9.0 INVESTIGATION OF ANADROMOUS FISH GENETICS IN THE KLAMATH HYDROELECTRIC PROJECT AREA

9.1 DESCRIPTION AND PURPOSE

The life history variation and existing population genetic studies of coho and Chinook salmon, coastal steelhead, and nonandromous *Oncorhynchus mykiss* (*O. mykiss*) *spp.* in the Upper and Lower Klamath River basins were reviewed. The primary goals of this section are to assess the evidence of reproductive isolation (genetic distinctiveness) between populations or groups of populations of salmonids throughout the Project area and evaluate whether there is sufficient evidence to determine if resident trout populations above Iron Gate dam are genetically similar to anadromous trout downstream of the Project. Additional information on the genetic impacts from operation of Iron Gate fish hatchery and disease-related information is presented.

This information, along with comments from interested stakeholders, were used to identify genetic data gaps and additional questions necessary for making scientifically informed management decisions about restoring anadromous runs above Iron Gate dam. Restoring salmonid runs in the Project area will require significant additional genetic studies, as well as an evaluation of the ecological factors underlying the feasibility of establishing self-sustaining salmonid populations above Iron Gate dam. Further genetic studies are necessary to identify (1) if independent lineages of *O. mykiss* are found in the Upper Klamath River basin, and (2) areas that will be opened to migration, if restoring steelhead is planned above Iron Gate dam. Long-term genetic monitoring of all anadromous salmon runs above Iron Gate dam will be critical to managing these new populations, given the potential for inbreeding depression (reduction in fitness) and for effective population sizes to drop below levels that can support viable salmonid populations. In the case of *O. mykiss*, long-term genetic sampling and monitoring also should be established to detect any negative genetic and disease-related impacts on native population of Upper Klamath River basin *O. mykiss newberri*.

After construction of the Copco No. 1 dam in 1918, anadromous fish passage ceased to occur in the Klamath River and its tributaries above RM 199. Historically, anadromous fish had the opportunity to migrate to the Upper Klamath basin. The later construction of the J.C. Boyle and Keno hydroelectric facilities included fish ladders built for upstream passage of resident trout. *O. mykiss* are found throughout the Project area. However, it is unclear if these fish are more closely related to resident upper basin stocks or to anadromous steelhead previously found in the area. The interest in genetics is related to the feasibility and potential genetic impacts to (1) resident trout populations from reintroducing steelhead above Iron Gate dam and at hydroelectric facilities, and (2) the selection of anadromous fish stocks that would have the highest likelihood of success if reintroduced above Iron Gate dam.

9.2 OBJECTIVES

The following are key questions raised by stakeholders:

1. Are resident trout populations in the Project area genetically different?
2. What are the genetic similarities among resident trout populations, anadromous steelhead, and residualized trout in Project area reaches that historically supported anadromous salmonids?

3. Are any genetic differences between wild and hatchery anadromous fish related to Iron Gate fish hatchery operations?

4. To the extent possible, can information gathered during this study help address disease issues regarding the co-presence of resident and anadromous fish?

The following are objectives of the study:

- Assess the genetic distinctiveness of trout populations throughout the Upper Klamath basin.

- Determine if resident trout populations are similar to anadromous trout downstream of the Project. (Assist in identifying appropriate source populations for reintroduction of steelhead above the dams.)

- Determine if Iron Gate fish hatchery stocks are genetically similar to native fish that return to the Iron Gate area. Are hatchery stocks "influencing" native stocks?

9.3 RELICENSING RELEVANCE AND USE IN DECISIONMAKING

The information synthesized from this effort will assist in evaluating the current state of knowledge and on-going research addressing the genetics of *O. mykiss* subspecies and other anadromous salmonids (i.e., Chinook and coho). This knowledge will be considered for fish passage, specifically connectivity issues for resident trout and to gain confidence in identification of appropriate salmonid stocks for possible reintroduction above Iron Gate dam.

9.4 METHODS AND GEOGRAPHIC SCOPE

The geographic scope of the Project will focus on the Upper Klamath basin, specifically the Project area.

Existing literature and current research pertinent for the Klamath River basin will be summarized as a draft memorandum. Following a review of the memorandum, a workshop will be convened with local genetic experts (e.g., the fisheries staff at OSU, ODFW, Humboldt State University, University of California at Davis, and CDFG) and stakeholders to identify critical data gaps. Future studies would be identified to fill critical, priority data gaps pertaining to Project area management objectives. Comments on the draft memorandum are incorporated in this FTR. (see Appendix 9A).

9.5 RELATIONSHIP TO REGULATORY REQUIREMENTS AND PLANS

This study, together with the fish passage study (described in other sections of this FTR), provides information that will help inform decisions related to fish passage and anadromous fish reintroduction in the Project area.

9.6 TECHNICAL WORK GROUP COLLABORATION

PacifiCorp has worked with stakeholders to establish a more collaborative process for planning and conducting studies needed to support Project relicensing documentation. As part of this collaborative process, the Aquatic Work Group (AWG) has met approximately monthly to

develop appropriate study plans and discuss results. The AWG approved Study Plan 1.17 Investigation of Anadromous Fish Genetics in the Klamath Hydroelectric Project Area.

9.7 RESULTS AND DISCUSSION

9.7.1 Classification Methods for Klamath River Salmonid Populations

9.7.1.1 Evolutionary Significant Units

The NOAA Fisheries (formerly the NMFS) policy described by Waples (1991) explains how the Endangered Species Act (ESA) definition of "species" is applied to the determination of evolutionarily significant units (ESUs) for Pacific salmonids. This policy indicates that one or more naturally reproducing salmonid populations will be considered distinct and, hence, a species under the ESA, if they represent an ESU of the biological species. To be considered an ESU, a population must satisfy two criteria: (1) It must be reproductively isolated from other population units of the same species, and (2) it must represent an important component in the evolutionary legacy of the biological species. The first criterion, reproductive isolation, need not be absolute, but must have been strong enough to permit evolutionarily important differences to occur in different population units. The second criterion is met if the population contributes substantially to the ecological or genetic diversity of the species as a whole.

Chinook Salmon.

The NOAA Fisheries completed a comprehensive status review of Chinook salmon (*O. tshawytscha*) populations in California, Idaho, Oregon, and Washington and identified 15 ESUs within this range (Myers et al. 1998). Scientific disagreement over the proposed southern boundary of the Southern Oregon and Northern California Coastal Chinook salmon ESU (SONCC ESU) as well as disagreement over the listing status of Chinook salmon within the SONCC ESU prompted further evaluation. In particular, there was uncertainly regarding the rationale for a separate Upper Klamath and Trinity rivers Chinook salmon ESU within the range of the larger SONCC ESU, and the inclusion of spring-run Chinook in the SONCC ESU on the proposed list of populations designated as threatened under the ESA (NOAA Fisheries, FR 64, no. 179).

In 1998 and 1999, the NOAA Fisheries, CDFG, USFWS, and USFS collected samples of spawned adult Chinook salmon carcasses from 13 rivers and hatcheries in the Central Valley and Klamath River basin while analyzing new genetic data for California Chinook salmon. These new samples were analyzed along with allozyme data for California and southern Oregon Chinook salmon that were previously used in the NOAA Fisheries coast-wide Chinook status review (Myers et al. 1998). The population genetic structure revealed by the new analysis of allozyme data was consistent with the delineations of major genetic groups described in previous genetic studies of California and southern Oregon Chinook salmon (Utter et al. 1989; Bartley et al. 1992). The original ESU boundaries, based on genetic and ecological differences between Chinook salmon populations to the north and south of the Klamath River, were not changed. Currently, the SONCC salmon ESU includes the mainstem and tributaries of the Klamath River upstream to its confluence with the Trinity River, and the Upper Klamath-Trinity Rivers ESU occupies the mainstem and tributaries of these two basins up to Iron Gate and Lewiston dams (Myers et al. 1998). Currently, neither ESU is listed.

Coho Salmon

The Southern Oregon-Northern California Coast coho salmon ESU was listed as a threatened species on May 6, 1997 (NOAA Fisheries, FR 62, no. 87). The ESU includes all naturally spawned coho populations in coastal streams from Cape Blanco, Oregon, to Punta Gorda, California.

Coastal Rainbow Trout/steelhead

O. mykiss irideus in the Klamath River are part of the Klamath Mountains Province ESU. This ESU includes resident trout, and different run-timings of steelhead that enter the Klamath River and all anadromous watersheds between there and the Elk River in Oregon. The NOAA Fisheries (Busby et al. 1996) stated there is considerable uncertainty about the relationship between anadromous and non-anadromous trout throughout the Klamath River basin, especially in waters above Klamath Falls, Oregon. This ESU includes fish that go through a unique life history stage, where they outmigrate to the estuary before migrating upstream and overwintering in the mainstem. These fish, known as "half-pounders," are distinguished by a unique number of chromosomes (Thorgaard, 1983)

9.7.1.2 Distinct Population Segments

USFWS policy (USFWS, 1996) allows populations or groups of populations to be protected as distinct population segments (DPSs). The first criterion for defining a DPS is identifying the discreteness of the population segment in relation to the remainder of the species to which it belongs. The second DPS criterion is recognizing how significantly the population differs from other populations of the same species. While genetic data are not the only measure for discreteness, they are a valuable tool for quantifying the significance of differentiation between putative DPSs. No DPSs are recognized for the species discussed in the section of the FTR, although uncertainty exists about the discreteness of coastal rainbow trout and Upper Klamath redband trout populations in the Project area.

9.7.1.3 Gene Conservation Groups

The ODFW used gene conservation groups (GCGs) to describe fish populations that are unique in terms of life history, meristics (meaning physically measurable), disease resistance, and/or allozyme variation. ODFW recognized the following four GCGs in the Klamath River basin for Upper Klamath redband trout: Klamath Lake trout, Upper Williamson River trout, Jenny Creek trout, and Upper Sprague River trout. The Klamath Lake GCG extends south to the Oregon border below Klamath Lake, up the Sprague to Trout Creek, and up the Williamson River to the outlet of Klamath Marsh. This group the Klamath Lake GCG, isolates the Upper William River and Upper Sprague River GCGs to headwater reaches of these subbasins. Lastly, an impassable waterfall on Lower Jenny Creek isolates the Jenny Creek GCG from the Klamath Lake trout.

9.7.1.4 Metapopulations

The Klamath River Stock Identification Committee (KRSIC) used metapopulations as the scale for identifying groups of breeding populations of salmonids in the Lower Klamath basin (KRSIC, 1996). Metapopulations are population structures that undergo regular extinction and recolonization events (Frankham et al. 2002). The KRSIC identified 12 summer steelhead

breeding populations, which constituted two metapopulations (Klamath River and Trinity River), but because of the lack of information, was unable to accurately assess fall- or winter- run steelhead populations. Similarly, 12 breeding population of fall Chinook contributed to six metapopulations, two of which the entire KRSIC did not reach consensus about. The KRSIC determined there were seven breeding populations of spring Chinook that constituted two breeding populations of the Klamath and Trinity rivers. Finally, coho salmon were categorized to be part of a single metapopulation because of lack of evidence and a history of introductions and intrabasin transfers. This concept is probably better suited for evaluating the demographics, dynamics, and extinction risks associated with independent lineages than elucidating the stock structure of salmonids in the Klamath basin.

9.7.2 Genetic Methods

9.7.2.1 Allozymes

From the late 1960s to the 1990s, most of available data on genetic diversity were collected from proteins. Gel electrophoresis is able to effectively separate different protein variants (allozymes) in a mixture because of the distinct net electrical charges of each allozyme. Proteins from specific loci usually are detected by their unique enzymatic activity coupled with a histochemical stain. Variation at a single Mendelian locus is revealed as bands migrating at different rates in an electrical field across a gel matrix. Different bands are a reflection of DNA variation and correspond to different alleles at a single locus. When analyzed at many loci, often 20 to 30, sufficient variability could characterize discreteness of populations. Unfortunately, this method requires lethal sampling and many organisms of conservation and management importance can not be sampled because of the negative impact from collecting samples. In addition, organisms that suffer a long population decline have lowered genetic diversity, which limits the application of protein electrophoresis.

9.7.2.2 Mitochondrial DNA

A number of molecular methods can be used to evaluate sequence variability in the circular mitochondrial DNA molecule (mtDNA). These methods include cutting with restriction enzymes, which detect restriction fragment length polymorphisms (RFLPs). Each restriction enzyme used in the reaction cuts the mtDNA sequence at a site with a specific known sequence. The resulting fragments are separated with gel electrophoresis and a restriction map created that displays the location of restriction sites. These can be compared among groups and their differentiation determined. Sequencing also can be accomplished with mtDNA, and, using the polymerase chain reaction (PCR), can provide the actual nucleotide sequence of the molecule. Common regions that are sequenced include the control (D-loop), cytochrome b, and 12S rRNA regions. Sequences can be compared between groups. Because an entire mtDNA sequence can only be considered a single marker, its statistical utility is limited. Because mtDNA is maternally inherited, it can be useful for tracing specific female lines of descents and migration patterns.

9.7.2.3 Microsatellite DNA

Microsatellite DNA markers amplify regions of short DNA sequence repeats, typically two to six bases in length. This class of markers has rapidly become popular in the last decade, because of its reliance on non-invasive sampling, high variability, and co-dominant inheritance pattern. Using PCR and electrophoresis, microsatellite DNA alleles are separated on the basis of size of

the amplified fragments. Genotype information from each locus examined is considered to be a statistically independent test of the hypothesis for which the data are being used, provided the microsatellite locus is unlinked (inherited in a manner independent of other loci) to other such loci. Because microsatellite DNA is amplified from regions that are not under selective pressure, they are rapidly evolving molecules and may reveal higher levels of genetic diversity over the same spatial and temporal scales than the other two classes of markers. One disadvantage is that PCR primers must be developed for each genetic locus used. Fortunately, cross-species amplification is sometimes possible, and microsatellite loci developed in other *Oncorhynchus* species are useful in other salmonid species.

9.7.3 Life History Characteristics and Distribution of Klamath River Salmonids

9.7.3.1 Chinook Salmon (*O. tshawytscha*)

Life History Characteristics

There are two freshwater life history types of Chinook salmon: "ocean-type" fish that migrate to the ocean predominantly within their first year (age-0 migrant) and "stream-type" fish that reside in freshwater for a year or more (age-1 migrant) following emergence (Gilbert, 1912). These classifications have been broadened to incorporate life history traits, geographic distribution, and genetic differentiation to provide a frame of reference for comparison of Chinook salmon populations (Healey, 1983, 1991).

Ocean-type Chinook salmon exhibit a more varied and alterable life history than their stream-type counterparts. Ocean-type Chinook juveniles immigrate to the sea within 3 to 12 months as fry, sub-yearling juveniles (during their first spring or fall), or as yearling juveniles (during their second spring), depending on stream conditions. At sea, ocean-type Chinook engage in distinctive coastally oriented migrations. The timing of their return to freshwater and spawning activity is closely related to the ecological characteristics of a population's spawning habitat. Different ocean-type Chinook salmon populations may express five distinct run times: spring, summer, fall, late-fall, and winter. Populations that make use of high water flows during spring to ascend to headwater or interior regions generally are characterized by earlier run times in the spring and summer. Populations that use the lower reaches of main stem rivers and tributaries typically have later run times (fall, late fall). Ocean-type Chinook adults spawn soon after entering freshwater.

