

**Genetic Comparisons among Hatchery and Wild Populations of  
Spring Chinook Salmon in the Methow River Basin:  
ESA Controversies in the Columbia River Basin**

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## Introduction

The number of adult spring Chinook salmon (*Oncorhynchus tshawytscha*) returning to the Columbia River Basin during the spring and summer of 2000 represented a substantial increase over the previous 10 years (Columbia River Fish Passage Center: [www.fpc.org](http://www.fpc.org)). From 1990 to 1999, an average of 62,347 adults per year were counted at Bonneville Dam. In contrast, 178,302 adults were counted at Bonneville Dam during 2000. This latter increase in adult returns most likely reflected increased ocean survivals associated with a recent shift in ocean temperatures and marine productivity (Mantua et al. 1997; Brown 2000; Finney et al. 2000). However, the vast majority of those returning fish were believed to be of hatchery origin. High numbers of fish returning in 2000 exceeded broodstock requirements at most hatcheries propagating spring Chinook salmon in the Columbia River Basin.

Management decisions regarding the disposition of surplus, hatchery-origin adult spring Chinook salmon resulted in disagreements among federal and state agencies, the Columbia River tribes, and the general public during the summer of 2000. These disagreements were particularly controversial in the Methow River Valley in eastern Washington state where local citizens and the tribes objected to the sacrifice of surplus spring Chinook salmon returning to the Winthrop National Fish Hatchery (NFH). Hatchery-origin adults exceeding broodstock requirements have traditionally been given to the tribes, food banks, and prisons for human consumption. However, during the summer of 2000, Columbia River tribes and local citizens in the Methow Valley advocated the release of surplus hatchery-origin adults, or their hatchery-produced progeny, into natural spawning and rearing areas to potentially assist with recovery of spring Chinook salmon which are currently listed as *endangered* under the U.S. Endangered Species Act (ESA). The Washington Department of Fish and Wildlife (WDFW), National Marine Fisheries Service (NMFS), and the U.S. Fish and Wildlife Service (USFWS) did not concur. State and federal biologists noted that those hatchery-origin fish were not listed under the ESA and could pose unacceptable biological risks to listed natural populations of spring Chinook salmon in the Methow River.

A central issue in the Methow River controversy was the genetic origin and ancestry of fish returning to the Winthrop NFH. Those fish trace their ancestry to the Carson NFH on the Wind River, a tributary to the lower Columbia River approximately 20 km upstream from Bonneville Dam. Since the early 1960's, the Carson NFH has propagated and maintained an introduced run of spring Chinook salmon in the Wind River. That stock was developed from upstream-migrating adults trapped at Bonneville Dam in the late 1950's and early 1960's (Zimmer et al. 1963; Wahle and Chaney 1981). The origin of those trapped adults upstream of Bonneville Dam is unknown. Since that time, fish from the Carson NFH have been used to establish hatchery runs of spring Chinook salmon elsewhere in the Columbia River Basin,

including the Winthrop NFH on the Methow River (see Background section below). As a result, NMFS did not include Chinook salmon from the Winthrop NFH (the “Winthrop-Carson” stock) as part of the Mid-Columbia River ESU when these latter populations were listed as *endangered* under the ESA (Federal Register 1999).

Because spring Chinook salmon returning to Winthrop NFH represent an introduced hatchery stock, NMFS concluded - with concurrence from WDFW and USFWS - that those fish are not appropriate for recovering naturally spawning populations in the Methow River. Indeed, as part of their ESA obligations, NMFS issued a draft Biological Opinion in 2000 stating that the Winthrop-Carson stock at the Winthrop NFH should be phased out and replaced with the *Methow Composite* stock currently being developed by WDFW (see below). Under this management plan, surplus adults at the Winthrop NFH would be euthanized and then given to the tribes and prisons for ceremonial and consumptive purposes.

However, as noted previously, Columbia River tribes and local citizens in the Methow Valley fervently opposed the euthanization and sacrifice of surplus adults returning to the Winthrop NFH. Those tribes and citizens have made significant economic and personal sacrifices to comply with federal restrictions associated with ESA listings of salmon and steelhead (*Oncorhynchus tshawytscha*) in the Methow River Valley: the tribes are being forced to curtail their treaty-guaranteed fisheries, and local citizens are being forced to significantly reduce their use of surface water for irrigation and commercial use. In general, the tribes and citizens do not understand, or believe, the genetic arguments for preventing surplus Winthrop-Carson fish from spawning naturally in the Methow River (or otherwise being used for recovery purposes). Those groups object to the “destruction” of adult spring Chinook salmon returning to the Winthrop NFH when natural-origin, spring Chinook salmon in the Methow River are listed as *endangered* under the ESA. Those groups note that wild and hatchery spring Chinook salmon in the Methow River are morphologically indistinguishable and that stray Winthrop-Carson fish have been spawning naturally in the Methow River for many generations (based on scale analyses of recovered salmon carcasses; data from WDFW). Therefore, those citizens and tribes do not accept the genetic arguments for sacrificing surplus hatchery-origin adults or their hatchery-produced progeny.

Here, I compare existing genetic data for hatchery and wild populations of spring Chinook salmon in the Methow River Basin. I address two related questions: (1) to what extent are the aforementioned biological opinions and management decisions for spring Chinook salmon in the Methow River scientifically defensible and (2) are the existing genetic data consistent with the contentions of the tribes and citizens in the Methow River Valley. My goal is to provide an objective evaluation of these questions.

## Background and History of the Winthrop National Fish Hatchery

The Winthrop NFH was constructed 1939-1941 as part of the Grand Coulee Fish Maintenance Project (GCFMP) to mitigate for fish and habitat losses resulting from the construction of Grand Coulee Dam (Fish and Hanavan 1948; Mullen 1987; Utter et al. 1995). This dam blocks all upstream migration of salmon and steelhead, thus excluding those fish from 1,835 stream km of historical spawning and rearing habit. From 1939 through 1944, all upstream-migrating salmon and steelhead in the mid-Columbia River were trapped at Rock Island Dam (approximately 75 km downstream from the confluence of the Methow and Columbia rivers) as part of the GCFMP's fish salvage operations. This trapping operation essentially prevented any fish from returning directly to their natal streams upstream from Rock Island Dam over a five-year period. Those trapped fish were relocated to mid-Columbia tributaries between Rock Island and Grand Coulee dams, or they were spawned at the (then) newly-constructed Leavenworth NFH. The progeny of those spawned fish were then released or outplanted into several mid-Columbia rivers, including the Methow River. The net result of those *salvage* operations was that any native populations of spring Chinook salmon that existed in the Methow River, or other mid-Columbia tributaries, prior to 1939 were randomly mixed with adults destined for the mid-Columbia River above Grand Coulee Dam and then redistributed to the mid-Columbia tributaries. At the beginning of this salvage operation (late 1930's), most populations of salmon and steelhead in the mid-Columbia River were considered highly depressed due to significant overfishing in the lower River during the preceding 50 years (Mullen 1987).

The Winthrop NFH on the Methow River began operation in 1942 and first released spring Chinook salmon in 1944. Those initial releases represented the progeny of the GCFMP spawnings at the Leavenworth NFH. In years 1945-1962, the Winthrop NFH released the progeny of adults returning to the Methow River from the earlier (1939-1944) GCFMP releases (Mullen 1985). Artificial propagation of Chinook salmon was suspended at the hatchery for the brood years 1962-1973 but resumed in 1974 and 1975 with the importation of spring Chinook salmon from the Cowlitz River and Little White Salmon hatcheries on the lower Columbia River, where the latter fish representing the Carson stock.. All subsequent releases of spring Chinook salmon from the Winthrop NFH (1976-present) were Carson-derived fish obtained from either the Carson NFH, the Leavenworth NFH, the Little White Salmon NFH, or adult returns to the Winthrop NFH resulting from those earlier Carson-stock transfers and releases. As a result, spring Chinook salmon returning today to the Winthrop NFH are believed to be largely of Carson-stock ancestry.

A second hatchery, the Methow State Fish Hatchery (SFH), was built on the Methow River in the

early 1990's (completed in 1992) as partial mitigation for fish losses at Wells Dam, a Public Utility District (Douglas County) hydropower dam on the Columbia River approximately 12 km downstream from the mouth of the Methow River. The Methow SFH, which is only 3 km upstream from the Winthrop NFH, is operated by WDFW. This hatchery began developing *native* broodstocks of spring Chinook salmon in 1993 to help rebuild naturally spawning populations in the Methow River. An initial objective of the program was to establish three “native” broodstocks of spring Chinook salmon for fish spawning in three areas of the drainage: Twisp, Chewuch, and Methow rivers. However, because of extremely low adult returns in the late 1990's, that initial objective was changed to one of establishing a single *Methow Composite* stock representing an admixture of fish from the Methow and Chewuch rivers. Only the Twisp River stock continued to be propagated separately. One factor contributing to these decisions was the discovery, based on scale analyses, that the majority of adults spawned for the Methow River broodstock in brood years (BY) 1992-1997 were either unmarked hatchery-origin fish that had voluntarily strayed into the Methow SFH, most likely came from the Winthrop NFH (i.e. Winthrop-Carson fish), or the progeny of those unmarked fish that had been spawned at the Methow SFH in 1993 (see companion document summarizing the broodstock history of spring Chinook salmon at the Methow SFH). The Methow Composite stock was initiated with BY 1998 by spawning 149 natural origin adults (78 females, 72 males), with 104 hatchery-origin fish primarily representing returning adults from the Methow and Chewuch river broodstocks but including a few out-of-basin strays (e.g. seven adults from the Entiat NFH based on recovery of coded wire tags).

