ANALYSIS OF GENETIC VARIATION AND POPULATION GENETIC STRUCTURE IN LAHONTAN
CUTTHROAT TROUT (ONCORHYNCHUS CLARKI HENSHAWI) EXTANT POPULATIONS

FINAL REPORT

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PROJECT OBJECTIVES - SUMMARY

Here we used ten highly variable nuclear microsatellite markers developed specifically for

Lahontan cutthroat trout ((*Oncorhynchus clarki henshawi*; Peacock *et al.* 2004) to resolve

evolutionary and contemporary relationships among populations within and among watersheds

within each DPS designation and among DPS's, that were not resolved with morphological or

other genetic data (Loudenslager and Gall 1980; Gall and Loudenslager 1981; Williams *et al.*

1992; Williams *et al.* 1998; Nielsen and Sage 2002). We asked a series of questions with these

data regarding population dynamics and hierarchical phylogenetic relationships. Specifically,

**1) Are the current DPS designations that were determined with morphological, meristic,
allozyme, and mitochondrial genetic data consistent with data from microsatellite markers
and more extensive and systematic sampling of extant LCT populations?**

Patterns observed with microsatellite data were largely consistent with earlier data sets

delineating three DPSs. Microsatellite data also provided further support for conclusions drawn

from mtDNA data, which suggests that LCT in the Quinn River and Western basin drainages,

primarily the Truckee River basin populations, form a single evolutionary clade with the

Humboldt River populations having diverged from the western basin prior to the split between

the Quinn and remaining Western basin cutthroat populations. These patterns are consistent with

the extent of and inundation by pluvial Lake Lahontan of the Quinn River and the western most

edge of the Humboldt River. Human perturbations have left the Carson and Walker river

populations with low levels of heterozygosity and strong evidence for genetic bottlenecks

making reconstruction of phylogenetic relationships among these and other extant LCT

populations inconclusive.

2) Is there evidence for a metapopulation dynamic within the few remaining interconnected stream habitats within the Lahontan basin across habitat types?

Metapopulations, defined as groups of small, discrete, but interacting populations, are primarily characterized by an extinction and colonization dynamic. **A major assumption of metapopulation theory is one of independent population dynamics such that extinction and colonization probabilities are uncorrelated among subpopulations. Long-term persistence of the subpopulation assemblage is achieved through the juxtaposition of interconnectedness and independence with extinction risk spread across the landscape.** Marys River and Maggie Creek are two of the few remaining interconnected stream systems that support LCT. Patterns of population genetic structure in both of these stream networks show that LCT are divided into subpopulations within these drainages, the number of which are commensurate with habitat size and complexity. Genetic subdivision, effective population size differences among subpopulations, and genetic bottlenecks support assumptions underlying metapopulation theory.

3) What is the population genetic structure of extant populations within and among watersheds within each DPS?

The Western and Northwestern DPSs have lost the majority of their native LCT populations. Therefore what we can infer about historical population genetic structure within and among

watersheds comes primarily from the extant populations in the Eastern (Humboldt River) DPS, which has the greatest number of kilometers of occupied habitat. The Humboldt River subbasins for which we have data - Marys River, North Fork Humboldt River, Rock Creek, Maggie Creek, Little Humboldt River, and Reese River all flowed historically into the main stem Humboldt River. None of these subbasins are currently connected due to water diversions. However, we observe a hierarchy of genetic structure, which reflects past connectedness among streams within watersheds and among watersheds, such that stream populations within watersheds are genetically more similar to each other than to streams in neighboring watersheds which are in turn are more similar to geographically proximate watersheds than more distance watersheds. Stream populations in the North Fork Little Humboldt River – Gance Creek, Foreman Creek and the North Fork Humboldt River – which were interconnected prior to European settlement, show a pattern of genetic population structure that is similar to the LCT populations found in the interconnected streams systems of Marys River and Maggie Creek. The Quinn River populations are much more genetically distinct from each other, but most are also very small and genetically bottlenecked (e.g., Washburn and Crowley creeks).

There is a striking pattern that emerges when all streams populations are considered. Once stream habitat is of sufficient size fish begin to assort themselves within streams such that population genetic structure emerges – i.e., distinct genoptype clusters appear with streams. This pattern together with levels of genetic variability maintained within populations could be used as a monitoring tool for efficacy of restoration efforts.

4) What is the likely origin of out-of-basin transplanted LCT populations putatively from the Lake Tahoe-Truckee River basin (pre-extirpation) based upon genetic comparison with extant LCT populations and museum preserved samples of LCT collected from 1872-1911 from the lower Truckee River and multiple locations in Lake Tahoe?

LCT populations found in Morrison and Bettridge creeks in the Pilot Peak range in Utah, Macklin Creek in the Yuba River drainage of California, Edwards Creek in the Desatoya Mountains of central Nevada and O'Harrel Creek in the eastern Sierra Nevada Mountains are outplanted populations with hypothesized origins in the Truckee and Walker (O'Harrel Creek) river basins respectively. The Pilot Peak broodstock and Bettridge creek populations were derived from the Morrison Creek population and are *definitively* of Truckee River basin ancestry. Phylogenetic analyses place these LCT populations as the most closely related populations to the known Truckee River basin historical samples obtained from museum collections with high bootstrap support (71%). All LCT populations that were sampled range-wide for this study were included in the phylogenetic analyses conducted for this study. The majority of extant native populations were sampled for this analysis, which is a *critical point* as no prior analyses had included multiple populations from all DPS designations. Macklin Creek LCT were most closely related to the Willow-Whitehorse populations in Oregon. Stocking records indicate that Macklin Creek LCT were originally from the Alpine hatchery which was a Lake Tahoe stock and in the population-level phylogeny Macklin does group with other Truckee basin populations but the bootstrap support is very weak (18%). The origin of Willow-Whitehorse populations thought to be Quinn River remains ambiguous at this time. Edwards Creek LCT are more closely aligned with Reese River LCT than the Truckee River basin and O'Harrel Creek aligns with the Carson

River populations. Thus the Pilot Peak strain represents the only outplanted population

considered in this study with Truckee River basin ancestry.

5) Is there historical evidence for population genetic structure of the now extinct LCT populations from within the Lake Tahoe-Truckee River basin based upon genetic analyses of museum preserved samples collected from multiple locations within Lake Tahoe and the lower Truckee River?

The results of Bayesian genotype clustering analysis revealed multiple genotype clusters within

the historic Truckee River basin museum specimens sampled from multiple locations in Lake

Tahoe, upper and lower Truckee River and Pyramid Lake. Three genotype clusters were found in

Lake Tahoe with only two of the clusters extending into the Truckee River and down to Pyramid

Lake. Proportional membership in the two genotype clusters in Lake Tahoe that extend down the

river and to Pyramid Lake changes as you move down the watershed showing both connectivity

between the two lakes but also local subpopulation structure. These data are consistent with

patterns seen in extant populations in the Humboldt River suggesting that interconnected

watersheds were historically comprised of groups of semi-independent subpopulations with

intermittent gene flow among them. These data also suggest that LCT populations have

characteristics of metapopulations that may have facilitated long term persistence of LCT in a

highly variable desert environment.

6) How can the level and pattern of genetic diversity within extant populations inform priority ranking for recovery activities?

Observed patterns of genetic variability and population genetic structure on both small (within

watersheds) and large (landscape level) spatial scales can inform our understanding of the

historic dynamic of unperturbed LCT populations networks. The goal of recovery and/ or

restoration should be naturally reproducing populations capable of being self-sustaining.

Interconnected stream systems were historically large enough to support multiple life history

strategies, i.e., resident and migratory life histories, as well as provide habitat complexity for all

age classes. Patterns observed in the interconnected streams systems of Marys River and Maggie

Creek provide a restoration template as representative of a once wide-spread population

dynamic. Phylogenetic analyses reveal that when dispersal corridors were available fish could

move not only among streams within watersheds but also among watersheds as evidenced by the

patterns seen in the large Humboldt River drainage.

- Restoration activities should focus on restoring stream networks.

- As habitat size, quality and complexity increase through restoration efforts population

   genetic structure should emerge consistent with subpopulation formation.

- Genetic variation and partitioning on the landscape can be used as an effectiveness

   monitoring tool.

INTRODUCTION

The "evolutionarily significant unit" or "distinct population segment" concept proposes the use

of genetic and/or ecological data to identify populations or groups of populations within a

species range that represent distinct evolutionary lineages. The ESU concept was formulated in

order to refine recovery objectives for threatened and endangered species (Moritz 1994; Waples

1995). As defined by the National Marine Fisheries and U. S. Fish and Wildlife Services [Bull

trout federal register notice (61FR4722, Feb 7, 1996) final listing determination for Klamath

River- Columbia River bulltrout DPS], this concept provides a useful framework for delineating

distinct groups of populations within a species range and ensuring conservation of the full range

of genetic and adaptive diversity within a species. This approach also allows specific

management objectives and activities to be drafted independently within each distinct population

segment (DPS).

Inland salmonid species of the intermontane western United States historically were found in

diverse habitats including multiple order stream systems and large terminal lakes. This habitat

diversity within a species range is likely responsible for genetic divergence among populations,

which may represent adaptation to different environments (Taylor 1991; Waples 1995; Healey

and Prince 1998). The post-Pleistocene dry down of the large pluvial lake systems (e.g., Lakes

Lahontan and Bonneville) further isolated watersheds. In the 20th century anthropogenic

disturbance has resulted in fragmented stream systems, potentially disrupting a metapopulation

dynamic that was inherent in many of these formerly interconnected systems and isolating

populations into headwater reaches (Gresswell *et al.* 1994; Dunham *et al.* 1997; Gresswell *et al.*

1997; Cegelski *et al.* 2006; Neville *et al.* 2006). As a result, many of these species are listed as

sensitive by state fish and game agencies or as threatened under the federal Endangered Species

Act (ESA) (Peacock and Kirchoff 2004). Successful recovery strategies will incorporate

consideration of within species diversity including life history variation, population dynamics

and adaptation to distinct environments (Harig *et al*. 2000; Dunham *et al*. 2002).

LAHONTAN CUTTHROAT TROUT

Lahontan cutthroat trout, *Oncorhynchus clarki henshawi*, endemic to the hydrographic Lahontan

basin of northeastern California, southeastern Oregon and northern Nevada (Figure 1), is one of

approximately 14 allopatrically distributed subspecies of cutthroat trout (*O. clarki*; Behnke

1992). Behnke (1992) proposed that the Lahontan subspecies be split further into separate

Lahontan and Humboldt (*O. Clarki* subsp.) subspecies, which would better reflect the lacustrine

and fluvial life histories of these fish and be consistent with morphological differences

[Humboldt fish also have fewer gill rakers and tend to have fewer scales in the lateral series and

above the lateral line (Behnke 1992)]. Classification of cutthroat trout from the Quinn River

system in northeastern Nevada and southeastern Oregon is more ambiguous. Morphological data

suggest that fish in the Quinn River drainage are more similar to Humboldt fish, while mtDNA

data suggest a common origin with western basin or "Lahontan" cutthroat. Currently there is no

formal recognition of the Humboldt subspecies. Based upon morphological, genetic, and

ecological differences Lahontan cutthroat populations have been divided into three DPSs by the

USFWS for recovery activities (Coffin and Cowan 1995): Western (Truckee, Carson, and

Walker Rivers), Eastern (Humboldt River), and Northwestern (Quinn River/Black Rock Desert).
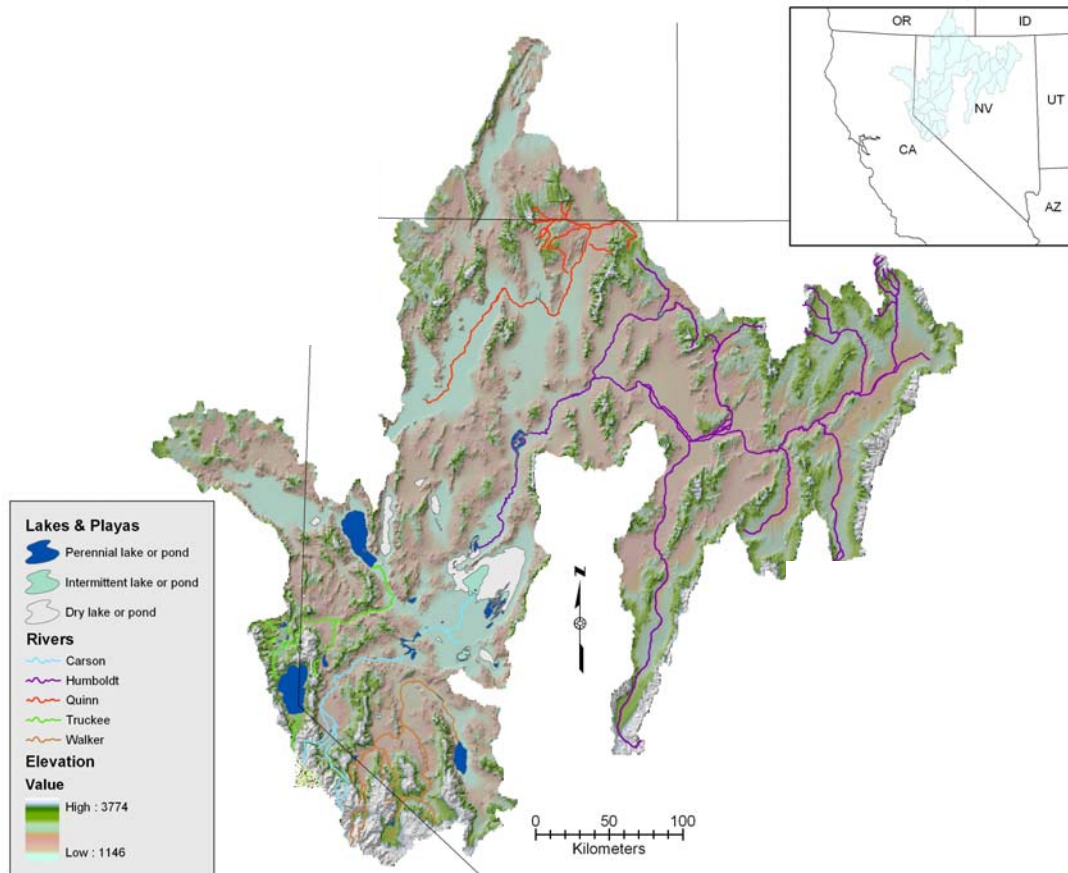
Figure 1. The hydrographic Lahontan basin of northern Nevada, southeastern Oregon and northeastern Califonia, which encompasses the entire range of Lahontan cutthroat trout, *Oncorhynchus clarki henshawi*. (map created by Robert Elson, GIS specialist, BRRC, University of Nevada, Reno).

