Analyses of Genetic Variation in Natural and Re-introduced Populations of Oregon Chub (*Oregonichthys crameri*)

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Summary

Oregon chub are a small floodplain minnow native to the Willamette River Valley of western Oregon. Historically Oregon chub were abundant throughout the Willamette River system and dispersed among off-channel habitats during periodic flood events. Extensive modification of the Willamette River Basin for flood control and navigation purposes and the introduction of non-native fishes led to substantial declines of Oregon chub and the species was listed as endangered by the U.S. Fish and Wildlife Service in 1993. Since the time the species was listed, the status of the species has improved due to habitat modifications and improvements, the discovery of new populations, and the re-introduction of several populations. Data suggest that Oregon chub are recovering and the species was downlisted to threatened status in 2010. Genetic data can provide important information for assessing the present status of Oregon chub populations as well as a means of evaluating population introduction strategies. In this study we used microsatellite DNA markers to examine the level of variation within and among Oregon chub populations and to evaluate four introduced chub populations. The levels of genetic diversity observed within Oregon chub populations were relatively consistent across the species distribution and were equal to or greater than those observed in other species of cyprinids. Levels of genetic diversity appear to be stable over time with no significant relationship between population size and genetic diversity. We observed significant genetic variation among all chub populations surveyed, indicating that gene flow among populations is limited. Populations in close geographic proximity were most genetically similar to one another suggesting that historical gene flow followed a stepping stone pattern. Levels of genetic diversity observed in three of four introduced populations were similar to natural populations. One introduced population that was founded from only 50 fish had lower levels of genetic diversity than populations founded using greater numbers of individuals and/or multiple source populations. When populations were introduced using multiple source populations, individuals from the different source populations did interbreed and the genetic signature of each of the founding populations was evident in the introduced population. Information presented in this report provides important baseline data for monitoring Oregon chub populations as well as information that will be useful for planning future introductions.

Introduction

Genetic data have become increasingly more important for conserving and managing threatened and endangered species (Allendorf and Luikart 2007). Genetic data have been used for identifying species and populations that have reduced genetic diversity that may face a greater risk of extinction (Quattro and Vrijenhoek 1989; Saccheri et al. 1998). Genetic data can also be used to infer the number of populations or evolutionary groups present (Waples 1995; Parker et al. 1999; Spruell et al. 2003; Waples and Gaggiotti 2006; Currens et al. 2009; Ardren et al., *in press*), information that can be useful in defining management and recovery units. Recently genetic data have been used to address more complex conservation issues including the estimation of effective population size (Peterson and Ardren 2009; Small et al. 2009), levels of migration and gene flow among populations (Howes et al. 2009), and the effects that barriers and other landscape features have on populations (Neville et al. 2006; Narum et al. 2008; Boizard et al. 2009).

Another area of endangered species conservation where genetic data have proven useful is for implementation and monitoring of population introductions and re-introductions. Consideration of genetic data is important when selecting donor stocks for population introductions. Donor stocks should contain adequate levels of genetic diversity in order to prevent inbreeding effects in introduced populations and ensure that introduced populations will be able to adapt to changing environmental conditions (Minckley 1995; Drauch et al. 2008; George et al. 2009). Genetic data can also be used to evaluate previous introduction efforts to determine if levels of genetic variation observed in the source populations have been maintained in introduced populations (Mock et al. 2004; Stephen et al. 2005; Drauch and Rhodes 2007) and to determine how introduction strategies have affected the genetic structure of native and introduced populations (Matala et al. 2008; Williams and Scribner 2010).

Oregon chub *Oregonichthys crameri* (Snyder 1908) are small floodplain minnows endemic to the Willamette Valley of western Oregon (Markle et al. 1991). Oregon chub grow to 75-90 mm TL, mature at age 2 (>40 mm), and are relatively long-lived (up to 9 years). Oregon chub prefer off-channel habitats with minimal or no flow, an abundance of vegetation, and depositional substrate (Pearsons 1989; Scheerer 2002). They spawn in aquatic vegetation from May through July when water temperatures exceed 15°C (Scheerer and McDonald 2003). Historically, Oregon chub were widely distributed throughout the Willamette Valley (Markle et

al. 1991). 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). Floods provided the mechanism of dispersal and periodic genetic exchange among isolated off-channel habitats for Oregon chub populations.

Today, the Willamette River is a highly altered system. 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, large scale removal of snags for navigation, channelization and revetments, and the drainage of wetlands to increase the land available for river bottomland agriculture (Sedell and Froggatt 1984; Benner and Sedell 1997). Floods in the winter and spring months were common prior to the construction of the dams (1941-1969), averaging 14 floods above bankfull per decade from about 1884-1969 (US Army Corps of Engineers 1970). A 10-year flood event prior to construction of the dams now has a 100-year return interval (Benner and Sedell 1997). Channelization and the construction of flood control dams restricts or eliminates many of the linkages and interactions between the river and its floodplain (Gabriel 1993). Flood suppression alters the hydrologic cycle of riverine environments and impacts native fish that rely on floodplain habitats (Bayley 1991, Osmundson and Burnham 1998, Modde et al. 2001). The connectivity of off-channel habitats to the river can be important for the persistence of local populations of fish, and when substantial habitat fragmentation occurs, metapopulations can undergo severe declines (Hanski and Gilpin 1997).

The fish fauna of the Willamette basin is highly altered as well. 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 (Lemly 1985; Moyle 1976; Newman 1993; Rinne and Minckley 1991; Simon and Markle 1999).

Studies conducted in the 1970s and 1980s (Bond 1974; Bond and Long 1984; Markle et al. 1991) found the distribution of Oregon chub to be severely restricted. The primary factors implicated in the species' decline were the loss of habitat due to the activities described above and the introduction of nonnative species. Markle et al. (1991) found nonnative fishes were common in historic Oregon chub habitats that no longer contained Oregon chub. 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 when their habitats were invaded by nonnative fishes following flood events or stocking. The loss of Oregon chub habitat and the species' restricted range led to its listing as endangered under the Endangered Species Act in 1993 (U.S. Fish and Wildlife Service [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). In 2007, all downlisting criteria were met and the US Fish and Wildlife Service reclassified the species from endangered to threatened status in 2010 (USFWS 2010).

A major effort for Oregon chub recovery has focused on introducing Oregon chub into suitable habitats within their historic range and 12 new populations have been established since 1988. The Oregon Chub Recovery Plan states that a minimum of 500 fish are to be used when establishing new populations to avoid genetic bottlenecks and a maximum of 10% of a population may be removed for an introduction in any one year. The recovery plan also states that donor stocks should be located in the same subbasin as the introduction site, whenever possible. Potential Oregon chub introduction sites were identified and evaluated using the following guidelines: 1) Restrict introductions to the historic distribution of Oregon chub; 2) Restrict introductions to protected sites that are secure from imminent or future threats of habitat destruction (invasion by warm water fish is included in this category); 3) Restrict introductions to sites where the potential for dispersal has been determined and is acceptable; 4) Restrict introductions to sites that likely fulfill life history requirements (includes shallow ponds that are less than 2 meters deep and less than 1,000 meters in elevation with depositional substrate, gradually sloping banks, varied and abundant aquatic vegetation, little or no water velocity, limited use or access by the public, no non-native fish species, and summer water temperatures exceeding 15°C); 5) Restrict introductions to sites that contain sufficient habitat to support a genetically viable population; and 6) Prohibit introductions into areas where other rare or endemic taxa could be adversely affected.

Although the status of Oregon chub has been improving since the species was initially listed as endangered, and the understanding of the species biology and life history has increased, genetic data are important to validate assumptions used for conservation planning and to provide

important information for recovery planning that is currently lacking. The Oregon chub recovery plan specifies that re-introduced populations be genetically viable and that genetic data be considered when choosing donor stocks for re-introductions (USFWS 1998). The purpose of this study was to provide genetic data for Oregon chub populations that can be used to aid ongoing recovery efforts for the species. Specifically, we were interested in examining levels of genetic variation within and among Oregon chub populations distributed throughout the species range. This information will be important for identifying populations with reduced genetic diversity, for inferring the level of gene flow among populations, and for clarifying the delineation of management units. We were also interested in comparing levels of genetic diversity in introduced populations of Oregon chub to natural populations and determining how different introduction strategies may influence levels of genetic diversity and population structure of introduced populations. This information has important implications for planning future introductions of Oregon chub, as well as planning future introductions for other threatened and endangered species.

Methods

Sample collection

We collected tissue samples (caudal fin clips) during annual population surveys (Scheerer et al. 2005). We collected samples from 16 naturally occurring populations representing the geographic range of Oregon chub distribution (Figure 1; Table 1). We collected most samples during 2004 and 2005. For some locations (Geren Island, EF Minnow Creek Pond, Hospital Pond, and Shady Dell Pond), we also obtained tissue samples from specimens stored at the Oregon State University Museum. These specimens were collected during population surveys in 1997 and 1998. In addition, we collected tissue samples from four introduced populations in 2004 and 2005 (Tables 1 and 2). These introduced populations included two that originated from a single donor source (Display Pond and Wicopee Pond), two with multiple donor sources (Dunn Wetland, Fall Creek Spillway Pond), and one that was founded with a small number of individuals (Wicopee Pond; n=50 fish). We also collected tissue samples from a population of Umpqua chub (*O. kalawatseti*) in Cow Creek, Oregon for use as an outgroup.