Populations with the stream-type life history appear to have an extended juvenile residency time in freshwater (usually more than 1 year), undertake long offshore ocean migrations, and typically return to spawn in freshwater as spring- or summer-run fish. Stream-type Chinook adults migrate upstream before they reach full reproductive maturity. Stream-type populations are found in northern British Columbia and Alaska, and east of the Cascades in the headwater regions of the Fraser River and Columbia River interior tributaries (NMFS, 2003) It is possible that extirpated, native Chinook above Iron Gate dam displayed "stream-type" life history variations because the Upper Klamath basin is east of the Cascade Crest, however, no peer-reviewed or gray literature sources were found to validate this statement.

The Klamath-Trinity River system provides spawning and rearing habitat for fall-, late-fall-, and spring-run ocean-type Chinook salmon. Historically, the spawning period for spring-run Chinook salmon that migrated past the site of Lewiston dam occurred from September through the second

week in October (USDI, 1980; T. Mills, CDFG, personal communication in Bartley et al. 1990). The fall-run enters freshwater in late summer and early fall in sexually mature condition, migrates to the lower reaches of mainstem rivers and their tributaries, and spawns within a few days or weeks upon arriving at the spawning grounds. Historically, the first fall-run Chinook appeared in mid-July and spawned from October through early November (Moyle, 2002). However, in the late 1990s the fall-run peak migration into Iron Gate fish hatchery was occurring 1 to 4 weeks later than historic run timing at the Klamathon Racks, potentially because of the operation of Iron Gate hatchery's fish ladder (Shaw et al. 1997). Fall-run juveniles from the Klamath River apparently move into the estuary in larger numbers in years when river flows are low and temperatures higher than they are when in-river rearing conditions that are more suitable (Wallace and Collins, 1997). Table 9.7-1 illustrates the considerable overlap of both freshwater migration and spawning periods of fall- and spring-run Chinook salmon in the Klamath-Trinity River system.

Table 9.7-1. Freshwater migration (hatched areas) and spawning timing (gray areas) of Chinook salmon from Klamath-Trinity River basin. Runs designations are Sp- spring, F- fall, LF- late fall. The designation "**P**" represents peak spawning. Because of variability in spawning times within a stock, some fish still may be entering freshwater during the spawning time intervals. Adapted from Myers et al. (1998).

		Month												
Stock	**Run**	**March**	**April**	**May**	**June**	**July**	**August**	**Sept.**	**Oct.**	**Nov.**	**Dec.**	**Jan.**	**Feb.**	**References**
Klamath R. Basin	Sp													HVIC 1997
Klamath R. Basin	F													HVIC 1997
Klamath R.	Sp								P					Snyder 1931; USFWS 1994; Tuss et al. 1987
Klamath R.	F									P				Leidy and Leidy 1984; Snyder 1931; USFWS 1994
Klamath R.	LF										P			Snyder 1931
Shasta R.	F								P					CDFG 1995
Trinity R.	Sp													Moffett and Smith 1950; CDFG 1995; Dean 1995
Trinity R.	F									P				USFWS 1994

Distribution

Chinook salmon once ascended the Klamath River into Upper Klamath Lake, Oregon, to spawn in the major tributaries of the lake (Williamson, Sprague, and Wood rivers; Moyle, 2002). Fortune et al. (1966) also suggested the presence of Chinook in Upper Klamath Lake subbasins including the Williamson and Sprague rivers. However, access to this region has been blocked almost continuously for more than 100 years. Around the turn of the 20th century, passage was eliminated upstream of the Klamathon area, first by log crib dams from 1889 to 1902, then by the Bureau of Fisheries installing a fish rack at the same location starting in 1910. In 1917, Copco dam was completed, forming a complete barrier to upstream migration (Fortune et al. 1966). Currently, fall-run Chinook are known to spawn in the mainstem Klamath River and its tributaries including, but not limited to, Bogus Creek, Shasta River, Scott River, Indian Creek, Elk Creek, Clear Creek, Salmon River (including major forks and Wooley Creek), Bluff Creek, Blue Creek (supports only late fall-run fish), and the lower reaches of some of the smaller tributaries to the mainstem. Fall-run Chinook also spawn in the mainstem Trinity River throughout the basin and numerous tributaries including, but not limited to, Willow Creek, Horse Linto Creek, and Hayfork Creek (Moyle, 2002). The Trinity River fish hatchery, North Fork Trinity River, Canyon Creek, New River, and South Fork Trinity River also support populations

of both fall- and spring-run Chinook salmon. Spring-run Chinook also are found in the Salmon River and Wooley Creek watersheds.

9.7.3.2 Coho Salmon *(O. kisutch)*

Life History Characteristics

Coho salmon that spawn in California have a fairly strict 3-year life cycle, with the first 15 to 20 months spent in streams and rivers. The 3-year life cycle combined with a strong homing instinct means that each stream has three temporally distinct and spatially distinct populations. Grilse or jack males, essentially 2-year-olds, and rare precocial females appear to be the primary exceptions to the 3-year rule and keep runs from becoming genetically isolated from one another (Moyle, 2002).

The Klamath River coho spawning migration period occurs between September and late December, peaking in October and November. During the early part of the run, males predominate on the spawning grounds, with females returning in greater numbers during the later part of the run (USFWS, 1979). Spawning activities occur primarily later in the run period (November to January). It should be noted that when coho salmon begin spawning during the first week in November, temporal and spatial overlap of spawning by Chinook with coho salmon is possible, and has been observed (USDI, 1980; T. Mills, CDFG, personal communication in Bartley et al. 1990).

Distribution

According to Moyle (2002), coho salmon once ascended the Klamath River and its tributaries at least as high as Klamath Falls, Oregon, but now are restricted from the uppermost river by Iron Gate dam. Similarly, in the Trinity River they can ascend only to the point of the Lewiston dam. Historical numbers of coho spawning in the Klamath River system have been estimated at 15,400 to 20,000, with 8,000 of these in the Trinity River. Returns to Klamath basin fish hatcheries were only 1,700 in 1990 (A. Baracco, CDFG, personal communication in Moyle, 2002) and 3,100 in 1991 (CDFG, 1992). Coho continue to be produced at both Iron Gate and Lewiston fish hatcheries, and those spawning in the Trinity River are primarily of hatchery origin (CDFG, 2001). Coho salmon are known to occur in tributaries of the Scott River (CDFG, 2002), but data on distribution are limited in other Klamath River subbasins.

9.7.3.3 Coastal Rainbow Trout/Steelhead *(O. mykiss irideus)*

Life History Characteristics—Non-Anadromous Phenotype

O. mykiss irideus in the Klamath-Trinity River basins display one of the most diverse sets of life history patterns found in the *Oncorhynchus* genus. This species encompasses two distinct phenotypes. Typically, the resident form (called a rainbow or redband trout) spends its entire life in freshwater isolated above natural barriers (e.g., waterfalls, landslides, subsurface stream flows). This natural form of *O. mykiss irideus* is apparently uncommon in the Klamath River below Iron Gate dam. However, non-anadromous trout have been observed in the summer below Iron Gate dam and are potentially late releases of hatchery steelhead progeny that residualized (remained non-anadromous because smoltification stopped) within this reach of the river (CDFG, 2001). The cause for residualization of steelhead progeny in the Klamath River is poorly

understood. Possible hypotheses on this phenomenon include accelerated growth rate of fish in hatcheries or excessively high water temperatures downstream delaying outmigrant behavior in these fish (Healey, 1991; Viola and Schuck, 1995). Steelhead also likely have residualized above recent manmade barriers in the basin such as Lewiston, Iron Gate, and Dwinnell dams, although the genetic integrity of these fish is questionable given the stocking on non-native rainbow trout into the waterbodies. These fish may remain migratory and may use tributaries to these reservoirs for spawning.

Life History Characteristics—Anadromous Phenotype

The second phenotype of coastal steelhead is the more common anadromous form. In the Klamath River basin, these fish display a variety of life history patterns constituting different freshwater and saltwater rearing strategies (ODFW, 1995). The differences between these different life history patterns are not well understood, and scientists group anadromous steelhead "races" depending on the timing of adult migration into the Klamath River. The classification of different adult migratory run-timings is not agreed upon (Table 9.7-2). Snyder (1931) found the timing of fall steelhead into the Shasta River to be earlier than other rivers.

Table 9.7-2. Classification of different run-timings and reproductive ecotypes of steelhead found in the Klamath River basin.

Steelhead Race	KRSIC (1993)	Hopelain (1998)	USFWS (1979)	Busby et al. (1996)	Moyle (2002)
Spring/Summer	May- July	March-June	April-June		April- June
Fall	August- October	July-October	August-November		
Winter	November- February	November-March	November-February		November-April
Stream-maturing				April- October	
Ocean-maturing				September-March	

The NOAA Fisheries does not classify Klamath River basin steelhead "races" based on run-timing of adults (phenotype), but instead recognizes two distinct reproductive ecotypes (populations of the same species that are adapted to different ecological conditions) of coastal steelhead in the Klamath River basis based on their reproductive biology and freshwater spawning strategy (Busby et al. 1996; Table 9.7-3). Burgner et al. (1992) identified the stream-maturing type as entering the river sexually immature and still requiring several months before maturing to spawning condition. In the Klamath River, Busby et al. (1996) called these fish summer steelhead and found they migrated upstream between April and October with a peak in spawning behavior during January. The second type, ocean-maturing, enter the Klamath River between September and March with a peak in spawning in March. These fish enter the river sexually mature and spawn shortly after reaching spawning grounds (Busby et al. 1996). The overlap in migration and spawning periods makes differentiating these ecotypes difficult (Roelofs, 1983).

Table 9.7-3. Deviations from random mating genotypic proportions over seven microsatellite loci (F_{IS} and associated p-value), proportion of di-loci pairs showing linkage disequilibrium (LD), and number of individuals (N) from Iron Gate and Trinity River fish hatchery coho salmon populations after pooling for homogeneous samples. The population representing a pooled set of samples is marked with an asterisk (*). Reference numbers are provided as a reference for Figure 9.7-8. Adapted from Hedgecock et al. (2002).

Population	Reference #	N	F_{IS}	p	LD
IGH - large adult, no mark*	1	30	0.061	0.032	1/21
IGH - small males	2	15	0.019	0.362	4/21
IGH - large adult, left maxillary	3	36	0.073	0.028	3/21
TRH - small males	4	17	0.024	0.266	1/21
TRH - Large adults	5	7	0.062	0.004	3/21

Most steelhead (86 percent) in the Klamath River basin spend 2 years in freshwater before undergoing smoltification (the physiological process of preparing to survive in ocean conditions) and migrating to sea (Hopelain, 1998). Kesner and Barnhardt (1972) determined that steelhead rearing in freshwater for longer periods made their seaward migration more quickly. Klamath River basin steelhead remain in the ocean for 1 to 3 years before returning to spawn. Their ocean migration patterns are unknown. It is believed that steelhead use their excellent homing sense to return to spawn in the same area where they lived as fry (Moyle, 2002).

The presence of half-pounder steelhead in the Klamath River basin is a distinguishing life history trait of steelhead found in the Klamath Mountains Province ESU. Half-pounder steelhead are subadult individuals who have spent 2 to 4 months in the Klamath estuary or nearshore marine waters before returning to the river to overwinter. They overwinter in the lower and mid-Klamath regions before returning to the ocean the following spring. The presence of half-pounders is uncommon above Seiad Valley (Kesner and Barnhardt, 1972). The occurrence of half-pounders is greater in spawning fish of mid-Klamath region tributaries (86 to 100 percent) when compared to the Trinity River (32 to 80 percent). There is a negative linear relationship between rates of half-pounder migration and first-time spawning size. The lowest occurrence of half-pounders was from Lower Klamath River winter-run steelhead (17 percent), which also demonstrated the greatest first-year growth rate (Hopelain, 1998). The proportion of half-pounders that become stream- or ocean-maturing ecotypes is not known.

Iteroparity (the ability to spawn more than once) is an important character of steelhead that makes them different from most all other *Oncorhynchus* species. Hopelain (1998) reported that repeat spawning varied between different run-timings. The frequencies of steelhead having undergone multiple reproductive events varied in range from 17.6 to 47.9 percent for fall run, 40.0 to 63.6 percent for spring run, and 31.1 percent for winter run. Females are the majority of repeat spawners (Busby et al. 1996), and lay between 200 and 12,000 eggs (Moyle, 2002). Non-anadromous coastal rainbow trout typically contain fewer than 1,000 eggs, while steelhead contain about 2,000 eggs per kilogram of body weight (Moyle, 2002).

Distribution

Snyder (1931) suggested that steelhead used tributaries above Upper Klamath Lake. A review of county documents, newspaper reports, and biological investigations by Fortune et al. (1966) raised the possibility that these steelhead may have been large adfluvial rainbow or redband

trout. Fortune et al. (1966) identified multiple runs of *O. mykiss* in the Project area before its isolation from the Lower Klamath River basin by manmade barriers (see Section 9.7.3.1). Moyle (2002) suggested that steelhead invaded the upper Klamath basin during the Pleistocene and that non-anadromous coastal rainbow trout are present above Klamath Lake. Currently, coastal rainbow trout and steelhead occupy tributaries open to migration in the Klamath and Trinity basins including, but not limited to, the Salmon River, Scott River, South Fork Trinity River, North Fork Trinity River, Elk Creek, Clear Creek, Indian Creek, Independence Creek, and Blue Creek. Summer steelhead reside in, but are not limited to, the Salmon River, Wooley Creek, Redcap Creek, Elk Creek, Bluff Creek, Dillon Creek, Indian Creek, Clear Creek, South Fork Trinity River, North Fork Trinity River, New River, and Canyon Creek. Above Iron Gate dam, coastal rainbow trout also may inhabit Fall Creek (Currens, 1990) and Spencer Creek. Behnke (1992) compared meristic characteristics to determine that trout in Spencer Creek were most similar to steelhead described in Snyder (1931) from the Lower Klamath River.

9.7.3.4 Upper Klamath Redband Trout (*O. mykiss newberri*)

Life History Characteristics

Redband trout from the Upper Klamath River basin reflect multiple life history patterns that represent the interwoven physical isolation and biological invasion of multiple strains of *O. mykiss* subspecies during the past 15,000 years. These fish exhibit migratory and resident patterns of distribution, and can be thought to express two distinct ecotypes. One ecotype is specialized for lacustrine conditions in Klamath Lake (Behnke, 1992), and displays higher gill raker numbers than riverine redband trout of the Columbia and Sacramento basins. In comparing additional fish from Trout and Whitworth Creek in the Upper Klamath basin, Behnke (1992) classified a second distinct ecotype of stream resident redband trout in Klamath Lake tributaries.

The environment in which these fish persisted likely led to the expression of a diversity of life history strategies. As the prehistoric large alkaline Lake Modoc (currently Klamath Lake) desiccated and expanded, the current day lake/marsh/stream complexes were formed. The redband trout established adfluvial life histories between the highly productive rearing areas in the lakes and marshes and adjacent spawning areas in the streams (ODFW, 1995). The environmental stability of the streams during times of drought led to the founding of refugial populations that may have been the predecessors to resident populations.

Spawning of trout in Upper Klamath River and Upper Klamath Lake tributaries has been recorded during almost all months of the year. On Spring Creek (Williamson River), trout spawned from October to August (Buchanan et al. 1991). Using scale data, these fish were determined to live up to 8 years and spawn up to six times (Buchanan et al. 1990). Rainbow trout from Spencer Creek spawned only between March and May. From mark-recapture data collected on Spencer Creek juvenile fish, no upstream migration over Keno dam was observed, although these fish were observed downstream of J.C. Boyle dam. The ODFW (Buchanan et al. 1990) determined that most of the Spencer Creek adult trout spent the majority of their life in the Keno reach.

