Shady Dell Pond, Hospital Pond, and Geren Island. To examine the relationship between abundance estimates (calculated as in Scheerer 2002) and estimates of genetic diversity, we calculated Pearson product-moment correlation coefficients between Oregon chub abundance estimates and measures of genetic diversity including allelic richness and observed heterozygosity. When genetic samples were collected over the course of subsequent years (e.g., Buckhead Creek), we averaged abundance estimates over the collection years. Oregon chub abundance estimates can vary widely from year to year (Bangs et al. 2009), so we also calculated correlation coefficients between the average abundance over 5 years and allelic richness and observed heterozygosity. Abundance estimates for the year in which genetic samples were collected and the 4 years immediately previous to collection were averaged. When abundance estimates were available for fewer than 5 years at the time genetic samples were collected (Shetzline South Pond, Big Island, and Hospital Pond in 1998; Geren Island in 1997), average abundance estimates were based on the year when samples were collected and all previous annual abundance estimates available.

We tested fish from all sample locations for evidence of recent genetic bottlenecks (within the past 0.2–4.0 effective generations) by using the program BOTTLENECK (Piry et al. 1999). This method tests for an excess of heterozygotes relative to the frequency of alleles in the population (Cornuet and Luikart 1996). We assumed a two-phased model of mutation with 90% step-wise mutations and 12% variance. We used a one-tailed Wilcoxon test to evaluate the significance of genetic bottleneck tests.

We estimated the effective population size (N_e) by examining temporal changes in allele frequencies at microsatellite alleles with a frequency greater than 0.02 within the four sampling locations that were sampled in multiple years (EF Minnow Creek Pond, Shady Dell Pond, Hospital Pond, and Geren Island). Standardized variance in allele frequencies (F) was calculated using the method of Pollak (1983). The generation interval for Oregon chub was assumed to be 3 or 4 years based on age structure information collected at Hospital pond (Scheerer et al. 2005a); we therefore estimated the elapsed time in generation between sampling events (t) using both 3- and 4-year generation intervals. Because multiple generations separated sampling events within each population, we were able to assume a discrete generation model (Jorde and Ryman 1995). The 95% confidence intervals

(CIs) for N_e were calculated with equation (16) in Waples (1989). The arithmetic mean of abundance (population size) (N) over the sampling period was used to estimate the ratio N_e to N.

A number of methods have been proposed for using genetic data to determine the number of populations or management units for a species (Waples and Gaggiotti 2006), and we employed several of these to examine the number of Oregon chub populations and determine the major evolutionary groupings. We first used traditional genetic distance based methods to determine the overall level of genetic variation among all sampling locations (F_{ST}; Weir and Cockerham 1984). Global F_{ST} and the associated 95% CI based on 1,000 bootstrap replicates were estimated by using the program FSTAT version 2.9.3.2 (Goudet 2001). We included only the most recent sample from locations that had been sampled multiple times for our overall estimate of F_{ST}. We also used FSTAT to estimate the level of genetic variation among each sampling location (pairwise F_{ST}). We estimated the level of genetic variation within the major subbasins of the Willamette River when multiple locations were sampled within a subbasin (i.e., Santiam, McKenzie, and Middle Fork [MF] Willamette). Using GENEPOP, we performed chi-square contingency tests of allele frequency heterogeneity to determine whether there were significant differences in allele frequencies among the different sampling locations. We adjusted P-values for multiple comparisons by using a Bonferroni correction (Rice 1989) and the Benjamini and Yekutieli false discovery rate (B-Y FDR) correction described in Narum (2006). To examine the spatial genetic relationship among sampling locations, we constructed a consensus neighbor-joining (NJ) tree. Using the program PHYLIP version 3.6 (Felsenstein 1993), we first generated 1,000 replicate data sets by using a bootstrap procedure. We then estimated Cavalli-Sforza and Edwards' (1967) chord distances between all sampling locations in each data set and generated a consensus NJ tree with these values.

Before dam construction, Oregon chub probably dispersed among geographically proximate habitats during flood events. To test the relationship between genetic distance and geographic distance, we used GENEPOP to conduct an analysis of isolation by distance by comparing the natural log of geographic distance in river kilometers between sampling locales to the pairwise genetic distance between sampling locations measured as $F_{ST}/(1 - F_{ST})$. We performed a Mantel test (1,000 permutations) to determine whether there was a significant isolation-by-distance relationship. Because Oregon chub are believed to be poor swimmers, dispersal is more likely to occur among locations within a subbasin; therefore, in addition to the basin-wide isolation-by-distance analysis, we conducted isolation-by-distance analyses separately for sampling locations from the Santiam and MF Willamette river basins. We did not analyze the mid-Willamette, Coast Fork (CF) Willamette, and McKenzie river subbasins because Oregon chub were only collected from one or two locations in these subbasins.

We conducted an analysis of molecular variance (AMOVA, Excoffier et al. 1992) to determine how genetic variation was partitioned among sampling locations. We grouped sampling locations according to their subbasin of origin (Santiam, mid-Willamette, CF Willamette, McKenzie, and MF Willamette) to determine whether there is more genetic variation among locations or among the different subbasins. The AMOVA was conducted with the program ARLEQUIN version 3.11 (Excoffier et al. 2005).