MATERIALS AND METHODS

Populations Sampled. Fin clips from adult Lahontan cutthroat trout were collected from 40

populations sampled throughout the three designated DPSs (Table 1). Paiute cutthroat (*O. clarki*

seleniris) and rainbow trout (*O. mykiss*) samples were also included as outgroups in the

construction of phylogenetic trees. LCT from the Humboldt River basin were sampled

throughout the occupied reach of all study streams ($N = 10$ streams) by University of Nevada

personnel as part of a larger project on population viability (Peacock *et al.* 1999, Ray *et al.*

2000). Trout Unlimited biologists provided samples from the Maggie Creek drainage in the

Humboldt River basin (Harig *et al.* 2004). LCT samples from the other DPSs were provided by

California, Nevada and Oregon state game and fish agencies as well as U. S. Federal agencies

(USFWS, USFS and BLM). Paiute cutthroat trout samples were provided by Dr. Bernie May,

University of California, Davis. Rainbow trout samples were provided by the Nevada

Department of Wildlife Mason Valley hatchery and Utah Division of Wildlife Resources Mantua

hatchery. Additional rainbow trout were collected from naturalized populations in McDermitt

Creek (Quinn River system; DPS members), and Cottonwood and Trout Creeks (Humboldt

River basin; NDOW). Thirty-eight museum preserved tissue samples collected from LCT in the

Lake Tahoe-Truckee River basin (1872-1913) were provided by the Smithsonian Institution and

California Academy of Sciences (Table 2).

Table 1. Lahontan cutthroat trout populations included in this study listed by DPS and basin within DPS designations. *N* is the number of individuals sampled per population for this study. Abbreviations for each population that are used elsewhere in the document are listed in parenthesis after each population name. Paiute cutthroat and rainbow trout populations were sampled as outgroups in the phylogenetic analyses.

LAHONTAN CUTTHROAT TROUT   **_N_**

Western Basin DPS
    Truckee River Basin
        Independence Lake (IND)   21
        Heenan Lake (Independence Lake strain) (HEL)   47
        Pyramid Lake (PYL)   34
        Truckee System Museum (TRM)   38
    Carson River Basin
        East Carson River (CAR)   42
        Murray Creek (MUC)   20
        Poison Flat Creek (POC)   40
        Pacific Valley River (Mokelumne River, Calif.) (PAC)   30
        Marshall Canyon Creek (Mokelumne River, Calif.) (MAC)   41
        Milk Ranch Creek (Mokelumne River, Calif.) (MIC)   36
    Walker River Basin
        By-Day Creek (BDC)   37
        Slinkard Creek (By-Day transplant) (SLC)   38
        Wolf Creek (Slinkard transplant) (WOC)   30
        Mill Creek (Slinkard transplant) (MILL)   30
        Silver Creek (Slinkard transplant) (SILV)   30
Humboldt River DPS
    Marys River Basin
        East Marys River (EMR)   36
        West Marys River (WMR)   48
    North Fork Humboldt
        Foreman Creek (FOC)   24
        Gance Creek (GAC)   26
        North Fork Humboldt River (NFH)   48
    Rock Creek Basin
        Fraser Creek (FRC)   54
    Maggie Creek
        Little Jack Creek (LJC)   39
        Coyote Creek (COC)   54
        Beaver Creek (BVC)   55
    Little Humboldt River
        Abel Creek (ABC)   36
        Indian Creek (INC)   33
    Reese River drainage
        Mohawk Creek (MHK)   36
        Tierney Creek (TIC)   30
Quinn River/Black Rock Desert DPS
        Line Canyon Creek (LIC)   28
        Threemile Creek (3MI)   46
        Washburn Creek (WAC)   25
        Crowley Creek (CRC)   15

     Summit Lake/Mahogany Creek (SUL)   47
Coyote Lakes Basin (Oregon)
     Willow-Whitehorse   31

OUT-OF-BASIN LCT POPULATIONS OF UNKNOWN ORIGIN
  Putative Lake Tahoe-Truckee River Basin
     Bettridge Creek (Pilot Peak Mtns) (BEC)   30
     Morrison Creek (Pilot Peak Mtns, Utah) (MOC)   31
     (Pilot Peak derived hatchery stock) (PPH)   26
     Macklin Creek (MAK)   40
     Edwards Creek (EDC)   33

Putative Walker Basin
     O'Harrel Creek (OHC)   42

PAIUTE CUTTHROAT TROUT (PAIUTE)   48

RAINBOW TROUT
  Cottonwood Creek (CC)   10
  Eagle Lake Hatchery strain (NDOW Mason Valley hatchery) (EL)   6
  Erwin Ralls Hatchery strain (NDOW Mason Valley hatchery) (ER)   6
  Mantua Hatchery Utah (MH)   32
  McDermitt Creek (MCD)   30
  Trout Creek (TC)   1

Table 2. Historical Lahontan cutthroat trout samples from Lake Tahoe and Truckee River collected prior to extirpation of these populations and obtained from preserved museum collections. Museum, sampling location, year of collection and collector are included for each sample. Curators at each museum provided us with muscle and/or skin tissue from the individual cutthroat trout listed here.

Museum Collection	Sampling location	Year	Collector
California Academy of Sciences (N=8)			
CAS-SU 13298	Truckee River at Derby dam	1911	J.O. Synder

CAS-SU 13299	Truckee River at Derby dam	1911	J.O. Synder
CAS-SU 13300	Truckee River at Derby dam	1911	J.O. Synder
CAS-SU 13301	Truckee River at Derby dam	1911	J.O. Synder
CAS-SU 13302	Pyramid Lake, the Willows	1911	J.O. Synder
CAS-SU 13303	Truckee River at Derby dam	1911	J.O. Synder
CAS-SU 13304	Lake Tahoe	1911-1913	J.O. Synder
CAS-SU 13305	Lake Tahoe	1911-1913	J.O. Synder
Smithsonian Institution (N = 28)			
USNM 015496	Lake Tahoe	1872	L. Stone
USNM 017085	Lake Tahoe	1876	H.W. Henshaw
USNM 017086	Lake Tahoe	1876	H.W. Henshaw
USNM 021083	Lake Tahoe	1876	H.W. Henshaw
USNM 023467	Lake Tahoe	1876	H.W. Henshaw
USNM 075165	Tallac – near Tahoe	1911	J.O. Synder
USNM 075166	Tallac – near Tahoe	1911	J.O. Synder
USNM 075167	Tallac – near Tahoe	1911	J.O. Synder
USNM 075168	Tallac – near Tahoe	1911	J.O. Synder
USNM 075169	Tallac – near Tahoe	1911	J.O. Synder
USNM 075170	Tallac – near Tahoe	1911	J.O. Synder
USNM 075171	Tallac – near Tahoe	1911	J.O. Synder
USNM 075172	Tallac – near Tahoe	1911	J.O. Synder
USNM 075173	Tallac – near Tahoe	1911	J.O. Synder
USNM 075174	Tallac – near Tahoe	1911	J.O. Synder
USNM 075175	Tallac – near Tahoe	1911	J.O. Synder
USNM 075187	Lake Tahoe	1876	H.W. Henshaw
USNM 075188	Lake Tahoe	1876	H.W. Henshaw
USNM 075189	Lake Tahoe	1911	J.O. Synder
USNM 075190	Lake Tahoe	1911	J.O. Synder
USNM 075191	Lake Tahoe	1911	J.O. Synder
USNM 075192	Lake Tahoe	1911	J.O. Synder
USNM 075193	Lake Tahoe	1911	J.O. Synder
USNM 075194	Lake Tahoe	1911	J.O. Synder
USNM 075195	Lake Tahoe	1911	J.O. Synder
USNM 075196	Lake Tahoe	1911	J.O. Synder
USNM 075197	Lake Tahoe	1876	J.O. Synder
USNM 107328	Lake Tahoe	1876	J.O. Synder
University of Michigan (N = 2)			
UM 176347	Cascade Creek	1913	J.O. Synder
UM 176347	Cascade Creek	1913	J.O. Synder

Laboratory. Modern samples. DNA was isolated from fin clips using Qiagen DNeasy tissue

extraction kits and quantified using a Labsystems Fluoroskan Ascent fluorometer. Polymerase

chain reactions (PCR) were carried out for all individuals included in the study at ten species-

specific microsatellite loci (Peacock *et al.* 2004) on a Perkin Elmer Gene amp 9600 thermal

cycler in 15 µl volumes. Twenty ng of template DNA, 0.23 µM of forward and reverse primers

(forward primer fluorescently labeled, OPERON and Applied Biosystems) and 0.4 mM dNTPs

were used in each reaction. Amplitaq Gold buffer (1X) was used for OCH 5-10, 16 and 17 with

$MgCl_2$ added (1.6 mM, OCH 5-10; 1.8 mM, OCH 16 and 17) to these reactions. Titanium Taq

buffer (1X), which includes approximately 3M $MgCl_2$, was used for OCH 11, 13, 14 and 15.

PCR conditions for OCH 5-11 consisted of 30 cycles of 30s 95^0C, 30s 55^0C, 30s 72^0C, followed

by 10 min extension at 72^0C. PCR conditions for OCH 13-17 consisted of 26 cycles of 30s 95^0C,

68^0C 1 min 45s, followed by 10 min extension 72^0C.

Museum preserved samples. Extracting DNA from formalin preserved samples presents unique

challenges. Formalin penetrates tissues and cross-links the proteins associated with the

chromosomes (Ren *et al.* 2000). This cross-linking makes DNA extraction difficult and

necessitates multiple extractions and PCRs. At least four different PCR reactions are typically

necessary to achieve results consistent enough to reliably assign alleles. Microsatellite

amplification is usually more successful with small microsatellite inserts [100-200 base pairs

(bp)]. The museum LCT samples were preserved in formalin for an unknown period of time and

then transferred to 70-75% ethanol. Small pieces of muscle and skin tissue were removed by

museum curators and sent to UNR in 2-4 ml of 70% ethanol. Approximately 25 mg of tissue was

removed from each sample and placed in 1.0 ml of phosphate buffered saline solution and

incubated at room temperature for one hour. Samples were inverting frequently to wash the

preservatives off of the sample. This process was repeated twice. Two aliquots of tissue were

removed and DNA was extracted separately for each portion where one sample was primarily

skin tissue, and the other muscle to increase probability of getting reliable data. However, no

significant differences were found in PCR success using these two tissue types.

The recommended amounts of lysis buffers and proteinase K (Qiagen DNeasy extraction kits)

were doubled to maximize lysis of the tissues. The washed tissues were transferred to 360 µl

Buffer ATL (Qiagen) and 40 µl proteinase K and incubated minimally overnight to ensure

complete digestion of the tissue. After digestion 400 µl of Buffer AL (Qiagen) was added and the

samples inverted rapidly for 15 seconds to yield a homogenous solution. The lysate was

transferred to a second 1.5 ml microcentrifuge tube that contains 400 µl ice cold 90% ethanol for

precipitation of DNA. Samples were mixed thoroughly and incubated in at 4°C. The

ethanol/lysate mix was transferred to Qiagen DNeasy columns and centrifuged at 6000g for one

minute to bind the DNA to the filter in the column. Columns were washed 2X with AW1 and

AW2 (Qiagen) and allowed to dry. Fifty µl of elution buffer heated to 70°C was applied to the

column and incubated at room temperature for one hour. A second elution step was carried out

using the same protocol. This process yields 100 µl solutions of various concentrations and

quality of DNA.

PCR was carried out multiple times to insure precise and accurate allele assignment. Each

reaction uses 5 µl of template DNA. Both multiplex (3-5 microsatellite primer pairs) and

individual PCR reactions were carried out. Multiplex PCR is an efficient way to screen the

DNA for contamination or failures across all loci. All PCR products were diluted in 50 µl

deionized water and 1 µl of diluted PCR product was added to 16 µl ROX/Formamide ladder.

Fragment analysis was carried out on a PE Applied Biosystems 3730 Genetic Analyzer. Alleles

were scored and binned using Genemapper software (version3.7, Applied Biosystems). Allele

calls were compared between the duplicate samples and duplicate PCR reactions. If samples did

not match, PCR was repeated on both samples.

Statistical Analyses. All populations were characterized for gene diversity (heterozygosity; *H*),

genotypic diversity, and allelic richness (R_S; FSTAT, version 2.9.3.2, Goudet 2001). We also

calculated Wright's *F* statistics (Wright 1969) in order to test for genetic population structure

(F_{ST}) and quantify average levels of inbreeding (F_{IS}) within populations using FSTAT. F_{ST}, a

measure of gene flow from allele frequency differences among populations, represents average

gene flow over many generations (Wright 1969). Values range from 0, indicative of no genetic

differentiation, to 1.0, which indicates complete differentiation. This statistic was used to

reconstruct likely historical patterns of movement among streams that are now isolated with no

possibility of natural gene flow.

As a general rule of thumb F_{ST} values from 0-0.05 indicate *low* levels of genetic differentiation,

0.05-0.15 indicate *moderate* levels of differentiation, 0.15-0.25 *great* levels differentiation and

above 0.25 *very great* levels of differentiation (Hartl and Clark 1997). Although these are

theoretical estimates derived from allozyme data, F_{ST} estimates from both allozyme and

microsatellite data are often compared directly (Estoup *et al.* 1998; Allendorf *et al.* 2000;

Innocentiis *et al.* 2001). Estoup *et al.* (1998) found that the higher level of polymorphism

observed at microsatellite loci resulted in higher power of statistical tests for differentiation

among population samples and for genotypic linkage disequilibrium, but multilocus F_{ST}

estimates computed over the an entire set of brown trout populations were not significantly

different for both categories of markers.

For populations in streams systems that are still interconnected we calculated historical gene

flow using *F* statistics and assessed contemporary gene flow using a Bayesian genotype

clustering method (Pritchard *et al.* 2000; STRUCTURE version 2.0). For isolated populations we

used this method to look for within stream population genetic structure. This approach is a type

of assignment test (Paetkau *et al.* 1995, 2004; Rannala and Mountain 1997; Waser and Stroebeck

1998; Cornuet *et al.* 1999; Piry *et al.* 2004), which uses genotype likelihoods within populations

versus allele frequencies to assign individuals to a probable population of origin and identify

first generation immigrants. The utility of the assignment approach is largely due to (1) reliance

on genotype frequencies, which are formed anew each generation, (2) no assumption of drift-

migration equilibrium and (3) effectiveness even when genetic differentiation is low (Paetkau *et

al.* 2004). Genotype clustering methods can be used to assess historical gene flow as well by

identification of contemporary genotype clusters. Proportional membership of individuals within

clusters across known isolated populations can give an indication of historical connectedness and

gene flow.