Distribution

Before construction of Copco dam in 1917, steelhead migrated up to the falls at the outlet of Klamath Lake (Snyder, 1931; Fortune et al. 1966). Behnke (1992) suggested that *O. mykiss*

irideus did not reside above this location and designated the migratory Upper Klamath trout as a separate subspecies, *O. mykiss newberrii*. It is likely that redband trout moved downstream of the outlet falls. Redband trout occupy suitable habitat throughout tributaries in subbasins draining into Upper Klamath Lake including the Williamson, Sprague, and Sycan rivers. Isolated trout in Jenny Creek, above a waterfall, and in the upper Williamson and upper Sprague rivers have meristic characteristics and biochemical characters that suggest a common origin, but are quite distinctive from all other trout (Behnke, 1992).

9.7.4 Review of Hatchery-Related Genetic Information on Klamath Salmonids

9.7.4.1 Coho Salmon Broodstock Practices

Three significant genetic concerns remain for hatchery populations of coho salmon in the Klamath basins. First, the potential for domestication selection in hatchery populations, such as the Trinity River fish hatchery where there is no or little infusion of wild genes. Also, concern exists about out-of-basin straying by large numbers of hatchery coho. However, hatchery production of coho salmon at the Mad River and Rowdy Creek facilities was ceased after the 1999 brood year, thereby eliminating potential genetic introgression associated with hatchery releases from these facilities (NOAA Fisheries, 2003). The NOAA Fisheries is concerned about the origins of the current coho stocks in the Klamath River. The NOAA Fisheries (NOAA Fisheries, 2003) stated that there is evidence that several of the large river basins in the SONCC-ESU, including the Rogue, Klamath, and Trinity rivers, are heavily influenced by hatchery releases of coho salmon. Historical and reciprocal transfers of stocks between SONCC and the Central California ESU streams were common (see below), and SONCC streams also have received inter-basin plants from hatchery stocks in the Lower Columbia River/Southwest Washington, Puget Sound/Strait of Georgia, and Oregon Coast ESUs. Two of the four hatcheries still producing and releasing coho salmon in the SONCC ESU are Iron Gate fish hatchery and Trinity River fish hatchery.

Iron Gate fish hatchery was founded in 1965 from Klamath River coho salmon returning to the hatchery. Cascade Hatchery (Columbia River) coho salmon stock were released in 4 of the first 5 years of the hatchery's operation via Trinity River fish hatchery and Mt. Shasta fish hatchery. In 1977, two-thirds of the coho released from Iron Gate fish hatchery were planted from the Trinity River (Rushton, pers. comm., 2003). Since 1977, only Klamath River basin fish have been released from Iron Gate fish hatchery. Adult returns averaged 1,120 fish between 1991 and 2000, and an average of 161 females have been spawned at Iron Gate fish hatchery annually during the same period (NOAA Fisheries, 2003).

Accurate estimates of the relative contributions of wild and hatchery-produced fish are not available for the Klamath River (CDFG, 2001). Beginning in 1995, Iron Gate fish hatchery coho salmon have been marked with left maxillary clips. Data on hatchery returns, which have only been published for 2000, indicate that 80 percent of the 1,353 adults returning to Iron Gate fish hatchery were marked as hatchery fish. Of those fish, 98 percent were Iron Gate fish hatchery releases and the remaining were from the Trinity and Cole River (Rogue River, Oregon) fish hatcheries. Fish from the Trinity River fish hatchery have right maxillary clips, while those from the Cole Hatchery have had their adipose fin clipped. Cole River fish have been verified by recovered pit tags (Iron Gate fish hatchery staff, personal communication in Hedgecock et al. 2002). Since Iron Gate fish hatchery is located near the upper end of accessible habitat, the

significance of the high percentage of hatchery fish with respect to the total production in the Klamath basin is uncertain (NOAA Fisheries, 2003).

Trinity River fish hatchery began releasing coho salmon in 1960. Originally, Trinity River fish were used as broodstock, although coho salmon from the Eel River (1965), Cascade River (1966-7, 1969), Alsea River (1970), and Noyo River (1970) have been reared and released at the hatchery as well as elsewhere in the Trinity basin. Outmigrant trapping on the lower Trinity River indicates that marked Trinity River fish hatchery fish comprised 65 to 97 percent of the catch between 1998 and 2000. In addition, an estimated 85 to 95 percent of the estimated coho salmon run upstream of Willow Creek weir between 1997 and 2001 were of Trinity River fish hatchery origin (Wade Sinnen, personal communication cited in CDFG, 2002). Additional analysis of carcass data suggests that straying of Trinity River fish hatchery coho is high and a large percentage of in-river spawners are of Trinity River fish hatchery origin.

9.7.4.2 Hybridization of Chinook and Coho Salmon at Trinity River Hatchery

Bartley et al. (1990) identified Chinook and coho salmon hybrids from Deadwood Creek, a tributary to the Trinity River, and from rearing ponds at Camp Creek, a tributary to the Lower Klamath River. Forty alleged juvenile out-migrant (smolt) coho salmon were collected in August 1984 from Deadwood Creek. A sub-sample of 120 putative juvenile Chinook salmon was collected from rearing ponds at Camp Creek. Juveniles from the rearing ponds were the progeny of presumable Chinook salmon that had been captured at Camp Creek. The spawning location of the broodstock of these fish is unclear. Bartley et al. (1990) reported fish were spawned at Iron Gate hatchery and transported back to Camp Creek to be reared. Hatchery personnel reported that all Camp Creek broodstock were spawned at Camp Creek from 1986 to 1990 (Rushton, pers. comm., (2003). Previous analysis of populations of Chinook and coho salmon in California (Bartley, 1987; Bartley and Gall, 1990) revealed seven allozyme loci whose allelic products could be used to distinguish between the two species. Electrophoretic analysis of 21 allozymes was performed and the likelihood of hybridization for individuals expressing alleles of both parental species was evaluated using the hybrid index described by Campton and Utter (1985).

Genotypes and hybrid index scores of the 40 juvenile salmon from Deadwood Creek suggested that 11 were Chinook salmon, 26 were coho salmon, and three were presumptive Chinook-coho hybrids. The hybrids were heterozygous for the common alleles of both Chinook and coho salmon at five out of seven distinguishing allozyme loci. Fourteen of 120 fish from the Camp Creek rearing ponds were heterozygous for the common alleles of both species at all seven distinguishing loci. Observed hybrids were considered to be first generation because all of the distinguishing loci, with the exception of one locus in a single individual, were heterozygous, containing one allele from Chinook salmon and the other allele from coho salmon.

While a quantitative estimate of the level of Chinook-coho hybridization in California was not possible because of the non-random sampling of fish within populations, the Deadwood Creek and Camp Creek samples represented the only occurrences of putative hybridization revealed in an analysis of 36 Chinook populations and 27 coho populations from northern California (Bartley, 1987; Bartley and Gall, 1990). Moreover, no evidence of Chinook-coho hybridization was reported in a study of 86 populations of Chinook between the Babine River, British Columbia, and the Sacramento River, California (Utter et al. 1989).

Bartley et al. (1990) suggest that the 14 hybrids from the Camp Creek rearing ponds were the result of hatchery personnel inadvertently crossing pure Chinook and coho salmon during spawning operations and hybrid offspring were placed with pure Chinook in the rearing ponds. Hatcheries designed to offset losses of salmon spawning habitat through artificial spawning could contribute to the erosion of the genetic resource of Chinook and coho salmon through direct or indirect production of hybrid salmon. All fish, including alleged hybrid individuals, held at the Camp Creek rearing ponds were released into the Klamath River system under the presumption they were pure Chinook salmon. If hybrid Chinook-coho salmon return to spawn, genetic resources within naturally spawning populations of both species may erode in the Klamath River system.

Disruption of the natural spawning environment and crowded spawning sites may result in hybridization of fish (Hubbs, 1955). When interspecific mating occurs between coho and Chinook salmon, gametes (genetic information passed on to the next generation in one particular species or the other) and/or spawning sites are no longer available for other population members for each species (Utter, 1989). Since mating between coho and Chinook produce offspring that are sterile (Johnson, 1988), the genes those parents carried (that component of genetic diversity for the population as well as the species as a whole) are no longer perpetuated in generations subsequent to the sterile offspring produced. In addition, as the rate of hybridization between the two species increases, there will be fewer individuals in the population who will pass their genetic information further than one generation. Interspecific hybridization, when the hybrid offspring are sterile, lowers the numbers of successfully reproducing fish (individuals that produce offspring that return to reproduce successfully themselves) in the population. As the number of successfully reproducing fish relative to all breeding members (not necessarily reproductively successful) of the population decreases, the proportion of the entire gamut of genetic diversity harbored by that population and that is passed onto the next generation also decreases. This phenomenon is called genetic drift and may contribute to erosion of genetic resources of small, fluctuating populations like Pacific salmonids.

Since the construction of Lewiston dam, Deadwood Creek and the tailwaters of the dam comprise the last available spawning habitat for fall- and spring-run Chinook and fall-run coho salmon in the Trinity River (Beddell and West, personal communication in Bartley et al. 1990). This situation may have forced the two salmon species to interbreed at the base of the dam or to return downstream to spawn in Deadwood Creek (Bartley et al. 1990).

9.7.4.3 Chinook Salmon Broodstock Practices

To mitigate the loss of spawning habitat caused by the installation of the Copco dam in 1917, a CDFG hatchery was constructed on Fall Creek approximately 4 river kilometers below the dam. The hatchery was supplied with eggs procured from the Klamathon egg collection site (Shebley, 1922). The Fall Creek fish hatchery was closed in 1948, and although egg collections continued, no artificial rearing facilities were available on the Klamath River until the beginning of spawning operations at the Iron Gate fish hatchery in 1966 (KRBFTF, 1991).

Attempts to maintain a spring-run of Chinook salmon at the Iron Gate fish hatchery were intermittent and eventually abandoned (Myers et al. 1998). Eggs from fall-run fish for Iron Gate fish hatchery have been collected from adult spawners returning to the hatchery, and occasionally from spawners collected from Bogus Creek. Runs of both fall- and spring-run

Chinook have been maintained successfully at the Trinity River fish hatchery. As with the Iron Gate fish hatchery, the Trinity River fish hatchery has relied on returning fall- and spring-run adults for egg collection. Since the mid-1960s, approximately 7.3 and 2.6 million fall-run Chinook salmon juveniles have been released into the Upper Klamath River and Trinity River per year, respectively (Myers et al. 1998).

9.7.4.4 Coastal Rainbow Trout/Steelhead Broodstock Practices

Iron Gate hatchery produces about 200,000 winter smolts per year, while the Trinity River hatchery produces about 800,000 smolts per year. Busby et al. (1994) reported that before 1973, both hatcheries received transfers of steelhead stocks from the Cowlitz and Washougal fish hatcheries, and fish from the Sacramento, Willamette, Mad, and Eel river basins. According to the CDFG (Rushton, pers. comm., 2003), Iron Gate fish hatchery received Trinity River fish hatchery steelhead eggs only in 1966, and received Cowlitz fish hatchery eggs in 1969. Busby et al. (1996) suggested most natural spawning populations experienced an infusion of naturally spawning hatchery fish each year and were unable to identify any population that was naturally self-sustaining. In addition, during the past 10 years, steelhead returns to Iron Gate fish hatchery have been low. Hatchery staff members observed that returning fish often resemble trout in size and coloration. Recent recoveries of tagged fish suggest that hatchery steelhead stocks may be residualizing, and a high correlation was observed between number of hatchery fish caught by the CDFG and the proximity to Iron Gate fish hatchery (Lamson, 2002).

The CDFG and NOAA Fisheries (CDFG, 2001) recommended the development of Hatchery Genetic Management Plans for all California fish hatcheries, which would include Iron Gate and Trinity River fish hatcheries. One option presented by the CDFG and the NOAA Fisheries regarding the dwindling Iron Gate fish hatchery steelhead returns was to identify an appropriate, naturally spawning population of steelhead from which broodstock could be taken to reinitiate the Iron Gate fish hatchery steelhead program. The Hatchery Genetic Management Plans should be developed to include practices that preserve genetic resources and increase survival through critical life history stages. They can provide the framework for monitoring and providing fish for reintroduction where native fish have been extirpated (CDFG, 2001).

Rainbow trout populations in Fall Creek, Shoat Springs, and Jenny Creek populations may have been genetically influenced by hatchery rainbow trout planted in these streams from the Spencer Creek fish hatchery in the late 1800s and early 1900s (Currens, pers. comm., 2003). Currens (1990) showed significant frequencies of allozyme alleles common in domesticated hatchery strains, yet uncommon throughout other parts of the Klamath basin. Since early-winter spawning (a characteristic of many hatchery strains that is extremely rare in wild populations) has been documented in Jenny Creek (ODFW, unpublished data in Currens [1990]), it is possible that hatchery fish interbreeding with wild fish may have influenced run-timing of these trout.

9.7.5 Review of Disease Related Genetic Information for Klamath River Salmonids

9.7.5.1 Coho Salmon

In coho salmon, several studies (Hjort and Schreck, 1982; Olin, 1984; Solazzi, 1986; Bartley et al. 1992) have used data for the highly variable transferrin locus. Transferrins are iron-binding proteins found in plasma and interstitial spaces. They transport iron from sites of absorption to sites of utilization in the body playing an important role in iron metabolism and resistance to

bacterial infection in a variety of organisms (Ford et al. 1999). The high binding affinity of transferrin for iron keeps free iron at low concentrations (Loehr, 1989). Because iron is often a limiting nutrient for bacterial growth (Guerinot, 1994; Gray-Owen and Schryvers, 1996), transferrin plays an immunological role by scavenging free iron that otherwise would be used by bacteria for growth inside an infected organism. Differential resistance to bacterial kidney disease (BKD) among transferrin genotypes has been reported (Suzumoto et al. 1977; Winter et al. 1980). Data for the transferrin locus are difficult to interpret in terms of population structure because polymorphism at this locus may be retained through a selective mechanism (Ford et al. 1999) and may reflect adaptive properties of various genotypes rather than the degree of genetic similarity between populations (Weitkamp et al. 1995).

Bartley et al. (1992) showed significant variation in the frequency of transferrin alleles exists between samples from California and Oregon (Figure 9.7-1). Combining data with that from Olin (1984), revealed a north-south cline in the frequency of transferrin alleles. Bartley et al. (1992) pointed out that the fact that the cline exists in the presence of the homogenizing effects of stock transfers may indicate a selective advantage for certain transferrin genotypes in California.