We also used the Bayesian clustering method of STRUCTURE version 2.3.2 (Pritchard et al. 2000) to investigate the number of Oregon chub populations (K). We applied the admixture model that assumes gene flow among populations and allows for correlated allele frequencies across populations. This model assigns a proportion of each individual's genome to each of the populations or genetic clusters pursuing solutions that maximize HWE and linkage equilibrium within clusters. Twenty replicated STRUCTURE runs were performed for each K from 1 to 16. All runs had a burn-in of 300,000 preliminary iterations followed by 300,000 iterations of data collection. The symmetric similarity coefficient (SSC) was used to determine the similarity of outcomes among the 20 replicate STRUCTURE runs for each K. By 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 greater than 0.9. Two methods were used to determine 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 determine 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 this method often leads to an overestimation of K and recommended using the second-order rate of change between K and $K + 1$ clusters, delta K (ΔK), as a more effective identifier of the correct K for the data set. Graphical displays of STRUCTURE results were generated using the DISTRUCT software (Rosenberg 2004) with the membership of each individual representing the mean membership over the replicate runs.

RESULTS

Genetic Diversity within Populations

Oregon chub samples from all locations conformed to HWE at all loci, with the exception of Big Island at the locus *Ocr111*, Dexter Reservoir RV Alcove at the locus *Ocr106*, EF Minnow Creek at the locus *Ocr105*, and Geren Island (1997 sample) at the locus *Ocr109*. All four deviations from HWE were due to an excess of heterozygotes. We observed 11 locus pairs (out of 756 total pairs) that showed evidence of linkage, but these pairs were randomly distributed among sampling locations and loci. These results suggest that our sampling efforts were representative of the true allele frequencies at each location (i.e., collections did not contain groups of siblings or multiple spawning populations). All estimates of genetic diversity were lowest in Shetzline South Pond (mean number of alleles per

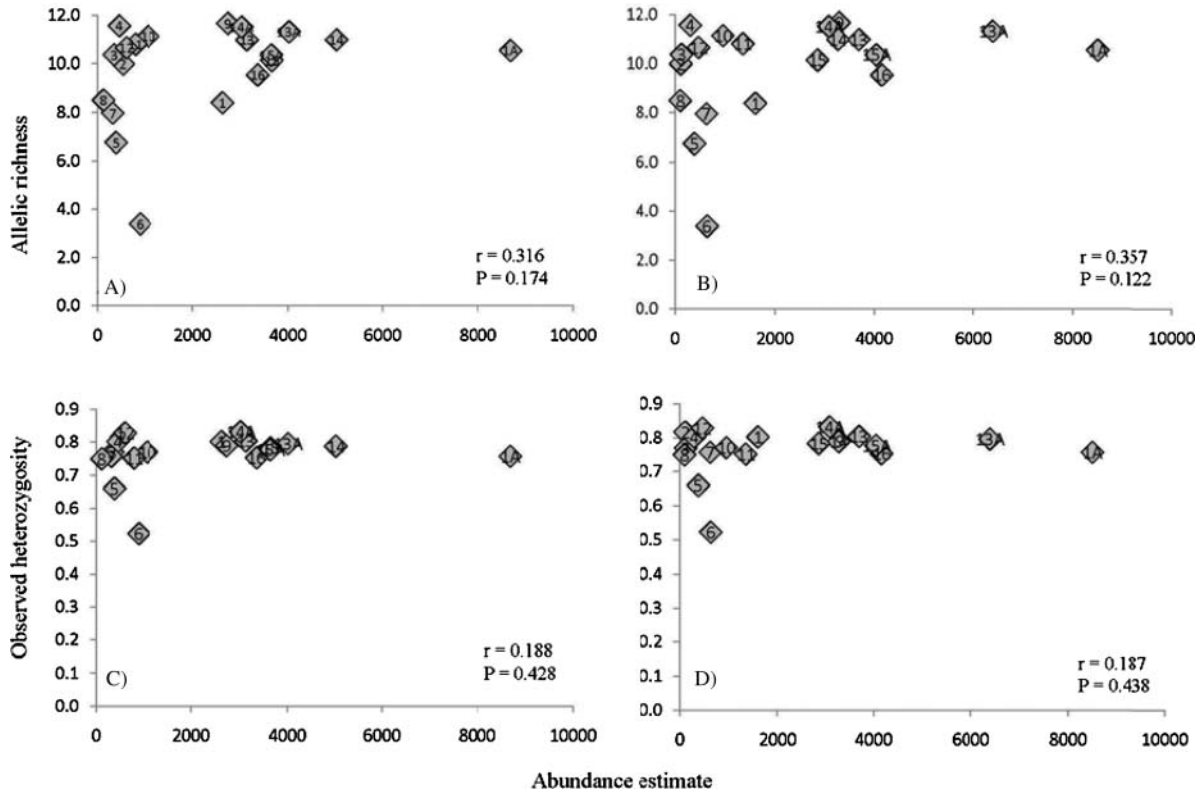

FIGURE 2. Relationships between Oregon chub abundance estimates and estimates of genetic diversity. Abundance estimates in Figure 2A, C are based only on the year that the genetic samples were collected at each site, and abundance estimates in Figure 2B, D represent the average abundance over 5 years except where noted in the text. Numbers on the points correspond to the sampling location numbers in Table 1 and Figure 1.

locus [A] = 3.444, allelic richness [A_R] = 3.381, estimated heterozygosity [H_e] = 0.558, and observed heterozygosity [H_o] = 0.523; Table 1). Shetzline South Pond was also the only location that showed evidence of a recent genetic bottleneck (P = 0.007). Mean number of alleles and allelic richness were greatest in Elijah Bristow Berry Slough (12.222 and 11.677, respectively), and expected and observed heterozygosities (H_e and H_o) were greatest in the 1998 sample from Hospital Pond (0.806 and 0.832, respectively; Table 1). When we compared genetic diversity among temporal replicate samples, the only significant difference observed was in samples from Geren Island, where allelic richness was significantly lower (P = 0.012) in the 2005 sample compared with the 1997 sample. We observed weak, nonsignificant correlations between estimates of genetic diversity and abundance estimates by using both the abundance estimate for the year when genetic samples were collected at each site and the 5-year average abundance estimate (Figure 2).