The Bayesian approach has been shown to be consistently more accurate at assigning individuals

to their population of origin both with an infinite alleles (IAM) and single step (SMM) mutation

models (Cornuet *et al.* 1999) and makes no apriori assumptions about population boundaries

when estimating prior allelic distributions. The grouping criteria include Hardy-Weinberg

equilibrium and gametic phase equilibrium between loci within groups. In STRUCTURE we used

an admixture model where individuals with novel genotypes can be identified and assigned.

STRUCTURE uses a Markov chain Monte Carlo (MCMC) re-sampling algorithm. We specified

anywhere from 30,000-1,000,000 burn-in period followed by three to five 30,000-1,000,000

MCMC replicates per *K* (number of genotype clusters) to approximate posterior allelic

distributions against which individual genotypes were compared and assigned to a cluster

(Pritchard *et al.* 2000). The natural log of the probability of the data [LnP(D)] is used to

determine the best fit of the data.

We also used the program GeneClass2 (version 2.0, Piry *et al.* 2004) to identify first generation

immigrants (F_0) into each of the three tributary populations in the interconnected Maggie Creek

basin, in order to assess degree of population isolation and rates of contemporary gene flow

(Paetkau *et al.* 2004) in the face of stream culverts. We used the likelihood computation of L =

L_(home)/ L_(max), which is the ratio of the likelihood computed from the population where the

individual was sampled (L_home) over the highest likelihood value among all population

samples including the population where the individual was sampled (L_max) (Paetkau *et al.*

2004). We also used a Bayesian computation model and probabilities were determined using a

Monte Carlo resampling algorithm (Rannala and Mountain 1997), 1000 simulated individuals

and an alpha level of 0.01.

The programs BOTTLENECK (Cornuet and Luikart 1996) and *M* ratio (Garza and Williamson 2001) were used to detect recent effective population size reductions. BOTTLENECK uses allele frequency data and heterozygosity excess criteria to determine reductions in population size. Rapid reductions in effective population size results in excess heterozygosity as allele numbers per locus are reduced faster than gene diversity (H_E) (Luikart *et al*. 1999). We tested for bottlenecks using the three mutation models – infinite alleles (IAM; Maruyama and Fuerst 1985), two-phase (TPM; Di Rienzo *et al*. 1994), and single step (SMM; Ohta and Kimura 1973) available in BOTTLENECK. Although variation in mutation mode has been demonstrated for loci with different repeat motifs (Valdes *et al*. 1993; Di Rienzo *et al*. 1998; Kruglyak *et al*. 1998), there is no consensus on a general mutation model for all microsatellite loci although the two-phase model has the most empirical support (Di Reinzo *et al*. 1998). We used the variance for TPM and proportion of SMM in TPM recommended by the authors (Cornuet and Luikart 1996). Here we compare all mutation models with special emphasis on the two-phase results as this model is most likely the best-fit model.

Bottlenecks and/or founder events were also assessed using the *M* ratio *(M = k/r)*, where *k* is the number and *r* is the size distribution of alleles per locus. The ratio is expected to decrease when a population is small and subject to random genetic drift as the random loss of alleles will reduce *k* more quickly than *r* (Garza and Williamson 2001). The decline in *M* will be most pronounced in those populations that remain small for prolonged periods. The *M* ratio has a longer temporal signal than other methods that use heterozygous excess (BOTTLENECK, Cornuet and Luikart

1996), as recovery of M depends upon a proportionally greater increase in k than r (Garza and

Williamson 2001). Garza and Williamson (2001), using both empirical data from species known

to have gone through severe bottlenecks and computer simulations, show that M values of less

than 0.68 indicate severe bottlenecks. The M ratio method was used to compare the

interconnected Maggie Creek stream systems to the Marys River where only the M ratio was

used by Neville *et al*. (2006).

Phylogenies were constructed at various spatial and temporal scales using Cavalli-Sforza genetic

distance measure and a neighbor-joining tree-building algorithm (POPULATIONS, version 1.2.26,

http://www.cnrs-gif.fr/pge). Trees were visualized using the program TREEVIEW, version 1.6.6

(http://taxonomy.zoology/gla.ac.uk/rod/rod.html).

RESULTS

Genetic variation. Stream populations – Eastern DPS Among the naturally reproducing stream

populations included in the study, the Humboldt River populations have the highest overall gene

diversity (averaged over all loci, $\overline{H} = 0.697$; **Appendix 1**). This is likely the result of relatively

large population sizes compared to populations in other DPSs due to both length (km) of

available habitat in isolated streams, interconnectedness among some stream reaches, and

absence of nonnative salmonids in these waters (Figure 2). LCT populations sampled in the

Reese River subbasin (Mohawk and Tierney creeks) and on the eastern side of the Santa Rosa

Mountains in the Little Humboldt River subbasin (Abel and Indian creeks), which historically

drained into the main stem Humboldt River, are now found in small isolated streams either

above barriers or pushed into headwater reaches by brook trout encroachment and have low to

moderate levels of gene diversity ($\overline{H}$ = 0.185 and 0.485) and concomitant small populations.

Significant heterozygote deficiency at the OCH 5 locus was found in Coyote Creek (F_{IS} = 0.384;

Appendix 1) in the Maggie Creek basin and OCH 13 in West Marys River (F_{IS} = 0.377).

However, there was no systematic deviation from Hardy-Weinberg equilibrium (HWE) at any

locus across all populations or all loci in a single population in the Humboldt and Reese river

drainages (7600 randomizations, adjusted P = 0.00015 for multiple comparisons).
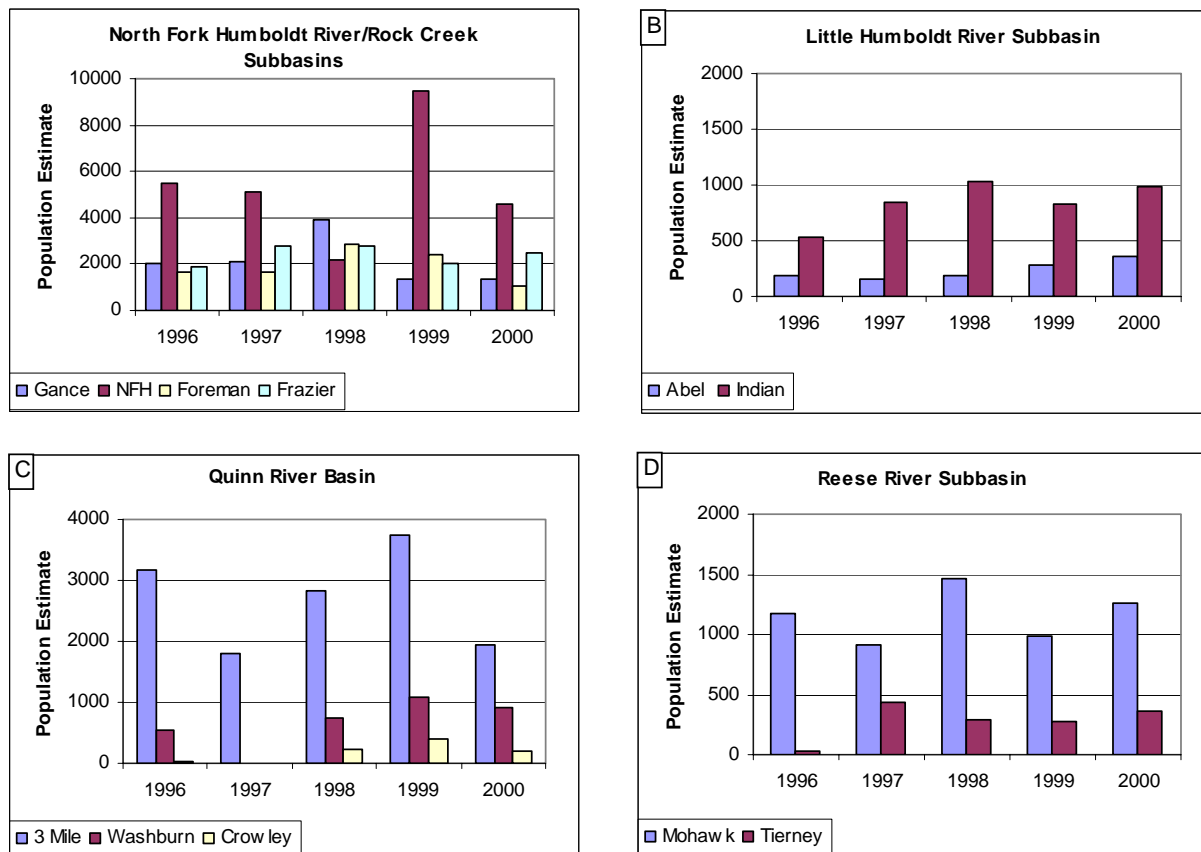
Figure 2. Population size estimates for 1996-2000 (LCT ≥ 1 year; Peacock, *unpublished data*) for representative populations from Eastern (Humboldt and Reese rivers) and Northwestern (Quinn River) DPSs (Note scale differences for population estimates in the different

basins/subbasins).

The <u>Northwestern DPS</u> has 11 extant stream populations, reduced from at least 46 present during post pluvial Lake Lahontan dry-down. Anthropogenic disturbances have resulted in at least six extirpation events since 1988 (Table 3; Sevon *et al.* 1999). Most of the extant Quinn River populations are also small and isolated and average gene diversity across the populations sampled is moderately low, $\overline{H} = 0.453$ (**Appendix 1**). Significant heterozygote deficiency was found at OCH 9 and OCH 15 in Crowley Creek, and OCH 14 and OCH 15 in 3 Mile Creek. Crowley Creek had a significant deviation from HWE ($F_{IS} = 0.318$, $P = 0.00015$) summed over all loci, suggesting a small effective population size and inbreeding. However, there was no systematic deviation from Hardy-Weinberg equilibrium in the other Quinn River populations (HWE; 7600 randomizations, adjusted $P = 0.00015$ for multiple comparisons).

Table 3. Recently extirpated populations in the Quinn River basin reported in Lahontan Cutthroat Trout Species Management Plan for the Quinn River/Black Rock Basins (Sevon *et al.* 1999).

Stream	Surveyor	Survey Year	Status	Non-native trout
Sage Creek	DPS team	2000	invasion	RBT/LCT hybrids 20%, pure LCT 80%
Riser Creek	NDOW	1995	extinct	RB/LCT hybrids
Indian Creek	DPS team	2000	extinct	RB/LCT hybrids, no pure LCT found in sample of 50 individuals
SF Flat Creek	NDOW	1995	extinct	BK, no LCT
Raster Creek	NDOW	1988	extinct	RB/LCT hybrids
Rodeo Creek	NDOW	1988	extinct	RB/LCT hybrids

Willow-Whitehorse populations have high average heterozygosity ($\overline{H} = 0.62325$) and allelic richness (**Appendix 1**). However, Willow-Whitehorse populations did have a significant

deviation from HWE (F_{IS} = 0.216, P = 0.00015, based upon 6720 randomizations) summed over

all loci, and show evidence of bottlenecks under IAM (P = 0.04) suggesting a small effective

population size and inbreeding.

The naturally reproducing extant LCT stream populations found in the Carson and Walker river

drainages in the Western DPS or known to be transplants from these drainages have low to

moderate levels of gene diversity ($\overline{H}$ = 0.366, 0.315), which likely reflects small habitats, small

founder population size and/or small extant population sizes. There are no extant LCT stream

populations native to the Truckee River basin.

Lake Populations. Independence, Heenan, Pyramid and Summit lakes all show high levels of

gene diversity ($\overline{H}$ = 0.677, 0.704, 0.7025, 0.6249 respectively). Whereas Independence and

Summit lakes are self-sustaining populations, Heenan and Pyramid are hatchery-based fisheries.

Significant heterozygote deficiency was found at OCH 11 in the Heenan Lake population (F_{IS} =

0.446, P = 0.00015) and OCH 13 (F_{IS} = 0.423, P = 0.00015) in Pyramid Lake. However, no

systematic deviations from HWE were observed at single loci across these populations or at all

loci within any single population.

Out-of-Basin LCT populations of unknown origin. Bettridge, Morrison, and Macklin creeks of

putative Truckee basin origin and O'Harrel Creek (putative Walker basin) have moderate to high

levels of gene diversity ($\overline{H}$ = 0.489 0.531, 0.549 and 0.4011 respectively), while Edwards Creek

in the Desatoya Mountains (putative Truckee basin) has very low gene diversity ($\overline{H}$ = 0.232).

Significant heterozygote deficiency was found at OCH 11 in Macklin Creek ($F_{IS} = 0.559$) and

OCH 17 in Morrison Creek ($F_{IS} = 0.427$, $P = 0.00015$). However, no systematic deviations from

HWE were observed at single loci across populations or at all loci within populations.

Phylogenetic analysis at the DPS level

1) Are the current DPS designations determined with morphological, meristic, allozyme,

and mitochondrial genetic data consistent with data from microsatellite markers and more

extensive and systematic sampling of extant LCT populations?

In the 1995 USFWS Recovery Plan for Lahontan cutthroat trout (Coffin and Cowan 1995), three

distinct population segments (DPS) were identified; Northwestern DPS (Quinn River drainage

and Summit Lake basin), Eastern DPS (Humboldt and Reese river drainages) and the Western

DPS (Truckee, Carson and Walker River drainages). These DPS designations were based upon

morphological, meristic, genetic and ecological data (Loudenslager and Gall 1980; Gall and

Loudenslager 1981, Behnke 1992; Williams *et al.* 1992; Coffin and Cowan 1995; Williams *et al.*

1998). Here we examine these DPS designations using nuclear microsatellite markers and data

from extensive sampling of populations known to be native (not transplanted) to their respective

DPS (see Table 1).

Behnke (1992) proposed that the Lahontan subspecies be split into separate Lahontan and

Humboldt (*O. Clarki* subsp.) subspecies, hypothesizing that this would better reflect their

lacustrine versus fluvial life histories of these fish and be consistent with morphological

differences (Humboldt fish also have fewer gill rakers and tend to have fewer scales in the lateral

series and above the lateral line). In contrast, the classification of cutthroat trout from the Quinn

River system in northeastern Nevada and southeastern Oregon has remained problematic.

Morphological data suggest that fish in the Quinn River drainage are more similar to Humboldt

fish, while mtDNA data suggest a common origin with western basin or "Lahontan" cutthroat

(Williams *et al*. 1992; Williams *et al*. 1998). Although the phylogeny presented here based upon

microsatellite data is not definitive due to moderate bootstrap support for the western

basin/northwestern DPS node (51%), the tree does provide support for conclusions drawn from

mtDNA data, which suggests that LCT in the Quinn River and Western basin drainages form a

single evolutionary clade with the Humboldt River populations having diverged from the

western basin prior to the split between the Quinn and remaining Western basin cutthroat

populations (Figure 3). Furthermore, the Willow-Whitehorse cutthroat populations in southern

Oregon are clearly distinct from all other Lahontan cutthroat populations. LCT was thought to

have colonized the Willow-Whitehorse basin from Quinn River populations when these systems

might have been connected by high water during the Pleistocene. However, the Lahontan

cutthroat trout is the only fish species in the Willow-Whitehorse drainage, which suggests that

this was a fishless basin and LCT were planted here anthropogenically (Cynthia Tait, *personal

communication*, BLM, Vale, Oregon).