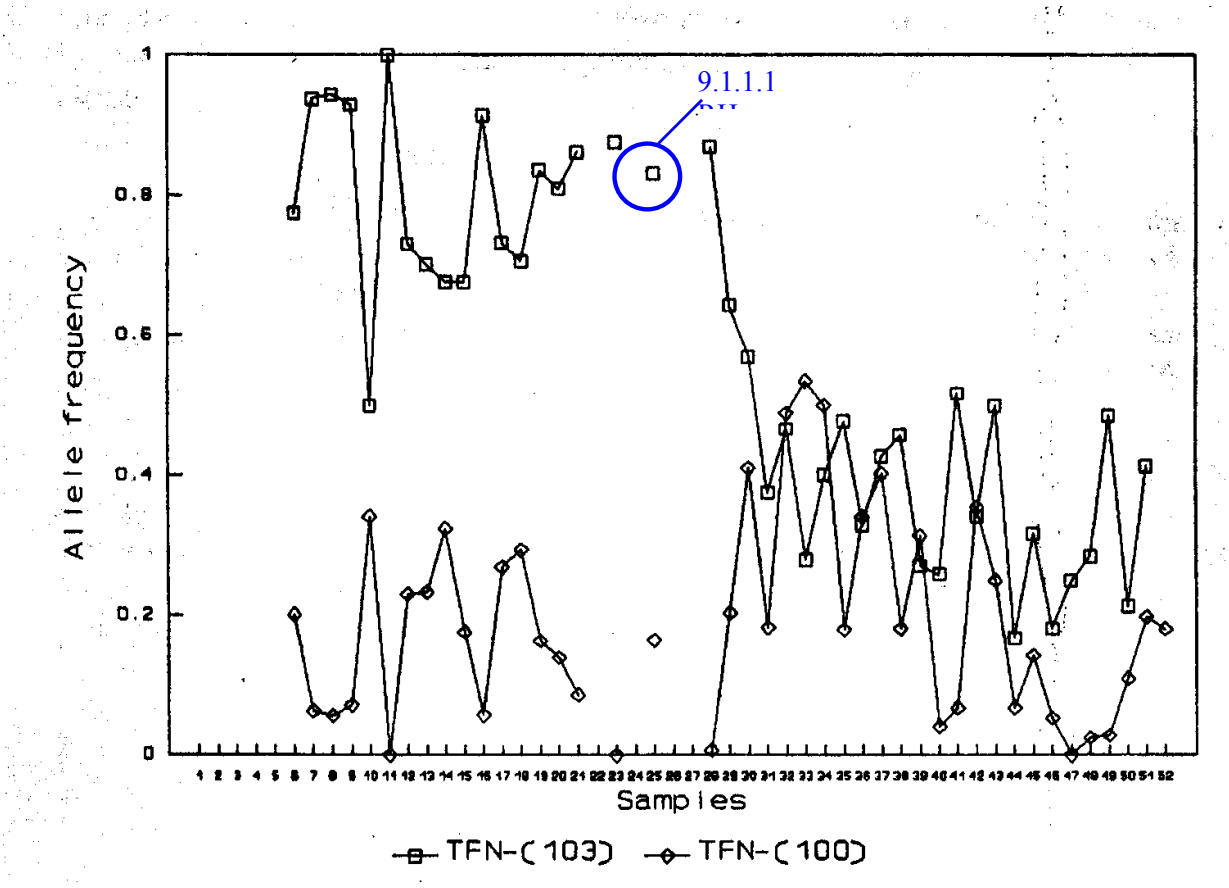

Figure 9.7-1. Clinal variation in transferring (TFN) allele frequencies, alleles 103 (squares) and 100 (diamonds) in 27 samples of California coho salmon evaluated by Bartley et al. (1992) and 25 samples of Oregon coho salmon evaluated by Olin (1984). Sample numbers are arranged from south to north, with number 1 the most southern sample and 52 the most northern sample. Trinity River fish hatchery (TRH) sample is circled. Gaps in line drawing indicate no data. Adapted from Bartley et al. (1992).

Winter et al. (1980) exposed different stocks of juvenile coho salmon from hatcheries in Oregon to either BKD or vibriosis causative bacteria under identical or similar environmental conditions. The first goal of the disease challenge tests were to determine if there were differences in resistance to BKD and vibriosis among coho stocks and whether these differences have a genetic basis. The second goal of the study was to determine if there was differential resistance among the transferrin genotypes to BKD or vibriosis. Fish from each strain were genotyped for transferrin. Winter et al. (1980) noted that the differential resistance of coho salmon stocks to BKD probably has a genetic component because the stocks were reared in similar environments. In addition, juveniles with an AA transferrin genotype (62 percent mortality) were significantly (p less than 0.01) more susceptible to BKD than those fish with either the AC (28 percent mortality) or CC (24 percent mortality) genotypes. Genotypes were interpreted according to Utter et al. (1970). Not only was there no differential resistance shown by transferrin genotypes in the vibriosis challenge, there were conflicting results regarding differential resistance of the two coho stocks so tested. Winter et al. (1980) state that the conflicting results demonstrate that the environment has a strong effect in determining susceptibility to vibriosis.

Winter et al. (1980) also discuss why it is possible that stocks with different transferrin genotypes may be resistant to one disease and not to another. There are several possible scenarios under which this may occur. First, stock resistance to acute diseases, such as vibriosis, depends more on which stock has an environmental advantage at the time of infection, rather than genetic make-up. Second, differences among transferrin genotypes may be more significant in chronic diseases such as BKD. Third, it is possible that *V. anguillarum* is more efficient at removing iron from transferrin than the organism that causes BKD. More efficient removal would explain to some extent the lack of differential resistance to vibriosis among transferrin genotypes within coho salmon. Fourth, the association of BKD resistance with transferrin genotype may be the result of a gene linkage, in which case transferrin is only a marker.

Hjort and Schreck (1982) evaluated transferrin gene frequencies of hatchery coho salmon from Washington to California in 1976-77. Samples from both the Iron Gate and Trinity River fish hatcheries have relatively high frequencies (more than 92 percent) of the A allele. Hatcheries evaluated from the Columbia River basin also had relatively high frequencies of the A allele (Figure 9.7-2). It is interesting to note that inter-basin releases of Cascade (Columbia River) stock were made in 4 of the first 5 years of the Iron Gate fish hatchery's operation. Perhaps the high frequency of the A allele in hatchery stocks in the Klamath-Trinity basin is the result of a founder event followed by accelerated genetic drift in a small population when stock was transferred from the Columbia River basin.

9.7.5.2 Chinook Salmon

Infectious hematopoietic necrosis virus (IHNV) and BKD caused by *Renibacterium salmoninarum* are pathogens that have been detected in juvenile and returning adult spring-run Chinook from the Trinity River fish hatchery (PFMC, 1994). The success of the hatchery programs in the Klamath River basin may have been significantly limited because of the presence of these pathogens. The loss of 20 percent of the spring-run Chinook juveniles reared at the Trinity River fish hatchery has been associated with IHNV infection (PFMC, 1994).

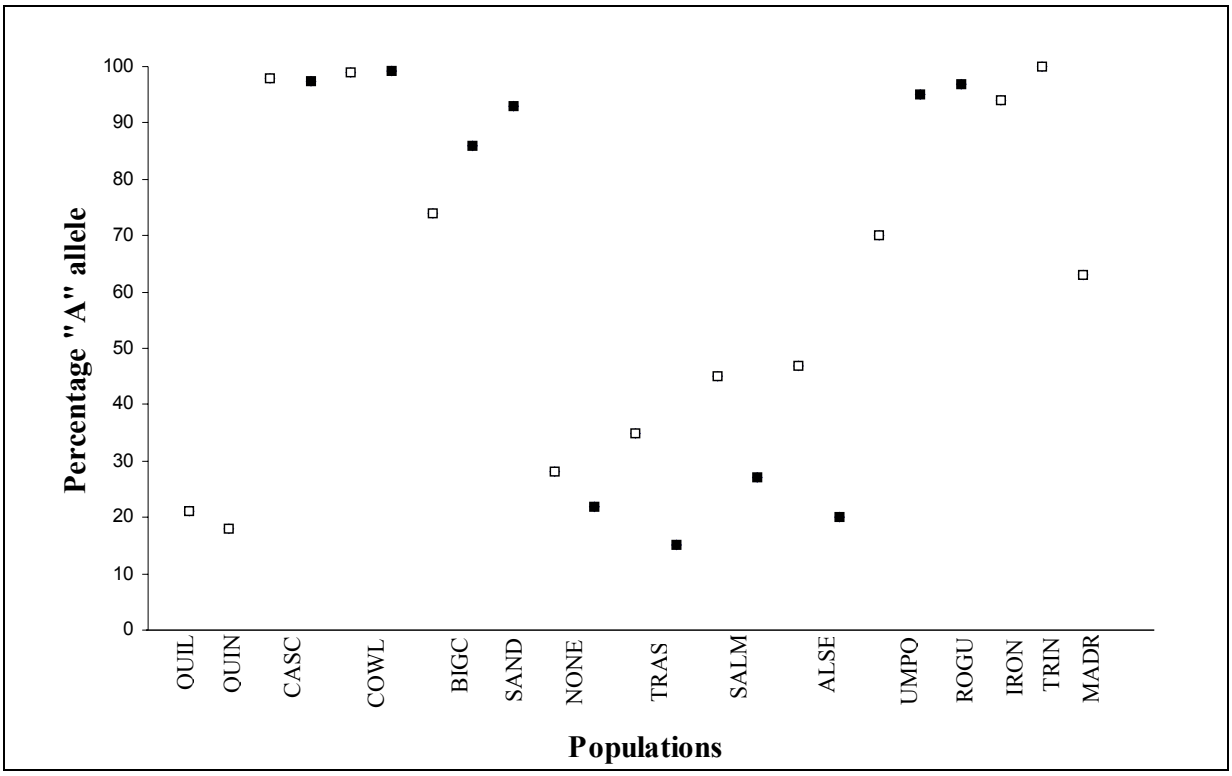

Figure 9.7-2. Transferring gene frequencies of hatchery coho salmon in 1976 (open) and 1977 (closed circles). Samples are arranged from north to south. Vertical lines represent 95 percent confidence intervals; numbers above the line show sample sizes. Location codes are as follows: Quilocene River (QUIL), Quinalt River (QUIN), Cascade (CASC), Cowlitz (COWL), Big Creek (BIGC), Sandy River (SAND), North Nehalem River (NONE), Trask River (TRAS), Salmon River (SALM), Alsea River (ALSE), Umpqua River (UMPQ), Rogue River (ROGU), Iron Gate (IRON), Trinity River (TRIN), Mad River (MADR). Adapted from Hjort and Schreck (1982).

9.7.5.3 Klamath River Salmonids and *Ceratomyxa shasta*

Several studies have been completed on *Ceratomyxa shasta* that demonstrated resistance to this myxosporean parasite is heritable (Hemmingsen et al. 1986; Wade, 1987). Infection by this parasite and the resulting ceratomyxosis can cause increased mortality in a number of salmonid species including Chinook salmon (Zinn et al. 1977), coho salmon (Hemmingsen et al. 1986), and summer steelhead (Buchanan et al. 1983). Limited information is known about *C. shasta* and susceptibility of Chinook and coho salmon in the Klamath River (see Section 8.0 of Fish Resources FTR).

Hemmingsen et al. (1988) determined that the upper limit of *C. shasta* in the Upper Klamath basin extended to Agency Lake and the lower Williamson River. Buchanan et al. (1990) exposed native trout from different Upper Klamath River and Upper Klamath Lake watersheds to *C. Shasta* to determine their resistance to the parasite. Experiments found some Klamath basin trout populations above Iron Gate dam did not die when exposed to the parasite and were resistant to *ceratomyxis*, the disease caused by *C. Shasta*. These population included Spencer Creek in the Project area and Spring Creek, a tributary of the Lower Williamson River. Other populations that died from exposure to the parasite included Jenny Creek and its tributaries; Cold and Johnson creeks; and Deming Creek, a tributary of the Upper Sprague River. Interestingly, native trout from Trout Creek, a Sprague River tributary downstream of Deming Creek, displayed an

intermediate level of resistance to infection by *C. shasta,* suggesting Trout Creek fish may have been progeny of matings between a susceptible, resident population and a resistant, migratory population (Buchanan et al. 1990; Hemmingsen et al. 1986). Because *C. shasta* susceptibility is a heritable trait, the distribution of this characteristic may be valuable for evaluating the reproductive isolation of native trout populations on different rivers and creeks.

Behnke (1992) suggested that the original genetic identity of Upper Klamath Lake redband remains intact because of the difficult environmental and biological conditions fish must survive. The ODFW study (Buchanan et al. 1990), described above, supports Behnke assertion about disease-resistance having a role in structuring the Upper Klamath River and Upper Klamath Lake *O. mykiss* complex. One possibility is that *C. shasta* colonized the Upper Klamath basin via resistant, coastal steelhead mating with and displacing susceptible, interior redband trout (Currens, pers. comm., 2003). However, it appears from *C. shasta's* distribution in the Upper Klamath River that it was not able to invade into all waters, and susceptible, native *O. mykiss newberri* may persist in these isolated reaches. It is unknown whether the migratory, resistant trout carrying *C. shasta* were isolated from the disease-susceptible waters by physical barriers or biological incompatibilities with the redband trout. The existence of a stock with intermediate resistant on Trout Creek, a tributary of the Sprague River (Buchanan et al. 1990), suggests that regions of overlap may exist between reaches with different disease-tolerance, which also may delineate divergent lineages of *O. mykiss*.

9.7.6 Review of Population Structure of Klamath River Basin Salmonids

9.7.6.1 Chinook Salmon

Utter et al. (1989) examined population genetic structure of 86 populations of Chinook salmon from British Columbia, Canada, to the Central Valley of California. A principal component analysis (PCA) of allelic frequencies and a unweighted pair-group arithmetic mean (UPGMA) cluster analysis of Nei's (1972) genetic distances between samples, based on 25 allozyme loci, indicated the existence of nine geographically distinct regional groups of populations. Samples from the Klamath River system included fall- and spring-run samples of fish from the Trinity River fish hatchery and fall-run fish from the Iron Gate fish hatchery. Klamath River populations were genetically similar to groups of Snake River populations (McCall Creek and Johnson Creek summer-runs, and Rapid River and Valley Creek spring-runs) from which they were geographically isolated. Both Klamath and Snake river populations were characterized by low average heterozygosities. These contrasted with the most proximal coastal populations for which the highest heterozygosities among all populations observed. Allele frequency data collected subsequent to the analyses conducted for the Utter et al. (1989) study indicated that fall-run fish from the Shasta and Scott rivers populations, two wild populations of the Klamath River, were statistically identical with allele frequencies of fish from the Iron Gate fish hatchery.

Chinook salmon native to the Klamath River and Snake River are well differentiated from nearby populations at several allozyme loci (Utter et al. 1989; Bartley and Gall, 1990; Waples et al. 1991). However, Utter et al. (1989) failed to distinguish four spring-run and two summer-run Snake River populations from two fall- and one spring-run Klamath River populations. This result was quite surprising given the substantial life history differences between Chinook salmon from the two rivers, the mouths of the Klamath and Snake rivers are separated by nearly 600

ocean miles, and that several ancestrally distinct populations are found in the areas between these rivers.

Utter et al. (1992) investigated the puzzling apparent genetic similarity between the two geographically separated groups of Chinook salmon using an additional 15 allozyme loci, data from Bartley et al. (1992) for Klamath River populations, data from Waples et al. (1991) for Snake River populations, as well as samples from another ten and 11 areas from the Klamath River and Snake River drainages, respectively. A map of the samples used in the Utter et al. (1992) study is presented in Figure 9.7-3. Genetically distinct groups of populations from the two regions were clearly identified using data from 30 allozyme loci. The data summarized in a UPGMA phenogram resulting from clustering of pairwise genetic distances shows a clear separation between population groups from the Klamath River and Snake River drainages (Figure 9.7-4). The mean genetic distance between populations from each river (0.014) was double that of the maximum within-river genetic distance for Klamath River populations. The data summarized by the UPGMA phenogram show that the topologies of clustering within Klamath River and Snake River groups and the relative genetic distances between the river drainage systems are similar to those distinguishing Klamath River populations from other genetically distinct populations of the California and Oregon coast based on a similar set of allozyme loci (Bartley et al. 1992).