When we assumed a 3-year generation interval, estimates of N_e ranged from 160.8 to 215.6 (Table 2). When we assumed a 4-year generation interval, estimates of N_e ranged from 120.2 to

TABLE 2. Temporal estimates of effective population size (N_e), associated 95% confidence limits (CLs), and N_e/N ratios for four Oregon chub populations. Estimates were calculated assuming both a 3-year and a 4-year generation interval. Negative estimates of N_e for Shady Dell Pond suggest that there was little genetic drift among sampling years and that the effective size of this population is too large to accurately estimate using the temporal method. Population estimates were averaged over the sampling period; NA = not applicable.

			3-year generation interval				4-year generation interval			
Population	Sampling years	Mean population estimate	N_e	Lower CL	Upper CL	N_e/N	N_e	Lower CL	Upper CL	N_e/N
Geren Island	1997, 2005	2,191	160.8	42.9	411.3	0.073	120.2	32.1	308.1	0.055
EF Minnow Creek	1997, 2004	3,966	194.9	46.3	1,185.5	0.049	146.2	34.8	890.4	0.037
Hospital Pond	1998, 2005	3,180	215.6	54.1	1,207.5	0.068	161.7	40.6	906.9	0.051
Shady Dell Pond	1998, 2005	3,086	−1,275.2	NA	NA	NA	−956.4	NA	NA	NA

TABLE 3. Pairwise estimates of genetic variation (F_{ST}) among Oregon chub sampling locations. The estimates are based on nine microsatellite loci. Values in bold italics represent nonsignificant comparisons after Bonferroni and B-Y FDR corrections. Abbreviations are as follows: CF = Coast Fork, MF = Middle Fork, and EF = East Fork.

Location identification number	Location name	Location identification number																			
		1	1A	2	3	4	5	6	7	8	9	10	11	12	13	13A	14	14A	15	15A	16
1	Geren Island																				
1A	Geren Island	***0.000***																			
2	Stayton PW Pond	0.040	0.047																		
3	Warren Gray Slough	0.019	0.025	0.029																	
4	Santiam I-5 Channel Pond	0.044	0.050	0.057	0.036																
5	Finley NWR Gray Creek Swamp	0.148	0.139	0.138	0.138	0.141															
6	Shetzline South Pond	0.177	0.169	0.215	0.186	0.181	0.250														
7	Big Island	0.069	0.067	0.096	0.073	0.064	0.135	0.130													
8	CF Willamette	0.079	0.071	0.099	0.067	0.079	0.150	0.156	0.079												
9	Elijah Bristow Berry Slough	0.069	0.062	0.072	0.049	0.054	0.089	0.154	0.058	0.059											
10	Elijah Bristow North Slough	0.059	0.052	0.063	0.040	0.051	0.103	0.164	0.059	0.055	0.010										
11	Dexter Reservoir RV Alcove	0.072	0.064	0.083	0.051	0.068	0.122	0.169	0.085	0.053	0.028	0.017									
12	Dexter Reservoir Alcove - The Pit	0.060	0.055	0.063	0.047	0.054	0.101	0.157	0.064	0.059	0.016	0.012	0.025								
13	EF Minnow Creek Pond	0.056	0.053	0.063	0.056	0.047	0.112	0.173	0.056	0.074	0.024	0.031	0.058	0.031							
13A	EF Minnow Creek Pond	0.052	0.046	0.069	0.054	0.049	0.113	0.149	0.051	0.063	0.017	0.026	0.050	0.023	***0.001***						
14	Hospital Pond	0.055	0.044	0.073	0.047	0.049	0.116	0.162	0.054	0.056	0.019	0.014	0.037	0.021	0.020	0.014					
14A	Hospital Pond	0.049	0.037	0.070	0.044	0.051	0.101	0.152	0.054	0.052	0.024	0.017	0.033	0.023	0.024	0.020	***0.001***				
15	Shady Dell Pond	0.071	0.056	0.086	0.066	0.074	0.112	0.156	0.060	0.069	0.023	0.031	0.047	0.033	0.035	0.025	0.020	0.021			
15A	Shady Dell Pond	0.071	0.058	0.085	0.063	0.069	0.120	0.156	0.052	0.056	0.025	0.031	0.050	0.035	0.031	0.023	0.021	0.022	***0.001***		
16	Buckhead Creek	0.087	0.072	0.113	0.086	0.094	0.131	0.167	0.070	0.081	0.037	0.050	0.062	0.048	0.040	0.028	0.025	0.027	0.014	0.018	

161.7 (Table 2). Our estimates of N_e for Shady Dell Pond were negative values suggesting that the change in allele frequencies between the two samples was less than would be expected owing to sampling error and that the effective size was infinitely large. Ratios of N_e/N ranged from 0.037 to 0.073 (Table 2).

Analysis of Population Structure

The overall level of genetic variation among sampling locations (F_{ST}) was 0.078 and was significantly different from 0.000 (95% CI = 0.068–0.088). Estimates of F_{ST} by subbasin were 0.037 for the Santiam, 0.130 for the McKenzie, and 0.030 for the MF Willamette. Pairwise estimates of F_{ST} ranged from 0.000 for the comparison between the two EF Minnow Creek samples to 0.250 for the comparison between the Shetzline South Pond and the Finley National Wildlife Refuge (NWR) samples (Table 3). After Bonferroni and B-Y FDR corrections, contingency tests of allele frequencies showed that there were significant differences in allele frequencies among all sampling locations but not among temporal replicate samples (i.e., Geren Island 1997 and Geren Island 2005, EF Minnow Creek 1997 and EF Minnow Creek 2005, Hospital Pond 1998 and Hospital Pond 2005, and Shady Dell Pond 1998 and Shady Dell Pond 2004–2005 [Table 3]).