Bayesian genotype clustering analysis. We used the genotype clustering approach to ask whether

populations cluster into three major groupings of streams that reflect current DPS designation.

Based upon this analysis populations do not group strictly along DPS geographical designations,

but groupings do reflect *both* historical connectedness and contemporary perturbations to

isolated LCT populations (Figure 4).
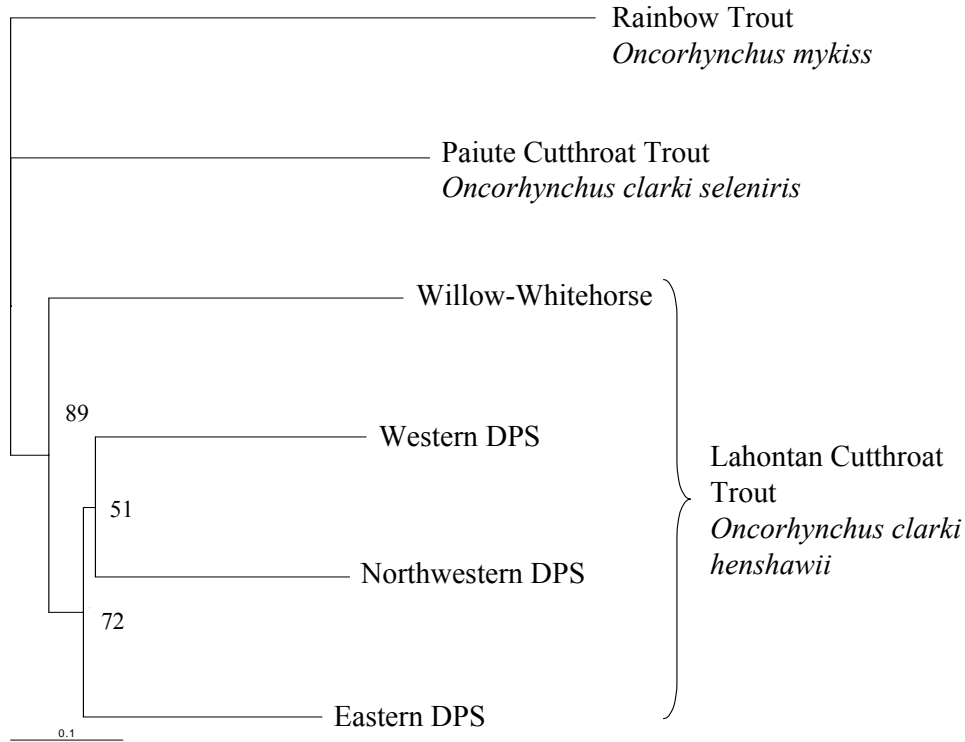
Figure 3. Phylogenetic analysis of DPS designation, using a Cavalli-Sforza genetic distance measure and neighbor-joining tree, with Rainbow trout and Paiute cutthroat as outgroups. 2000 iterations were conducted in the program POPULATIONS (version 1.2.6). Populations comprising each DPS are listed in Table 1. Scale represents genetic distance.

The three genotype clusters are as follows: (1) populations from the Reese River, Abel Creek

(see below) in the Little Humboldt drainage, the Quinn and Truckee rivers tended to form a

single genotype cluster with some exceptions. Bettridge, Morrison and the Pilot Peak broodstock

were in this first cluster. The historical Truckee River samples showed all individuals as having

proportional membership in both this cluster and the following cluster (Figure 5); (2) Heenan

and Independence Lake, LCT from Indian Creek in the Little Humboldt River drainage, all of the

Humboldt River populations, Willow-Whitehorse and Pyramid Lake formed the second cluster.

Pyramid Lake individuals were also split in proportional membership between clusters 2 and 3;

(3) The Carson and Walker river populations formed the third distinct genotype cluster with the

Pyramid Lake and Able Creek populations split in membership between the Carson River and

main Humboldt River clusters.

The Reese and Little Humboldt rivers are in the western portion of the large Humboldt River

drainage and are geographically closer to each other than to the other tributaries to the Humboldt

River sampled for this study. Most of the Quinn River drainage and the lower portions of the

Little Humboldt River drainage were inundated by pluvial Lake Lahontan during its high stand

(~13,750 years ago; Figure 6) providing a dispersal corridor for fish from the Quinn River into

the Humboldt River which may account for the cluster analysis results grouping Quinn River

fish with Reese and Little Humboldt river(s) LCT.

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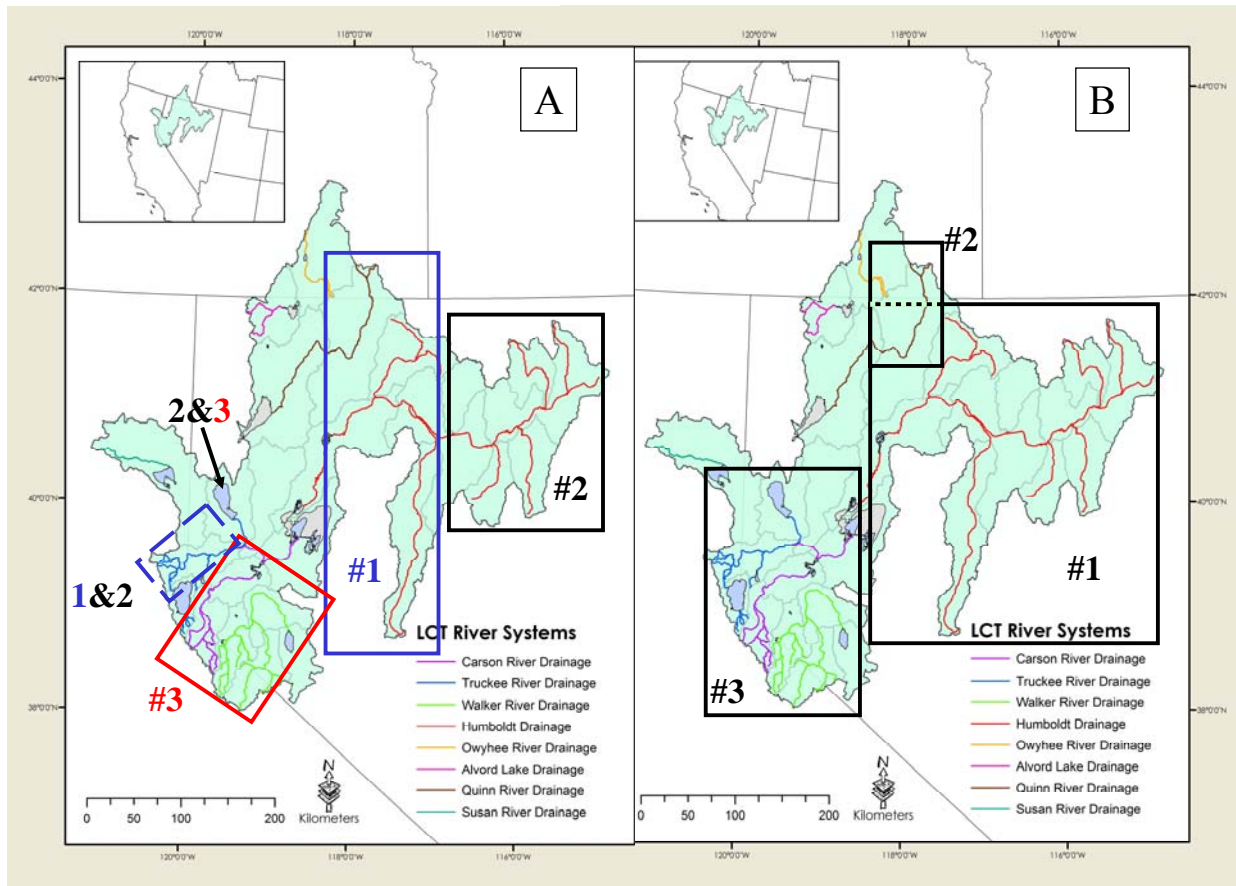
Figure 4. Map of Lahontan hydrographic basin showing panel (A) boxes represent distinct genotype cluster membership when $K = 3$ and panel (B) boxes represent current DPS designation.
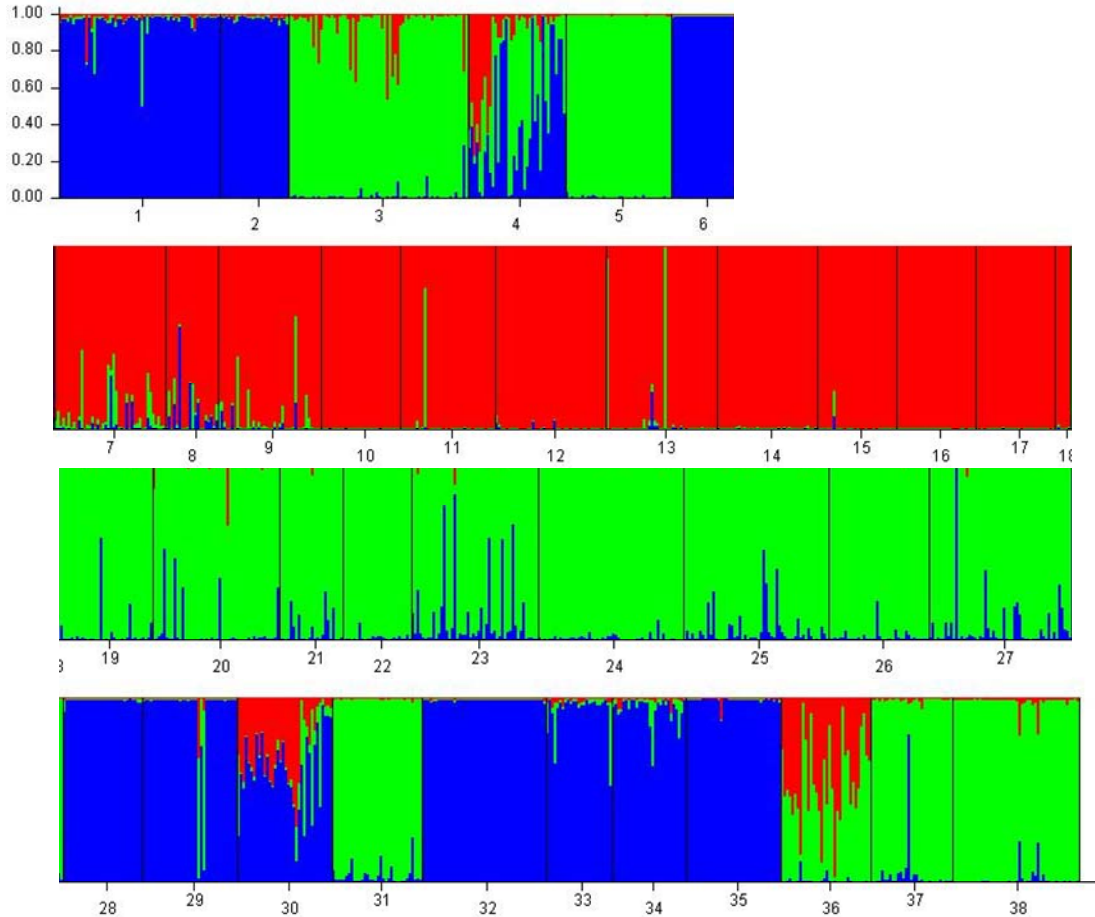
Figure 5. Bayesian clustering analysis showing three separate genotype clusters indicated by color. Populations are designated by number on the X axis and separated by black vertical lines. Populations 1-6 are Truckee basin in origin – 1&2 are Bettridge, Morrison and Pilot Peak brood stock, 3 is Heenan and Independence and 4 is the historical Truckee River basin samples. Populations 7-18 are Carson and Walker river populations, 19-27 are Humboldt River, 28-29 are Reese River, 30-31 are Little Humboldt River, 32-35 are Quinn River, 36 is Pyramid Lake, 37and 38 are Willow-Whitehorse (see text for details on analysis).

Figure 6. High stand of pluvial Lake Lahontan (~13,750 years before present)
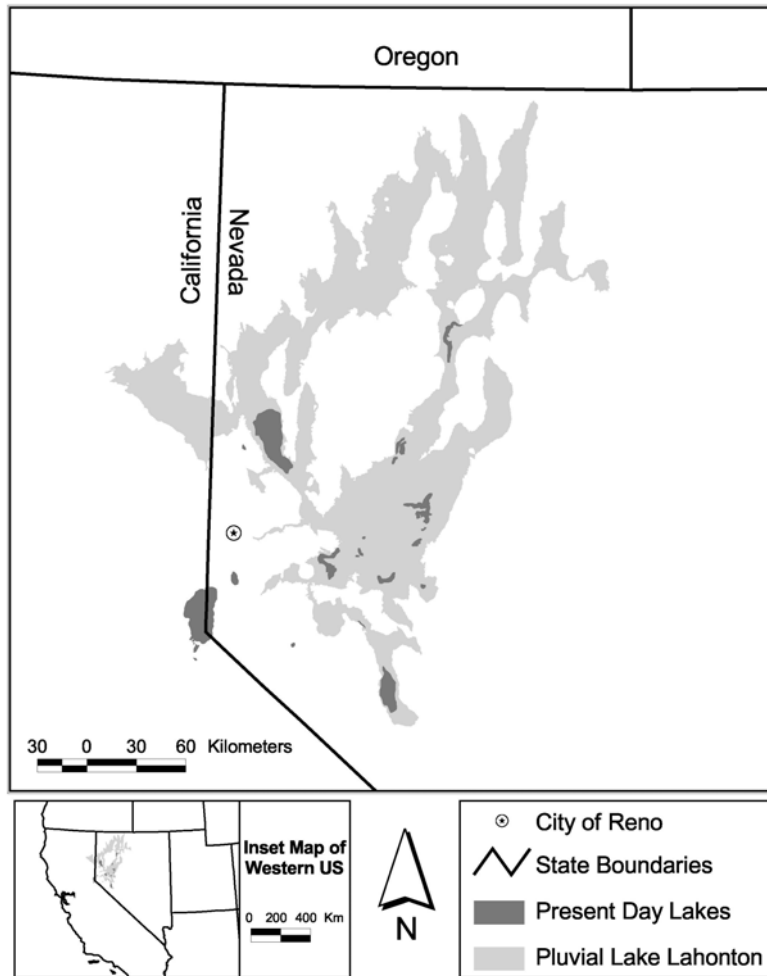
There are only seven extant LCT populations of Carson River origin - six were sampled for this study. Three of these are outplanted populations in the Mokelumne River drainage in California and three are isolated populations in the headwaters of East Carson River. All of the outplanted populations are significantly bottlenecked under all mutation models ($P \leq 0.001$). The in situ populations in the Carson River drainage also show evidence of genetic bottlenecks under the IAM and TPM mutations for two of the populations and all three mutation models for the third ($P \leq 0.04$). Walker river populations are bottlenecked under all mutation models, have very low levels of heterozygosity which makes it difficult to interpret their historical relationship with the rest of the extant LCT populations.