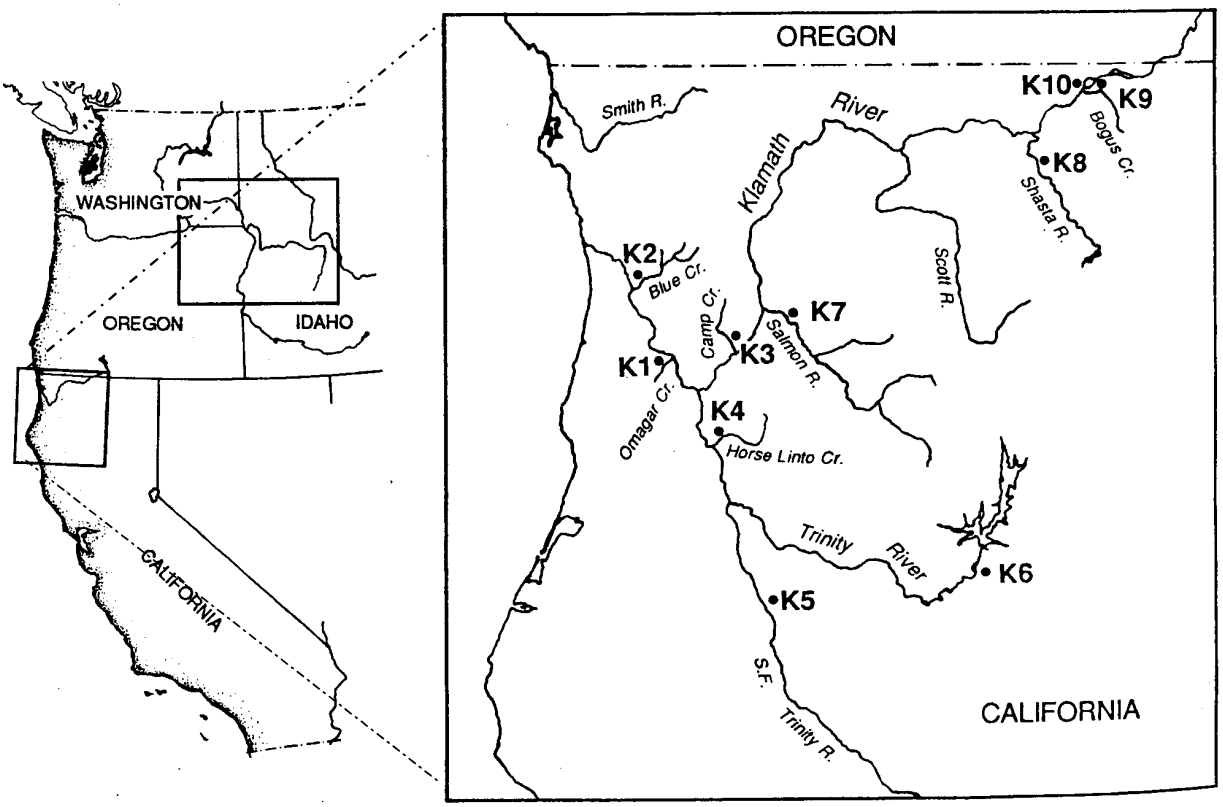

Figure 9.7-3. Sampling locations of Chinook salmon in the Klamath River-Trinity River basin. Adapted from Utter et al. (1992).

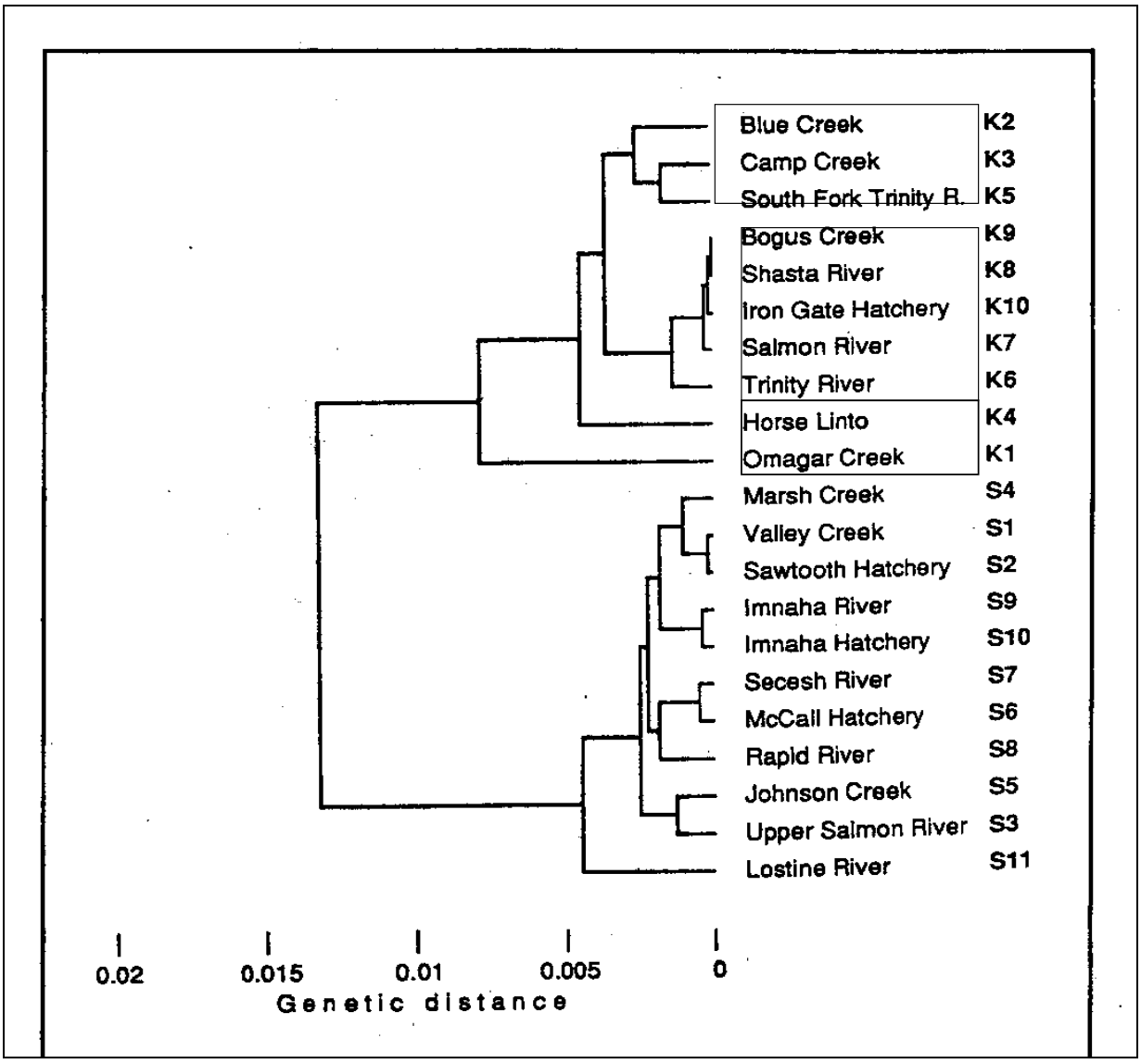

Figure 9.7-4. UPGMA phenogram of Nei's genetic distances using 30 allozyme loci between fall-run Klamath River and spring-run Snake River populations of Chinook salmon. Boxed samples are those from the Klamath-Trinity River system (Utter et al. 1992).

Banks et al. (2000) used seven microsatellite loci to investigate genetic relationships among three spring-run and 11 fall-run Chinook salmon populations from the Klamath River drainage. Samples of fall-run fish were collected between 1992 and 1999 included fish from Blue Creek, Horse Linto Creek, McGarvey Creek, Salmon River, Scott River, Shasta River, South Fork Trinity River, Trinity River, Pine Creek, New River, and the Iron Gate fish hatchery. Samples of spring-run fish were collected during 1997 from the Salmon, Trinity, and South Fork Trinity Rivers. Microsatellite alleles were amplified via PCR assays as described in Banks et al. (1999). Multilocus genotype data then were used to construct UPGMA trees (Sneath and Sokal, 1973) using the Cavalli-Sforza and Edwards (1967) cord distance measure of genetic divergence. This measure ranges from 0.0 (identity) to 1.0 (complete dissimilarity). The clustering of the UPGMA phenogram (Figure 9.7-5) demonstrates that Chinook salmon of the Klamath-Trinity rivers drainage system harbor substantial genetic structure between subpopulations. Indeed, the genetic distance between some fall populations was greater than that between some fall and spring

populations from the same tributary. The strong correlation between geographic origin and genetic relationship in coupling with no strong clustering within either fall- or spring-run life history types indicates that geography appears to play a larger role than life history in determining population genetic structure in the Klamath system. The results of the study by Banks et al. (2000) corroborated preliminary study results from Banks et al. (1999). The fall-run populations farthest upstream on the Klamath River (Iron Gate fish hatchery, Shasta River, and Scott River) were genetically differentiated from subclusters of fall-and spring-run populations from the Trinity River system and the Salmon River. The fall-run Chinook from Blue Creek had the greatest genetic divergence from all other Klamath-Trinity populations and was found to be more genetically similar to southern Oregon and California coastal Chinook populations (NOAA Fisheries, FR 64, No. 179).

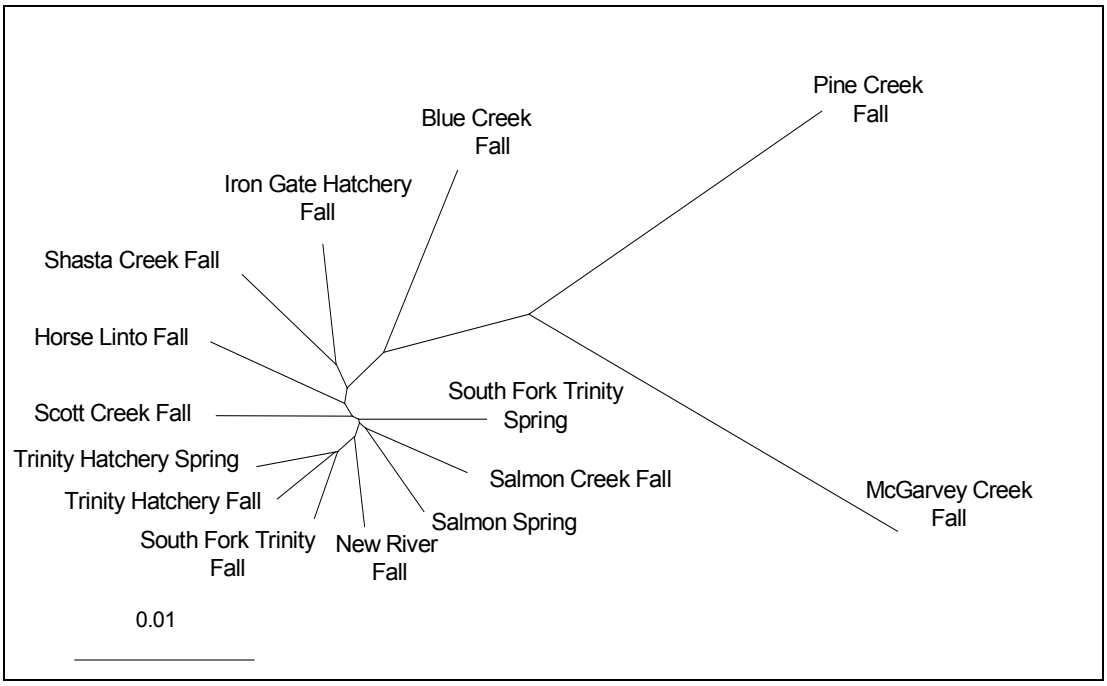

Figure 9.7-5. UPGMA phenogram of Cavalli-Sforza and Edwards (1967) cord distance for population samples from fall and spring Chinook of the Klamath and Trinity basins characterized at seven microsatellite loci (Banks et al. 2000).

The UPGMA phenograms from both Utter et al. (1992) and Banks et al. (2000) demonstrate similar patterns of genetic differentiation between fall-run Chinook in the Klamath-Trinity system. First, both indicate substantial genetic structure between subpopulations. Second, genetic distances were reflected by the geographic distribution of populations.

9.7.6.2 Coho Salmon

Multiple population genetic studies have found evidence for population structure in coho salmon using allozymes, transferrin, or DNA characters. However, these studies were limited to specific geographical regions and did not examine geographically broad patterns of genetic relationships. In addition to having few molecular markers to analyze, small sample sizes, as well as use of a molecular marker possibly under selection may have limited the conclusions that could be drawn from these studies (Weitkamp et al. 1995).

Before 1988, published allozyme studies used less than half of the ten most polymorphic loci that had been identified for coho salmon (Milner, 1993). These previous studies generally reported a lack of genetic variation and relatively low levels of population subdivision. A characteristic of allozyme loci is that they are relatively less polymorphic than other classes of molecular genetic markers. This makes them less useful for revealing fine scale population genetic structure in species such as Pacific salmonids. Given the fact that the allozyme loci used in the previous studies had lower levels of polymorphism compared to subsequently developed allozyme markers (Milner, 1993), it is not surprising that only low levels of population subdivision were revealed. Many of these studies were limited by small sample sizes. The use of small sample sizes may be a particular problem for coho salmon because of the large number of loci, which are variable at low levels (Reisenbichler and Phelps, 1987; Bartley et al. 1992). In such a case, without large sample sizes one would not have an adequate representation of allele frequencies for populations, thereby limiting the potential resolution to discern genetic differences between those populations.

Bartley et al. (1992) used 22 variable allozyme loci and the transferrin locus to investigate population genetic structure of 27 coho salmon populations from California. They reported low levels of variability and little evidence of geographic pattern to the observed genetic variability. However, Bartley et al. (1992) found significant allele frequency differences among all samples as well as within six regional groupings. Both Bartley et al. (1992) and Weitkamp et al. (1995) pointed out that the genetic analyses could be greatly improved by increasing the samples sizes, which averaged only 34 fish per sample.

The NOAA Fisheries examined the genetic relationships of California and southern Oregon coho salmon populations by combining data from the five most southern samples from its Genetic Stock Identification (GSI) studies (Milner, 1993) and two previous ESA status reviews (Johnson et al. 1991; Weitkamp et al. 1995) with the Iron Gate fish hatchery sample from Olin (1984) and 20 coho salmon samples from Bartley et al. (1992). The geographic coverage of samples in this analysis is shown in Figure 9.7-6.