Sampling locations from the same subbasin grouped together on the NJ tree with high bootstrap support for the different subbasin groupings (Figure 3). The Finley Gray Swamp and CF Willamette samples were the only collections from their respective subbasins, and these two locations grouped independently on the NJ tree with relatively long branch lengths. All temporal replicate samples grouped together with high bootstrap support

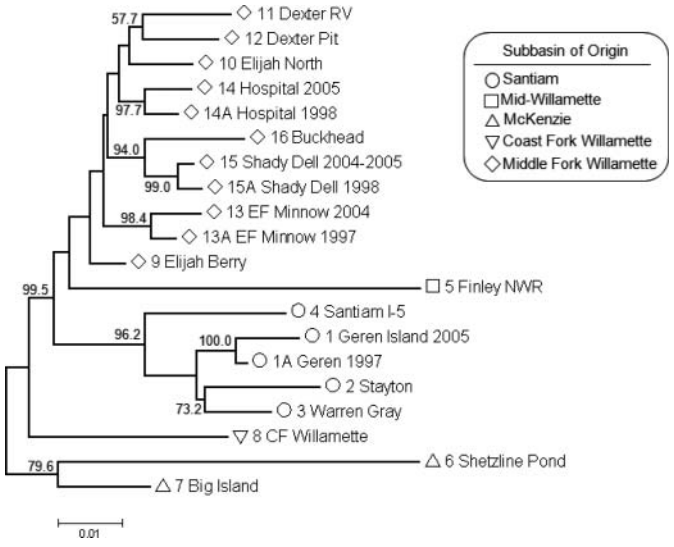

FIGURE 3. Consensus neighbor-joining tree based on Cavalli-Sforza and Edwards' (1967) chord-distances for 16 Oregon chub sampling locations. Values at the nodes represent the percentage of 1,000 bootstrap replicates that showed the displayed arrangement. Only bootstrap values greater than 50.0% are shown. Shapes indicate the different subbasins where each sampling location is located. Sampling years are given for locations that were sampled multiple times.

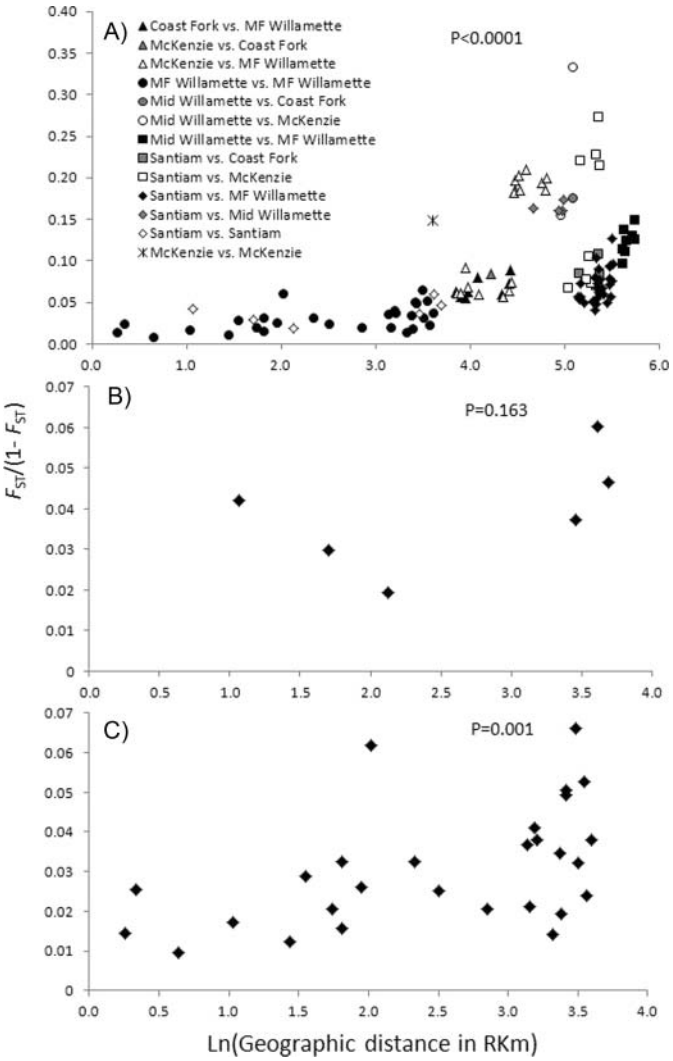

FIGURE 4. Analysis of isolation by distance for Oregon chub sampling locations. Geographic distance was measured as the natural log of the fluvial distance between sampling locations (measured in kilometers) and genetic distance was measured as $F_{ST}/(1 - F_{ST})$. (**A**) The relationship among all sampling locations in the Willamette River basin. The different shapes designate comparisons among locations in the different subbasins. (**B**) The relationship among sampling locations within the Santiam River subbasin only. (**C**) The relationship among sampling locations within the MF Willamette River subbasin only. *P*-values are based on Mantel tests (1,000 replicates).

(Figure 3). At the basin-wide scale, we observed a significant isolation-by-distance relationship (Mantel test: $P < 0.00001$; Figure 4A). We did not observe significant isolation by distance in the Santiam subbasin (Mantel test: $P = 0.163$; Figure 4B), but we did observe significant isolation by distance in the MF Willamette subbasin (Mantel test: $P = 0.001$; Figure 4C). The AMOVA showed that 91.1% of the total genetic variation was among individuals within sampling locations, 5.0% of the total genetic variation was among subbasins, and 3.9% of the genetic variation was among locations within the different subbasins.