All populations sampled for this study showed evidence of genetic bottlenecks under at least the IAM mutation model. The western basin populations (Truckee. Carson and Walker rivers) have

been highly impacted by human development. Few natural populations remain in these

watersheds and tend to be small and very isolated. Random loss of genetic variation through the

genetic bottleneck process can erode information critical to reconstructing historical

phylogenetic relationships. This is likely the cause of the low bootstrap values seen in the

constructed phylogenetic trees and lack of clear genetic cohesion among the western basin

drainages. Never-the-less the pattern seen in the Bayesian clustering analysis supports the

phylogenetic analysis which supports a historical connection between the western (Truckee

River) and northwestern DPSs. Overall, despite the significant human impact to these

populations, geographic proximity and inundation by pluvial Lake Lahontan largely explains the

observed genetic relationships.

Population genetic structure within and among watersheds

<u>Eastern basin DPS</u>. The majority of naturally sustaining fluvial LCT populations are found in

the Humboldt River watershed (Figure 7). The Humboldt River is a large main stem river that

connected 1000's of kilometers of stream habitat pre-European settlement of the Lahontan basin.

Historically, fluvial LCT populations were interconnected at various temporal and spatial scales

facilitating wide-ranging movement. Although water still flows into the main stem Humboldt

River from ancillary drainages, water diversions have largely isolated LCT within headwater

systems in either single streams or small groups of tributaries. Here we use patterns of

population genetic structure on the landscape among both the remaining interconnected streams

and streams once connected but now isolated to assess the natural population dynamics of steam

living Lahontan cutthroat trout.
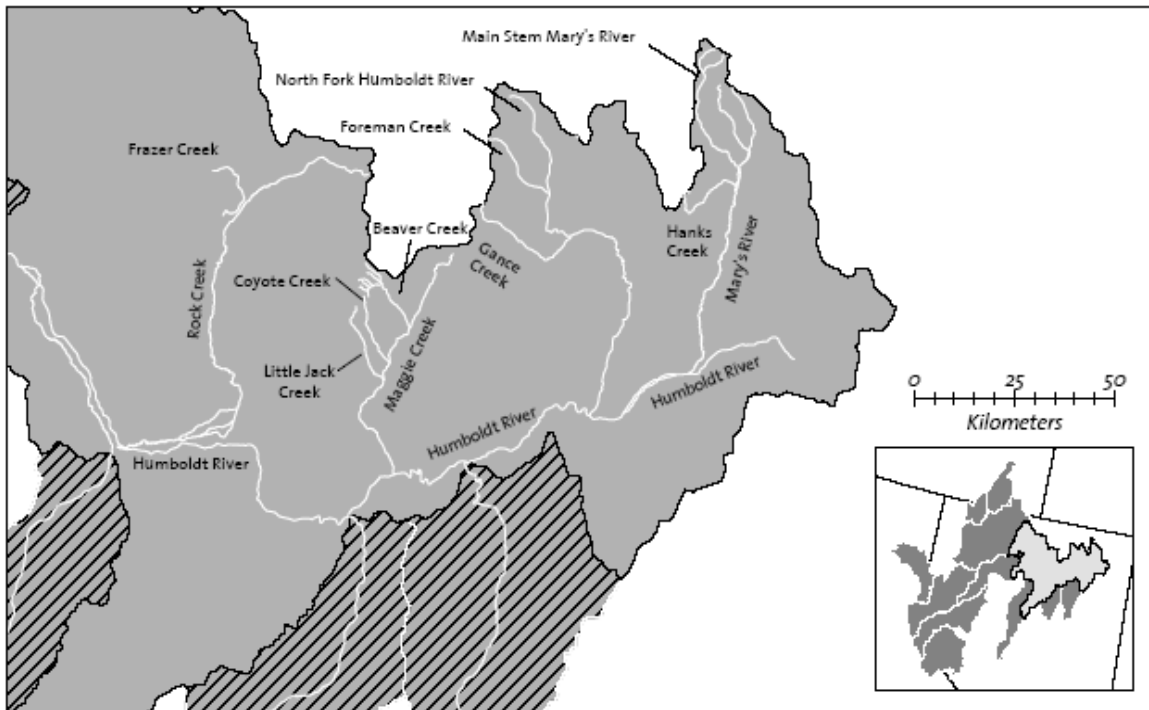
Figure 7. The Humboldt River basin shown in light shading on smaller inset map of the entire Lahontan basin. The larger map shows the Marys River, North Fork Humboldt River, Maggie Creek and Rock Creek (Frazer Creek) drainages (Figure reprinted from Peacock and Kirchoff 2004, *Transactions of the American Fisheries Society* 133:309-325).

Population level phylogenetic analysis shows that the two most geographically proximate

watersheds, the Marys and North Fork Humboldt rivers, form a single evolutionary clade that

clusters with the next geographically closest watershed, Maggie Creek, with strong bootstrap

support (**70**, Figures 8). Frazer Creek in the Rock Creek subbasin, the neighboring watershed to

the west, clusters with the Maggie Creek, Marys River and North Fork Humboldt River

populations with equally strong bootstrap support (**69**). Populations sampled in the Reese River

(Tierney and Mohawk creeks) cluster weakly with Abel Creek, while Indian Creek groups with

the Humboldt populations but also with weak bootstrap support (Figure 8). Overall, phylogenetic

patterns reflect spatial structuring of populations on the landscape both within and among

watersheds. Low bootstrap values within clades likely reflect the effects of genetic bottlenecks

and low rates of gene flow from both anthropogenic and natural causes and concomitant loss of

gene diversity and phylogenetic signal.
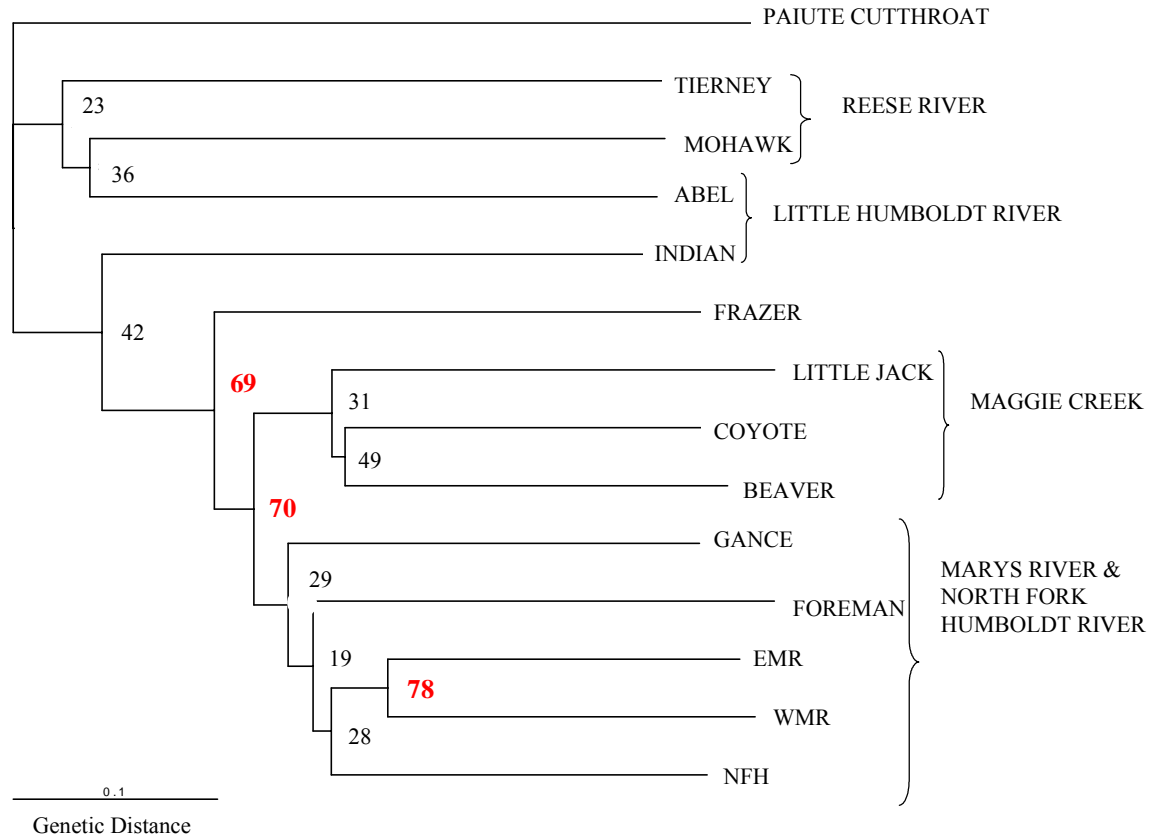
Figure 8. Phylogenetic analysis of Humboldt River LCT populations using a Cavalli-Sforza genetic distance measure and a neighboring-joining tree with Paiute cutthroat trout as the outgroup. 2000 iterations were conducted in POPULATIONS (version 1.2.26) with bootstrap values indicated at the tree nodes. Red highlighted bootstrap values (**69** and **70**) indicate major grouping of geographically proximate populations in the Humboldt River drainage (See Table 1 for population abbreviations). EMR and WMR (**78**) are in the interconnected Marys River basin.

Interconnected watersheds

2) Is there evidence for a metapopulation dynamic within the few remaining interconnected stream habitats within the Lahontan basin across habitat types?