The Methow Composite stock was included with the *endangered* listing of spring Chinook salmon in the Methow River because those hatchery fish were considered “essential for recovery” of the naturally spawning populations (Federal Register 1999). Conferring federal ESA protection to the Methow Composite stock has further contributed to controversy in the Methow River because unknown numbers of unmarked Winthrop-Carson fish were, most likely, inadvertently included in the broodstock. The Columbia River tribes and local citizens in the Methow Valley believe that naturally spawning fish in the Methow River, the Winthrop-Carson stock, and the Methow Composite stock essentially represent a single gene pool or genetic population. Those groups believe that all returning adults, regardless of parentage or immediate ancestry, should be used to help recover naturally spawning populations in the Methow River. They do not agree with the decision, based on a *Biological Opinion*, to phase out the Winthrop-Carson stock and replace it with the Methow Composite stock at the Winthrop NFH. A complete and objective analysis of existing genetic data for all wild and hatchery populations of spring Chinook salmon in the Methow River is thus necessary to ascertain the scientific defensibility of those biological opinions and management decisions

## Materials and Methods

Existing genetic data for spring Chinook salmon consist almost entirely of allozyme genotypes and corresponding allele frequencies for a large number of population samples (Myers et al. 1998). The most complete allozyme data set for populations in the mid Columbia River is maintained by the Genetics Unit of the Washington Department of Fish & Wildlife (Robin Waples, NMFS, pers. comm.). This data set was provided to me by Anne Marshall and Craig Busack of WDFW (Table 1). Some of those data have been published previously in the scientific literature, particularly for natural populations in the Wenatchee River drainage (Utter et al. 1995). However, data for Methow River populations have not been published previously.

### *Population samples*

**Mid-Columbia River populations** - Samples of spring Chinook salmon were collected by WDFW from several localities in the Wenatchee and Methow River watersheds from 1989 to 1998 (Table 1; Fig. 1). Limited sampling was performed also in the Entiat River. Eye, liver, muscle, and heart tissues were obtained from carcasses in streams or from adults used for hatchery broodstocks. Most wild populations are represented by multiple samples collected in different years. This repeat sampling of populations is considered a major strength of the data set because it provides a temporal component to which spatial genetic variation can be compared. The data set also included samples from the following hatcheries (adult return year in parentheses): Carson NFH (1989), Leavenworth NFH (1991), Entiat NFH (1994, 1997, 1998), Winthrop NFH (1992), and Methow Composite stock (1998). Genotypes of each fish at 44 enzyme-coding loci were determined by standard procedures (Appendix 1; Aebersold et al. 1987). Some additional details, described below, are necessary for understanding the origin of samples collected from the Twisp, Chewuch, and Methow Rivers (Table 1).

Samples of spring Chinook salmon from the Chewuch and Twisp Rivers in 1992-1994 represented natural-origin (i.e. "wild") adults trapped for hatchery broodstocks at rkm 2.4 and 8.0 in the Chewuch and Twisp rivers, respectively (Bartlett 1995-1998). The 1993 sample from the Twisp River (n=48) consisted also of 16 natural spawners plus 32 fish trapped for broodstock.

Samples of spring Chinook salmon from the Methow River in 1993 and 1994 represented mixtures of natural and hatchery-origin adults, the latter believed to be unmarked Winthrop-Carson fish from the Winthrop NFH (Bartlett 1995, 1996). In 1993, 99 adults voluntarily entered the Methow SFH, and 62 of those fish had hatchery-origin scale patterns. All 99 of those fish were spawned together to produce the 1993 brood. The 1993 genetics sample for the Methow River (n=93) represented 90 of those 99 adults

plus three natural spawners (carcasses). In 1994, only 17 adults (age 4, 5, or 6) and 1 jack (age 3) entered the Methow SFH, all of which appeared to be unmarked hatchery-origin fish, presumably from the Winthrop NFH (Bartlett 1996). Fourteen of those fish were sampled for genetic analysis.

In 1996, all adults destined for the Twisp, Chewuch, and Methow Rivers were trapped at Wells Dam on the Columbia River. A total of 464 adults were trapped, of which 126 were classified as “wild” based on scale patterns (Bartlett 1998). Elemental scale analyses were performed on 100 of those 126 fish to determine their river of origin (Elemental Research, Inc., Vancouver, BC). Based on the criteria of a minimum 60% threshold probability for the most likely river of origin and approximately equal probability for the other two rivers, 46 wild adults were assigned to the Methow River, 11 to the Chewuch River, and 13 to the Twisp River. Those adults were then used for propagating each of the three hatchery broodstocks at the Methow SFH. In addition, coded-wire tagged hatchery-origin adults from the Twisp, Chewuch and Methow River broodstocks (1992 and 1993 brood-year releases) were also trapped at Wells Dam in 1996 and used for those respective broodstocks. Collectively, fish constituting the 1996 genetic samples were comprised of the following adults for each of the three rivers (i.e. broodstocks): (a) Twisp River (n=37): 11 wild adults (including 2 jacks), 20 BY92 hatchery adults, and 6 BY93 hatchery jacks; (b) Chewuch River (n=55): 7 wild adults (including 4 jacks), 34 BY92 hatchery adults, and 14 BY93 hatchery jacks; (c) Methow River (n=43): 40 wild adults (including 1 jack) and 3 BY93 hatchery jacks. No unmarked hatchery-origin adults were used for the Methow River stock in 1996. This was not the case in 1993 or 1994 when the majority of adults used for broodstock (all in 1994) were unmarked, hatchery-origin adults, most-likely from the Winthrop NFH.

Sample sizes for some populations in some years (e.g. 1994) were substantially less than desired (i.e. <30 fish) reflecting very low adult returns to the mid-Columbia River that year. I pooled samples with less than 15 individuals with samples collected in the immediately preceding or subsequent year from the same population to achieve minimum sample sizes of 20 individuals. However, I did not pool the 1994 sample from the Methow River (n =14) with another sample because I wanted to retain three independent samples (1993, 1994, 1996) for that population in the analysis (Table 1).

The genetic data file obtained from WDFW included a sample of 25 fish indicated as “Methow?” 94 in the data set. The origin of those fish in the Methow River Basin is uncertain (Anne Marshall, WDFW, per. comm.). Based on scale readings, 15 of those fish were designated hatchery origin (13 BY89, 2 BY90) and were most likely from the Winthrop NFH, five fish were designated wild (4 BY89, 1 BY90), and five fish had unreadable scales. After reviewing reports from WDFW (Bartlett 1996) and USFWS (Kelly and Hamstreet 1996), including a personal conversation with Anne Marshall of the Genetics Unit of WDFW, I was unable to ascertain the actual source of that “Methow 94” sample. That sample may

represent fish trapped at the Winthrop NFH plus fish recovered as carcasses from the Methow River (Anne Marshall, WDFW, pers. comm.; see also Table 5 of Kelly and Hamstreet 1996). Although the exact source of this sample is unknown, the majority of those fish most likely represent adults from the Winthrop NFH, but I could not ascertain the exact location of capture. Nevertheless, I included this unknown sample with the analyses presented here for the sake of completeness. As the genetics data will show, that sample clustered very closely with the Winthrop NFH sample (Sample #11, Table 1).

**Snake River populations** - I also compared allele frequencies for spring Chinook salmon in the mid-Columbia River to those for spring/summer Chinook salmon in the Snake River (Appendix 2; data courtesy of Robin Waples, NMFS, Seattle, Washington). The purposes of these latter comparisons were to (1) show levels of allele frequency divergence between Snake River and mid-Columbia River populations and (2) compare similarities in allele frequencies between Carson-derived hatchery populations and natural populations in the mid-Columbia and Snake River drainages. Spring chinook salmon trapped at Bonneville Dam to initiate the Carson NFH stock may have been derived from adults destined to either or both of those regions.

### ***Statistical analyses***

G-tests were used to test for homogeneity of allele frequencies among population samples from the Methow River basin (Sokal and Rohlf 1981). Significance probabilities associated with a specific hypothesis or set of comparisons were adjusted for the number of tests or loci by the sequential Bonferroni correction (Rice 1989). Genetic chord distances (Cavalli-Sforza and Edwards 1967) between all mid-Columbia River samples (Table 1) were used to construct a *Neighbor-Joining* (NJ) tree with the program MEGA (Kumar et al 1993). This latter analysis was performed largely to confirm previous, unpublished assessments by WDFW. Genetic distances among population samples from the Methow River and associated hatcheries (populations 1-14 only, Table 1) were used as input for a principal coordinates analysis (Gower 1966; Everitt 1978). Values of the first two principal coordinates for each sample were then used to generate a two-dimensional biplot of the hatchery and wild populations to visualize their pairwise genetic distances in multivariate space. In this latter analysis, eigenvector components were scaled such that their sums of squares equaled the corresponding eigenvalue (i.e., variance) associated with the corresponding principal coordinates axis.

## Results

Genetic distance analyses of mid-Columbia River populations yielded three major clusters, with a few samples outside these clusters (Fig 2). For example, samples from the White River (Wenatchee River drainage) clustered together and had the highest overall mean genetic distance from the other samples. A second clustered was composed primarily of natural-origin or wild fish, but included the Methow Comp 98 and Methow River 96 samples. The 1996 Methow River sample was composed primarily of natural-origin adults trapped at Wells Dam. Within this “wild” fish cluster, all three samples from the Twisp River grouped together as a distinct population. A third cluster was composed exclusively of hatchery-origin fish that included the Carson NFH and other stocks that can trace their ancestry back to the Carson stock. This third cluster included the Methow River 93 sample which was composed of 67% unmarked hatchery-origin strays, presumably from the Winthrop NFH. Allele frequencies for the three temporal samples from Nason Creek (upper Wenatchee River watershed) were highly variable, suggesting a very small effective population size and/or varying levels of straying from elsewhere (e.g. White River in 1992, Leavenworth NFH in 1993).