Initial STRUCTURE analysis showed that a K of 10 had the highest likelihood, that is, $L(K) = -26051.8$ (Figure 5A), and K of 3 was the highest ΔK with a value of 33.3 (Figure 5B). The STRUCTURE plot for $K = 3$ showed chub samples from sampling locations from the Santiam River formed one genetic cluster, those from sampling locations from the CF Willamette and MF Willamette formed a second genetic cluster, those from sampling locations from the McKenzie River formed a third genetic cluster, and those from Finley NWR Gray Creek Swamp in the mid-Willamette shared membership with both the McKenzie and the MF Willamette clusters (Figure 5C). Most individuals in the $K = 3$ plot exhibited high membership to one specific genetic cluster (i.e., high q-values) with the exception of Finley NWR Gray Creek Swamp and Big Island (Figure 5C). The STRUCTURE plot for $K = 10$ showed that three locations from the Santiam (Geren Island North Channel, Stayton Public Works Pond, and Warren Gray Slough) formed one genetic cluster, Santiam I-5 Channel Pond, Finley NWR Gray Creek Swamp, Shetzline South Pond, Big Island, and CF Willamette each formed a distinct genetic cluster, and locations from the MF Willamette River comprised the remaining four clusters (Figure 5C). With the exception of the locations in the MF Willamette, most individuals in the $K = 10$ plot had high membership to one specific genetic cluster. In the MF Willamette, most individuals exhibited membership to multiple clusters, although individuals from Shady Dell Pond and Buckhead Creek appeared to form a somewhat distinct cluster.

DISCUSSION

Genetic Diversity within Populations

Several species of cyprinids are currently listed as threatened or endangered, and many have low levels of genetic diversity (see Table A.1 and references therein). Maintaining adequate levels of genetic diversity is important for endangered species, particularly in cases where species exist largely as a collection of isolated populations. Populations with higher levels of genetic diversity will be better able to adapt to changing environmental conditions, and are likely to have increased fitness and a lower risk of extinction (Quattro and Vrijenhoek 1989; Saccheri et al. 1998; Reed and Frankham 2003). Our survey of genetic variation within Oregon chub populations included locations from the entire species range. Despite declines in distribution and abundance and isolation among several of the sampling locations, levels of genetic diversity remain relatively high in nearly all locations and most of the locations we sampled do not appear threatened by the effects of low genetic diversity. Furthermore, estimates of genetic diversity observed in this study were often greater than, or equivalent to, those observed in several other species of cyprinids, many of which are also listed as threatened or endangered (Table A.1). It is important to consider that all locations were sampled several decades after major dam construction and flood control activities on the Willamette River had been completed, and it is unknown whether levels of