Marys River basin. EMR and WMR sampled for this study are part of interconnected stream habitat in the Marys River basin. This is the largest of the few remaining intact interconnected stream networks occupied by LCT in the Lahontan basin. In an expanded study (Neville-Arsenault 2003; Neville *et al*. 2006), which included the 11 tributary streams in the east and west basins of the Marys River, microsatellite data revealed that limited and asymmetric gene flow among LCT populations within this basin. Genetic bottlenecks and small effective population sizes have resulted in significant population genetic structure both within and among tributaries in the Marys River basin (system-wide F_{ST} estimate of 0.12 [95% CI: 236 0.09-0.15]; Figure 9; Neville *et al.* 2006). Population-level phylogenic analysis revealed geographically proximate populations were not always the closest genetically (Figure 10). Estimates of effective population size (Figure11) and evidence of genetic bottlenecks demonstrate the complexity of population dynamics in large intact stream networks with dynamic movement patterns and fluctuating population sizes. Bayesian clustering analysis (STRUCTURE) revealed additional genetic structure as 20 distinct genotype clusters were identified in the overall stream system which further elucidates the extent of gene flow within and among tributaries (Figure 12). This analysis corroborated the F_{ST} analysis, which showed that that some populations are very isolated with all individuals assigning to only one or two genotype clusters. Populations which exchanged more individuals not only have more genotype clusters but individual assign to multiple clusters indicating extensive gene flow.
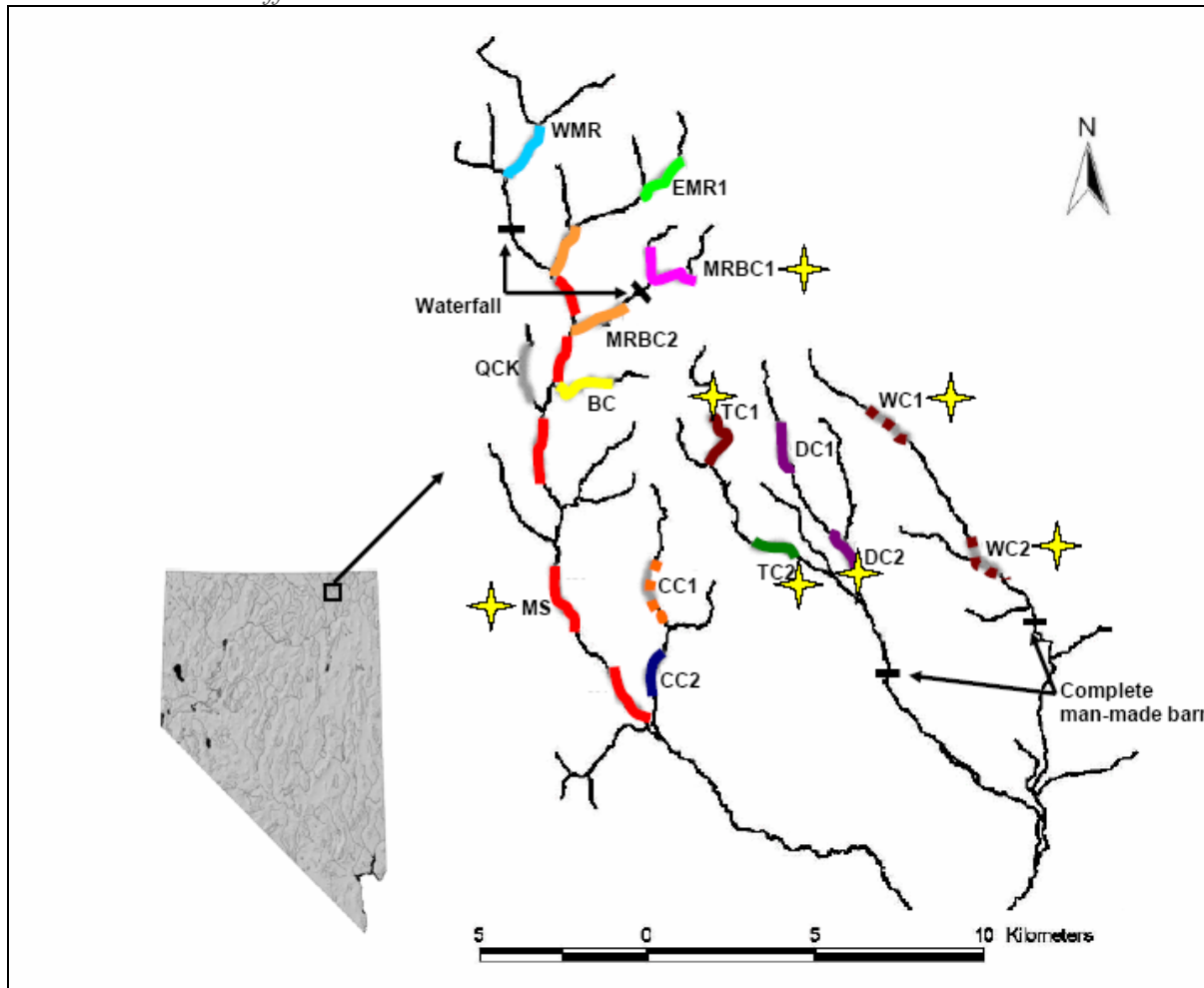
Figure 9. Map of Nevada inset at left with the location of the Marys River boxed. At right is the study site; sample sites are indicated by bold colored lines. Pairs of samples with the same colors had F_{ST} values that were not statistically different from each other, indicating panmixia, whereas samples with different colors were significantly differentiated from all other samples. Stars indicate samples for which a significant genetic bottleneck was detected using the M ratio. Locations of waterfalls and man-made barriers within the stream network are indicated. Population abbreviations; WMR = West Marys River, EMR = East Marys River, MRBC = Marys River Basin Creek, QCK = Question Creek, BC = Basin Creek, CC = Cutt Creek, MS = Main stem Marys River, TC = T Creek, WC = Wildcat Creek, DC = Draw Creek (reprinted from the original Figure 1 in Neville, H. M., J. B. Dunham and M. M. Peacock (2006) Landscape attributes and life history variability shape genetic structure of trout populations in a stream network. *Landscape Ecology* 21:901-916, with kind permission of Springer Science and Business Media).
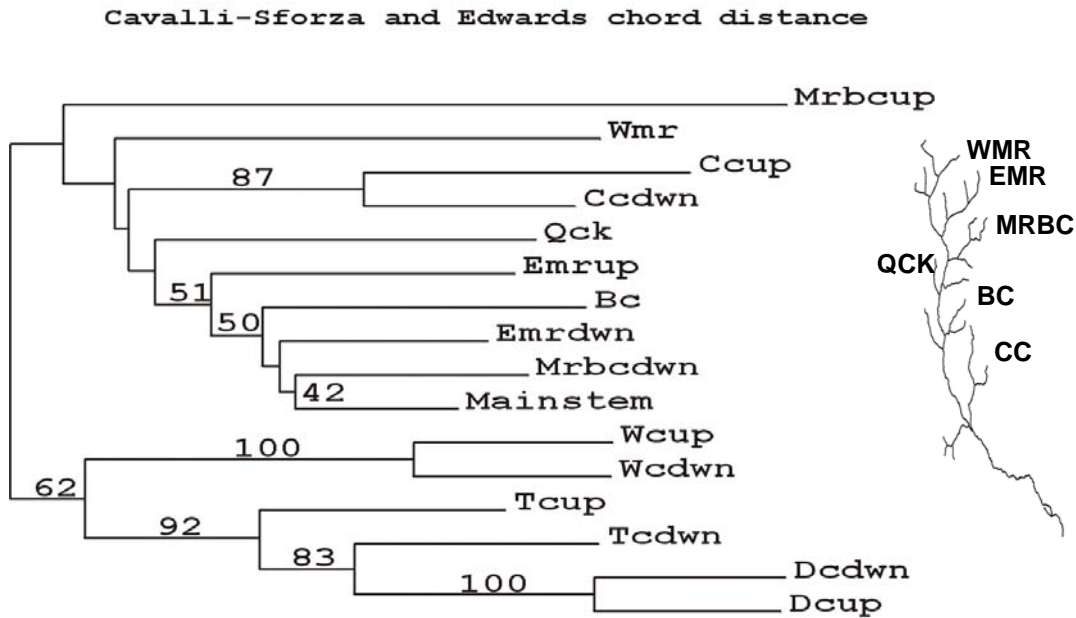
Figure 10. Population-level phylogeny using a Cavalli-sforza genetic distance measure and a neighbor-joining tree building algorithm of main stem and tributary populations in the Marys River basin (reprinted from Neville-Arsenault 2003).
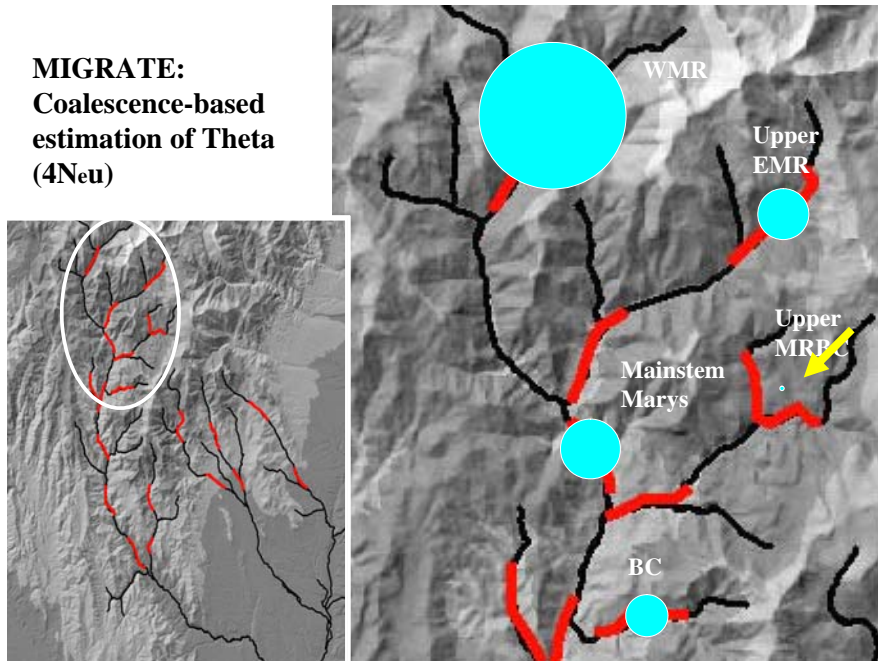
Figure 11. Upper western Marys River basin tributary populations (inset). Blue circles represent the relative effective population sizes (theta), determined using MIGRATE (VERS 1.6; Beerli 2002), for tributary populations in the western Marys River basin. Circle size correlates with N_e. The yellow arrow indicates upper Marys River Basin Creek with a very small N_e, (reprinted from Neville-Arsenault 2003).
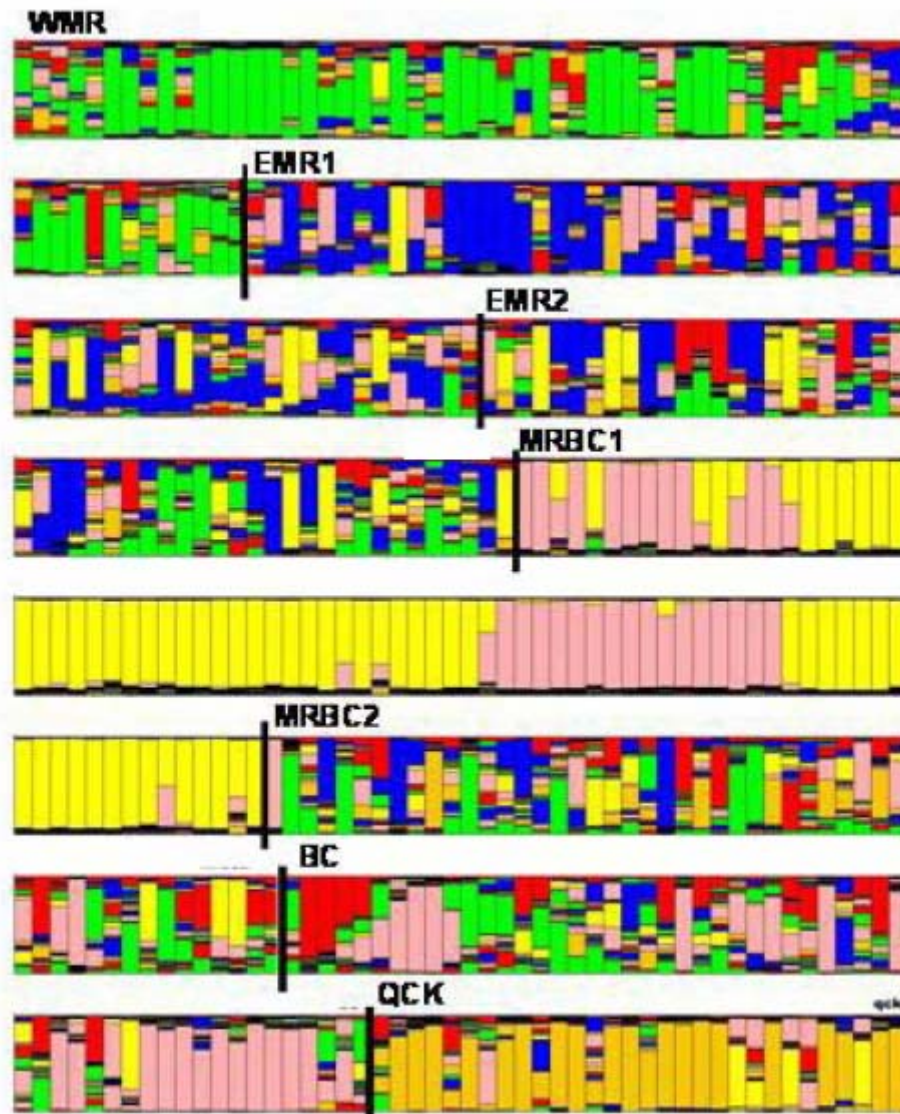
Figure 12. Results of bayesian genotype clustering analysis (STRUCTURE), for a subset of streams, in the western Marys River basin. Colors represent distinct genotype clusters and each column represents a single individual. Creeks are separated by black vertical lines. MRBC1 represents the upper MRBC, which has small N_e and two genotype clusters, whereas MRBC2 represents lower MRBC that flows into the main stem river without barriers. MRBC2 has multiple clusters with individuals assigning to more than one cluster indicating gene flow and ancestry originating from multiple clusters (reprinted from Neville, H. M., J. B. Dunham and M. M. Peacock (2006) Landscape attributes and life history variability shape genetic structure of trout populations in a stream network. *Landscape Ecology* 21:901-916, with kind permission of Springer Science and Business Media).

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Overall, the genetic patterns observed in this basin reflect local diversity in landscape and

perhaps population characteristics, which are more likely to emerge in larger basins like the

Marys River and may represent patterns that were once common to LCT populations prior to

widespread anthropogenic fragmentation of stream networks (Neville Arsenault 2003; Neville *et*

al. 2006).

Maggie Creek basin. Similar to the Marys River basin, patterns of complex population structure

were observed in the smaller, but interconnected stream system in the Maggie Creek basin

(Figure 13). In the Maggie Creek basin only three tributaries have (historically and

contemporaneously) suitable habitat for LCT; Beaver, Coyote and Little Jack creeks. These

tributaries are connected via main stem Maggie Creek. The main tributary of Beaver Creek, the

largest and most diverse tributary in the Maggie Creek system, occupies ~10 km of habitat

during base flows, including multiple smaller headwater reaches that flow into Beaver Creek

proper (e.g., Toro Canyon and Williams Canyon creeks, Figure 13; Harig *et al.* 2004). However,

the headwater reaches can be seasonally disjunct from main stem Beaver Creek during base flow

conditions; for example Toro Canyon was isolated from main stem Beaver Creek by

approximately 5 km of dry streambed during the 2002 sampling season. Coyote and Little Jack

creeks are smaller, have fewer kilometers of occupiable stream (~ 7 km each at base flow), and

have less diverse habitats, with only single headwater tributaries flowing into the main stem

creeks. All streams have the potential to be isolated seasonally, as lower sections of the creeks

and of the main stem Maggie Creek can dry completely during low flow periods. Little Jack

Creek is physically the most isolated of the occupied streams in the system. during base flow

conditions the occupied reaches in Beaver and Coyote creeks are separated by ~ 30 km and their

confluences with Maggie creek are separated by ~ 10 km. In contrast, the occupied reaches in

adjacent Coyote and Little Jack creeks are also separated by ~ 30 km, but their confluences with

Maggie Creek are only 4 km apart. Little Jack Creek is a smaller tributary habitat and during

base flows the occupied reach is farther from the confluence with the main stem Maggie Creek.

As such the LCT in Little Jack may be more isolated than LCT in the other tributaries.

The tributaries to Maggie Creek may be currently isolated by a series of man-made, potential

barriers to dispersal movements (Note: these barriers were present at the time of sampling but

have now been removed). Because our interests include both historical and contemporary

patterns of movement we assessed whether these structures function as barriers to contemporary

movement as evidenced by levels of current gene flow. There are two road culverts in the system

that act as partial/seasonal barriers to upstream movement; one each on Coyote and Little Jack

creeks near the confluence with main stem Maggie Creek (see Figure 13). Two additional

structures, a road culvert on Beaver creek and a culvert and irrigation diversion on main stem

Maggie creek, may act as complete barriers to contemporary movement. There have been road

culverts of some kind on these creeks on and off for an undetermined length of time, however,

the current tributary culverts were put in place in mid-1980's. The irrigation barrier on the main

stem Maggie Creek was installed in 1995. At the time of sampling, fish were found in the upper

headwater reaches of these three tributaries and one individual was found in the main stem

Maggie Creek. All Maggie Creek tributary populations had a full complement of size

classes,suggesting resident populations (see Harig *et al.* 2004; Figure 14).

Global F_{ST} within Maggie Creek basin was 0.067 and all tributary populations were significantly genetically differentiated from one another ($P \leq 0.016$, adjusted P value for multiple comparisons; Peacock *et al. in review*). Pairwise F_{ST} values varied from 0.045 between Beaver and Coyote, 0.07 between Beaver and Little Jack and 0.093 between Coyote and Little Jack, indicating low to moderate levels of genetic differentiation. As seen in the Marys River basin, genetic structure was evident even within tributary habitats. Of the 1-6 clusters modeled, five genotype clusters had the best statistical support [highest LnP(D); - 4385.63, $P = 1$; Figure 15]. The average Bayesian posterior probability across the five runs for $K(5)$ was 1.0 versus $3.16e^{-20}$ for $K(3)$, the next most likely number of clusters. Three primary clusters were identifiable in Beaver creek (Figure 16). Samples from Toro Canyon ($N = 22$) in the upper watershed, which was isolated from the rest of the Beaver Creek drainage at the time of sampling by dry sections of creek, assigned primarily to only two of the three clusters (1 and 2) with approximately half of the individuals assigning to cluster 1 (43%, $N = 10$, Table 4) and only one individual assigning to cluster 5 (Little Jack Creek). The Beaver Creek samples collected in main stem Beaver Creek lower in the watershed also assigned primarily to two clusters, 1 (28% assignment) and 3 (45% assignment). Membership in cluster 3 was confined almost completely to main stem Beaver Creek, as there was very low proportional membership in this cluster in Toro Canyon (0.081), Little Jack, or Coyote creeks (~0.015).

We sorted all individuals sampled within Beaver Creek according to their highest proportion cluster membership and computed pairwise F_{ST} estimates among the three genotype clusters confined primarily to Beaver Creek [pink (1), yellow (2), and blue (3) clusters, see Figure 16].