Laboratory Analyses

Total genomic DNA was extracted from fin clips following a modified Chelex extraction protocol (Miller and Kapuscinski 1996). A small piece of fin tissue (approximately $1mm^2$) was placed in 190µl of a 5% Chelex (Chelex 100, Sigma) solution and boiled for eight minutes. All individuals were then genotyped at nine microsatellite loci: *Ocr100, Ocr103, Ocr104*, *Ocr105*, *Ocr106*, *Ocr109*, *Ocr111*, *Ocr113*, and *Ocr114* (Ardren et al. 2007). PCR was carried out in 15µl volumes and contained 2µl supernatant from the Chelex extractions and final concentrations of 1X PCR buffer (10mM Tris-HCl, 50mM KCl, 0.1% Triton x-100), 1.5mM $MgCl_2$, 0.2mM of each dNTP, 0.5µM of forward and reverse primer, and 0.2U *Taq* DNA polymerase (Promega). Reaction conditions were as follows: initial denaturation at 94ºC for 2.5 minutes, followed by 38 cycles of 94 ºC for one minute, one minute at primer specific annealing temperature (see Ardren et al. 2007), one minute at 72ºC, and PCR concluded with a final extension at 72ºC for seven minutes.

Following PCR, we pooled reactions for automated electrophoresis on an Applied Biosystems 3100 genetic analyzer (Applied Biosystems, Inc.). Electropherograms for each individual were analyzed using GENOTYPER v3.7 NT software (Applied Biosystems, Inc.). All individuals were double scored by multiple laboratory personnel. In order to assess genotyping error rate, 10% of the individuals were re-extracted and re-genotyped by a separate laboratory member following the procedures described above.

Statistical Analyses

For statistical analyses we grouped individuals into populations according to their sampling location. We split locations that had been sampled over multiple generations (Table 1) into two populations according to sampling year. Unless otherwise noted, we also included the Umpqua chub samples from Cow Creek in all statistical analyses. We tested populations for conformance to Hardy-Weinberg expectations (HWE) using exact tests implemented in the program GENEPOP v4.0 (Raymond and Rousset 1995). We also used GENEPOP to test each population 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 using a sequential Bonferroni adjustment (Rice 1989). We estimated measures of genetic diversity, including mean number of alleles per locus, and observed and expected heterozygosity, using the program GDA (Lewis and Zaykin 2001). Additionally, we used the

program HP-Rare v1.0 (Kalinowski 2005) to estimate allelic richness for each population, 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 among populations. Because of the relatively small sample size (n=21), we excluded the Umpqua Chub sample from allelic richness estimates.

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 to determine if there was a significant difference in allelic richness and expected heterozygosity among the temporal samples from E.F. Minnow Creek Pond, Shady Dell Pond, Hospital Pond, and Geren Island. We also tested whether populations introduced from multiple sources had greater levels of genetic diversity than introduced populations with a single source. We used permutation tests in the program FSTAT v2.9.3.2 (Goudet 2001) to determine if there were significant differences in allelic richness, and observed and expected heterozygosity between introduced populations with a single source (Wicopee Pond and Finley NWR Display Pond) and introduced populations with multiple sources (Fall Creek and Dunn Wetland).

Previous studies have shown a link between population size and levels of genetic diversity, with larger populations showing increased levels of variation (Frankham 1996; Bazin et al. 2006). Population sizes of Oregon chub vary from year to year and population to population (Bangs et al. 2009). We examined the relationship between population size and estimates of genetic diversity for Oregon chub. We calculated Pearson product-moment correlation coefficients between chub population estimates and measures of genetic diversity including allelic richness and observed heterozygosity. For each measure we calculated correlation coefficients using all collections from natural and introduced populations and then using the most recent collections from natural populations only. When genetic samples were collected over the course of multiple years (e.g. Buckhead Creek), we averaged estimates of population size over the collection years.

We tested populations for evidence of recent genetic bottlenecks (within the past 0.2 – 4.0 N_e generations) using the program BOTTLENECK (Cornuet and Luikart 1996). This method tests for an excess of heterozygotes relative to the frequency of alleles in the population (Luikart and Cornuet 1998). 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 population bottleneck tests.

We used FSTAT to estimate the overall level of genetic variation among populations (F_{ST}; Weir and Cockerham 1984) and the associated 95% confidence interval based on 1000 bootstrap replicates. We excluded the Umpqua chub from Cow Creek and the earliest samples (i.e. 1997 and 1998) from populations that had been sampled over multiple generations from the overall estimate of F_{ST}. We also estimated the level of genetic variation among natural origin populations from which we had multiple population samples (i.e. Santiam, McKenzie, and MF Willamette) within the major sub-basins of the Willamette River. We also used FSTAT to estimate the level of genetic variation among each population pair (pairwise F_{ST}) and to determine if pairwise estimates of F_{ST} were significantly different from 0.0. We included Umpqua chub and temporal replicate samples when estimating pairwise F_{ST}. We used a Bonferroni correction (Rice 1989) to adjust significance values of pairwise F_{ST} estimates for multiple comparisons. Using GENEPOP, we performed chi-squared contingency tests to determine if there were significant differences in allele frequencies among the different spawning tributaries. We adjusted P-values for multiple comparisons using a sequential Bonferroni correction (Rice 1989) and the B-Y FDR correction described in Narum (2006).

Oregon chub are poor swimmers and presumably migrate in a downstream direction during floods and high water events. If migration proceeds mainly downstream, we would expect increased levels of genetic diversity in populations located further downstream. To test this theory, we estimated the Pearson product moment correlation between upstream distance and allelic richness. We measured the upstream distance in kilometers relative to the I-5 side channel population (the furthest downstream population). We only included natural populations in the analysis and we based allelic richness estimates on the most recent sample for populations that had been sampled multiple times. Given the relatively large geographic distance among some populations at the basin wide scale, it may be more reasonable to assume that chub disperse primarily among populations within a sub-basin. Therefore, we examined this relationship for the Santiam and MF Willamette sub-basins (sub-basins where multiple natural populations had been sampled). We did not include the McKenzie River sub-basin in this analysis because we only sampled two populations, one of which showed evidence of a recent genetic bottleneck (see below). For the Santiam sub-basin, we used geographic distance relative to the I-5 side channel

population. For the MF Willamette sub-basin, we used geographic distance relative to the Elijah Bristow Berry Slough population.

We constructed a consensus neighbor joining (NJ) tree to examine the spatial genetic relationship among populations. Using the program PHYLIP v3.6 (Felsenstein 1993), we first generated 1000 replicate datasets using a bootstrap procedure. We then estimated Cavalli-Sforza and Edwards (1967) chord distances between all population pairs in each dataset and generated a consensus NJ tree using these values. 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 tributaries measured as $F_{ST}/(1- F_{ST})$. We performed a Mantel test (1000 permutations) to determine if there was a significant isolation by distance relationship. Because dispersal is more likely to occur among chub populations within a subbasin, we also conducted isolation by distance analysis separately for populations from the Santiam and MF Willamette basins only. We did not analyze the mid-Willamette, Coast Fork Willamette and McKenzie subbasins because only one or two population samples were available from these subbasins.

We conducted an analysis of molecular variance (AMOVA; Excoffier et al. 1992) to determine how genetic variation was partitioned among the natural Oregon chub populations. We grouped populations according to their subbasin of origin (Santiam, mid-Willamette, Coast Fork Willamette, McKenzie, and MF Willamette) to determine if there is more genetic variation among populations or among the different subbasins. AMOVA analysis was conducted using the program ARLEQUIN v3.11 (Excoffier et al. 2005).

Oregon chub populations in Fall Creek and Dunn Wetland were introduced from multiple source populations (Table 2). We used the Bayesian methods implemented in the program STRUCTURE v2.2 (Pritchard et al. 2000) to determine the contribution of each source population to the present populations in Fall Creek and Dunn Wetland. STRUCTURE determines the most appropriate number of clusters or populations (K) for a dataset without taking prior population designations into account. Structure can be used to determine the most likely number of clusters or populations for a dataset given a range of options (e.g. K = 1-10 possible populations) or the program can be used to partition individuals into a pre-defined number of populations (e.g. K = 2). We performed two independent STRUCTURE analyses using the source populations and the introduced populations; the first analysis partitioned

individuals from E.F Minnow Creek, Shady Dell Pond, and Fall Creek into 2 clusters and the second analysis partitioned individuals from Geren Island, Elijah Bristow Slough, Shady Dell Pond, and Dunn Wetland into 3 clusters. We used the allele frequency model that assumes gene flow among clusters and allows for correlations of allele frequencies across clusters. STRUCTURE analyses consisted of 30,000 burn-in iterations followed by 100,000 iterations. Each analysis was run 10 times to determine the proportion of each individual's genotype attributed to each population/cluster and the overall genetic composition of the two introduced populations. We used the program CLUMPP (Jakobsson and Rosenberg 2007) to determine the consensus results of the 10 STRUCTURE runs.