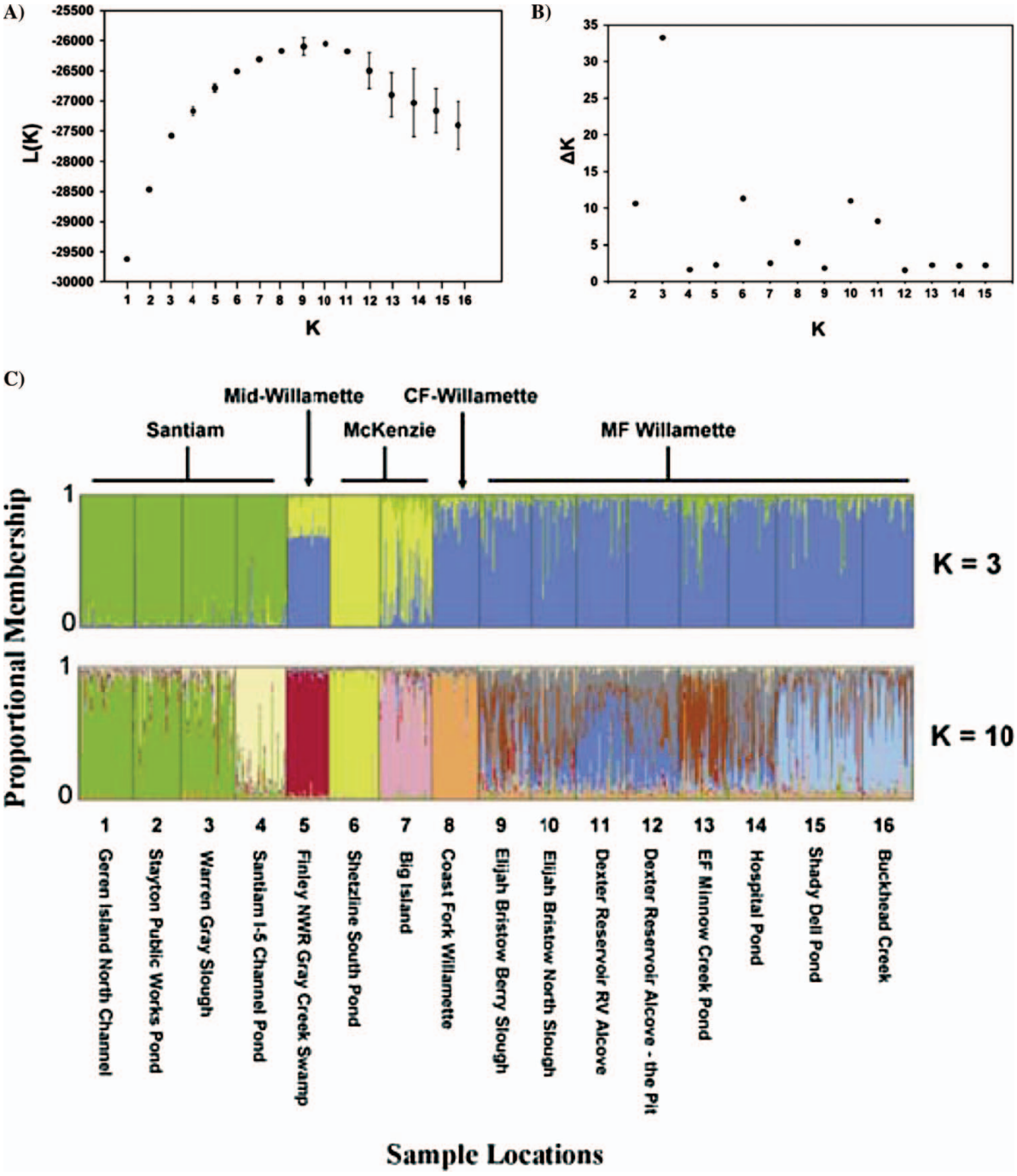

FIGURE 5. Results from the program STRUCTURE to determine the most likely number of Oregon chub populations including (**A**) likelihood values for each K ($L[K]$) from 1 to 16, (**B**) ΔK values, and (**C**) the mean membership for each individual in each genetic cluster when $K = 3$ (the highest ΔK) and $K = 10$ (the highest likelihood). (Color figure available online).

genetic variation we observed at each location were consistent with those that existed before the extensive alteration of the Willamette River system. One sample location, Shetzline South Pond, had significantly lower levels of variation and showed evidence of a recent genetic bottleneck. When Shetzline South Pond was sampled in 2004 and 2005, Scheerer et al. (2005b) estimated abundance at this location at 1,050 and 730 individuals, respectively. Although abundance was relatively high, no data exist for this location before the time genetic samples were collected, and our genetic data suggest that it experienced a severe decline before our sampling efforts.

Previous studies have demonstrated that larger populations often show greater levels of nuclear genetic diversity (Frankham 1996; Bazin et al. 2006). This has important implications for threatened and endangered cyprinids that often experience large fluctuations in population size from one year to the next; genetic diversity could vary substantially from year to year as population sizes fluctuate. Although abundance for the locations we sampled ranged from a few hundred to several thousand individuals, we observed little correlation between abundance estimates and estimates of genetic diversity. Four locations—Geren Island, EF Minnow Creek, Hospital Pond, and Shady Dell Pond—were also sampled at multiple time periods, two to three Oregon chub generations apart. When we compared estimates of genetic variation among temporal replicates, we observed no significant differences in measures of diversity between replicate samples. Samples from Geren Island provided an exception, however; we observed a significant decline in allelic richness from 1997 to 2005. Abundance estimates for Geren Island chub declined from an estimate of over 8,000 individuals in 1997 to approximately 2,600 individuals in 2005, a likely explanation for the reduction in allelic richness we observed. Unlike many species that maintain relatively constant population size from one year to the next, Oregon chub abundance can vary widely from year to year, even at sites that are stable over the long term (Bangs et al. 2009). Our data provide evidence that for Oregon chub, genetic diversity generally remains stable over time. This same trend of stable genetic diversity, despite fluctuations in population size, was observed in a study of another threatened cyprinid, the Pecos bluntnose shiner *Notropis simus pecosensis* (Osborne et al. 2010). These data, along with our results, suggest that abundance is not always associated with genetic diversity, and biologists need to consider both types of information when assessing population viability.

Estimates of effective population size can provide another means of evaluating population viability (Luikart et al. 2010). Establishing guidelines for minimum effective population sizes for threatened and endangered species can be difficult (Allendorf and Luikart 2007). As a general guideline, Franklin (1980) proposed that populations with an N_e of fewer than 50 may face immediate risks of inbreeding and genetic drift. Following this rule, our estimates of N_e do not suggest that the locations we sampled face an immediate genetic risk. Franklin (1980) also suggested that an N_e of more than 500 may be necessary to avoid long-term genetic risks. Three of the locations we surveyed had N_e estimates of fewer than 500, and ratios of N_e/N were somewhat lower than those reported for other wild populations (Frankham 1995). When N_e is low, connectivity among populations and the potential for gene flow is important for maintaining genetic variation and ensuring long-term population persistence (Rieman and Allendorf 2001; Palstra and Ruzzante 2008). Pairwise estimates of F_{ST} as well as STRUCTURE analysis suggest that these locations do have some degree of connectivity with other nearby sampling locations and this connectivity will probably help to maintain genetic variation. Many other locations where Oregon chub are found are presently isolated from one another and there could be concerns associated with isolation and low effective population size at these locations.

Analysis of Population Structure

An understanding of how populations are delineated is important for developing effective management and recovery plans for threatened and endangered species (Pearse and Crandall 2004; Vähä et al. 2007; Hasselman et al. 2010). A number of methods exist for using genetic data to infer the number of populations (Waples and Gaggiotti 2006) and we employed several of these methods to determine the number of populations represented by the sampling locations in our data set. Analyses based on genetic distance presented here suggest a significant level of genetic variation among the 16 sampling locations. Furthermore, pairwise estimates of genetic variation and contingency tests of allele frequency heterogeneity indicated that all sampling locations contained genetically distinct populations. Generally we observed much greater levels of variation among locations from different subbasins than we did among locations within the same subbasin, particularly in the Santiam and the MF Willamette rivers. This probably reflects more recent divergence between sampling locations within subbasins, as well as contemporary gene flow among locations within the same subbasin. Significant isolation by distance at the basin-wide scale and among locations within the MF Willamette River provides additional evidence that gene flow primarily occurs among geographically proximate populations.