The pairwise F_{ST} estimates ranged from 0.0337 to 0.0549, indicating low levels of genetic

differentiation. In the case of the two clusters found in Toro Canyon [pink (1) and yellow (2)],

these were significantly genetically differentiated from each other ($F_{ST} = 0.0337$, $P = 0.008$,

obtained after 120 permutations). The blue cluster (cluster 3) in main stem Beaver Creek was

significantly differentiated from the pink ($F_{ST} = 0.0549$), but not the yellow cluster found in Toro

Canyon ($F_{ST} = 0.0385$, $P = 0.008$), which may have to do with small sample size ($N = 11$). The

F_{ST} estimates between clusters within Beaver Creek were comparable to the estimate between

Beaver and Coyote creeks. The genetic results (genotype clustering and significant pairwise F_{ST}

estimates) are consistent with the hydrologic dynamics seen in this tributary, i.e., temporal

isolation of sections of Beaver Creek corresponding with seasonal and annual stream flow.

Individuals from Little Jack and Coyote creeks assigned primarily to single genotype clusters

found largely confined to each tributary respectively (Figure 16). Assignments of individuals
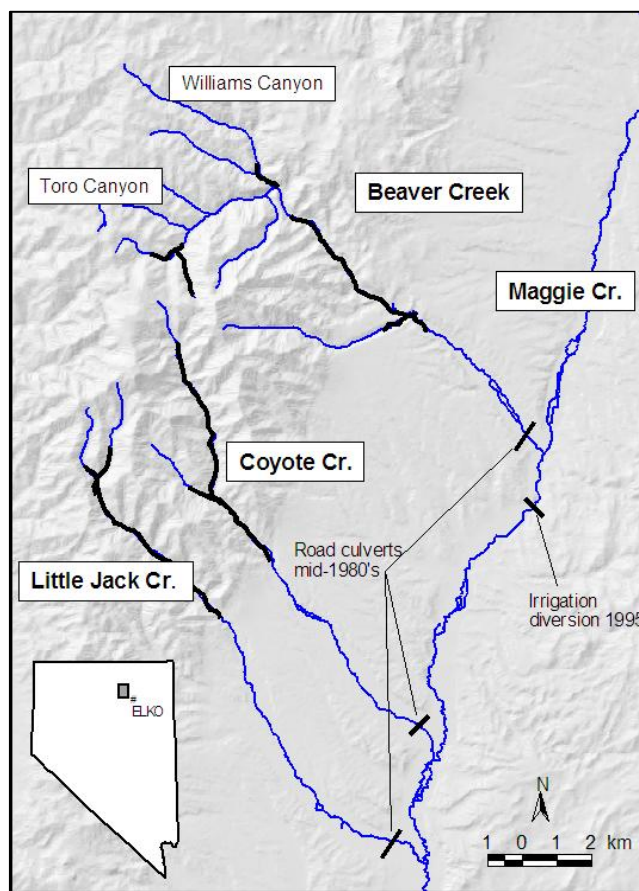
from Beaver and Coyote creeks, to multiple genotype clusters suggest a more fluid movement dynamic between these creeks as suggested by the lowest pairwise F_{ST} estimate (0.045). In contrast, Little Jack individuals tended to form a very distinct genotype cluster with little evident immigration.

To determine the effects of road culverts on

current movement among tributary populations we used the "detection of first generation

migrants" module in GeneClass2. We determined that three individuals sampled from Beaver

Creek were first generation immigrants from neighboring Coyote Creek ($P < 0.002$). One of

these individuals was sampled in Toro Canyon in the upper Beaver Creek drainage. Five

individuals sampled in Coyote Creek were immigrants from Beaver Creek ($P < 0.007$) and

additional immigrant assigned to Little Jack Creek ($P > 0.000$). One immigrant was identified in

Little Jack Creek and assigned to Coyote Creek ($P > 0.000$). N_e was not calculated for the

Maggie Creek streams, but the M ratio test showed all three tributary populations to have

experienced genetic bottlenecks (M for Beaver = 0.596, Coyote = 0.655, and Little Jack =

0.625).

Figure 13. Maggie Creek basin with tributaries identified and position of road culverts indicated.
Extent of occupied habitat during base flow is represented in each tributary by highlighted area
within the stream channel.
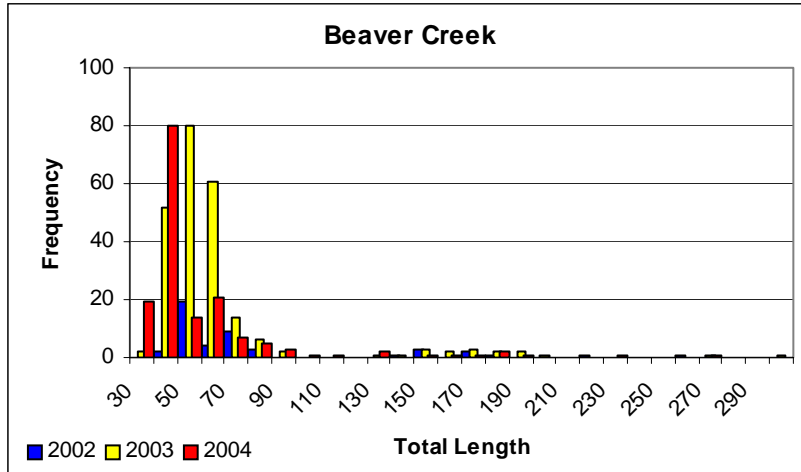
Figure 14. Total length and frequency histograms for fish sampled in each of the tributary populations of the Maggie Creek drainage (2002-2004; data from Harig *et al.* 2004).
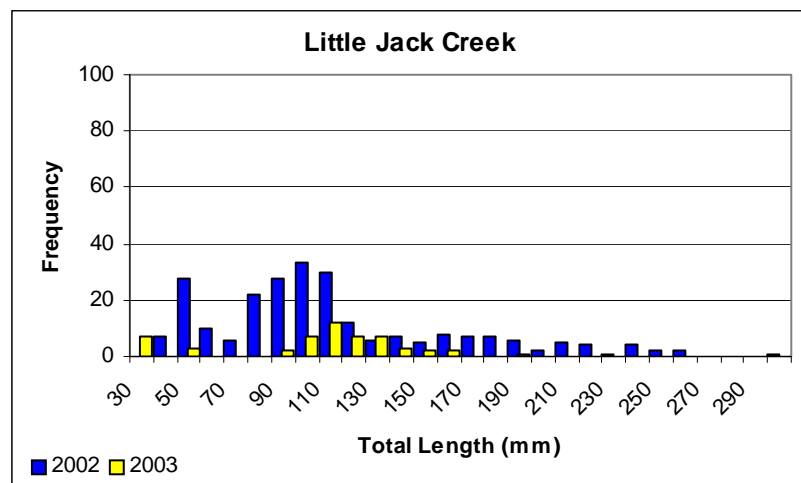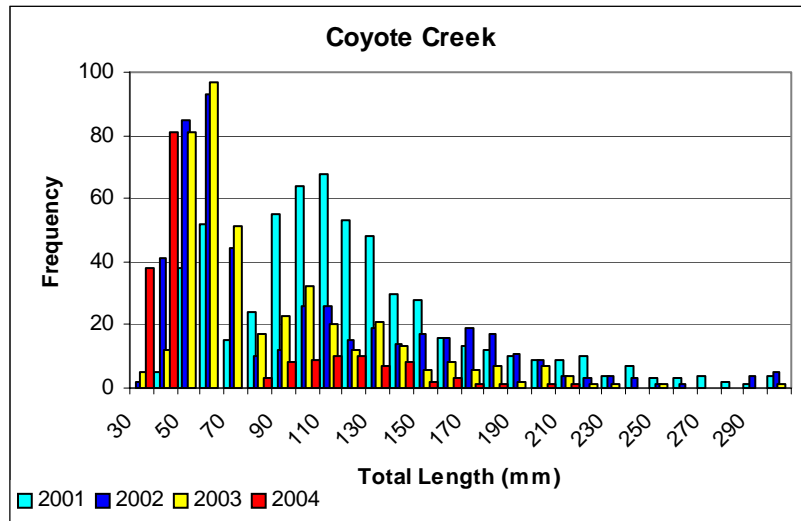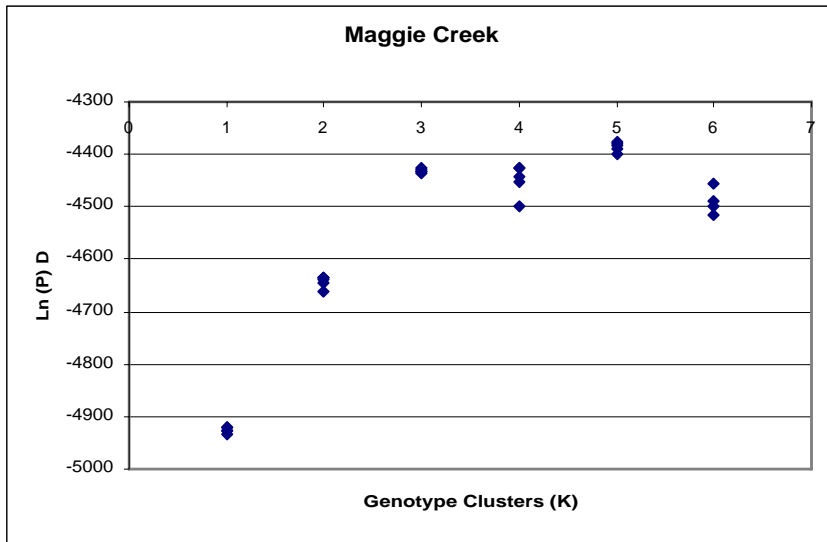
Figure 15. Results of STRUCTURE analysis for the Maggie Creek drainage showing the LnP(D), for each iteration per genotype cluster (*K*) designated (1-6 clusters modeled). The best fit of the data is five clusters, which has the highest LnP(D) = - 4385.63 (see text).
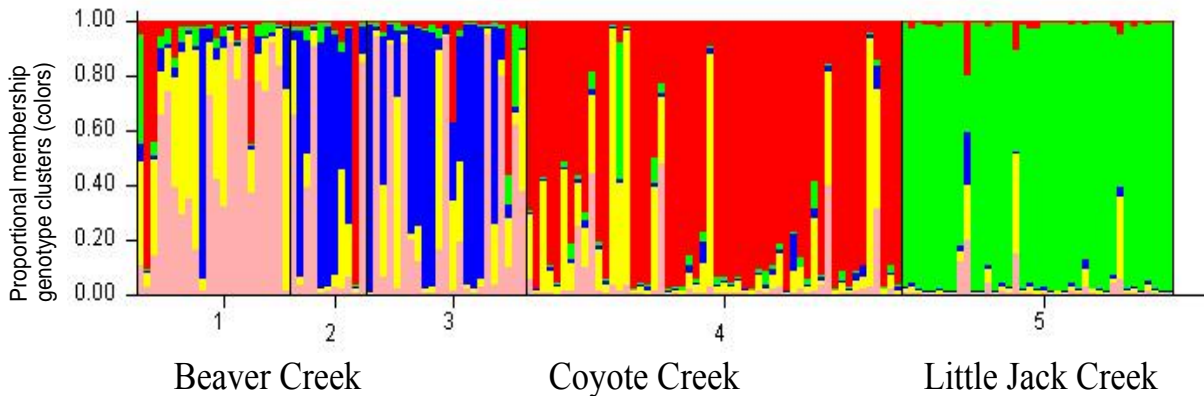
Figure 16. Results of STRUCTURE analysis for the Maggie Creek drainage. The colors represent each of the five distinct genotype clusters. Each individual fish included in the analysis is represented in this figure. Each individual and their proportional membership in each of five genotype clusters (*K*) are represented by the histogram. The colors represent each of the five distinct genotype clusters. The geographic location of each of these clusters is indicated on the X-axis (1-3 represent Beaver Creek, 4 Coyote Creek and 5 Little Jack Creek).

Table 4. Proportion of individuals of each population assigned to each of 5 genotype clusters. Results represent the average over the five, 100,000 iterations runs conducted in STRUCTURE [LnP(D) = -4384.3]. Highest proportional memberships are bolded for each population.

	Genotype Clusters						
	1	2	3	4	5	Sum	N
Population							
Toro Canyon	**0.432**	**0.333**	0.081	0.108	0.046	1.0	22
Main stem Beaver	0.284	0.158	**0.450**	0.083	0.025	1.0	34
Coyote	0.050	0.140	0.016	**0.755**	0.030	1.0	54
Little Jack	0.024	0.033	0.015	0.018	**0.910**	1.0	39

Isolated stream populations

3) What is the population genetic structure of extant LCT populations within and among watersheds within each DPS?

North Fork Humboldt subbasin. The upper North Fork Humboldt River and Foreman and Gance creeks were interconnected historically (see Figure 7) and may have behaved as a metapopulation or networked stream system (*as per* Ray *et al.* 2000; Neville *et al.* 2006), but have been isolated from one another and from other Humboldt River subbasins for the past 60-100 years. Pairwise F_{ST} estimates, however, suggest long term patterns of gene flow prior to habitat fragmentation not only among North Fork Humboldt River tributary populations but also among neighboring subbasins (Table 5). STRUCTURE results show four contemporary genotypic clusters within the North Fork Humboldt subbasin [LnP(D); -4528.11; $P = 1.0$; Figure 16&17] with two distinct clusters present in the North Fork Humboldt River. The upper North Fork Humboldt River has 19 km of occupied stream habitat compared to 3.54 km in Frazer, 5.93 in

Foreman, and 8.55 in Gance creeks and concomitantly the largest LCT population among these

four streams (see Figure 2). Current isolation of gene pools within the North Fork Humboldt

River subbasin is evident, as there is very little overlap in cluster membership among extant

tributary populations. Frazer Creek forms a singular distinct genotype cluster consistent with its

isolated status (Figure 18). These four populations show evidence of genetic bottlenecks under

the infinite alleles mutation model (IAM; Wilcoxon Sign Rank test, $P \leq 0.001$), but not under the

TPM or SMM.

Table 5. Pairwise F_{ST} estimates between LCT populations within and among the geographically proximate subbasins of the main stem Humboldt River: North Fork Humboldt River, Maggie Creek and Rock Creek subbasins. All populations are significantly genetically differentiated. *P*-values were obtained after 420 permutations. Indicative adjusted nominal level (5%) for multiple comparisons is 0.00238. North Fork Humboldt River subbasin tributaries are currently physically isolated - Foreman Creek, Gance Creek, and North Fork Humboldt River (NFH)from each other, whereas Maggie Creek subbasin tributaries (Beaver, Little Jack, and Coyote creeks) are interconnected. Frazer Creek is the only occupied stream sampled in the Rock Creek subbasin (see Figure 7).