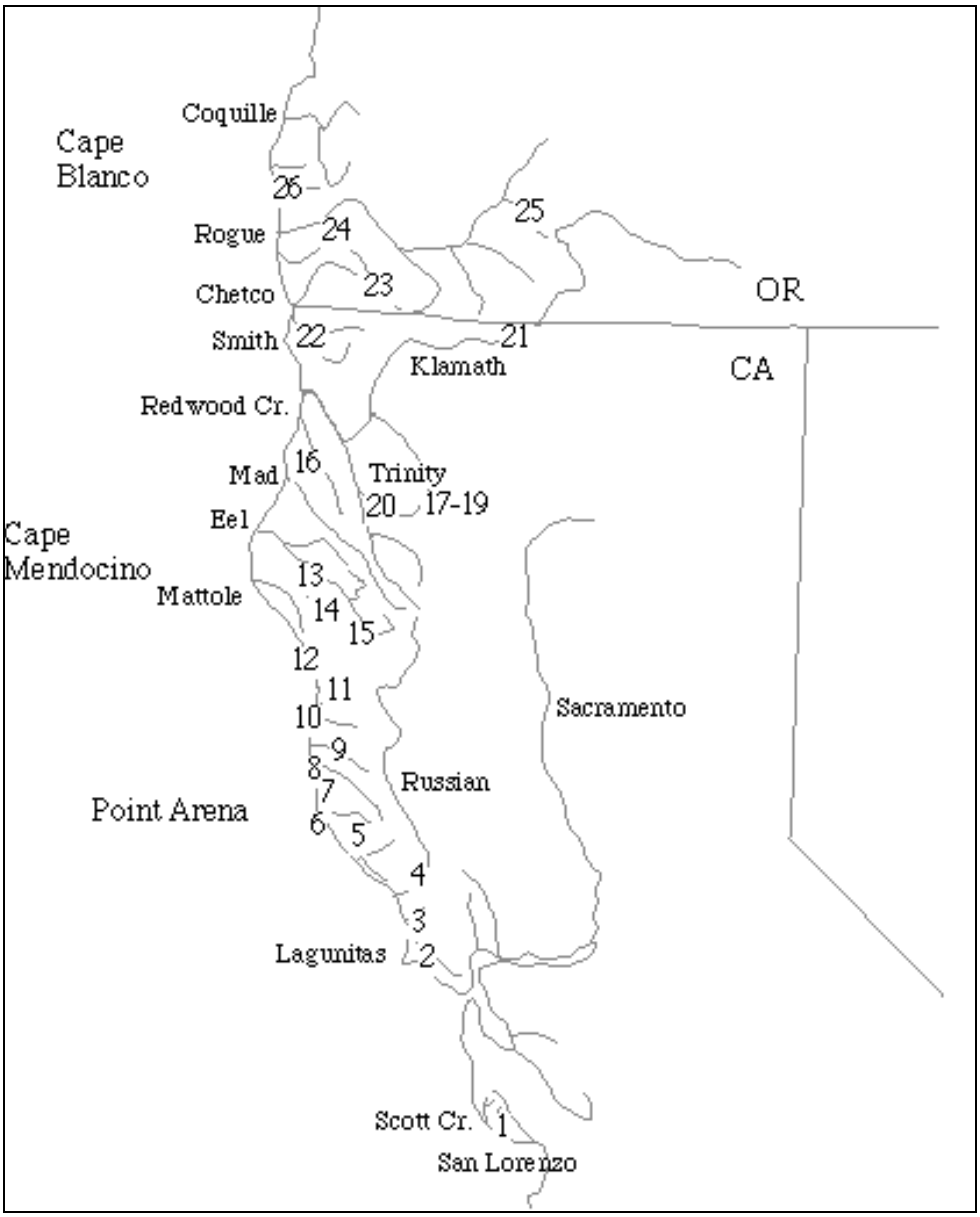

Figure 9.7-6. Map of coho salmon samples used in additional study by Weitkamp et al. (1995) that included samples from the Klamath-Trinity River system.

Ideally, inferences about genetic relationships based on genetic distances should be based on a set of gene loci common to all pairwise comparisons of populations. Even after exclusion of the smallest samples, however, few loci were scored in all samples in the combined dataset of Weitkamp et al. (1995). Genetic distances using the Cavalli-Sforza and Edwards (1967) chord distance for each pair of populations using 13 polymorphic allozyme loci common to all samples was computed. The UPGMA phenogram (Figure 9.7-7) indicates two major geographic clusters that are separated by a relatively large genetic distance (CSE = 0.126). The northern (and primarily large-river) group includes 11 samples from the Elk River (near Cape Blanco) to the Eel River (just north of Cape Mendocino). The southern (and primarily small- river) group includes nine samples spanning a geographic range from Fort Bragg to Tomales Bay (Lagunitas

Creek), in addition to three samples from north of Cape Mendocino. Considerable genetic diversity among populations is apparent within both groups (Weitkamp et al. 1995).

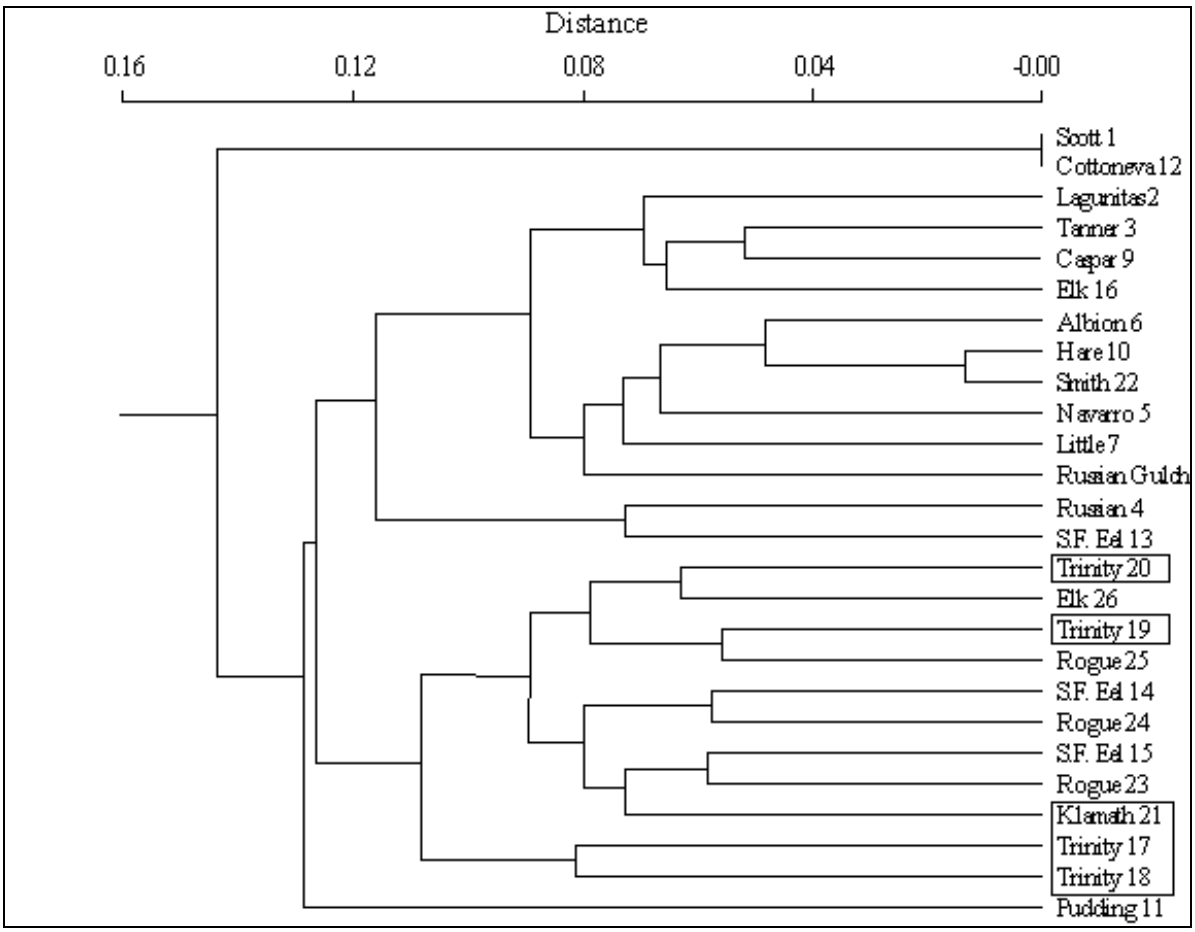

Figure 9.7-7. Dendrograms based on pairwise genetic values Cavalli-Sforza and Edwards (1967) between 26 samples of coho salmon from southern Oregon and California. Distances were calculated using data for 13 polymorphic gene loci. Sample numbers correspond to map codes in Figure 9.7-6 and samples from the Klamath-Trinity River basin are boxed. Data sources include Olin (1984), Bartley et al. (1992), and samples collected by the NMFS for this status review (Weitkamp et al. 1995). Source: Weitkamp et al. (1995).

Hedgecock et al. (2002) used seven microsatellite markers to describe genetic diversity of coho populations along the central and northern coast of California. Samples of adult coho salmon were collected in 1997 from mid-November to December at the Iron Gate and Trinity River fish hatcheries as well as 55 samples from populations of coho across the California Coast and South San Francisco ESUs. Fundamental quantitative population genetic data, allele frequencies, observed and expected numbers of heterozygotes, *F*-statistics (F_{IS} and F_{ST}), significance of pairwise linkage disequilibrium (LD) as well as testing the fit of genotypic proportions within populations to the Hardy-Weinberg equilibrium proportions were calculated. Tests of LD (gametic-phase disequilibrium) extend the principle of random mating equilibrium to multiple genes considered simultaneously. Specifically, the significance of departures from random association of alleles expected under random mating given the observed allele frequencies in the sample are tested statistically for pairwise combinations of loci. The statistical significance of LD and F_{IS} were calculated for each sample by performing 500 permutations of alleles among

individuals within a population. For F_{ST} , the permutations were of multi-locus genotypes among individuals from all populations. Observed values then were compared to the frequency distribution of permuted values. Observed values that were as large or larger than the 2.5 percent largest permutations were considered significant.

Adult samples that departed significantly from random mating expectations were evaluated for evidence of admixture, specifically, whether deficiencies of heterozygotes in a sample may have been artificially induced by the unwitting admixture of individuals from random mating, yet genetically differentiated, local populations (Wahlund effect). Samples from the Klamath River and Trinity River fish hatcheries were subdivided on the basis of FL and identification marks. After correcting for admixture of samples or family structure, in the case of juvenile samples, a UPGMA tree (Sneath and Sokal, 1973) was constructed using the Cavalli-Sforza and Edwards (1967) cord measure.

Hedgecock et al. (2002) found high LD in the Iron Gate fish hatchery sample. Eight out of 21 possible pairs of loci combinations indicated significant (P less than 0.05) LD. An excess of homozygotes relative to H-W expectations was revealed as well (F_{IS}=0.076, p=0.0). The Iron Gate fish hatchery sample was subdivided via collection date, size, and hatchery mark type to evaluate putative sub-structure within the original sample. There were no significant differences among samples based on collection date (F_{ST}=0.0038, P less than 0.159). Four separate subpopulations based on size and mark type were created and tested for genetic heterogeneity. The subpopulations included small (FL less than 56 cm) males, large (FL more than 56 cm) adipose fin clipped, left maxillary clipped, and non-clipped adults. Tests for heterogeneity among all four putative subpopulations indicated that only the adipose-clipped and non-clipped fish could be combined. Non-significant departures from H-W equilibrium expectations within two of the sub-samples, but significant F_{ST} among sub-samples as well as reduction of LD to low levels in sub-samples is evidence that the original sample of Iron Gate hatchery fish was an admixture (Table 9.7-3).

There were no significant differences among samples from the Trinity River fish hatchery based on collection date (F_{ST}=0.0024, P less than 0.253) in Hedgecock et al. (2002). However, testing for genetic heterogeneity among different size classes indicated a discrete separation between small males (FL: 36 to 44 cm) and large (FL: 53 to 74 cm) adults of both sexes (F_{ST}=0.0131, P less than 0.022).

Temporal variation in allele frequencies was statistically significant between jacks and adults in the Iron Gate fish hatchery samples. Large deviations from random mating equilibria in these samples complicate the interpretation of temporal differences. Despite statistically significant differences between temporal samples they generally cluster closest on the phenogram (Figure 9.7-8). This suggests that temporal variation, though often significant, is of smaller magnitude than the geographic component of genetic structure in the coho salmon populations examined (Hedgecock et al. 2002).

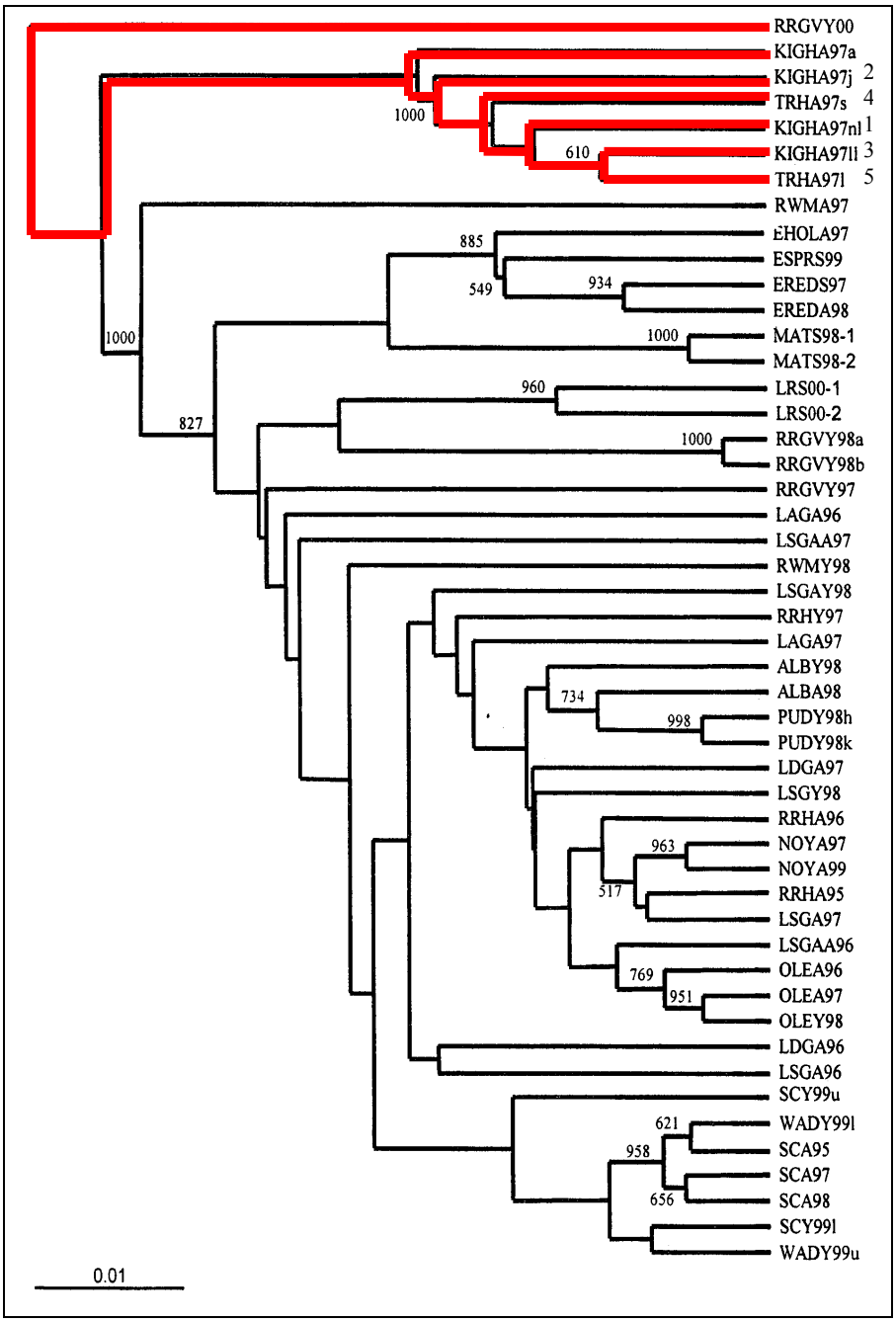

Figure 9.7-8. Unrooted UPGMA phylogram, showing Cavalli-Sforza and Edwards (1967) cord distances among 49 California coho salmon populations of sample size greater than 15 individuals. Nodes supported by bootstrap values greater than 500 out of 1,000 are shown. Klamath-Trinity samples in red and referenced to Table 9.7-3 by numbers to the right of phylogram. RRGVY00 = Green Valley Creek, a tributary to the Russian River, collected in 2000. Adapted from Hedgecock et al. (2002).