Alternatively, STRUCTURE analysis suggests that there are either 10 (highest likelihood) or 3 (highest ΔK) populations. The plot of $K = 3$ suggests that the Santiam subbasin represents one population, the MF Willamette and CF Willamette represent a second population, the McKenzie represents a third population, and Finley NWR Gray Creek Swamp in the mid-Willamette is similar to both the McKenzie and the MF Willamette populations. The plot of $K = 10$ shows five of the sampling locations as distinct clusters or populations (locations 4–8), three of the Santiam locations as a single distinct cluster or population (locations 1–3), and individuals from the MF Willamette as a collection of fish from four different clusters or populations. These results suggest that Oregon chub from locations in the mid-Willamette, McKenzie, CF Willamette, and I-5 Channel Pond from the Santiam (location 4) are mostly isolated from one another and represent distinct populations, whereas those from locations in the Santiam and the MF Willamette rivers have greater levels of gene flow, and population boundaries are not as clearly defined. This is particularly true in the MF Willamette River where many individuals appear to have membership in multiple genetic clusters and no locations had individuals corresponding to a single genetic cluster. Although the ΔK method has been reported to provide a more accurate estimate of the number of populations for data sets with high levels of variation among populations (Evanno et al. 2005), other studies have found that this method did not perform as well when the level of

variation among populations was more moderate (Waples and Gaggiotti 2006; Hasselman et al. 2010). Based on the level of variation we observed (overall $F_{ST} = 0.078$), a K of 10 may be a better representation of our data set.

Differences in the number of populations inferred from distance-based methods and contingency analyses versus Bayesian methods may reflect the limited ability of STRUCTURE to detect the true number of populations for our data set. Waples and Gaggiotti (2006) found that contingency tests were more accurate than Bayesian analysis methods such as STRUCTURE for identifying the true number of populations when there were moderate to high levels of gene flow among populations. Furthermore, Latch et al. (2006) found that when F_{ST} was relatively low (0.01–0.02), STRUCTURE often could not identify the correct number of populations. The main differences between contingency analyses and the $K = 10$ STRUCTURE analysis were the number of populations detected in the Santiam and MF Willamette subbasins. Overall estimates of F_{ST} for these subbasins were approximately 0.030, although several pairwise estimates of variation within these two subbasins were much lower than this. Increased gene flow in these subbasins may be causing STRUCTURE to underestimate the true number of populations. STRUCTURE may also have difficulty determining the number of populations when unsampled populations exist (Waples and Gaggiotti 2006). Oregon chub have been collected at several additional locations that were not included in this study (Bangs et al. 2009), and genetic analysis of these locations may help to further clarify the number of populations that exist. Overall, our data suggest that each sampling location does in fact represent a genetically distinct population and that there are greater levels of gene flow among populations in the Santiam and MF Willamette subbasins. Our data highlight the need to consider multiple analysis methods when inferring the number of populations.

Genetic data can also play an important role in organizing populations of threatened and endangered species into management and recovery units (Parker et al. 1999; Currens et al. 2009; Ardren et al. 2011). At the time of listing, when no genetic data were available, Oregon chub had been documented in the main-stem (mid) Willamette, the MF Willamette, and the Santiam rivers, and these subbasins were identified as separate recovery areas (USFWS 1998). The recovery plan also established recovery goals for each area to achieve downlisting and delisting for Oregon chub. Since recovery goals are focused at the level of these recovery areas, it is important that they are accurately defined. Oregon chub populations grouped together on the NJ tree according to their subbasin of origin, which suggests that subbasins of the Willamette River represent the major genetic groupings for Oregon chub. The two major groups on the NJ tree represent the populations from the MF Willamette and Santiam subbasins. Despite the limited number of samples from the mid-Willamette, McKenzie, and CF Willamette, populations from these subbasins were isolated on the NJ tree and had relatively long branch lengths, and pairwise estimates of variation suggest that these three subbasins are distinct genetic groups as well. Furthermore, the AMOVA indicated that there were greater genetic differences among the subbasins than there were among the populations within the subbasins.

Data on the amount of variation among populations and sampling locations has additional implications for Oregon chub conservation. When populations are isolated and become small, genetic drift may result in significant changes in allele frequencies within a population over the course of only a few generations (Yamamoto et al. 2004; Demandt 2010). Despite fluctuations in abundance in the four populations that were sampled over multiple generations, pairwise estimates of variation and contingency tests showed no significant differences in allele frequencies among temporal replicate samples from the same population (e.g., Hospital Pond 1998 and Hospital Pond 2005), and temporal replicates always grouped together on the NJ tree with high bootstrap support. These data indicate that allele frequencies in these populations were stable over the course of the sampling period and did not change significantly owing to genetic drift.

Conservation Implications

When habitats are fragmented, fisheries managers are often faced with the dilemma that increasing connectivity to reduce the risk of extinction due to isolation can increase the probability that nonnative fishes will invade and increase the extinction risk due to competition, predation, or hybridization (Peterson et al. 2008; Fausch et al. 2009). Although floods were important to Oregon chub historically, they now pose a substantial risk through the dispersal of nonnative fishes (Scheerer 2002). The severe anthropogenic alteration of the Willamette River drainage has relegated biologists to managing many populations of Oregon chub in isolation (Scheerer 2002). This is contrary to their evolutionary life history and may have important genetic implications. Small isolated populations often show reduced levels of genetic variation (Yamamoto et al. 2004; Wofford et al. 2005; Neville et al. 2006) and may face a greater risk of extinction as a result. Although levels of genetic diversity observed in Oregon chub were greater than those observed in several other threatened and endangered cyprinid species (Table A.1), continued isolation of some populations will probably lead to an increase in genetic drift and a reduction in genetic diversity. In instances where connectivity cannot be restored owing to the risks associated with nonnative species, or because the natural connection to the floodplain has been lost, translocations of small numbers of individuals among populations (i.e., genetic rescue; Mills and Allendorf 1996; Tallmon et al. 2004) may provide a reasonable alternative. It is important to recognize that there are both demographic and genetic risks associated with this strategy and these risks should be carefully considered before any action is taken (Tallmon et al. 2004).