Results

Genetic Diversity within Populations

All populations 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 13 locus pairs (out of 900 total pairs) that showed evidence of linkage. The locus pairs that showed evidence of linkage appeared to be randomly distributed among populations. All estimates of genetic diversity (mean # alleles per locus, allelic richness, expected heterozygosity, and observed heterozygosity) were lowest in the Shetzline Pond population (A=3.444, A_R=3.381, H_e=0.558, and H_o=0.523; Table 1). Shetzline Pond was also the only population that showed evidence of a recent genetic bottleneck (P=0.007). The Dunn wetland introduced population showed the greatest estimates of genetic diversity (A=13.000, A_R=12.441, H_e=0.810, and H_o=0.834; Table 1). Among natural origin populations, mean number of alleles and allelic richness were greatest in the Elijah Bristow Berry Slough population (12.222 and 11.677, respectively) and expected and observed heterozygosity 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 population samples, the only significant difference observed was in the Geren Island population, where allelic richness was significantly lower in the 2005 sample (P=0.012). The mean estimates of allelic richness and observed and expected heterozygosity for introduced populations from a

single source were 6.028, 0.682, and 0.705, respectively. Mean estimates of these same measures for populations introduced from multiple sources were 9.467, 0.802, and 0.804, respectively. Permutation tests showed a significant difference in allelic richness among the two groups (P=0.034) but not in observed (P=0.131) or expected (P=0.173) heterozygosities. We observed weak, non-significant correlations between estimates of genetic diversity and population size (Figure 2).

Genetic Variation among Populations

The overall level of genetic variation among populations (F_{ST}) was 0.078 and was significantly different from 0.0 (95% C.I. = 0.068-0.088). Estimates of F_{ST} by basin were 0.037, 0.130, and 0.030 for the Santiam, McKenzie, and MF Willamette Basins, respectively. Pairwise estimates of $F_{ST,}$ which included all natural population samples, introduced populations, and Umpqua chub, ranged from 0.0 for the comparison between the two EF Minnow Creek samples to 0.367 for the comparison between the Finley NWR introduced population and the collection of Umpqua chub (Table 3). All pairwise estimates of F_{ST} were significantly different from 0.0 with the exception of: Geren Island 1997 and Geren Island 2005; EF Minnow Creek 1997 and EF Minnow Creek 2005; Hospital Pond 1998 and 2005; Shady Dell Pond 1998 and Shady Dell Pond 2004-2005; EF Minnow Creek 1997 and Fall Creek; EF Minnow 2005 and Fall Creek; and Elijah Bristow Berry Slough and Fall Creek. Following Bonferroni and B-Y FDR corrections, contingency tests of allele frequencies showed that there was a significant difference in allele frequencies among all population pairs with the following exceptions: Geren Island 1997 and Geren Island 2005; EF Minnow Creek 1997 and EF Minnow Creek 2005; Hospital Pond 1998 and Hospital Pond 2005; Shady Dell Pond 1998 and Shady Dell Pond 2004-2005; EF Minnow Creek 1997 and Fall Creek; and Elijah Bristow Berry Slough and Fall Creek (significant following B-Y FDR correction only).

In general, populations from the same subbasin grouped together in four main groups on the NJ Tree (Figure 3). Exceptions were the introduced Dunn Wetland sample (Mid-Willamette subbasin) which grouped with populations from the Santiam subbasin and the sample from the Coast Fork Willamette which clustered with the McKenzie samples. All temporal replicate samples grouped together with high bootstrap support (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), but we did observe significant isolation by distance in the MF Willamette subbasin (Mantel Test P = 0.001). AMOVA analysis showed that 91.1% of the total genetic variation was among individuals within populations, 5.0% of the total genetic variation was among subbasins, and 3.9% of the genetic variation was among populations within the different subbasins.

At the basin-wide scale, we did not observe a significant relationship between allelic richness and upstream distance (r = -0.0354, P = 0.8964; Figure 5A). In the Santiam and MF Willamette subbasins, we observed an increase in allelic richness from upstream to downstream populations (Figures 5B and 5C) and there was a high degree of correlation between the two variables; r = -0.8490 (P = 0.1510) for the Santiam subbasin and r = -0.7711 (P = 0.0251) for the MF Willamette subbasin.

Analysis of Introduced Populations

STRUCTURE results for the Fall Creek population showed that individuals from EF Minnow Creek and Shady Dell Pond formed two relatively distinct clusters. In Fall Creek we observed individuals that assigned primarily to one cluster as well as individuals that were a mixture of both clusters (Figure 6A). The overall proportion of each population in the sample from Fall Creek was 0.281 from Shady Dell and 0.719 from EF Minnow Creek (Figure 6B). We observed a high degree of consistency among the 10 STRUCTURE runs. Results of the Dunn Wetland STRUCTURE analysis showed that the three source populations formed distinct clusters. In Dunn Wetland we observed individuals that assigned primarily to one of these clusters as well as individuals that were a mixture of two or three clusters (Figure 7A). The overall proportion of each cluster present in the Dunn Wetland sample was 0.477 from Elijah Bristow, 0.367 from Geren Island and 0.156 from Shady Dell Pond (Figure 7B). Results were highly consistent among the 10 STRUCTURE runs.

Discussion

Historically, Oregon chub thrived in an unconstrained Willamette River, a dynamic riverine environment with frequent connectivity between off-channel habitats and the main river channel (Lewin 1978; Dykaar and Wigington 2000). Modifications to the Willamette River and its floodplain (Sedell and Froggatt 1984; Benner and Sedell 1997) led to the decline of Oregon

chub populations and concern for the continued persistence of this species (Scheerer 2002). Today Oregon chub populations are recovering as a result of habitat restoration efforts, creation of new habitat, isolating populations from non-native species in secure habitat, and introducing new populations into the Willamette River basin (Scheerer 2007). Information regarding levels of genetic diversity within chub populations, as well as the level of gene flow among remnant populations, will be important for guiding further recovery efforts for this species. Genetic analysis of the various re-introduction strategies that have been utilized to date will also be important for planning future re-introductions.

Genetic diversity within natural populations

Maintaining adequate levels of genetic diversity is important for endangered species, such as Oregon chub, that exist largely as a collection of isolated populations. Populations with greater levels of genetic diversity will be better able to adapt to changing environmental conditions than populations that lack diversity (Meffe 1995; Minckley 1995). Populations with increased genetic diversity are also likely to have increased fitness and a lower risk of extinction (Quattro and Vrijenhoek 1989; Meffe 1995; Saccheri et al. 1998; Reed and Frankham 2003). Furthermore, information on levels of genetic diversity within populations is important when selecting donor stocks for translocations and re-introductions (Meffe 1995; Drauch et al. 2008; George et al. 2009). Our survey of genetic diversity in Oregon chub included populations from the entire distribution of the species. Estimates of genetic diversity we observed for Oregon chub were greater than or equivalent to those observed in several other species of cyprinids (Appendix I), many of which are also listed as threatened or endangered (Burridge and Gold 2003; Saillant et al. 2004; Alo and Turner 2005; Skalski et al. 2008; Boizard et al. 2009). These data suggest that despite declines in abundance and isolation of many populations, adequate levels of genetic diversity still exist within Oregon chub populations.

In general, we found that levels of diversity were relatively consistent across the natural populations we surveyed, with somewhat higher levels observed in populations in the MF Willamette and Santiam River subbasins. Currently, greater numbers of chub are found in these two subbasins and historical records suggest greater numbers in these two subbasins as well (data from Oregon State University Collection, Dr. Douglas Markle, *personal communication*). Increased numbers and distribution of Oregon chub in the Santiam and MF Willamette subbasins

likely facilitated genetic exchange among populations in the past. Studies of creek chub (*Semotilus atromaculatus*) have demonstrated that populations with increased connectivity and genetic exchange show greater levels of genetic diversity than populations that are isolated or semi-isolated (Skalski et al. 2008; Boizard et al. 2009). Historic levels of exchange among populations likely helped to maintain slightly higher levels of genetic diversity within Oregon chub populations in the Santiam and MF Willamette subbasins. Genetic diversity was lowest in the Shetzline Pond population and this population also showed evidence of a recent genetic bottleneck. When this population was sampled in 2004 and 2005, Scheerer et al. (2004; 2005) estimated the size of the population at 1,050 and 730 individuals. Although the size of this population was relatively large at the time of sampling, little data exists for this population prior to the time genetic samples were collected and it is unknown how the size of this population in 2004 and 2005 compares to its past size. Genetic data suggest that this population may have experienced a severe decline prior to 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 endangered cyprinids that may experience fluctuations in population size from one year to the next. Although the size of the populations we sampled varied considerably, we observed little correlation between population size and estimates of genetic diversity (Figure 2). Four populations, Geren Island, EF Minnow Creek, Hospital Pond, and Shady Dell Pond were sampled at multiple time periods, three to four Oregon chub generations apart. When we compared estimates of genetic variation among temporal replicates, we observed no significant differences in measures of diversity between temporal replicates. Geren Island provided an exception however; we observed a significant decline in allelic richness from 1997 to 2005. The Geren Island population 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. Our data provide evidence that for endangered species with similar life history traits as Oregon chub, even though populations may fluctuate in size from year to year, genetic diversity remains somewhat stable over time. It is important to consider that all populations were sampled several decades after major dam construction and flood control activities on the Willamette River had been completed and it’s unknown whether levels of genetic variation we

observed were consistent with those that existed prior to the extensive alteration of the Willamette River system.