The results of the Hedgecock et al. (2002) study of microsatellite variation revealed genetic distances among coho samples correlated well with the geographic distances among populations and strongly supported the existing ESU designations. This congruence of genetic diversity and geography is surprising given the extensive intra- and inter-ESU stock transfers that have occurred. Two, not necessarily mutually exclusive, hypotheses could explain the present spatial genetic diversity of coho salmon stocks in northern California. First, stock transfers have not

propagated themselves successfully because of reduced fitness of salmon introduced from hatcheries. Second, the rate of population divergence has accelerated with the radical decline in the abundance of coho salmon in the region, because of acceleration in genetic drift and the reduction in the absolute number of migrants between watersheds (Hedgecock et al. 2002).

9.7.6.3 Coastal Rainbow Trout/Steelhead

While *O. mykiss irideus* displays multiple life history variations that may confound understanding of their population structure, the NOAA Fisheries has supported a policy to include resident populations above barriers to anadromy in the same ESU as anadromous fish that spawn in the same subbasin. The NOAA Fisheries classifies coastal rainbow trout and its more common ecotype, steelhead, in the Klamath River basin as part of the Klamath Mountains Province (KMP) ESU. This ESU designation is supported by the genetic differentiation detected with protein electrophoresis between populations south of the Klamath River and north of the Elk River (Hatch, 1990; Reisenbichler et al. 1992). Hatch (1990) observed sharp transitions in enzyme frequencies in the area south of the Coos River. Also, the pattern of some alleles becoming undetectable just north of Cape Blanco suggested a decrease in gene flow (straying) by steelhead into northern Oregon rivers of the KMP ESU. Reisenbichler et al. (1992) included samples of Trinity River summer-run and Mad River hatchery winter-run steelhead in a study using ten allozyme loci with 37 populations in the Pacific Northwest. The Trinity River sample was more genetically similar to the Rogue River sample than to the Mad River hatchery samples (Figure 9.7-9). An unpublished mtDNA study (Buroker in Busby et al. 1994) of steelhead from Oregon, reported the highest diversity of mtDNA was in Oregon steelhead in the KMP ESU.

One attempt to identify geographically distributed stocks of steelhead in the Klamath River basin using protein electrophoresis was conducted on the South Fork Trinity River. Baker (1988) compared South Fork Trinity River stock groups with those of the upper mainstem of the Trinity River and found significant difference between stocks in the two subbasins. Lesser differences were noted among steelhead juveniles in South Fork Trinity River tributaries, which Baker (1988) suggested was indicative of important local adaptations to environmental conditions. Also, KMP steelhead contain a unique chromosomal karyotype reported only in this ESU (Thorgaard, 1983). Northern California/Rogue River region steelhead displayed 59 or 60 chromosomes when compared to the 58-chromosome karyotype that was most common in the sample. The same larger number of chromosomes was observed in the Puget Sound/Strait region, however the karyotype was distinct.

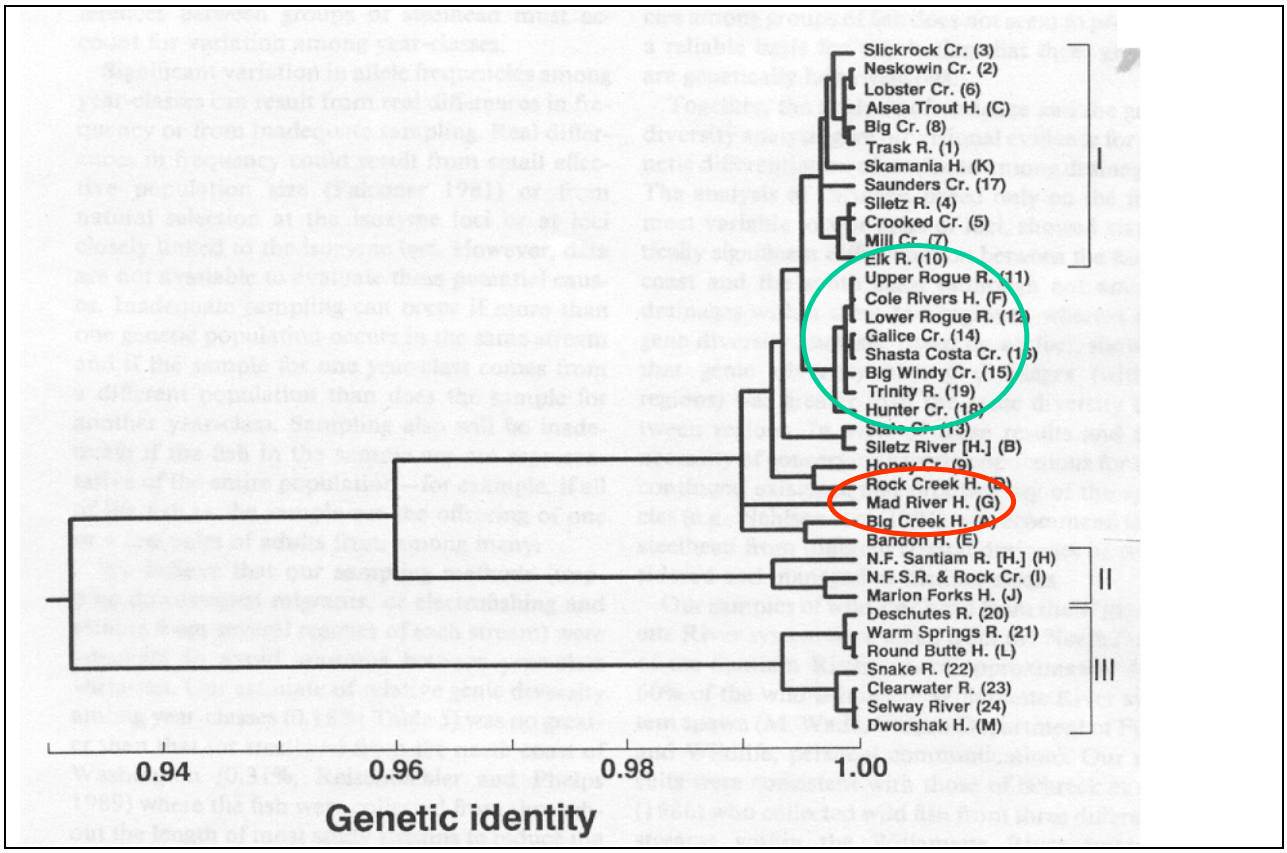

Figure 9.7-9. UPGMA dendrogram based on Nei's genetic identity (1972) between samples. Note the proximity of Trinity River and Hunter Creek samples to Rogue River samples. Mad River steelhead (to the south) are not on the same branch. Adapted from Reisenbichler (1992).

Two studies have evaluated the genetic differentiation of putative steelhead populations among the distinct run-timings of the Lower Klamath River. Reisenbichler et al. (1992) determined that different runs of steelhead within particular subbasins of the Klamath-Trinity system shared more genetic similarities than populations of similar run-timings in adjacent basins. Papa et al. (2003) investigated the genetic relationship among three winter-run and two summer-run collections of steelhead sampled through the mainstem Klamath River with seven microsatellite loci. All populations were out of Hardy-Weinberg proportions for at least one locus and significant LD was found, suggesting the existence of multiple independent populations in each sample. Additionally, a majority of pairwise F_{ST} comparisons suggested low, yet significant, genetic differences between run-timings (five of six samples; Table 9.7-4). Hierarchical cluster analysis was determined using the UPGMA algorithm (Sneath and Sokal, 1973) calculated using Nei's (1978) unbiased minimum distance with 1,000 bootstrapped permutations (Figure 9.7-10). Unlike other studies of sympatric steelhead reproductive ecotypes, these data demonstrate that there exist slight, yet significant, differences between winter and summer runs. While the differentiation between winter- and summer-runs appears strong, differentiation within either particular run is not supported. This study's data support the belief that multiple breeding populations of summer-run steelhead exist as part of a larger metapopulation, although the sampling strategy eliminated any opportunity to study spatially the reproductive isolation of stocks. This study lacked samples to gather information about steelhead on a basinwide geographic scale.

Table 9.7-4. Pair-wise F_{ST} values show differentiation between a majority of comparisons suggesting low, yet significant, genetic variability between run-timings (five of six samples) and also between the hatchery population and putative wild steelhead samples from two of the years (4 of 5 years). Significance values of pair-wise F_{ST} values indicated by * p=0.05, ** p=0.01, and *** p=0.001. Source: Papa et al. (2003).

	Winter 2000	Winter 2001	Summer 2001	Winter 2002	Summer 2002
Winter 2001	0.004				
Summer 2001	0.007**	0.004			
Winter 2002	0.002	0.004	0.005**		
Summer 2002	0.009***	0.011***	0.004	0.010***	
Hatchery 2002	0.021***	0.024***	0.023***	0.027***	0.002

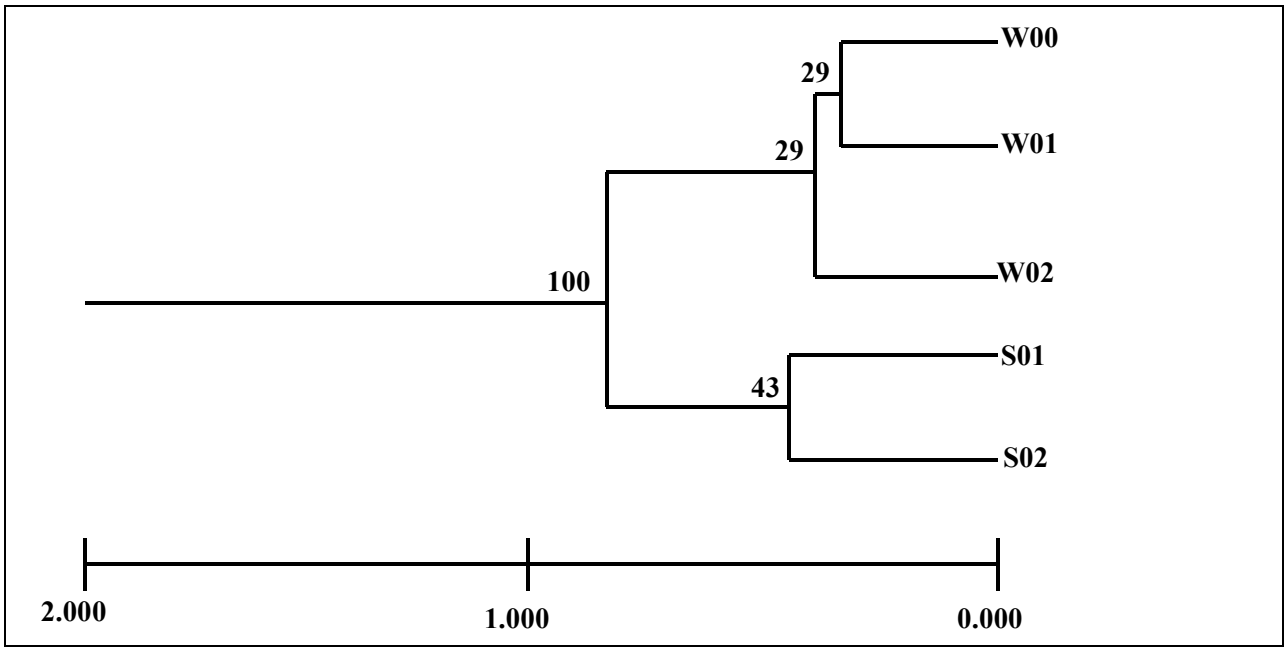

Figure 9.7-10. UPGMA tree of Nei's unbiased genetic distance (1978) for winter and summer samples from 2000-2002. Proportion of replicates resulting in similar node structure recorded next to node. Source: Papa et al. (2003).

9.7.6.4 Upper Klamath Redband Trout

The relationship of steelhead to non-anadromous Upper Klamath redband trout (*O. mykiss newberri*; Behnke, 1992) remains unknown, although trout inhabiting the Upper Klamath River basin in Oregon and California are isolated by a number of mainstem dams. The questions of potential reproductive interactions between the two forms, and whether the redband trout become anadromous remain questions requiring further ecological and genetic investigation. Before the construction of Copco dam in 1917, steelhead migrated up to the falls at the outlet of Klamath Lake. Behnke (1992) suggested that *O. mykiss irideus* did not reside above this location and designated the migratory Upper Klamath trout as a separate subspecies, *O. mykiss newberrii*. Isolated trout in Jenny Creek, above a waterfall, and in the Upper Williamson and Upper Sprague rivers have meristic characteristics and biochemical characters that suggest a common origin, but are quite distinctive from all other trout (Behnke, 1992). Moyle (2002) suggested that steelhead invaded the Upper Klamath River basin during the Pleistocene and non-anadromous

coastal rainbow trout are present above Klamath Lake. It is likely that redband trout moved downstream of Upper Klamath Lake.

The population structure of sympatric redband trout in tributaries above Bogus Creek and subbasins of Klamath Lake was evaluated using 28 allozyme loci (Currens, 1997). These fish were characterized as trout that retained plesiomorphic traits associated with cutthroat trout, *O. clarki,* that have been lost in coastal rainbow trout populations (Behnke, 1992). Samples from multiple watersheds were grouped into two populations: the Upper Klamath Lake basin and Upper Klamath Lake and Upper Klamath River (Figure 9.7-11). Fish in the Upper Klamath Lake basin group included samples from Jenny Creek, Sprague River, and Upper Williamson River. The Upper Klamath Lake and Upper Klamath River samples were collected from the Klamath River, Sprague River, and Lower Williamson River (Table 9.7-5). Together these groups were shown to be distinct from redband trout collected from the Sacramento and Columbia basins. In fact, the differences observed between these two clusters were greater than between coastal and redband trout in the Columbia River. The fact that many of the samples distinct from the Upper Klamath River group are from headwater reaches of subbasins suggests that spring-fed reaches throughout the basin harbor significant population structure.