Introductions and translocations can be effective methods for conserving threatened and endangered fishes, but these efforts should be carefully planned and should incorporate genetic data into the planning process (George et al. 2009). Information on

levels of genetic diversity within populations and the genetic relationship among populations are important when selecting donor stocks for translocations and reintroductions (Meffe 1995; Drauch et al. 2008; George et al. 2009). The use of source populations with low genetic diversity may result in introduced populations that are unable to adapt to changing environmental conditions, have low fitness, and a low probability of persistence (Quattro and Vrijenhoek 1989; Meffe 1995; Minckley 1995). Information on the level of genetic divergence among populations is also important since mixing genetically divergent populations may result in outbreeding depression and reduced fitness (Gharrett et al. 1999; Goldberg et al. 2005). Population introductions have been an effective means of increasing the numbers and distribution of Oregon chub and for achieving recovery goals (Scheerer 2007), but previous introduction efforts for Oregon chub were implemented before genetic data were available and donor populations were mainly selected based on abundance estimates and geographic proximity to donor sites. Genetic information presented in this study can be used to make more informed decisions regarding introduction strategies. For example, Shetzline South Pond was one of two known natural populations in the McKenzie River subbasin when it was sampled in 2004 and 2005 and had a population of Oregon chub that was larger than many others at the time. While this might make this population appear to be a good source population for introductions in the McKenzie subbasin, Shetzline South Pond had the lowest levels of genetic diversity we observed (allelic richness in all other populations was at least twice as great) and showed evidence of a recent genetic bottleneck. Clearly this population is not the most appropriate choice as a donor for future introduction efforts based on genetic data.

Genetic studies such as this one can provide important information for managing threatened and endangered species, but it is important to recognize that such studies often reflect a single point in time and that genetic variation often changes over time as a result of biological processes (Østergaard et al. 2003) and management actions (Palstra and Ruzzante 2010). When management actions such as population introductions and supplementation are used to conserve genetic diversity in natural populations, it is important to establish a genetic monitoring protocol that involves temporal replicate sampling so that the effects of these actions can be evaluated (Schwartz et al. 2007). Genetic monitoring can be used to track changes in levels of genetic diversity, estimates of effective population size, and gene flow among populations over time, as well as population responses to different management actions (Schwartz et al. 2007; Osborne et al. 2010; Palstra and Ruzzante 2010). Information presented in this study serves as a baseline for future genetic monitoring efforts for Oregon chub. Additional sampling of these populations in the future will allow biologists to track any changes in levels of genetic variation in natural populations, as well as population responses to future introduction efforts and management actions aimed at re-establishing connectivity among populations.

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Appendix: Genetic Diversity Estimates for Cyprinids

TABLE A.1. Published estimates of genetic diversity for a number of different cyprinid species. The values represent the minimum and maximum values of genetic diversity observed (averaged over all loci) for the different localities sampled in each study. Bold italics indicate the present study. Abbreviations are as follows: Min = minimum, Max = maximum, E = endangered, T = threatened, W = widespread and abundant, A = the mean number of alleles per locus, A_R = allelic richness, H_e = expected heterozygosity, H_o = observed heterozygosity, and NA = data not available.

Species	Current status	Number of microsatellite loci surveyed	Number of locations surveyed	A		A_R	
				Min	Max	Min	Max
Jarabugo *Anaecypris hispanica*	E	5	8	7.40	13.40	NA	NA
Portugese nase *Chondrostoma lusitanicum*	E	6	6	2.00	4.00	1.95	3.42
Rare minnow *Gobiocypris rarus*	E	11	1[b]	5.00	5.36	NA	NA
Rio Grande silvery minnow *Hybognathus amarus*	E	7	7	9.30	11.10	NA	NA
Cape Fear shiner *Notropis mekistocholas*	E	11	3	5.09	5.27	NA	NA
Cape Fear shiner	E	22	2	7.77	8.59	5.56	5.62
Pecos bluntnose shiner *Notropis simus pecosensis*	T	11	1[a]	NA	NA	20.70	22.56
Oregon chub Oregonichthys crameri	***T***	***9***	***16***	***3.44***	***12.22***	***3.38***	***11.68***
Gila topminnow *Poeciliopsis occidentalis*	E	5	4	1.20	3.80	NA	NA
Creek chub *Semotilus atromaculatus*	W	7	32	1.20	5.86	8.90	35.20
Creek chub	W	19	16	NA	NA	NA	NA
Squalius aradensis[b]	E	7	10	2.30	9.00	2.00	6.50

[a]In these studies one spawning population was sampled multiple times over the course of multiple years for genetic monitoring.

[b]No common name; found in Portugal.

TABLE A.1. Extended.

H_e		H_o		
Min	Max	Min	Max	Reference
0.590	0.780	0.440	0.730	Salgueiro et al. (2003)
0.230	0.354	0.233	0.333	Sousa et al. (2008)
0.560	0.620	0.420	0.520	He and Wang (2010)
0.680	0.750	0.530	0.720	Alo and Turner (2005)
NA	NA	0.491	0.600	Burridge and Gold (2003)
0.772	0.780	NA	NA	Saillant et al. (2004)
0.842	0.859	0.644	0.694	Osborne et al. (2010)
0.558	***0.806***	***0.523***	***0.832***	***The present study***
0.075	0.281	0.070	0.305	Parker et al. (1999)
0.604	0.644	0.039	0.671	Boizard et al. (2009)
0.417	0.572	0.424	0.570	Skalski et al. (2008)
0.236	0.637	0.250	0.616	Mesquita et al. (2005)