A two-dimensional principal coordinates plot of the genetic distances among samples from the Methow River Basin, and including the Carson and Leavenworth NFH stocks, yielded three groups (Fig 3): (1) Twisp River; (2) Chewuch River, Methow Composite 98, and the Methow River 96 sample; and (3) the Carson-derived hatchery stocks plus the Methow River 1993 and 1994 samples. The long branch length connecting the 1994 Methow River sample (#8) in the third group most likely reflects sampling error (n=14). In general, unique alleles characterizing specific populations were not observed except for the *sAH\*(86)* allele, observed only in the Chewuch River and Methow Composite populations (Table 2). Allele frequencies for the Twisp River population diverged from the other populations at several loci.

Allele frequencies for samples 1-12 (Table 1) differed statistically at six polymorphic loci (Table 3). Exclusion of the Twisp River samples from the analysis still yielded significant allele frequency differences at three loci among the other nine samples from the Methow River Basin. Allele frequencies for samples 7-12 only (i.e. after excluding both the Twisp River and Chewuch River samples) did not differ significantly at any single locus after significance levels were adjusted for the number of tests or polymorphic loci (Rice 1979); however, summing G-values and degrees of freedom for this latter comparison over all polymorphic loci (n=28), including those not shown in Table 3, yielded a summed G-statistic ( $G_{\text{total}} = 216.8$ ;  $df = 170$ ) that was statistically significant ( $P = 0.0088$ ). Variations in allele frequencies among only the Methow Composite, Winthrop NFH, and “Methow? 94” samples were

significant at one locus (*PEP-B*;  $P=0.025$ ) and for all loci combined ( $G_{\text{total}} = 99.47$ ;  $df = 62$ ;  $P = 0.0018$ ).

Examination of the data sets for Snake River (Appendix 2) and mid-Columbia River populations (Table 1) revealed three loci with a minimal amount of allele frequency overlap between the two sub-basin regions: *sIDHP-1*, *PEP-B1*, and *sSOD-1* (Figs. 4, 5, and 6; data sets available upon request from Anne Marshall, WDFW, and Robin Waples, NMFS). Allele frequencies for the Carson-derived hatchery populations at these three loci were generally in the intermediate or border zones between the two regions.

### Discussion

Allele frequencies for the Carson-derived hatchery stocks do not support previous speculation that spring Chinook salmon at the Carson NFH may have been derived primarily from fish destined for the Snake River (Myers et al. 1998). This latter speculation is based largely on the relative numbers of upstream-migrating spring Chinook salmon counted in the Snake River at Ice Harbor Dam (mean = 37,750 fish/yr) and the mid-Columbia River at Rock Island Dam (mean = 6,875 fish/yr) in those years (1958-1963) when fish were trapped at Bonneville Dam to initiate the spring Chinook population at the Carson National Fish Hatchery (Jim Myers, NMFS, pers. comm.). On the contrary, the genetic data are more consistent with the hypothesis that the Carson stock was derived from a mixture of mid-Columbia and Snake River populations. These interpretations assume, of course, the absence of allele frequency founder effects and constant allele frequencies since the Carson NFH stock was founded. In addition, populations not analyzed here - particularly spring Chinook salmon in the Deschutes, John Day, and Yakima Rivers - may have been part of the founding broodstock also. For example, the mean number of spring Chinook salmon counted at Bonneville Dam during the years 1958-1963 was 76,533 fish/yr, nearly 32,000 more than the sum of the means for Rock Island and Ice Harbor dams. The genetic data are thus most consistent with the hypothesis that the Carson NFH stock was founded from some unknown genetic admixture of fish from both the Snake and mid-Columbia rivers upstream from Bonneville Dam. More fine-scaled genetic analyses such as the use of microsatellite, nuclear DNA loci may be able to detect region- or river-specific alleles that may provide greater insights regarding the ancestry of the Carson NFH stock of spring Chinook salmon.

Populations of spring Chinook salmon in the Methow River Basin cannot be viewed as a single, random breeding population. Independent samples from the Twisp and Chewuch River populations clearly show greater similarity among samples from the same locality than samples collected in the same year from different localities. Moreover, these populations are not only diverged from each other, but

they also show equal (Chewuch River) or greater (Twisp River) genetic distances from the Carson-derived hatchery populations than they do to each other.

The genetic status of the mainstem Methow River population is unclear. Samples collected in 1993 and 1994 largely represented unmarked, hatchery-origin adults (presumably from the Winthrop NFH) that had voluntarily strayed into the Methow SFH. The genetic data for those two samples appear to reflect that ancestry. In addition, carcasses of hatchery-origin adults, also from the Winthrop NFH, are commonly found along the Methow River in the vicinity of the two hatcheries (Brian Cates, USFWS, pers. comm.). However, the 1996 sample for the Methow River population - which was comprised primarily of natural-origin adults trapped at Wells Dam and assigned to the Methow River population on the basis of the chemical composition of their scales - grouped with the *wild* Chewuch River population, and not Carson-derived hatchery stocks. This latter result is consistent with two alternative hypotheses: (1) significant numbers of Chewuch River fish trapped at Wells Dam in 1996 may have been misidentified as Methow River fish in 1996, as could occur if juveniles produced in the Chewuch River reside in the Methow River for an extended period prior to outmigration; or (2) allele frequencies for *natural* populations of spring Chinook salmon in the Methow and Chewuch rivers are actually very similar. If this latter hypothesis is true, then Winthrop-Carson fish would appear to have made little or no genetic contribution to the naturally spawning population in the Methow River, or - at the very least - their contribution in the Methow River population has not been significantly greater than their genetic contribution in the Chewuch River. Note, these interpretations are based on data that had only been collected through 1996.

The genetic data do not support the hypothesis that the Methow Composite and Winthrop NFH stocks are genetically homogeneous. The data are more consistent with the hypothesis that the Methow Composite stock, at least in the one year for which genetic data were obtained, was derived primarily from Chewuch River fish or a mixture of Chewuch River and natural-origin Methow River fish. The extent to which the Methow Composite stock may owe some of its ancestry to the Winthrop-Carson stock cannot be ascertained from the genetic data presented here. However, based on the broodstock records for the Methow SFH, I estimated that the 1998 brood year of the Methow Composite stock most likely had a genetic background composed of 17-25% Winthrop-Carson ancestry (see companion document of broodstock histories). Consequently, based on the data presented in this report and broodstock records, one cannot conclude that the Methow Composite stock is a Carson-derived population. This latter conclusion is not the case for the Leavenworth and Winthrop NFH populations.

Although carcasses of Winthrop-Carson adults are routinely recovered in the Methow River Basin (Brian Cates, USFWS, pers. comm.), the past reproductive success and actual genetic contributions of

those hatchery-origin adults to naturally-produced fry, smolts, and returning adults are unknown. The persistence of consistent and repeatable levels of genetic divergence (i.e. via temporal sampling) among the Twisp, Chewuch, and Carson-derived hatchery stocks would suggest little or no genetic influence of Winthrop-Carson fish to those two naturally spawning populations, at least to the extent to overcome genetic discreteness. The potential genetic contribution of Winthrop-Carson fish to naturally-produced fish in the mainstem Methow River is also unknown, as noted previously based on the 1996 "Methow River" sample collected at Wells Dam. Unfortunately, no genetic data appear to exist for natural-origin fry or smolts produced in each of the three sub-basins of the Methow River drainage. All genetic samples analyzed in this report are based on adults where questions of hatchery vs. wild origin were resolved primarily on the basis of freshwater growth patterns as revealed by scale analyses.

Several studies indicate that the natural reproductive success of non-native, hatchery-origin salmon and steelhead is generally very poor relative to natural-origin fish returning to the same streams. For example, in the Kalama River, Washington, non-native summer-run hatchery steelhead (Skamania stock) produced only 6-20% as many "wild" progeny per adult spawner as did natural-origin adults (Chilcote et al. 1986; Leider et al. 1990; Campton et al. 1991). Similarly, non-native winter-run hatchery steelhead (Chambers/Beaver Creek stock) produced an estimated 0-17% as many "wild" progeny per adult as did natural-origin winter-run steelhead spawning in the Kalama River (Hulett et al. 1996). Indeed, in two of the three years for which genetic data are available for winter-run steelhead in the Kalama River, hatchery-origin adults made no detectable genetic contribution to naturally-produced offspring. A number of other studies with Chinook and coho salmon have provided empirical evidence of similarly poor reproductive success of non-native, hatchery-origin adults relative to wild adults (Nickelson et al. 1986; Reisenbichler and Rubin 1999; Carmichael 2000). The controlled study of Nickelson et al. (1996) with coho salmon (*O. kisutch*) is particularly noteworthy because hatchery-origin juveniles displaced - via competition - their smaller, natural-origin counterparts in the test (treatment) streams, and this displacement inevitably drove down the abundance of those natural populations one generation later because of the very poor reproductive success of those hatchery-origin fish when they returned as adults.