Genetic variation among natural populations

Genetic data are important for identifying evolutionary units and management groups for threatened and endangered species (Waples 1995; Parker et al. 1999; Spruell et al. 2003; Currens et al. 2009). At the time of listing, when no genetic data were available, Oregon chub had been documented in the mainstem Willamette, the MF Willamette, and the Santiam Rivers, and these subbasins were identified as separate recovery units (USFWS 1998). The recovery plan also established recovery goals for each of these units in order to achieve downlisting and delisting for Oregon chub. Since recovery goals are focused at the level of these units, it is important that these units are accurately defined. Oregon chub populations generally grouped together on the NJ tree according to their subbasin of origin suggesting 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 (Figure 3). Fewer chub populations exist in the mid mid-Willamette, McKenzie, and Coast Fork subbasins and fewer populations from these subbasins were included in our study. Populations from these subbasins were isolated on the NJ Tree with long branch lengths, and pairwise estimates of variation (Table 3) suggest that these three subbasins are distinct genetic groups as well. Furthermore, the AMOVA results also indicated that there were greater genetic differences among the subbasins than there were among the populations within the subbasins. Recently, new populations of Oregon chub have been discovered in multiple subbasins (Bangs et al. 2009) and analysis of these new populations will help to further identify the major genetic groups of Oregon chub in the Willamette River system.

Pairwise estimates of genetic variation and contingency tests indicated that all sampling locations contained genetically distinct populations, suggesting that gene flow is limited among Oregon chub populations. Historically periodic flood events served as the primary means for Oregon chub to disperse among geographically proximate spawning habitats. The significant isolation by distance relationship we observed at the basin wide scale and within the MF Willamette subbasin suggest that historically, Oregon chub exhibited a stepping stone model of dispersal and gene flow, which is consistent with fish dispersing among proximate populations.

This same relationship was observed in populations of creek chub in an un-impounded area (Skalski et al. 2008). It is presumed that Oregon chub dispersed in a downstream direction during flood events. Within the Santiam and MF Willamette subbasins we did observe an increase in allelic richness from upstream to downstream populations, which would be expected if fish were dispersing downstream. However, levels of allelic richness could be influenced by past colonization events, founder events, genetic bottlenecks, and a number of other factors and these data should not be interpreted as definitive evidence of downstream migration patterns. Modifications of the Willamette River system have significantly reduced the frequency of flooding in the Willamette system (Sedell and Froggatt 1984; Benner and Sedell 1997) and have likely led to a reduction in gene flow among Oregon chub populations. Although we observed genetic differences among all populations surveyed, there was greater variation among the different subbasins. Unlike some species of stream dwelling salmonids such as bull trout (*Salvelinus confluentus*) and westslope cutthroat trout (*Oncorhynchus clarki lewisi*) that exhibit very high levels of variation among populations due to extremely limited geneflow (Costello et al. 2003; Taylor et al. 2003; Taylor and Costello 2006), Oregon chub populations showed greater variation among subbasins suggesting that while some gene flow may have occurred among populations, genetic exchange among subbasins occurred less frequently.

When populations are small, genetic drift may result in significant changes in allele frequencies within a population over the course of a few generations (Yamamoto et al. 2004; DeMandt 2010). Despite fluctuations in population size in the four populations that were sampled multiple 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). These data indicate that allele frequencies in these populations were stable over the course of the sampling period and did not change significantly due to genetic drift.

Analysis of introduced populations

Introductions and re-introductions have been utilized as a conservation tool for many threatened and endangered fish species (George et al. 2009) and they can be particularly useful for species such as Oregon chub that have a short generation time, are highly fecund, and can be established in relatively small habitat patches. Introduction and re-introduction programs for

fishes are often carried out with little information available regarding the biology and genetics of the source population, no knowledge of potential interactions between the introduced species and native species, and no specific strategy for introductions (i.e. numbers of fish to use, numbers of source populations to use) (George et al. 2009). Furthermore, the success of many introduction and re-introduction programs is not evaluated after the species has been established or success is simply evaluated based on presence and abundance of the introduced species. Genetic information can be particularly valuable when selecting donor stocks prior to initiating species introduction programs (Meffe 1995; Drauch et al. 2008; George et al. 2009), for evaluating whether levels of genetic variation within introduced populations are consistent with those observed in the natural source populations (Mock et al. 2004; Stephen et al. 2005; Drauch and Rhodes 2007), and for evaluating the genetic structure of populations introduced from multiple donor populations/stocks (Williams and Scribner 2010). Re-introduction efforts for Oregon chub followed specific guidelines regarding numbers of individuals used to establish populations, source populations used for introductions, and the number of individuals that could be taken from each population. In this study we evaluated four re-introduced populations; each one established using different source populations and different numbers of source populations. This allowed us to examine the genetic differences among re-introduction strategies, information that will be helpful when planning future re-introductions.

In general, levels of genetic diversity in introduced populations were equal to or greater than natural source populations (Table 1). The Wicopee Pond population showed somewhat reduced levels of variation compared to the source population, Dexter Reservoir Alcove – the Pit. Only 50 individuals were introduced to Wicopee Pond in the late 1980s and monitoring of this population in the 1990s documented very few chub (Scheerer et al. 2000) indicating that this population initially persisted at very low levels. Introductions that utilize small numbers of individuals often produce populations with relatively low levels of genetic diversity (Mock et al. 2004; Stephen et al. 2005). Data from Wicopee Pond suggest that the use of a small number of founding individuals can result in chub populations with reduced genetic variation compared to other natural populations. Introduced populations founded from multiple sources (Dunn Wetland and Fall Creek) showed greater levels of genetic diversity than populations founded from a single source (Wicopee Pond and Finley NWR Display Pond) as well as greater levels than their source populations. Permutation tests revealed that allelic richness was significantly higher in

populations founded from multiple sources; however we only sampled two populations in each category. Assuming that populations with increased genetic diversity have greater population fitness (Quattro and Vrijenhoek 1989; Reed and Frankham 2003) these populations may have an increased likelihood of long-term persistence. Our data indicate that introductions that utilize greater numbers of individuals and potentially multiple source populations, will exhibit greater levels of genetic diversity.

When populations are introduced into new habitats, the possibility exists that individuals in the introduced population will segregate during reproduction due to differences in habitat preferences or behavior (Hendry et al. 1996; Hendry et al. 2000). We observed no deviations from HWE and no pairs of linked loci in the two populations introduced from multiple sources, providing evidence that chub from different source populations do interbreed in the introduced populations. It's also possible that when multiple populations are used for introductions, one population may be more successful that the other(s) (Page et al. 2003; Wilson et al. 2007). Results from the STRUCTURE analysis showed that Dunn Wetland and Fall Creek contained genetic material from each of the donor stocks, suggesting that intraspecific competition had not occurred among donor stocks in the introduced populations. Furthermore, comparison between the STRUCTURE results and the number of individuals used to found these two populations (Table 2) showed that the current genetic structure of these populations is roughly equivalent to the proportions of fish from each source used as founders. Mating among individuals from genetically differentiated populations can lead to a reduction in population fitness through outbreeding depression (Gharrett et al. 1999; Goldberg et al. 2005). The Dunn Wetland introduced population was founded using fish from the Santiam and MF Willamette subbasins and pairwise F_{ST} estimates among these source populations were approximately 0.070. Dunn Wetland is presently the largest Oregon chub population and had the greatest levels of genetic diversity we observed. These data suggest that this population has not suffered any effects of outbreeding depression since it was introduced. Although previous studies have documented the effects of outbreeding depression within relatively few generations (Gharrett et al. 1999; Goldberg et al. 2005), the possibility exists that the effects of mating among chub populations from different subbasins may not be evident for several generations.

Conservation implications

Whereas natural perturbations like floods often favor native species over nonnative species, human perturbations typically favor the nonnative species. Floods now pose a substantial risk to chub populations through the dispersal of nonnative fishes (Scheerer 2002). The severe human alteration of the Willamette drainage has relegated us into managing 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 (Costello et al. 2003; 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 other threatened and endangered species of cyprinids (Appendix I), continued isolation will likely lead to an increase in genetic drift and a reduction in genetic diversity in some populations. For populations where natural connectivity cannot be restored due 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 as well, and these risks should be carefully considered prior to any action (Tallmon et al. 2004).