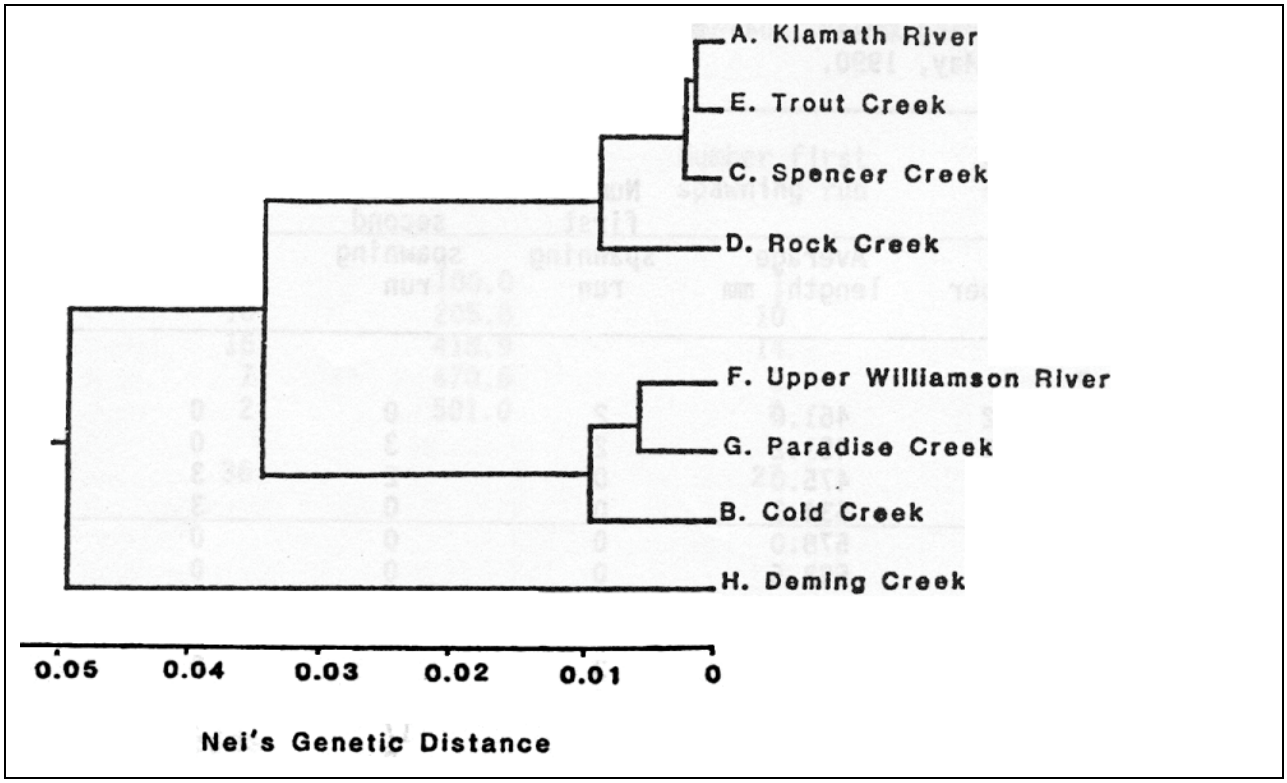

Figure 9.7-11. A UPGMA dendrogram from Hemmingsen et al. (1988) showing the genetic divergence of rainbow trout populations from the Upper Klamath River basin. Deming Creek later was determined to be part of the Upper Klamath Lake basin group with Upper Williamson River and Paradise Creek (Currens, 1997).

Table 9.7-5. Names of creeks sampled and subbasin samples that were pooled as part of Currens' (1997) groups.

Upper Klamath River and Upper Klamath Lake	Upper Klamath Lake Basin
Klamath River:	Jenny Creek:
Bogus Creek	Beaver Creek
Klamath River	Fall Creek
Spencer Creek	Jenny Creek
Rock Creek	Johnson Creek
Wood Creek	Shoat Springs
Lower Williamson River:	Willow Creek
Spring Creek	Sprague River:
Sprague River:	Deming Creek
Trout Creek	Paradise Creek
	Upper Williamson River:
	Deep Creek
	Williamson River

Similar to the results obtained with microsatellite DNA data on steelhead (Papa et al. 2003), nested analysis of allele frequencies from among tributary differences were large and seemed to indicate divergence of more than a single reproductive population within these regions (Currens, 1997). Using patterns of unique allele combinations, Currens (1997) determined that multiple headwater streams contained trout that have diverged from other trout populations. Trout in the Sprague River, Williamson River, and Jenny Creek appear to have diverged from trout populations associated with Upper Klamath Lake and Upper Klamath River or the Rogue River in the coastal Klamath Mountains. Interestingly, using cladistics and neighbor-joining trees showed that redband trout in interior, pluvial lake basins did not share a common lineage to Upper Klamath Lake trout populations, and that coastal Klamath Mountain and Upper Klamath Lake populations seem more closely related (Figure 9.7-12). Currens (1990) detailed meristic characteristics of Jenny Creek native trout that were typical of coastal rainbow trout and not *O. mykiss newberri*, thus increasing uncertainty about what to call trout that are genetically similar to trout from Upper Klamath River subbasins, yet quite physically distinct.

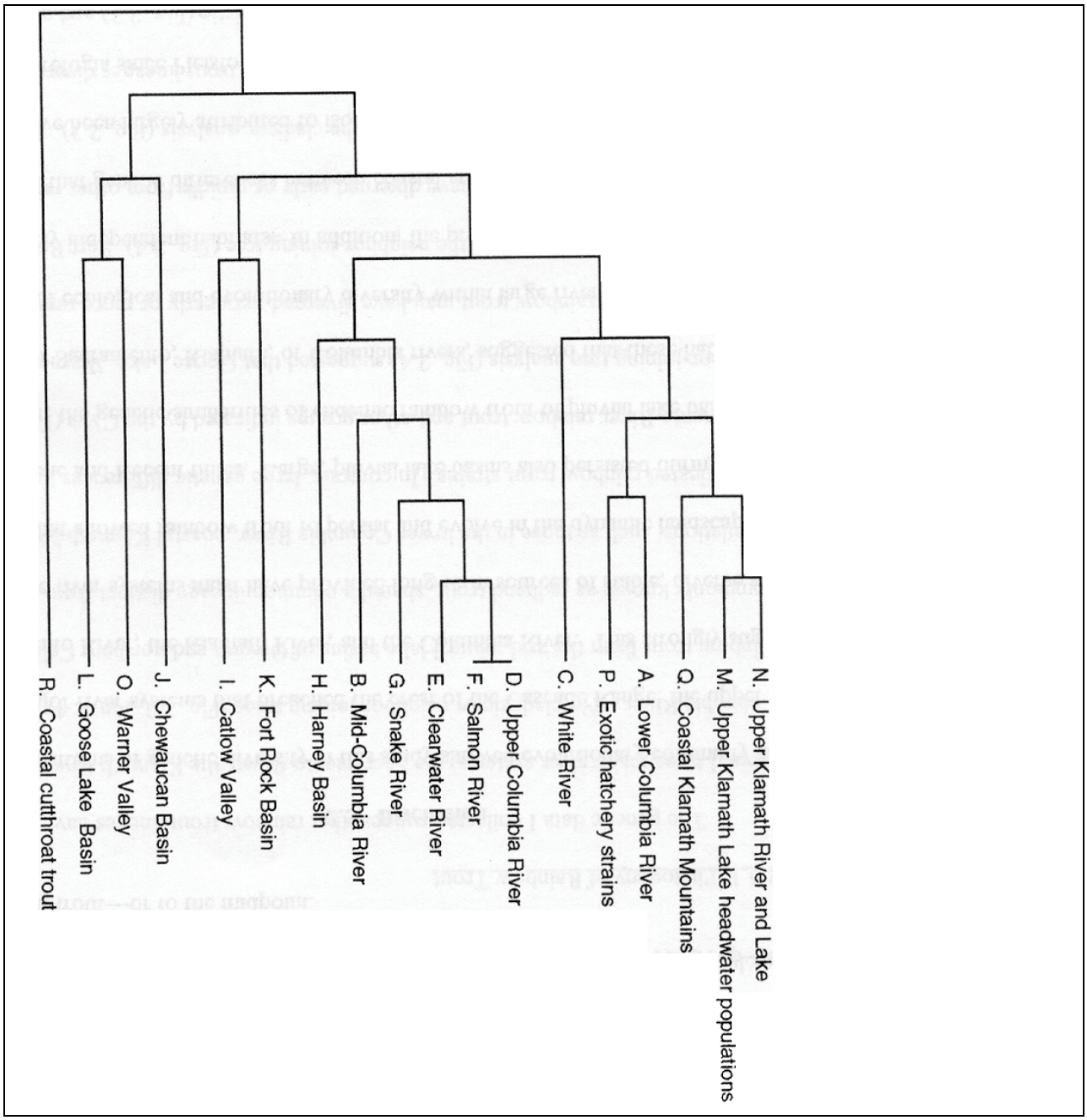

Figure 9.7-12. A neighbor-joining trees with all the groups analyzed by Currens (1997). The clustering of both Upper Klamath River and Upper Klamath Lake groups away from other interior redband trout populations suggests that Klamath trout do not share a common lineage with other trout.

9.7.7 Data Gaps and Further Studies

Issues developed in this section were recommended by participants at the PacifiCorp Genetic Workshop (convened November 3, 2003), received via e-mail by the authors, and determined relevant to discussing the utility of molecular research to the continued operation of dams in the Project area (Appendix 9A).

Issue: Insufficient ecological information exists about the stocks being considered for restoring runs above Iron Gate dam to determine what conservation strategy (reintroduction, recolonization, or translocation) will support self-sustaining runs of anadromous salmonids**.**

A comment of multiple reviewers was the need to evaluate environmental conditions and ecological factors in the Project area and their impact on the feasibility of the development of self-supporting anadromous salmonid populations. While that is not in the scope of this section, a review of the literature found that there is a high degree of uncertainty in reintroducing salmonids to their native ranges and it is a generally difficult process (Groot and Margolis, 1991). However, salmonids have been successfully established in native, fishless systems (Harig et al. 2000; Hepworth et al. 1997), as well as, basins outside of their historic ranges (Quinn et al. 2001). Genetic issues are most critically important when the local and regional environments provide only marginal conditions for survival and reproduction (Reisenbichler et al. 2003). Restoration of the processes necessary to create and maintain suitable habitats (Beechie and Bolton, 1999), and support gene flow and demographic support among populations (Rieman and Allendorf, 2001) will be critical to the long-term persistence of salmonids in the Project area.

One data gap seems to be a recent assessment, similar to Fortune et al. (1966), which would evaluate the suitability of each watershed targeted for restoration of anadromous runs above Iron Gate dam. This study would evaluate life-history periodicity, habitat, and water quality in these watersheds to determine carrying capacity and feasibility of restoring anadromous runs. A similar assessment of a watershed with Chinook and coho runs, which would permit a paired study design, may provide some quantitative criteria for what amount of habitat and conditions are necessary for maintaining self-supporting salmon populations. This type of study would aid in prioritizing watersheds for reintroduction efforts of both salmon species above Iron Gate dam. Many of the tributaries above Iron Gate dam still contain non-anadromous *O. mykiss* juveniles, thus there is certainty these subbasins are able to support *O. mykiss* juveniles. If *O. mykiss* from these watersheds were able to express anadromous life history patterns and return, it is possible their offspring would be able to survive in these subbasins, like their non-anadromous counterparts. It seems another data gap would be whether mainstem Klamath River conditions would support migration of salmonid smolts and adult spawning fish returning from the ocean.

Issue: There is not sufficient genetic baseline data to adequately identify independent populations of coho salmon or between anadromous and resident *O. mykiss* populations for restoring runs above Iron Gate dam.

Although an intrabasin study has been completed examining temporal- and geographic-scale genetic differences with microsatellites in Klamath River Chinook salmon (Banks et al. 1999, 2000), no such work has been completed for coho salmon or the *O. mykiss* lineages found in the basin. Samples are being collected by state, federal, and tribal agencies for resolving the appropriate units for conserving independent populations of Chinook and coho salmon in the Klamath and Trinity rivers, but funding has not secured for starting these studies (Garza, pers. comm., 2003). Because these species currently are extirpated above Iron Gate dam, establishing a baseline framework of independent populations is critical. This information would allow managers to monitor gene flow between the restored populations and other independent populations in the Klamath River basin, and evaluate the viability of the restored populations. Currens (1997) concluded that genetically differentiated clusters of *O. mykiss* exist in the Klamath River basin, however, the scale at which independent populations are distributed

through the Project area and basin is likely finer than allozymes were able to determine and has yet to be studied. The independent population units of *O. mykiss* in the Project area and throughout the Klamath basin remains unknown, and determining this information would be essential for monitoring the impacts of any steelhead restoration above Iron Gate dam.

For all three species, if there is determined to be sufficient population structure, a genetic stock identification study should be completed before connectivity is restored above Iron Gate dam. This information would allow managers to identify the population origin of fish migrating through fish passage facilities in the Project area. These data would be useful for studying the outmigration and spawning periodicity of runs originating from geographically distinct regions above Iron Gate dam. Genetic stock identification also would provide managers with a powerful tool for determining if adfluvial and anadromous *O. mykiss* returning to Iron Gate dam and other dams are from populations already residing in and above the Project area or downstream populations. This information would permit managers to selectively move fish upstream by collecting multilocus genotype data on individual migratory fish, while the fish is held, to determine its population of origin from a genetic stock identification baseline data set. This technique provides information to make a decision on upstream passage for each fish and limits gene flow between distinct, independent populations farther upstream. Currently, a similar protocol is being used for individual bull trout at a series of dams fragmenting the Lake Pend Oreille-Lower Clarks Fork River system (Ardren, pers. comm., 2003).

Issue: The impact of Iron Gate hatchery operation on the genetics of stocks in the Project area need to be examined before using them for restoring anadromous runs above Iron Gate dam.

Hatchery supplementation may be considered for restoring Chinook and coho salmon into watersheds from which they have been extirpated in the Project area. A conservation hatchery program is operating in California in an effort to restore coho salmon in the Russian River. Its operational guidelines, which are being used to minimize the hatchery's unnatural conditioning, should be evaluated for use at Iron Gate fish hatchery, if hatchery stocks were to be used for restoring coho and Chinook salmon runs above Iron Gate dam. Genetic data would be useful for evaluating if mating and rearing designs minimize genetic divergence of hatchery fish from their wild counterparts, thus maintaining long-term adaptive traits. These considerations and other genetic and physical effects caused by hatcheries should be developed in the context of a hatchery genetic management plan for Iron Gate fish hatchery.

Domestication selection in Iron Gate hatchery stocks may work against reintroduction goals. Coho salmon populations throughout the basin have been heavily introgressed from hatcheries outside the basin and stray rates are high. A hatchery genetic study could monitor whether straying between hatchery-supported stocks and wild populations close to the Project area is being minimized and whether domestication selection is influencing the fitness of fish that return to be part of the hatchery breeding population. In addition, marking of all Chinook salmon hatchery fish would permit managers to easily identify hatchery stocks passing through various fish passage facilities and track gene flow between Chinook populations being restored. Complete marking of coho salmon and steelhead should continue at hatcheries in the Klamath-Trinity basin, and could be used for the same purpose of tracking hatchery fish through fish passage facilities above Iron Gate dam.

Genetic bottlenecks may cause inbreeding effects and potentially lead to extirpation of populations. Iron Gate hatchery steelhead are known to have undergone a demographic bottleneck (CDFG, 2001). Genetic data should be examined to determine how a genetic bottleneck affected this stock's genetic diversity. Likewise, Iron Gate fish hatchery's Chinook and coho salmon have not been studied thoroughly to determine if these stocks have undergone a genetic bottleneck, which would be essential information if these fish were to be used for reintroduction or translocation. Microsatellite DNA data should be collected on stocks being used for restoring anadromous runs above Iron Gate dam to determine the recent demographic history of these populations.

Outbreeding depression causes a reduction in population fitness associated with interbreeding among divergent lineages, and it is a consideration that should be evaluated in each reintroduction alternative. Establishing a genetic stock identification baseline for each of the three anadromous salmonid species being restored above Iron Gate dam would be useful for avoiding outbreeding depression. This type of study would provide managers with the information necessary to avoid founding reintroduced populations from divergent independent lineages, thus avoiding a loss of locally adapted phenotypes. For *O. mykiss*, outbreeding depression also should be evaluated as part of the restoration alternatives, given the likelihood of multiple independent populations being reconnected by certain restoration strategies.