The ability of Winthrop-Carson fish to naturally reproduce and potentially confer a demographic benefit to naturally spawning populations in the Methow River is a major uncertainty. Reservations regarding the deliberate release of Winthrop-Carson fish into natural spawning areas of the Methow River have been based on (a) the unknown genetic ancestry of that stock, (b) their long history of artificial propagation (approximately 40 years or 8-10 salmon generations) at three different hatcheries (Carson, Leavenworth, and Winthrop NFHs), and (c) major uncertainties regarding the ability of those fish to reproduce successfully under natural conditions. Indeed, biologists for the state and federal agencies

have expressed concerns that extensive natural spawning of Winthrop-Carson fish in the Methow River Basin could actually reduce the overall abundance of spring Chinook salmon in subsequent generations by competing directly with natural-origin juveniles or reducing their reproductive success as adults through direct interbreeding. The consensus opinion of NMFS, WDFW, and USFWS is that the Winthrop NFH should propagate spring Chinook salmon from the Methow River (i.e. the Methow Composite stock or other locally-derived stock) - rather than the imported *Carson* stock- to potentially aid recovery of naturally spawning populations and potentially reduce negative, biological impacts.

### Conclusions

The genetic data presented here are consistent with management decisions to phase out the Winthrop-Carson stock at the Winthrop NFH and replace it with the Methow Composite stock or other locally-derived broodstock. The Winthrop-Carson and Methow Composite stocks do not appear to be genetically identical; on the contrary, the Winthrop-Carson stock, as predicted, is most similar ancestrally to spring Chinook salmon at the Carson and Leavenworth NFHs, whereas the Methow Composite stock - based on only one sample representing one year - is most similar to the Chewuch River population. Moreover, the Winthrop-Carson stock and naturally spawning populations in the Methow River basin are not genetically identical despite the inability of biologists (federal, state, and tribal) and local citizens to distinguish them morphologically. This is not to say that Winthrop-Carson fish do not have the ability to help restore naturally spawning populations in the Methow River Basin or elsewhere. On the contrary, management decisions to phase out the Winthrop-Carson stock are based on the *biological opinion* that the biological risks associated with those fish spawning *en masse* with listed fish in the Methow River basin are much greater than the probability that those hatchery-origin adults would spawn successfully and help recover naturally spawning populations. If the natural reproductive success of Winthrop-Carson fish in the Methow River is poor relative to natural-origin adults, then there would be very little opportunity for those hatchery-origin fish to confer a demographic or genetic benefit to naturally spawning populations. On the other hand, carcasses resulting from those hatchery-origin adults could be a significant nutrient input that would be expected to increase the survival of any naturally-produced fish, regardless of their parentage (Cedarholm et al. 1997). These are major biological and management uncertainties that may warrant direct experimentation of natural spawning success of Winthrop-Carson fish in appropriate test streams where spring Chinook salmon are now extirpated but occurred historically. An isolated sub-basin in the Methow River drainage or other mid-Columbia tributary may be an appropriate location to release surplus Winthrop-Carson fish. Such an *experiment*, if properly monitored

with fry, smolt, and adult abundance surveys, could add to our knowledge base on which controversial management decisions regarding the future fate of ESA-listed populations depend.

### **Summary of Results and Conclusions**

- (1) Development of the Carson NFH stock most likely included significant numbers of fish from areas outside the mid-Columbia River region. The initial broodstocks may have included significant mixtures of both Snake River and mid-Columbia River fish.
- (2) Naturally spawning populations of spring Chinook salmon in the Methow River Basin are not genetically homogeneous: Twisp and Chewuch river populations each show evidence of genetic divergence. As a result, naturally spawning populations in the Twisp and Chewuch Rivers appear to have resisted potential genetic influences from Winthrop-Carson fish.
- (3) The Methow Composite and Winthrop-Carson Hatchery populations are not genetically homogeneous. The one population sample for the Methow Composite stock (obtained in 1998) was genetically more similar to the Chewuch River population than to the Winthrop-Carson stock.
- (4) The Methow Composite stock most likely captures genetic or allelic variation from naturally spawning populations in the Methow River Basin not captured by the Winthrop-Carson stock.
- (5) Major uncertainties still exist regarding past and present genetic contributions of Winthrop-Carson fish to naturally spawning populations in the mainstem Methow River. The genetic data for wild adults trapped at Wells Dam in 1996, and assigned to the Methow River on the basis of their scale compositions, are consistent with the hypothesis that Winthrop-Carson fish have had little or no genetic contribution to naturally spawning populations in the Methow River. However, one cannot exclude the possibility that significant numbers of Chewuch River adults trapped at Wells Dam were incorrectly assigned to the Methow River. Additional studies or analyses are necessary to test these alternative hypotheses.
- (6) The “Draft” *Biological Opinion* of NMFS concerning the future disposition of Winthrop-Carson fish at the Winthrop NFH is appears to be consistent with existing genetic data for spring Chinook salmon in the Methow River, including uncertainties regarding the ability of Winthrop-Carson fish to reproduce successfully in the Methow River and help recover wild populations. However, this latter ability needs to be directly tested and evaluated, preferably in areas where potential risks to existing, naturally spawning populations are minimal.

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Table 1. Samples of spring Chinook salmon from the mid-Columbia River for which allozyme genetic data were analyzed. Preliminary analyses were performed on all populations, and more detailed analyses were performed on populations 1 through 14. Data for populations with the same number followed by a lower case letter (e.g. 3a, 3b) were pooled in the analyses because of small sample sizes resulting from extremely low adult returns, particularly in 1994. Allozyme frequency data were provided courtesy of Anne Marshall and Craig Busack, Washington Department of Fish & Wildlife, Olympia, Washington.

Sample	Locality	Year sampled	Life stage	Sample size
1	Twisp River	1992	Adults	59
2	Twisp River	1993	Adults	48
3a	Twisp River	1994	Adults	5
3b	Twisp River	1996	Adults	37
4	Chewuch River	1992	Adults	47
5	Chewuch River	1993	Adults	104
6a	Chewuch River	1994	Adults	7
6b	Chewuch River	1996	Adults	55
7	Methow River	1993	Adults	93
8	Methow River	1994	Adults	14
9	Methow River	1996	Adults	43
10	Methow? <sup>1</sup>	1994	Adults	25
11	Winthrop Nat'l Fish Hatchery	1992	Adults	100
12	Methow Composite Stock	1998	Adults	100
13	Leavenworth Nat'l Fish Hatchery	1991	Adults	100
14	Carson National Fish Hatchery	1989	Adults	100

<sup>1</sup> The exact source and location of these fish could not be ascertained. They are listed in WDFW's genetics datafile as "Methow? 94". The majority of these fish had hatchery scale patterns. They may represent a mixed sample of adults from the Winthrop NFH with carcasses from the Methow River based on the reports of Bartlett (1996) and Kelly and Hamstreet (1996), although these origins could not be confirmed.

Table 1 . Continued.

Sample	Locality	Year sampled	Life stage	Sample size
15	White River (Wenatchee R.)	1989	Adults/carcasses	55
16	White River (Wenatchee R.)	1991	Adults/carcasses	22
17	White River (Wenatchee R.)	1992	Adults/carcasses	36
18	White River (Wenatchee R.)	1993	Adults/carcasses	24
19	Nason Creek (Wenatchee R.)	1989	Adults/carcasses	30
20	Nason Creek (Wenatchee R.)	1992	Adults/carcasses	41
21	Nason Creek (Wenatchee R.)	1993	Adults/carcasses	51
22	Chiwawa River (Wenatchee R.)	1989	Adults/carcasses	62
23a	Chiwawa River (Wenatchee R.)	1990	Adults/carcasses	11
23b	Chiwawa River (Wenatchee R.)	1991	Adults/carcasses	40
24	Chiwawa River (Wenatchee R.)	1992	Adults/carcasses	20
25	Chiwawa River (Wenatchee R.)	1993	Adults/carcasses	101
26a	Chiwawa River (Wenatchee R.)	1994	Adults/carcasses	13
26b	Chiwawa River (Wenatchee R.)	1996	Adults/carcasses	18
27a	Little Wenatchee River	1992	Adults/carcasses	2
27b	Little Wenatchee River	1993	Adults/carcasses	16
28	Entiat Nat'l Fish Hatchery	1994	Adults	69
29	Entiat Nat'l Fish Hatchery	1997	Adults	100
30	Entiat Nat'l Fish Hatchery	1998	Adults	17
31	Entiat River	1997	Adults/carcasses	14

Table 2. Allele frequencies at loci contributing to the heterogeneity among spring Chinook salmon in the Methow River Basin (alleles in parentheses). Data were provided courtesy of Craig Busack and Anne Marshall, WDFW, Olympia, Washington.