Introductions and translocations can be an effective means for conserving threatened and endangered fishes, but these efforts should be implemented carefully (George et al. 2009). Introductions of new Oregon chub populations has been an effective means of increasing the numbers and distribution of Oregon chub and for achieving recovery goals (Scheerer 2007). Results from this study indicate that most introduced populations had levels of genetic diversity that were equivalent to natural populations. Low levels of genetic diversity observed in the Wicopee Pond introduced population highlight the importance of using adequate numbers of individuals when founding new populations, an action that is now specified in the Oregon chub recovery plan guidelines for population introductions (USFWS 1998). Our results indicate that the current guidelines for introducing new populations of Oregon chub are effective for establishing genetically viable populations.

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Tables and Figures

Table 1. Sample sizes, locations, years, and estimates of genetic diversity based on nine microsatellite loci for Oregon Chub populations sampled for this study.

Population #	Subbasin	Population 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	Coast Fork Willamette	Coast Fork 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
17	Umpqua	Umpqua Chub	2005	21	5.222	NA	0.434	0.435

Table 1. Continued

Population #	Subbasin	Population Name	Year(s) Sampled	n	A	A_R	H_e	H_o
Intro - 1	Middle Willamette	Finley NWR Display Pond	2005	48	6.222	6.094	0.654	0.646
Intro - 2	Middle Willamette	Dunn Wetland	2005	47	13.000	12.441	0.810	0.834
Intro - 3	MF Willamette	Wicopee Pond	2004	45	7.778	7.606	0.759	0.720
Intro - 4	MF Willamette	Fall Creek Spillway Pond	2004	45	11.778	11.383	0.798	0.769

n = Number individuals sampled
A = Mean number alleles per locus
A_R = Allelic richness
H_e = Expected heterozygosity
H_o = Observed heterozygosity

Table 2. Donor populations, numbers of fish transferred, and years of introduction for the four introduced Oregon chub populations.

Population #	Sub-basin	Population name	Donor population(s)	Number of fish	Year of introdution
Intro - 1	Middle Willamette	Finley NWR Display Pond	Finley NWR Gray Creek Swamp	314	1998
Intro - 2	Middle Willamette	Dunn Wetland	Geren Island North Channel	200	1997
		Dunn Wetland	Elijah Bristow Berry Slough	300	1997
		Dunn Wetland	Shady Dell Pond	73	1997
Intro - 3	MF Willamette	Wicopee Pond	Dexter Reservoir Alcove - the Pit	50	1988
Intro - 4	MF Willamette	Fall Creek Spillway Ponds	EF Minnow Creek Pond	350	1996
		Fall Creek Spillway Ponds	Shady Dell Pond	150	1996

Table 3. Pairwise estimates of genetic variation (F_{ST}) among Oregon chub populations sampled for this study. Estimates are based on nine microsatellite loci. All values were found to be significantly different from 0.0 except for those values in bold text.

Population #	Population Name	1	2	3	4	5	6	7	8	9	10
1	Geren Island										
2	Stayton PW Pond	0.040									
3	Warren Gray Slough	0.019	0.029								
4	Santiam I-5 Channel Pond	0.044	0.057	0.036							
5	Finley NWR Gray Creek Swamp	0.148	0.138	0.138	0.141						
6	Shetzline South Pond	0.177	0.215	0.186	0.181	0.250					
7	Big Island	0.069	0.096	0.073	0.064	0.135	0.130				
8	Coast Fork Willamette	0.079	0.099	0.067	0.079	0.150	0.156	0.079			
9	Elijah Bristow Berry Slough	0.069	0.072	0.049	0.054	0.089	0.154	0.058	0.059		
10	Elijah Bristow North Slough	0.059	0.063	0.040	0.051	0.103	0.164	0.059	0.055	0.010	
11	Dexter Reservoir RV Alcove	0.072	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	0.063	0.047	0.054	0.101	0.157	0.064	0.059	0.016	0.012
13	EF Minnow Creek Pond	0.056	0.063	0.056	0.047	0.112	0.173	0.056	0.074	0.024	0.031
14	Hospital Pond	0.055	0.073	0.047	0.049	0.116	0.162	0.054	0.056	0.019	0.014
15	Shady Dell Pond	0.071	0.086	0.066	0.074	0.112	0.156	0.060	0.069	0.023	0.031
16	Buckhead Creek	0.087	0.113	0.086	0.094	0.131	0.167	0.070	0.081	0.037	0.050
17	Umpqua Chub	0.258	0.262	0.239	0.264	0.343	0.352	0.265	0.227	0.273	0.259
1A	Geren Island	**0.000**	0.047	0.025	0.050	0.139	0.169	0.067	0.071	0.062	0.052
13A	EF Minnow Creek Pond	0.052	0.069	0.054	0.049	0.113	0.149	0.051	0.063	0.017	0.026
14A	Hospital Pond	0.049	0.070	0.044	0.051	0.101	0.152	0.054	0.052	0.024	0.017
15A	Shady Dell Pond	0.071	0.085	0.063	0.069	0.120	0.156	0.052	0.056	0.025	0.031
Intro - 1	Finley NWR Display Pond	0.181	0.158	0.170	0.171	0.020	0.280	0.167	0.196	0.126	0.139
Intro - 2	Dunn Wetland	0.018	0.048	0.021	0.030	0.107	0.145	0.044	0.046	0.021	0.017
Intro -3	Wicopee Pond	0.090	0.102	0.078	0.083	0.148	0.189	0.079	0.087	0.046	0.034
Intro - 4	Fall Creek Spillway Pond	0.057	0.061	0.044	0.053	0.101	0.156	0.057	0.058	**0.008**	0.016

Table 3. Continued

Population #	11	12	13	14	15	16	17	1A	13A	14A	15A	Intro - 1	Intro - 2	Intro -3
1														
2														
3														
4														
5														
6														
7														
8														
9														
10														
11														
12	0.025													
13	0.058	0.031												
14	0.037	0.021	0.020											
15	0.047	0.033	0.035	0.020										
16	0.062	0.048	0.040	0.025	0.014									
17	0.254	0.256	0.276	0.277	0.273	0.299								
1A	0.064	0.055	0.053	0.044	0.056	0.072	0.258							
13A	0.050	0.023	**0.001**	0.014	0.025	0.028	0.260	0.046						
14A	0.033	0.023	0.024	**0.001**	0.021	0.027	0.265	0.037	0.020					
15A	0.050	0.035	0.031	0.021	**0.001**	0.018	0.269	0.058	0.023	0.022				
Intro - 1	0.165	0.128	0.143	0.156	0.150	0.172	0.367	0.177	0.144	0.143	0.156			
Intro - 2	0.029	0.017	0.027	0.018	0.031	0.046	0.233	0.015	0.018	0.014	0.029	0.143		
Intro -3	0.048	0.039	0.058	0.037	0.040	0.052	0.308	0.084	0.061	0.041	0.043	0.186	0.050	
Intro - 4	0.040	0.024	**0.009**	0.013	0.016	0.025	0.261	0.047	**0.000**	0.016	0.015	0.135	0.020	0.051

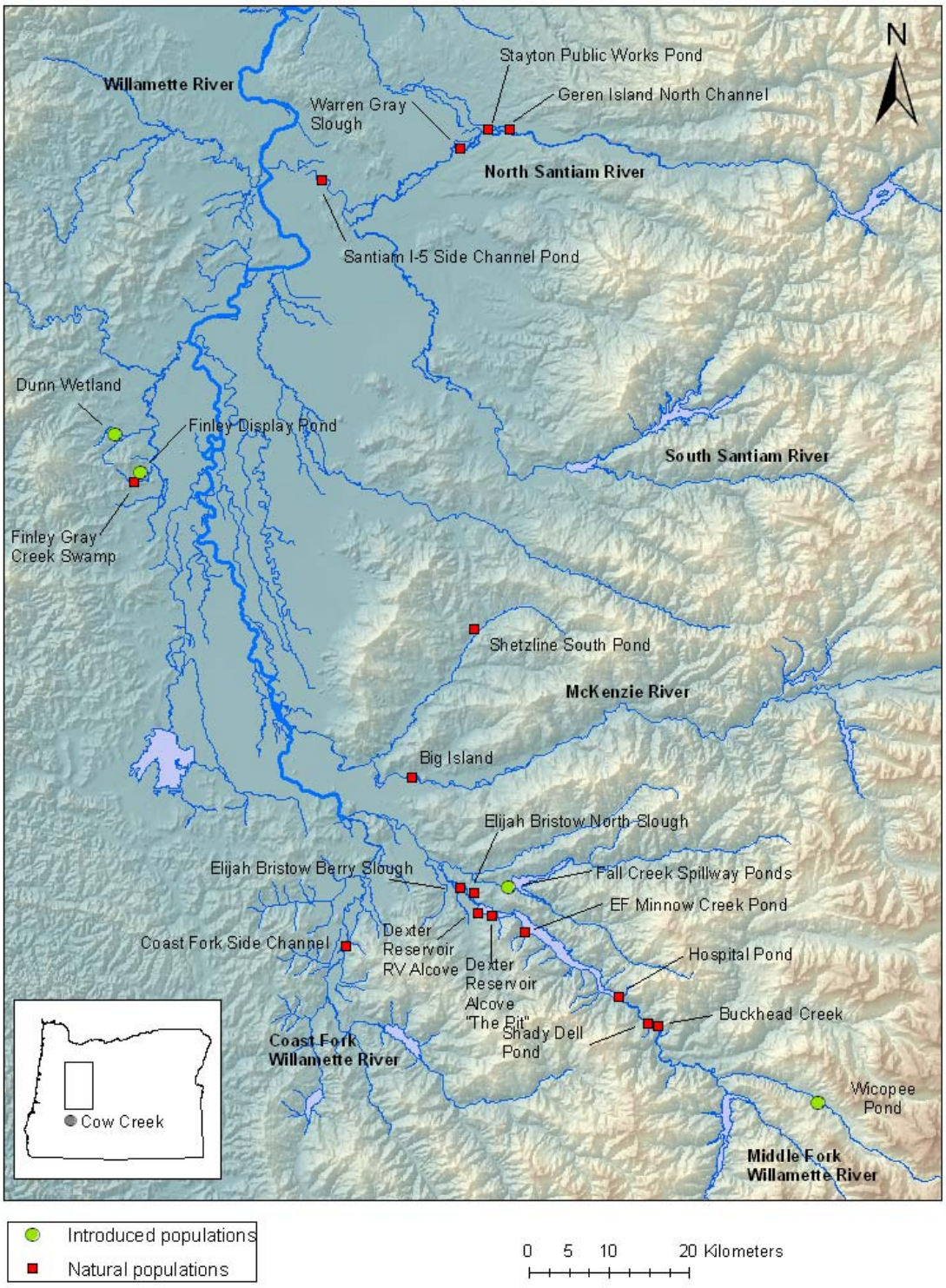


Figure 1. Oregon chub sampling locations within the Willamette River basin. Red squares represent naturally occurring populations and green circles represent introduced populations. Location of the Cow Creek site in the Umpqua River drainage is shown on the inset map.

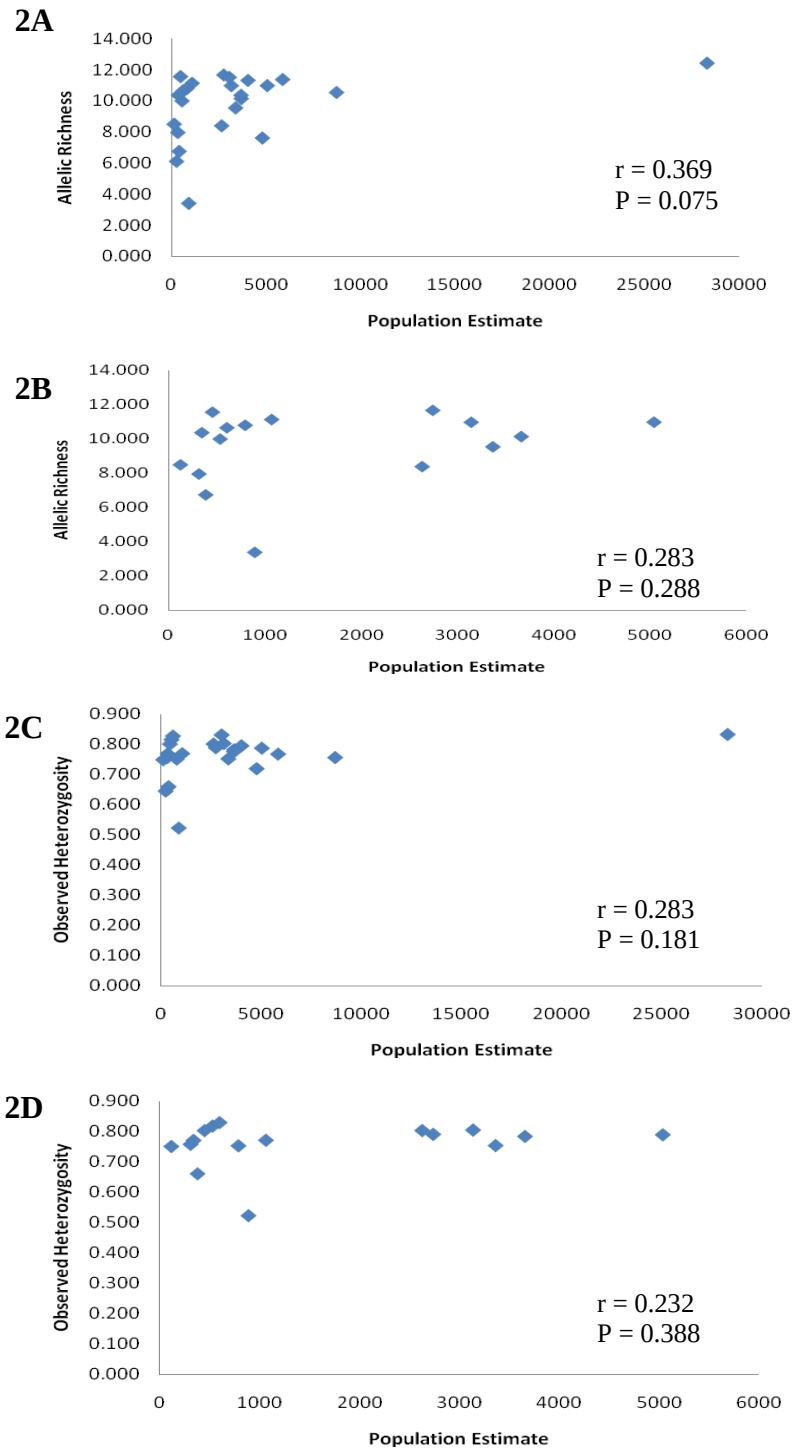


Figure 2. Relationships between estimates of genetic diversity and population size for Oregon chub. Graph 2A represents the relationship between population size and allelic richness for all populations sampled and 2B represents the relationship for natural populations only. Graph 2C represents the relationship between population size and observed heterozygosity for all populations sampled and 2D represents the same relationship for natural populations only.