Population sample	<i>mAAT-1</i> (100)	<i>sAAT-2</i> (100)	<i>ADA-1</i> (83)	<i>sAH</i> (86)	<i>HAGH</i> (100)	<i>sIDPH-1</i> (100)	<i>PEP-B</i> (-350)	<i>PEP-LT</i> (100)	<i>sSOD-1</i> (-260)
1. Twisp 92	0.991	1.000	0.129	0.0	0.966	0.661	0.319	0.991	0.314
2. Twisp 93	0.990	0.979	0.146	0.0	0.958	0.750	0.156	0.969	0.333
3. Twisp 9496	0.988	1.000	0.131	0.0	0.964	0.762	0.214	0.952	0.333
4. Chewuch 92	1.000	0.957	0.021	0.022	0.862	0.691	0.138	0.989	0.309
5. Chewuch 93	1.000	0.981	0.053	0.005	0.909	0.625	0.168	0.981	0.255
6. Chewuch 9496	1.000	0.895	0.008	0.0	0.839	0.702	0.121	0.992	0.282
7. Methow 93	1.000	1.000	0.022	0.0	0.882	0.758	0.134	0.924	0.210
8. Methow 94	1.000	1.000	0.036	0.0	0.923	0.786	0.077	0.929	0.107
9. Methow 96	1.000	0.988	0.035	0.0	0.895	0.721	0.151	0.917	0.256
10. Methow??94	0.960	0.980	0.0	0.0	0.860	0.820	0.100	0.920	0.220
11. Winthrop 92	0.990	1.000	0.025	0.0	0.900	0.810	0.050	0.914	0.245
12. Methow C98	1.000	0.995	0.035	0.025	0.920	0.740	0.180	0.960	0.260
13. Leavenw. 91	0.985	1.000	0.050	0.0	0.910	0.785	0.075	0.950	0.245
14. Carson 89	0.985	0.985	0.020	0.0	0.915	0.760	0.075	0.955	0.155

Table 3 . G-tests for homogeneity of allele frequencies at loci showing significant heterogeneity among samples of spring Chinook salmon from the Methow River, Washington. The analyses were first performed on all 12 samples (1-12, Table 1), and then on subsets of samples by sequentially deleting samples from the Twisp and Chewuch Rivers. The final comparison is among the two Winthrop-Carson samples (# 10, 11) and the Methow Composite stock (#12). The parametric significance probabilities for each locus are given first (“Probability”) followed by the table-wide probabilities ( $P_{adj.}$ ) adjusted for the number of tests or polymorphic loci (Rice 1979). The Total G-statistic values and corresponding degrees of freedom were obtained by summing the G-statistics and degrees of freedom over all polymorphic loci, including those loci shown below. Only those tests (loci) statistically significant after Bonferroni corrections in the first comparison (samples #1-12) are shown below.

Locus	No. of Samples	No. of Alleles	df	G-stat.	G/df	Probability	$P_{adj.}$
<b><i>Samples 1-12</i></b>							
<i>sAAT-2</i>	12	2	11	52.919	4.811	0.00000019	<0.001
<i>ADA-1</i>	12	2	11	54.664	4.969	0.00000009	<0.001
<i>sIDHP-1</i>	12	3	22	55.257	2.512	0.00010919	0.003
<i>PEP-B</i>	12	3	22	69.087	3.140	0.00000092	<0.001
<i>PEP-LT</i>	12	2	11	32.992	2.999	0.00052773	0.013
<i>PGK-2</i>	12	2	11	31.675	2.880	0.00085984	0.021
Total (28 loci)			374	706.5	1.889	<10 <sup>-8</sup>	
<b><i>Samples 4-12 (Twisp River deleted)</i></b>							
<i>sAAT-2</i>	9	2	8	45.180	5.647	0.00000034	<0.001
<i>ADA-1</i>	9	2	8	10.162	1.270	0.25381820	NS
<i>sIDHP-1</i>	9	3	16	43.787	2.737	0.00021248	0.006
<i>PEP-B</i>	9	3	16	34.513	2.157	0.00463049	NS
<i>PEP-LT</i>	9	2	8	26.644	3.330	0.00081437	0.021
<i>PGK-2</i>	9	2	8	19.285	2.411	0.01340924	NS
Total (28 loci)			272	467.7	1.719	<10 <sup>-8</sup>	

Table 3. Continued.

Locus	No. of Samples	No. of Alleles	df	G-stat.	G/df	Probability	P <sub>adj.</sub>
<b><i>Samples 7-12 (Twisp and Chewuch rivers deleted)</i></b>							
<i>sAAT-2</i>	6	2	5	5.824	1.165	0.32375590	NS
<i>ADA-1</i>	6	2	5	3.702	0.740	0.59312494	NS
<i>sIDHP-2</i>	6	3	10	10.955	1.095	0.36107110	NS
<i>PEP-B</i>	6	3	10	25.987	2.599	0.00375718	NS
<i>PEP-LT</i>	6	2	5	4.222	0.844	0.51798010	NS
<i>PGK-2</i>	6	2	5	12.714	2.543	0.02621195	NS
Total (28 loci)			170	216.8	1.275	0.00884887	
<b><i>Samples 10-12 (Winthrop-Carson and Methow Composite only)</i></b>							
<i>sAAT-2</i>	3	2	2	3.260	1.630	0.19594560	NS
<i>ADA-1</i>	3	2	2	3.213	1.606	0.20059230	NS
<i>sIDHP-2</i>	3	3	4	3.628	0.907	0.45875640	NS
<i>PEP-B</i>	3	3	4	18.589	4.647	0.00094638	0.025
<i>PEP-LT</i>	3	2	2	3.729	1.864	0.15500110	NS
<i>PGK-2</i>	3	2	2	2.133	1.067	0.34420810	NS
Total (26 loci)			62	99.47	1.604	0.00178502	

Appendix 1. Allozyme loci used for genetic comparisons among mid-Columbia River populations of spring Chinook salmon.

Enzyme	Loci	Number of alleles
Aspartate aminotransferase	<i>sAAT-1</i> <sup>a</sup>	1
	<i>sAAT-2</i> <sup>a</sup>	3
	<i>sAAT-3</i>	1
	<i>sAAT-4</i>	2
	<i>mAAT-1</i>	2
	<i>mAAT-2</i>	2
Adenosine deaminase	<i>ADA-1</i>	2
	<i>ADA-2</i>	1
Aconitate hydratase	<i>sAH</i>	3
	<i>mAH-3</i>	1
	<i>mAH-4</i>	2
Dipeptidase (Peptidase-A)	<i>PEP-A</i>	3
Glucose-6-phosphate isomerase	<i>GPI-A</i>	1
	<i>GPI-B2</i>	2
	<i>GPI-r</i>	1
Glutathione reductase	<i>GR</i>	2
Hydroxyacylglutathione hydrolase	<i>HAGH</i>	3
Iditol dehydrogenase	<i>IDDH-1</i>	2
Isocitrate dehydrogenase	<i>mIDHP-2</i>	1
	<i>sIDHP-1</i>	3
	<i>sIDHP-2</i>	3
Lactate dehydrogenase	<i>LDH-B1</i>	1
	<i>LDH-B2</i>	2
	<i>LDH-C</i>	2
Malate dehydrogenase	<i>sMDH-A1</i> <sup>a</sup>	1
	<i>sMDH-A2</i> <sup>a</sup>	3
	<i>sMDH-B1</i> <sup>a</sup>	1
	<i>sMDH-B2</i> <sup>a</sup>	3
	<i>mMDH-2</i>	2
	<i>mMDH-3</i>	2

## Appendix 1. Continued.

Enzyme	Loci	Number of alleles
Malic enzyme	<i>sMEP-1</i>	3
Mannose-6-phosphate isomerase	<i>MPI</i>	3
Proline dipeptidase (Peptidase D)	<i>PEP-D-2</i>	2
Leucyl-tyrosine dipeptidase	<i>PEP-LT</i>	2
Phosphogluconate dehydrogenase	<i>PGDH</i>	1
Phosphoglycerate kinase	<i>PGK-2</i>	2
Phosphoglucomutase	<i>PGM-1</i>	1
	<i>PGM-2</i>	1
Superoxide dismutase	<i>sSOD-1</i>	2
	<i>sSOD-2</i>	2
	<i>mSOD</i>	2
Tripeptide aminopeptidase (Peptidase B)	<i>PEP-B-1</i>	3
Triosephosphate isomerase	<i>TPI-3</i>	1
	<i>TPI-4</i>	2

<sup>a</sup>These loci occur as duplicated, *isoloci* at which allelic variation cannot be assigned to a specific locus of each pair. However, for the analyses described in this report, allelic variation was assigned to only one locus of each isolocus pair because observed levels of variation (i.e. number of individuals expressing two alleles at each isolocus pair) loci were very low. Consequently, the second locus of each pair was assumed to be monomorphic.

Appendix 2. Samples of spring/summer Chinook salmon from the Snake River for which allozyme genetic data were compared to data for populations from the mid-Columbia River (Figs. 4, 5, and 6). Allozyme frequency data were provided courtesy of Robin Waples, NMFS, Seattle, WA. Allozyme data for samples collected in 1989 and 1990 are presented in the annual report of Waples et al. 1993.

Pop. No.	Locality	No. of samples	Years sampled	Life stage	Sample sizes
32	Johnson Creek, S. F. Salmon R.	5	1989, 90, 91, 94	Juveniles	97, 80, 80, 60, 59
33	Secesh River, S. F. Salmon R.	3	1989, 90, 91	Juveniles	92, 80, 80
34	McCall State Hatchery, Idaho <sup>1</sup>	5	1989, 90, 91, 92, 94	Juveniles	100, 100, 100, 80, 60
35	Stolle Meadows, S.F. Salmon R.	3	1992, 93, 94	Juveniles	59, 58, 60
36	Poverty Flat, S.F. Salmon R.	3	1992, 93, 94	Juveniles	27, 60, 60
37	Trap/weir, S.F. Salmon R.	1	1992	Juveniles	60
38	Upper Salmon River	5	1989, 91, 92, 93, 94	Juveniles	99, 60, 59, 65, 60
39	Frenchman Creek, Salmon River	2	1991, 92	Juveniles	60, 60
40	Alturus Lake Creek, Salmon R.	1	1992	Juveniles	60
41	Valley Creek, Salmon River	5	1989, 90, 91, 92, 93	Juveniles	99, 99, 77, 111, 142
42	Sawtooth State Hatchery, Idaho <sup>2</sup>	5	1989, 90, 91, 92, 94	Juveniles	100, 100, 99, 80, 60
43	Marsh Creek, M. F. Salmon R.	4	1989, 90, 91, 92	Juveniles	100, 80, 78, 60
44	Chamberlain Creek, Salmon R.	3	1991, 93, 94	Juveniles	80, 50, 60
45	Lookingglass Hatchery, Oregon <sup>3</sup>	5	1990, 91, 92, 93, 94	Juveniles	100, 100, 80, 60, 60
46	Lookingglass Creek	2	1993, 1994	Juveniles	60, 60

## Appendix 2. Continued.

Pop. No.	Locality	No. of samples	Years sampled	Life stage	Sample sizes
47	Rapid River Hatchery, Idaho <sup>4</sup>	3	1989, 93, 94	Juveniles	100, 80, 60
48	Rapid River (wild)	2	1993, 94	Juveniles	30, 28
49	Lostine River	6	1989, 90, 91, 92, 93, 94	Juveniles	100, 99, 98, 60, 59, 60
50	Minam River	5	1990, 91, 92, 93, 94	Juveniles	100, 99, 60, 75, 60
51	Catherine Creek	5	1990, 91, 92, 93, 94	Juveniles	100, 87, 60, 60, 60
52	Grande Ronde River	4	1991, 92, 93, 94	Juveniles	85, 59, 59, 60
53	Wenaha River	4	1991, 92, 93, 94	Juveniles	101, 48, 60, 60
54	Imnaha River	6	1989, 90, 91, 92, 93, 94	Juveniles	100, 80, 100, 60, 60, 60
55	Imnaha River, hatchery-origin <sup>5</sup>	6	1989, 90, 91, 92, 93, 94	Juveniles	100, 100, 100, 80, 60, 60

<sup>1</sup>These fish represent a native broodstock derived each year from returning adults trapped in the South Fork of the Salmon River.

<sup>2</sup>These fish represent a native broodstock derived each year from returning adults trapped in the upper Salmon River (near Stanley, Idaho).

<sup>3</sup>Progeny of Rapid River stock adults returning to Lookingglass Hatchery.

<sup>4</sup>This stock was developed during the 1960's from adults returning to the upper Snake River but trapped at the base of Hell's Canyon Dam. This stock was sampled at the Rapid River Hatchery in 1993 and 1994 but at the Lookingglass Hatchery in 1989.

<sup>5</sup>These fish represent a native broodstock derived each year from returning adults trapped in the Imnaha River, Oregon.

Appendix 3. Genetic chord distances (Cavalli-Sforza and Edwards 1967) among samples of spring Chinook salmon from the Methow River Basin and associated hatchery populations.

Population Sample	Population sample No													
	1	2	3	3	5	6	7	8	9	10	11	12	13	14
1. Twisp 92	.0													
2. Twisp 93	.038	.0												
3. Twisp 94/96	.039	.036	.0											
4. Chewuch 92	.051	.050	.056	.0										
5. Chewuch 93	.040	.046	.051	.029	.0									
6. Chewuch 94/96	.062	.062	.071	.043	.048	.0								
7. Methow 93	.067	.068	.064	.066	.051	.064	.0							
8. Methow 94	.074	.067	.072	.078	.071	.072	.058	.0						
9. Methow 96	.051	.049	.054	.042	.038	.053	.051	.064	.0					
10. Methow? 94	.076	.070	.063	.064	.062	.062	.049	.071	.061	.0				
11. Winthrop NFH	.070	.065	.063	.062	.052	.061	.035	.058	.047	.045	.0			
12. Methow Composite	.048	.049	.052	.043	.036	.053	.040	.060	.042	.053	.045	.0		
13. Leavenworth NFH	.060	.057	.052	.057	.049	.056	.037	.060	.049	.045	.034	.045	.0	
14. Carson NFH	.073	.070	.071	.061	.053	.061	.046	.072	.054	.050	.035	.047	.041	.0

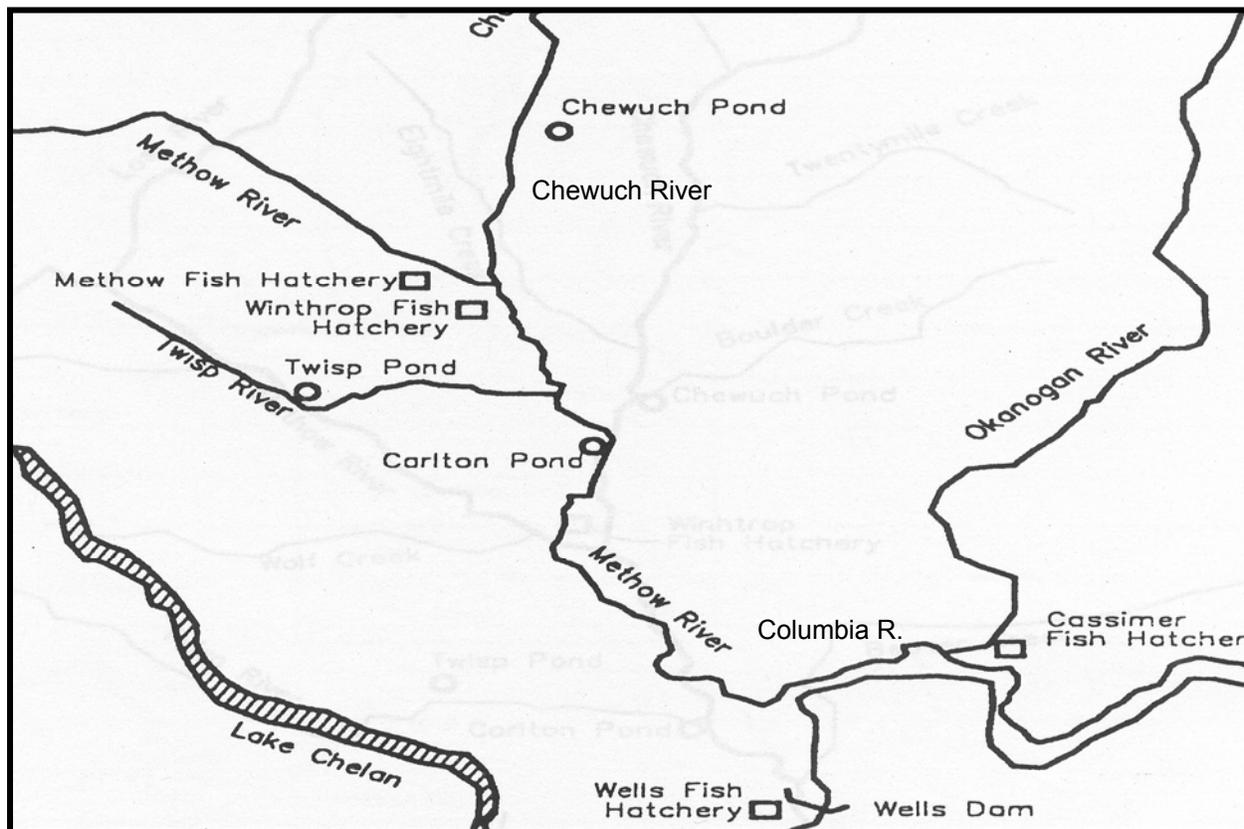


Figure 1. Methow River, mid-Columbia River. The Methow River Basin consists of three major tributaries: Twisp River, Chewuch River, and the upper mainstem Methow River upstream from the confluence of the Chewuch River.