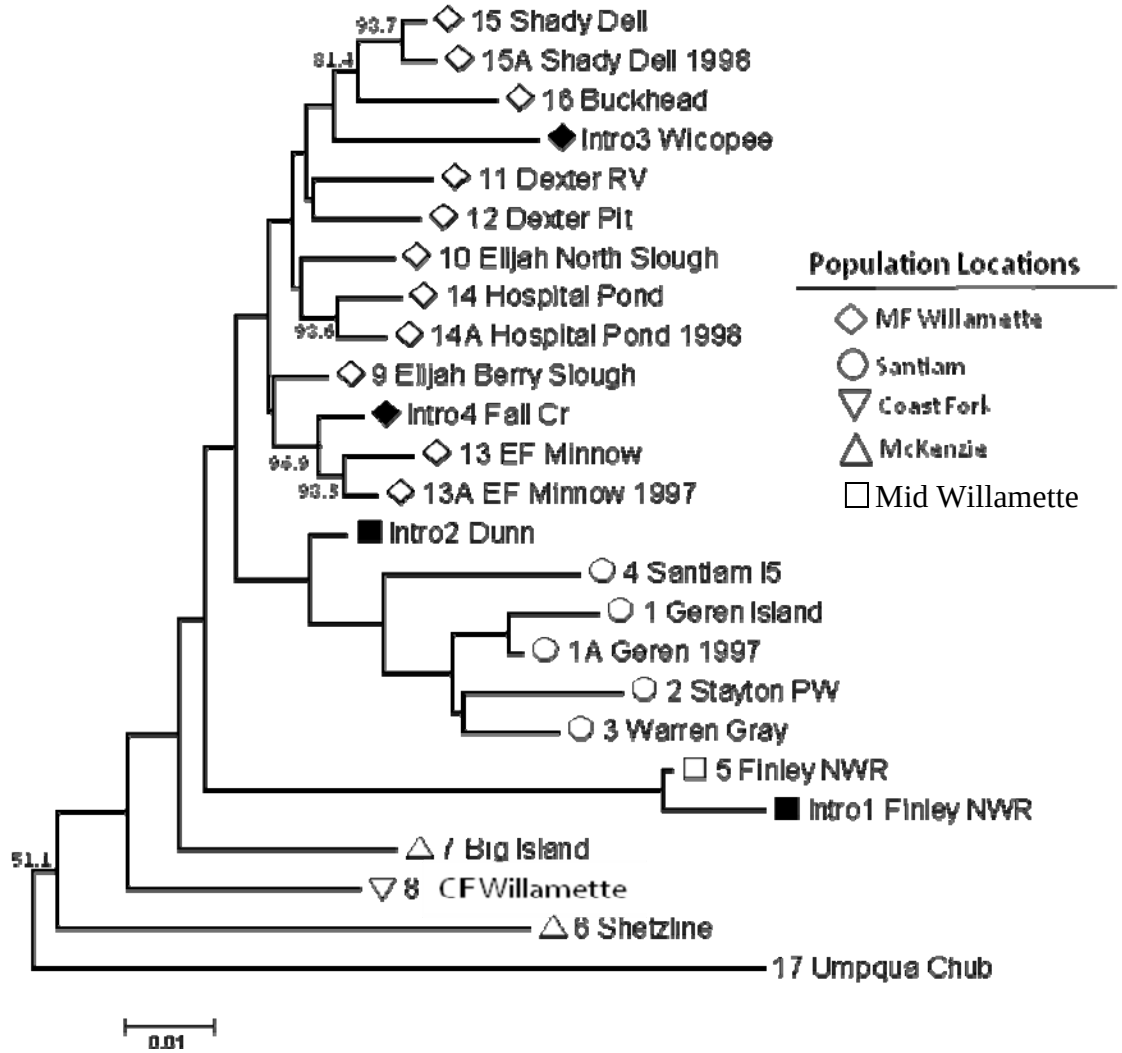
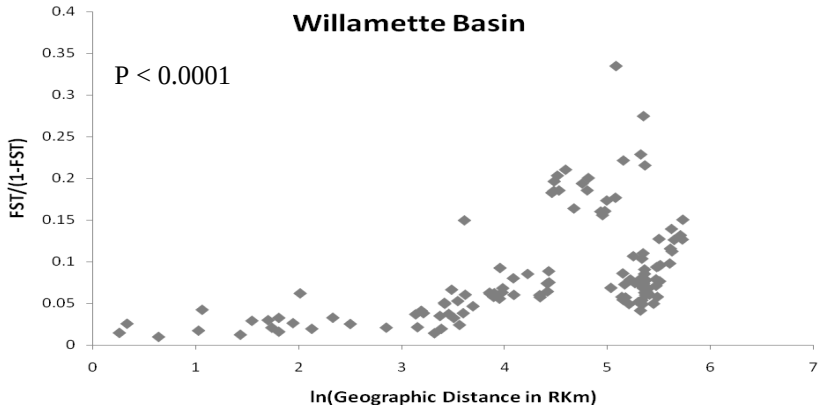
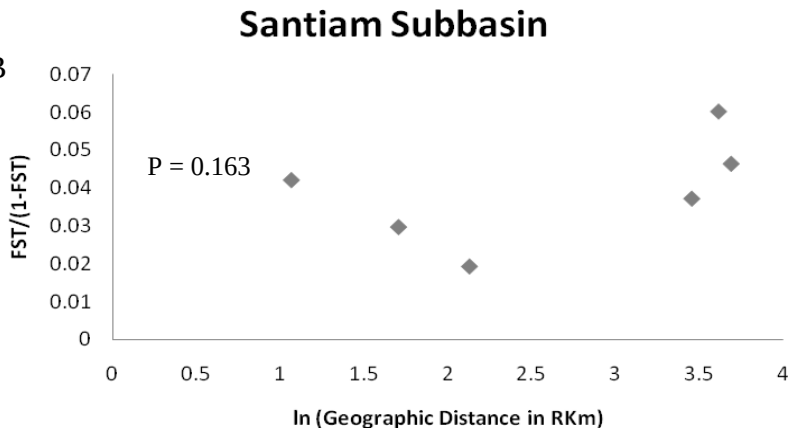


Figure 3. Consensus NJ tree based on Cavalli-Sforza and Edwards' (1967) chord-distances. Values at the nodes represent the percentage of 1000 bootstrap replicates that showed the displayed arrangement. Only bootstrap values greater than 50.0% are shown. Shapes indicate the subbasin of origin and shaded shapes represent introduced populations.

4A



4B



4C

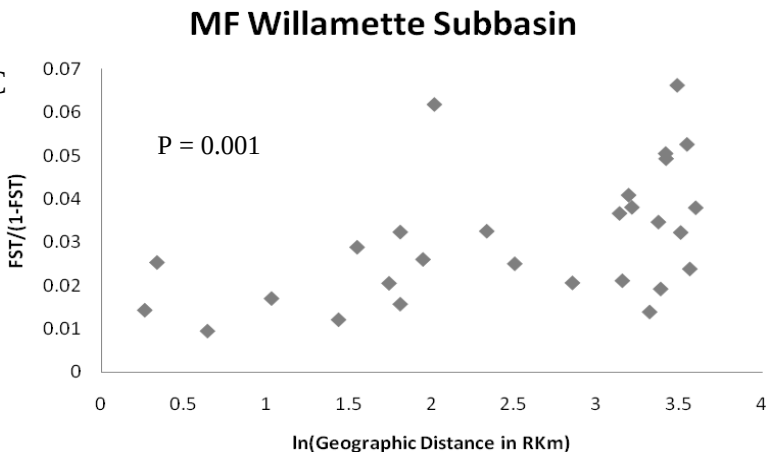


Figure 4. Analysis of isolation by distance for Oregon chub populations. Geographic distance was measured as the natural log of the fluvial distance between sampling locations (measured in km) and genetic distance was measured as $F_{ST}/1\text{-}F_{ST}$. Figure 4A shows the relationship for the entire Willamette Basin and Figures 4B and 4C represent the relationship for the Santiam and MF Willamette subbasins only. P values are based on Mantel tests (1000 replicates).

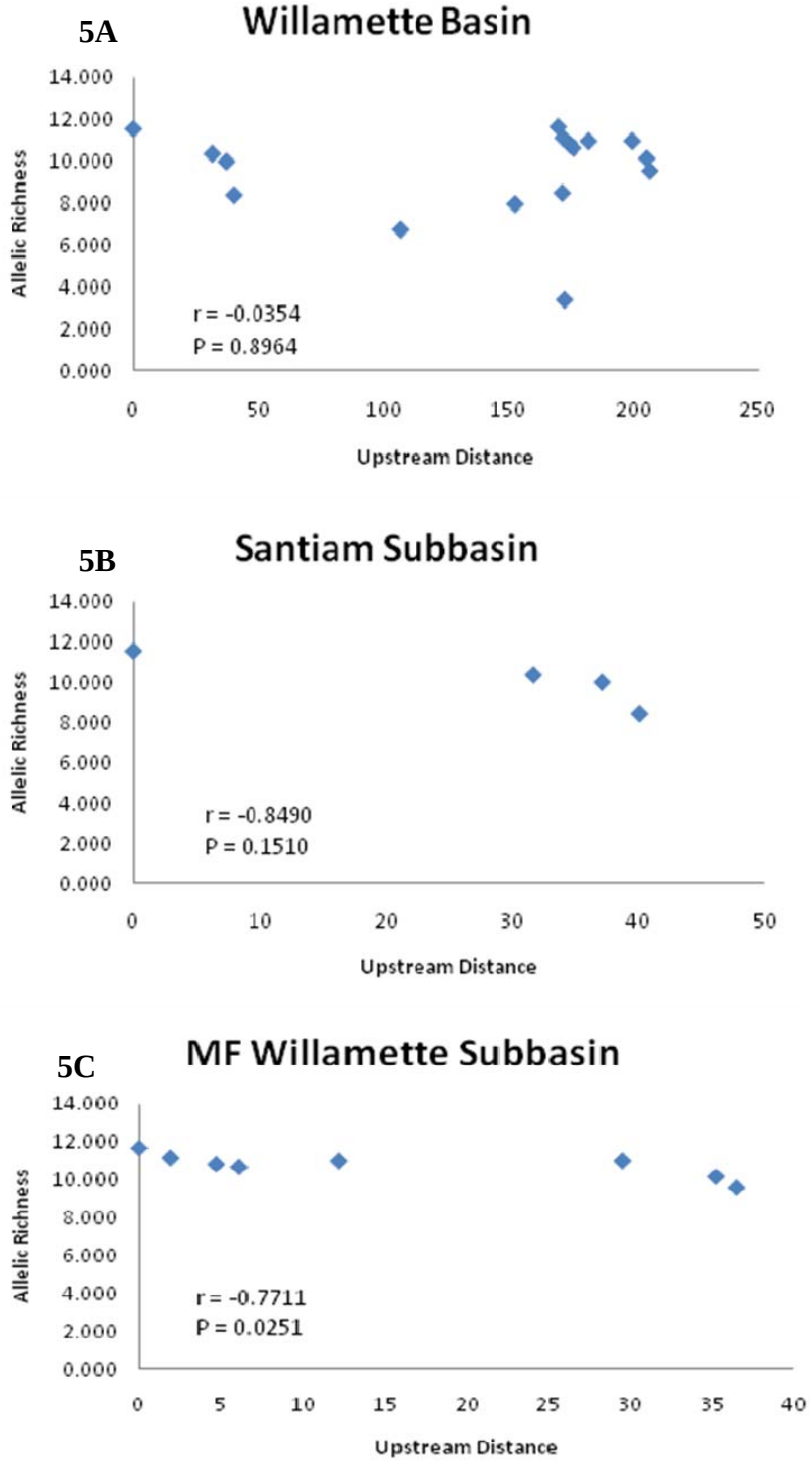


Figure 5. Relationship between upstream distance (relative to the furthest downstream population) and allelic richness. Figure 5A includes all natural origin chub populations sampled, Figure 5B represents only populations in the Santiam subbasin and Figure 5C represents only populations in the MF Willamette subbasin.

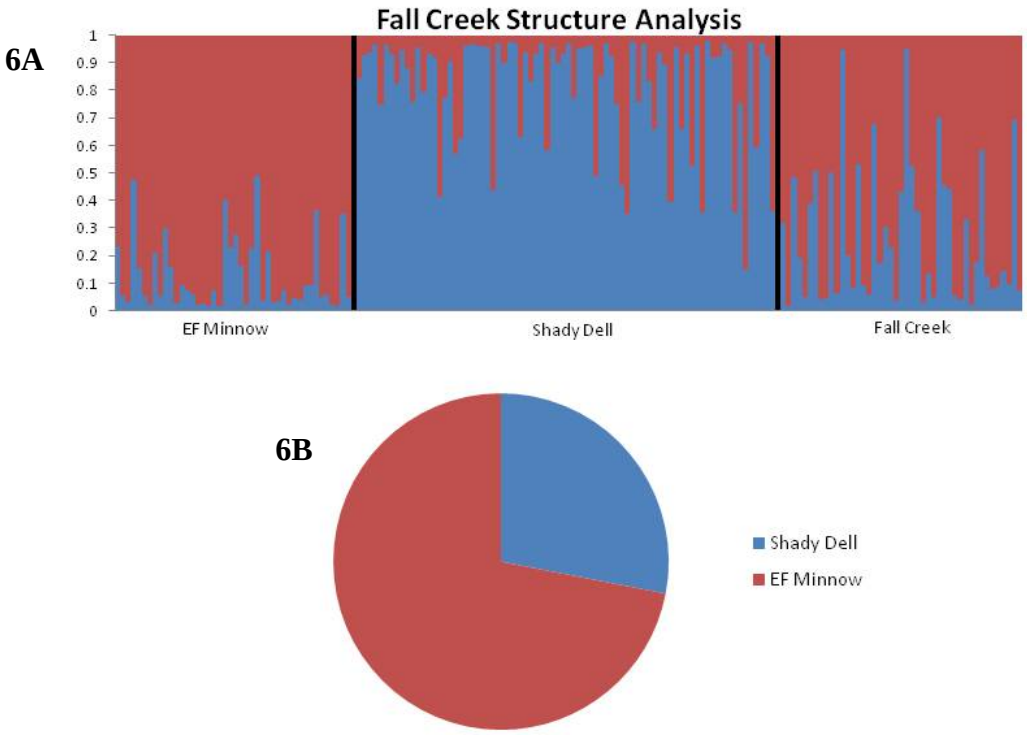


Figure 6. STRUCTURE results for the Fall Creek introduced population when K = 2. Figure 6A shows the individual results where each vertical bar represents an individual and the shading represents the proportion of that individual's genotype corresponding to each genetic cluster/population. Figure 6B shows the overall proportion of the Fall Creek sample that corresponds to each genetic cluster/population.

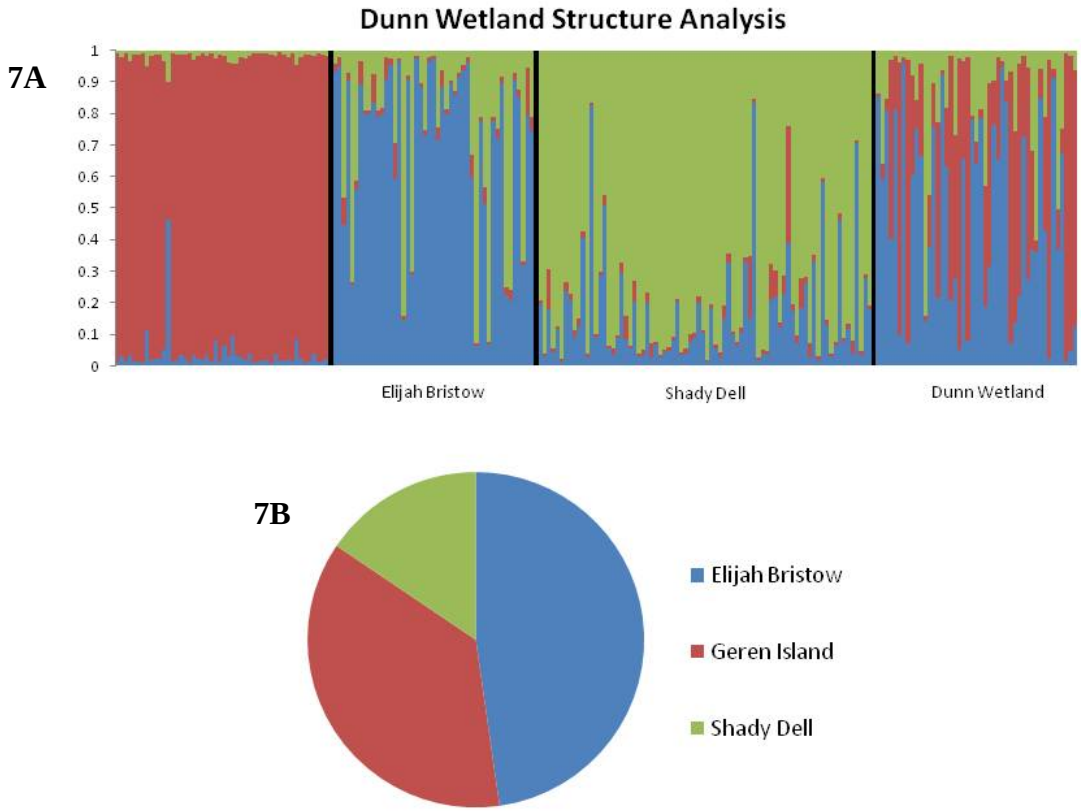


Figure 7. STRUCTURE results for the Dunn Wetland introduced population when K = 3. Figure 7A shows the individual results where each vertical bar represents an individual and the shading represents the proportion of that individual's genotype corresponding to each genetic cluster/population. Figure 7B shows the overall proportion of the Dunn Wetland sample that corresponds to each genetic cluster/population.

Appendix I. Estimates of genetic variation among a variety of cyprinid species, including Oregon chub populations analyzed for this study. Species values are ordered based on the mean number of alleles per locus. Superscripts refer to the present listing status of each species

Reference	Species	Common Name	Number of Loci		A	A_R	H_e	H_o
Alo and Turner 2005	*Hybognathus amarus*	Rio Grande silvery minnow[E]	7	Mean	11.1	n/a	0.72	0.63
				Minimum	9.3	n/a	0.68	0.53
				Maximum	13.0	n/a	0.75	0.72
Salgueiro et al. 2003	*Anaecypris hispanica*	Iberian cyprinid[E]	5	Mean	10.3	n/a	0.68	0.63
				Minimum	1.0	n/a	0.00	0.00
				Maximum	27.0	n/a	0.96	1.00
This Study	*Oregonichthys crameri*	Oregon chub[T]	9	Mean	10.2	9.83	0.77	0.77
				Minimum	3.4	3.38	0.56	0.52
				Maximum	12.2	11.68	0.81	0.83
Saillant et al. 2004	*Notropis mekistocholas*	Cape Fear shiner[E]	22	Mean	8.2	5.59	0.70	n/a
				Minimum	2.0	1.76	0.13	n/a
				Maximum	14.0	8.40	0.88	n/a
Skalski and Grose 2006	*Semotilus atromaculatus*	creek chub[W]	32	Mean	7.5	n/a	0.64	0.56
				Minimum	2.0	n/a	0.09	0.09
				Maximum	16.0	n/a	0.93	0.91
Turner et al. 2004	*Plaatygobio gracilis*	flathead chub[D]	5	Mean	7.4	n/a	0.74	0.73
				Minimum	3.0	n/a	0.49	0.33
				Maximum	14.0	n/a	0.93	1.00
Turner et al. 2004	*Hybognathus amarus*	Rio Grande silvery minnow[E]	7	Mean	5.4	n/a	0.64	0.69
				Minimum	2.0	n/a	0.08	0.08
				Maximum	10.0	n/a	0.94	1.00
Turner et al. 2005	*Rhinichthys cataractae*	longnose dace[W]	7	Mean	5.3	n/a	0.46	0.55
				Minimum	1.0	n/a	0.00	0.00
				Maximum	12.0	n/a	0.94	1.00
Burridge and Gold 2003	*Notropis mekistocholas*	Cape Fear shiner[E]	11	Mean	5.2	n/a	n/a	0.56
				Minimum	1.0	n/a	n/a	0.00
				Maximum	9.0	n/a	n/a	1.00
Parker et al. 1999	*Poeciliopsis o. occidentalis*	Gila topminnow[E]	5	Mean	2.5	n/a	0.21	0.21
				Minimum	1.0	n/a	0.00	0.00
				Maximum	12.0	n/a	0.81	0.80

[E] Endangered
[T] Threatened
[D] Not listed, thought to be declining
[W] Widespread and abundant