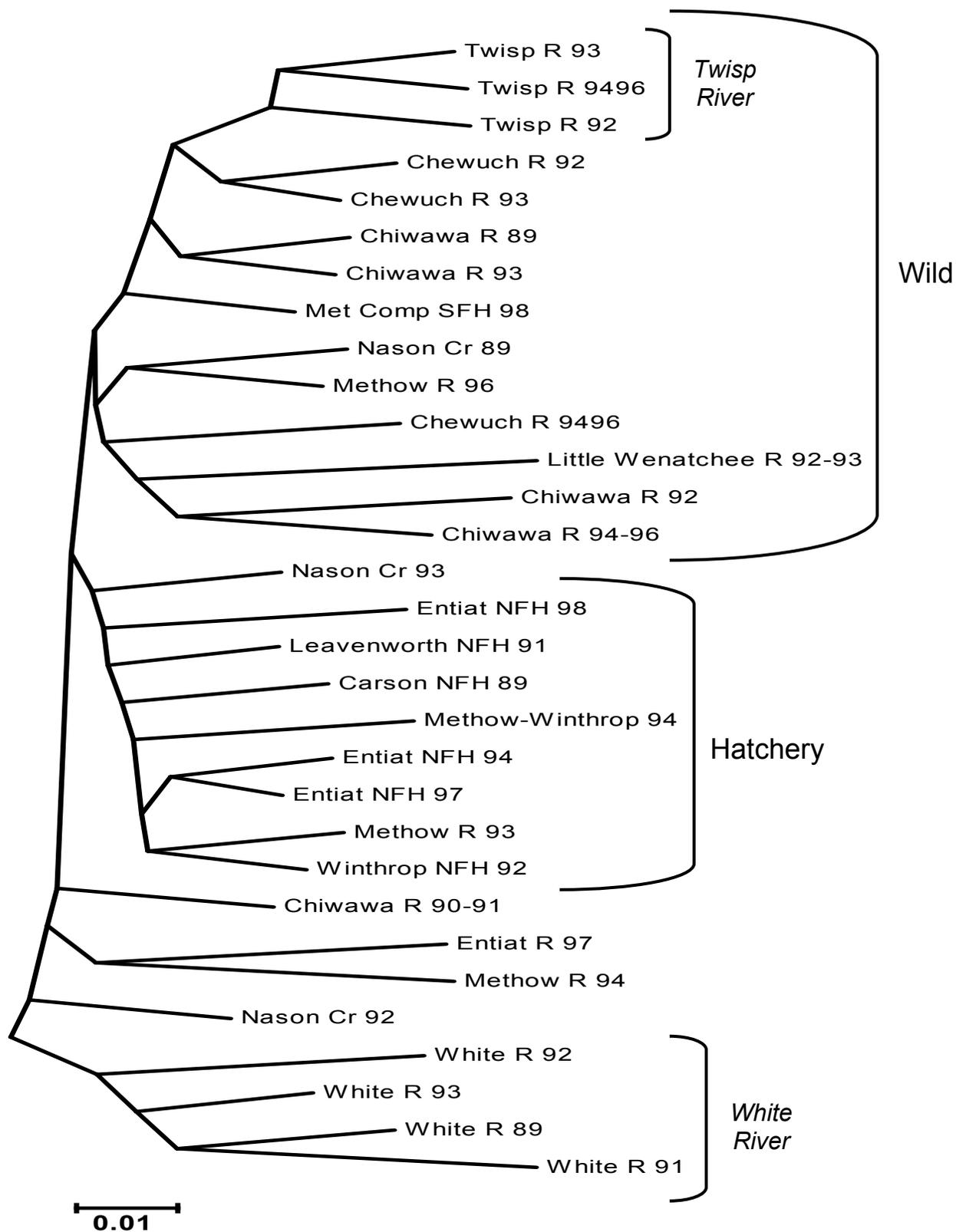


Figure 2. Neighbor-joining tree among samples of spring Chinook salmon from the mid-Columbia River (Table 1) based on allele frequencies at 44 allozyme loci (Appendix 2).

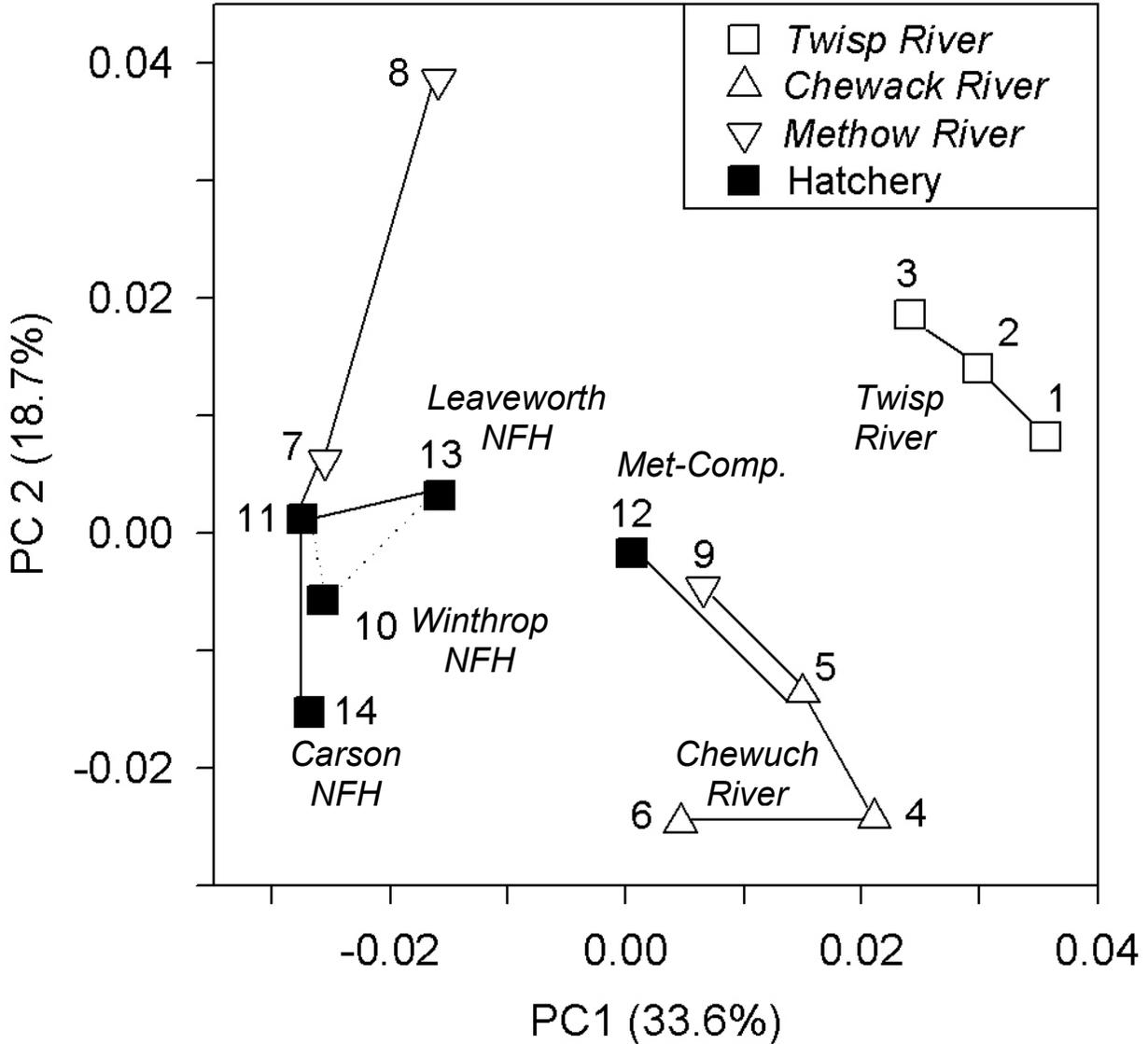


Figure 3. Projection of population samples 1-14 (Table 1) onto the first and second principal coordinate axes (Everitt 1979) derived from the matrix of pairwise genetic chord distances (Cavalli-Sforza and Edwards 1967) among those samples. Line segments connect each sample to its nearest neighbor in the data matrix (i.e. other sample with the smallest, shared genetic distance). Sample number 10 was equal distance to samples 11 and 13. The first two principal axes accounted for 33.6 and 18.7%, respectively, of the total variation among genetic distance values.

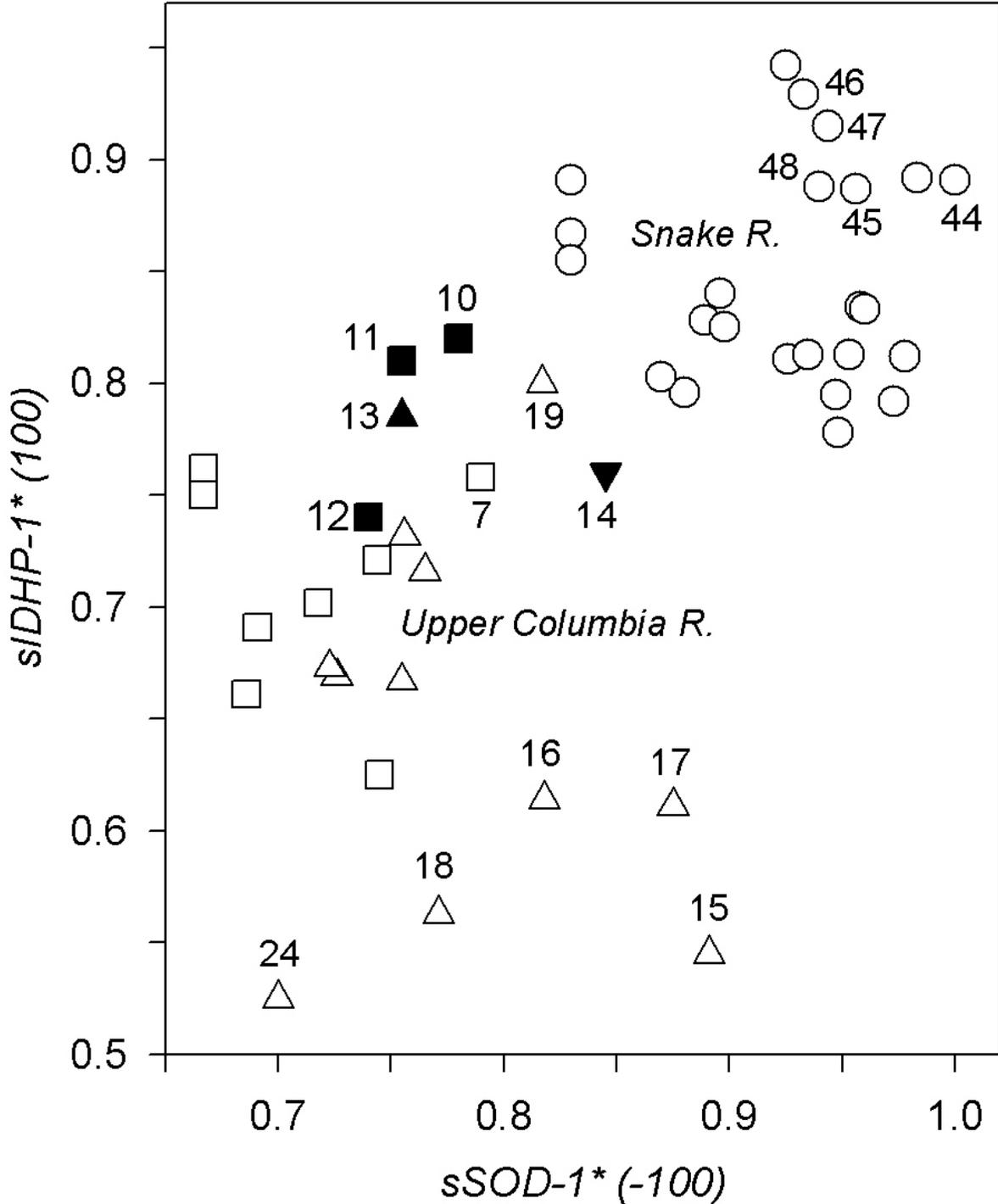


Figure 4. Biplot of common allele frequencies at two loci ( $sSOD-1$  and  $sIDHP-1$ ) distinguishing populations of spring Chinook salmon from the Snake River (open circles; Appendix 1) and the mid-Columbia River (squares and triangles; Table 1). Samples from the Entiat River (#28-31) and the 1994 sample from the Methow River (#8) are not included (Table 1). Hatchery populations in the mid-Columbia River, including the Carson NFH (14), are blackened. Methow and Wenatchee River populations are indicated by squares and upward triangles, respectively.

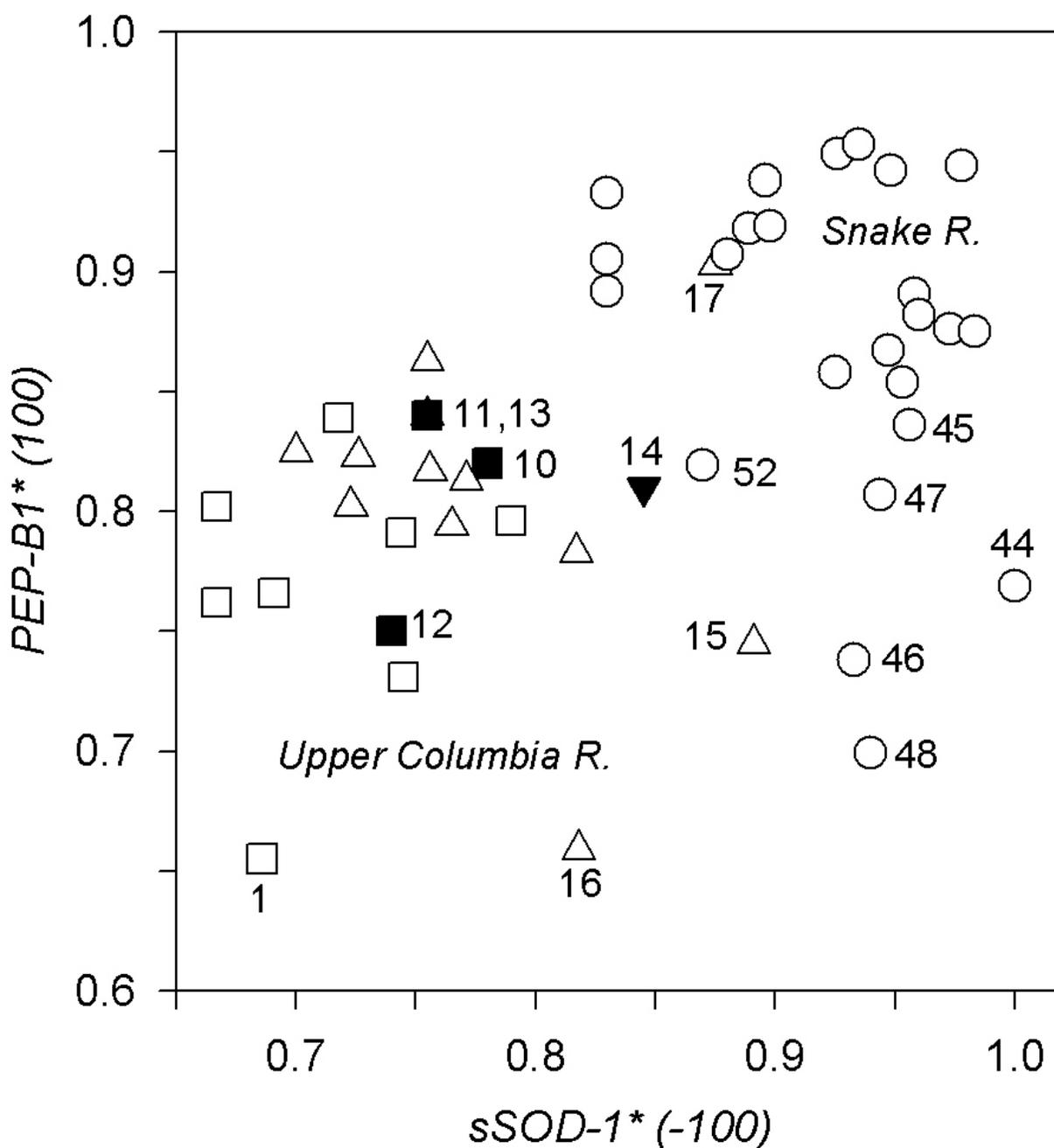


Figure 5. Biplot of common allele frequencies at two loci (*sSOD-1* and *PEP-B1*) distinguishing populations of spring Chinook salmon from the Snake River (open circles; Appendix 1) and the mid-Columbia River (squares and triangles; Table 1). Samples from the Entiat River (#28-31) and the 1994 sample from the Methow River (#8) are not included (Table 1). Hatchery populations in the mid-Columbia River, including the Carson NFH (14), are blackened. Methow and Wenatchee River populations are indicated by squares and upward triangles, respectively.

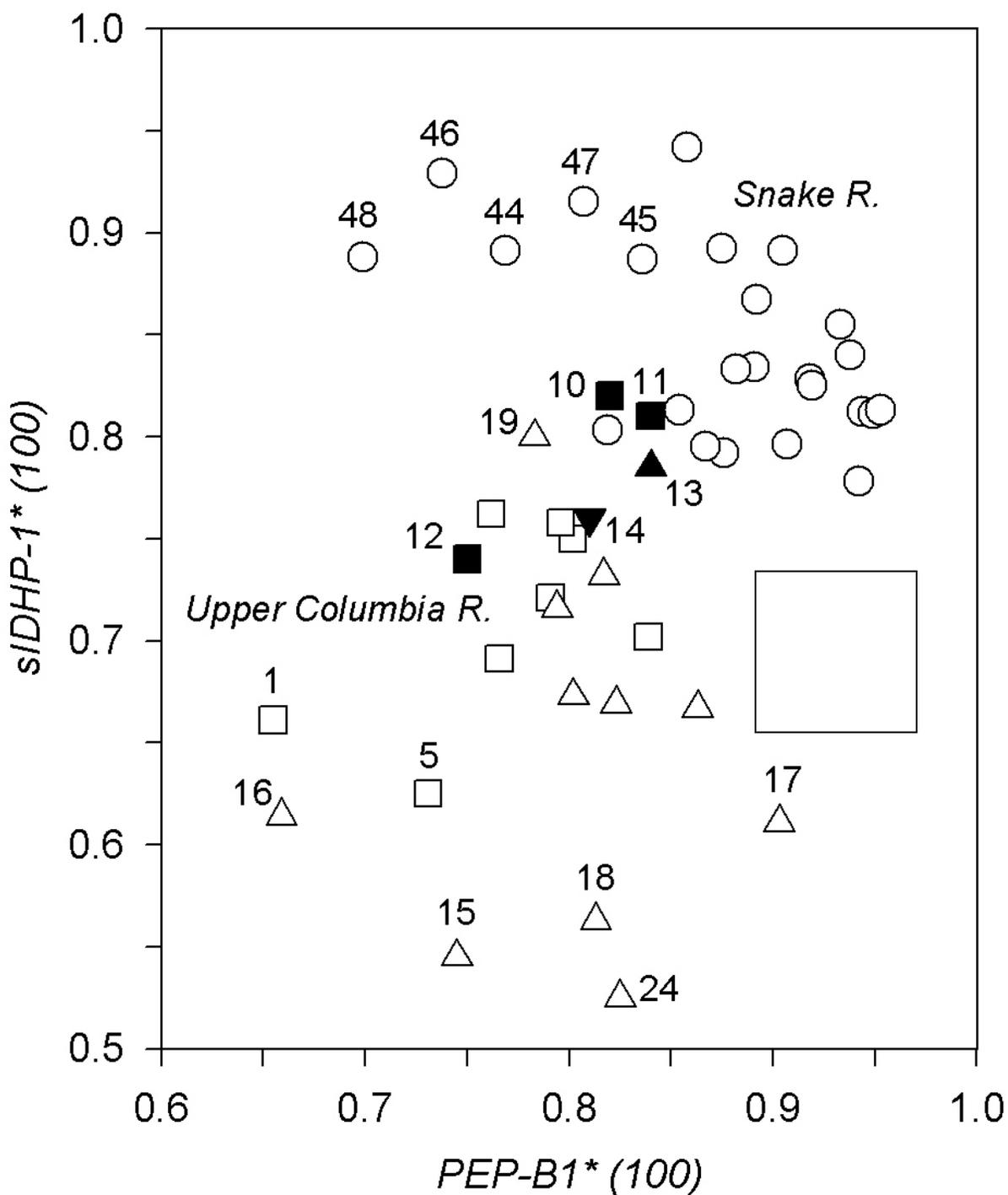


Figure 6. Biplot of common allele frequencies at two loci (*PEP-B1* and *sIDHP-1*) distinguishing populations of spring Chinook salmon from the Snake River (open circles; Appendix 1) and the mid-Columbia River (squares and triangles; Table 1). Samples from the Entiat River (#28-31) and the 1994 sample from the Methow River (#8) are not included (Table 1). Hatchery populations in the mid-Columbia River, including the Carson NFH (14), are blackened. Methow and Wenatchee River populations are indicated by squares and upward triangles, respectively.