	NF Humboldt River			**Maggie Creek**		
	Foreman	Gance	NFH	Little Jack	Coyote	Beaver
North Fork Humboldt River						
Gance	0.0906					
NFH	0.0787	0.0642				
Maggie Creek						
Little Jack	0.1316	0.1287	0.1007			
Coyote	0.0816	0.0896	0.0734	0.0936		
Beaver	0.0803	0.0693	0.0574	0.0699	0.0446	
Rock Creek Subbasin						
Frazer	0.1967	0.1443	0.1376	0.1962	0.1494	0.1414

North Fork Humboldt

Ln (P) D vs Genotype Clusters (K)
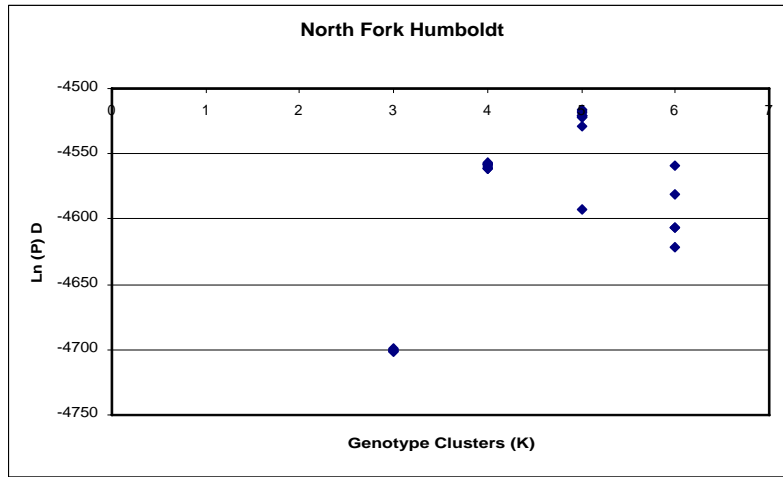
Figure 17. Results of
STRUCTURE analysis for the
North Fork Humboldt River and
Rock Creek subbasin populations
showing the LnP(D) for each
iteration per genotype cluster (*K*)
designated (1-6 clusters
modeled). The best fit of the data
is five clusters with the highest
LnP(D) (see text).
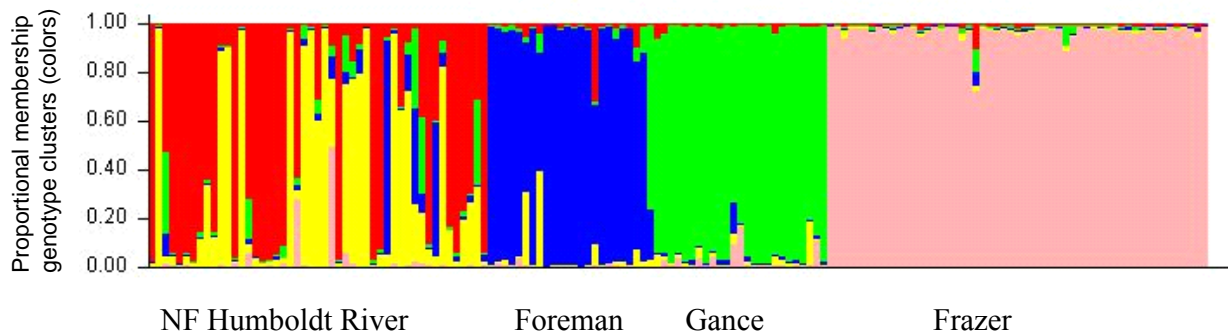
Figure 18. The proportional membership of each individual fish in each of five genotype
clusters can be assessed by the proportion of each color assigned to each individual column, e.g.,
individual number one in the North Fork Humboldt River is primarily red, whereas individual
two is primarily yellow, suggesting these individuals come from two separate breeding groups.
Individual three assigns to the red, green, blue and yellow clusters, which suggests mixed
ancestry from the different interbreeding clusters. The geographic location of each of these
clusters is indicated on the X-axis.

Reese River subbasin. Mohawk and Tierney creeks in the Reese River subbasin, which

historically drained into the larger Humboldt River, support relatively small populations

compared to streams in the North Fork Humboldt and Rock Creek subbasins despite similar

length (linear km) of occupied habitat (see Figure 2). Dunham *et al.* (2002) showed that LCT

density is related to stream channel morphology and regression quantile models indicate that

variation in LCT densities are inversely related to the width:depth ratio of streams. Mohawk and

Tierney creeks are small habitats where the stream channel tends to be narrow and stream depth

shallow. Anthropogenic impact on fish density is evident in Tierney Creek, which has

approximately 13 km of occupied habitat but a LCT population that is consistently smaller than

that found in Mohawk Creek with only 4.67 km of occupied habitat (Figure 19a). The riparian

zone of Tierney Creek has been highly impacted by cattle grazing and subsequent erosion

(Figure 19b) such that temperatures in portions of Tierney Creek are likely to exceed upper

lethal limit for LCT (Dickerson and Vinyard 1999; Dunham *et al.* 2003) during the summer

months.

The pairwise F_{ST} estimate (0.702) between LCT populations in Tierney and Mohawk creeks

shows them to be *highly* differentiated from each other and all other Humboldt River populations

(Table 6; $P = 0.0005$). Genetic bottlenecks were evident under all mutation models tested (IAM,

TPM and SMM; Mohawk, $P \leq 0.002$ and Tierney, $P = 0.0009$). Mohawk and Tierney creeks

have the lowest average heterozygosities ($\overline{H} = 0.138, 0.237$) of all the populations sampled with

the exception of three populations of Western DPS origin transplanted into out-of-basin

locations.
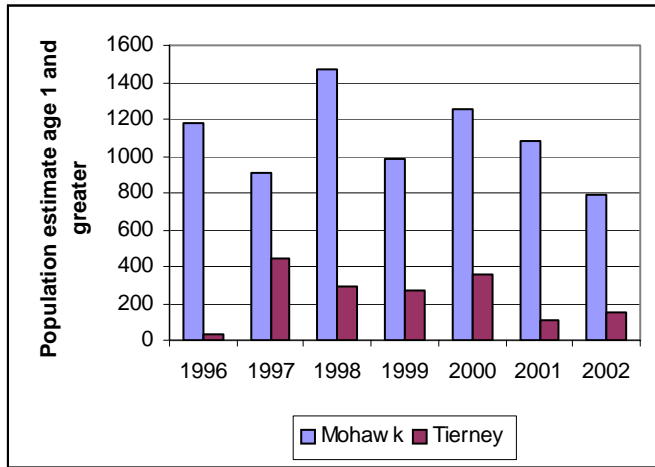
A.   **B.**

Figure 19. A. Population size estimates for LCT ≥ 1 year for occupied reaches in Mohawk and Tierney creeks in the Reese River subbasin from 1996-2002 (Peacock, *unpublished data*). **B.** Downcut banks and erosion on Tierney Creek (photo Jason Dunham).

Four genotype clusters were identified in the STRUCTURE analysis (LnP(D) = -449.883, P = 0.98; K = 2, 5, and 6 had little statistical support with $P = 1.0536e^{-9}$, $4.264e^{-13}$, and $3.119e^{-21}$ respectively, K = 3 had greater statistical support (P = 0.02), but was a lower probability than K = 4) with two distinct clusters in each creek (Figure 20&21). Together with the F_{ST} analysis, the STRUCTURE results suggest contemporary isolation of these gene pools and little if any historical gene flow between these populations. However, genetic bottlenecks can increase levels of apparent differentiation through chance loss of allelic diversity during the bottleneck process. Individuals in Mohawk Creek assign with high proportional membership primarily to one genotype cluster or the other suggesting little interbreeding among these two groups. This could reflect a spatial structuring of the genotype clusters within the occupied reach of Mohawk Creek.

Individuals in Tierney Creek assign to both clusters equally, which suggests interbreeding

among genotype clusters that may have once been spatially discrete.
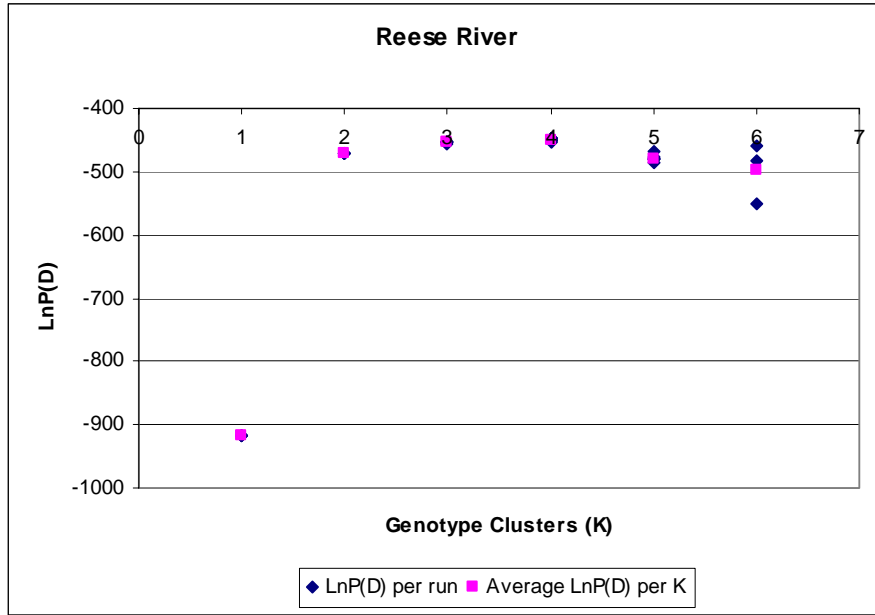
Figure 20. Results of STRUCTURE analysis for the Reese River drainage showing the LnP(D) for each iteration per genotype cluster (*K*) designated (1-6 clusters modeled). The best fit of the data is four clusters (LnP(D) = -449.883, *P* = 1; see text).
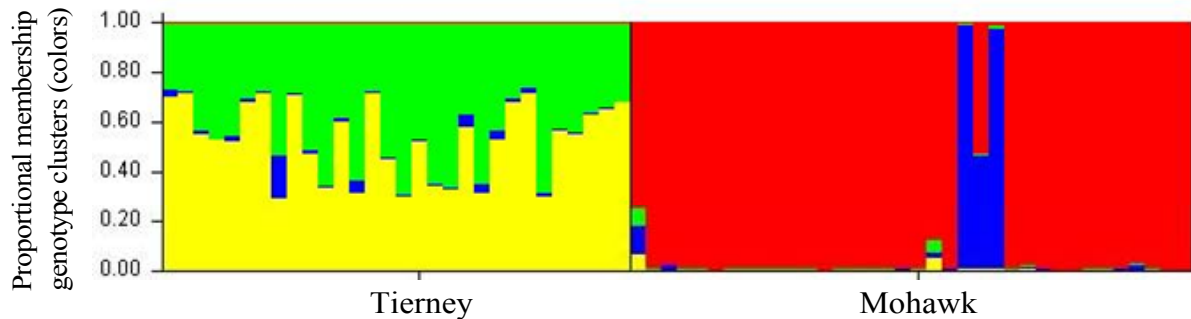
Figure 21. Results of STRUCTURE analysis for the Reese River drainage. The colors represent each of the four distinct genotype clusters. Each individual and their proportional membership in each of four genotype clusters (*K*) are represented in this histogram (*K* = 4, *P* = 0.98). The geographic location of each of these clusters is indicated on the X-axis.

Table 6. Pairwise F_{ST} estimates between Reese River populations and the Marys River, North

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Fork Humboldt River, Maggie Creek, Rock Creek, and Little Humboldt River subbasins. *P*-values were obtained after 1820 permutations. Indicative adjusted nominal level (5%) for multiple comparisons is 0.000549.

	Mohawk	Tierney
Marys River		
EMR	0.462	0.390
WMR	0.449	0.384
North Fork Humboldt River		
Foreman	0.522	0.451
Gance	0.485	0.424
NFH	0.412	0.369
Rock Creek		
Frazer	0.481	0.443
Maggie Creek		
Little Jack	0.486	0.455
Coyote	0.427	0.381
Beaver	0.433	0.374
Little Humboldt River		
Abel	0.527	0.484
Indian	0.552	0.506

STRUCTURE analysis of all populations (*N* = 11) sampled from the main stem Humboldt and Reese rivers revealed 14 distinct genotype clusters (LnP(D) = -12415.3, *P* = 1, Figures 22). Overlap in genotype cluster membership within the interconnected stream systems of the Marys River (EMR and WMR) and within Maggie Creek (Beaver, Coyote, and Little Jack creeks) drainages is evident (Figure 20). Overlap is also evident among genotype clusters found in the North Fork Humboldt River tributaries that are now physically isolated, but were connected historically. Long-term patterns of gene flow among subbasins is also evident from this analysis, as genotype cluster membership is not confined to single subbasins but spans multiple subbasins and populations therein. Genotype cluster membership among subbasins is largely predicted by geographic proximity, e.g., WMR in the Marys River subbasin has 20% membership in cluster 4 and Foreman Creek in the North Fork Humboldt River subbasin has ~15% membership in this

same cluster, whereas the Reese River populations are quite isolated and show very little overlap

with the main stem Humboldt River populations.
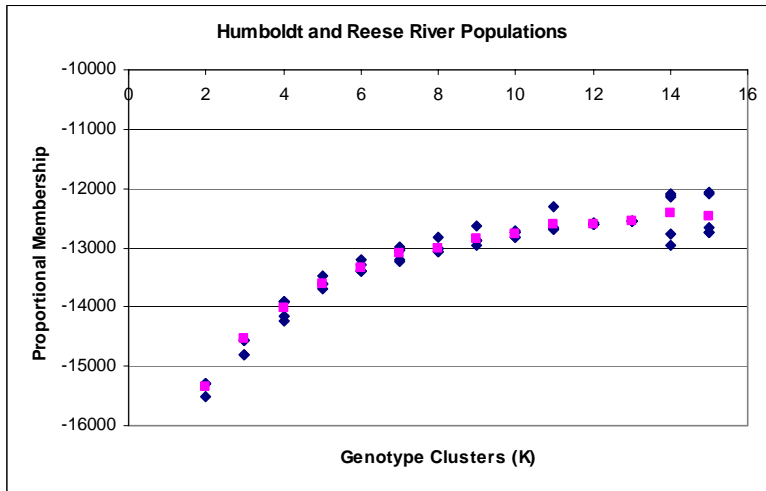
Figure 22. Results of STRUCTURE analysis for the Humboldt and Reese river populations showing the LnP(D), for each iteration (blue diamonds) per genotype cluster (*K*) designated (1-15 clusters modeled), pink squares represents average LnP(D) per K. Fourteen clusters was the best fit of the data [LnP(D) = -12415.3, *P* = 1, see text].
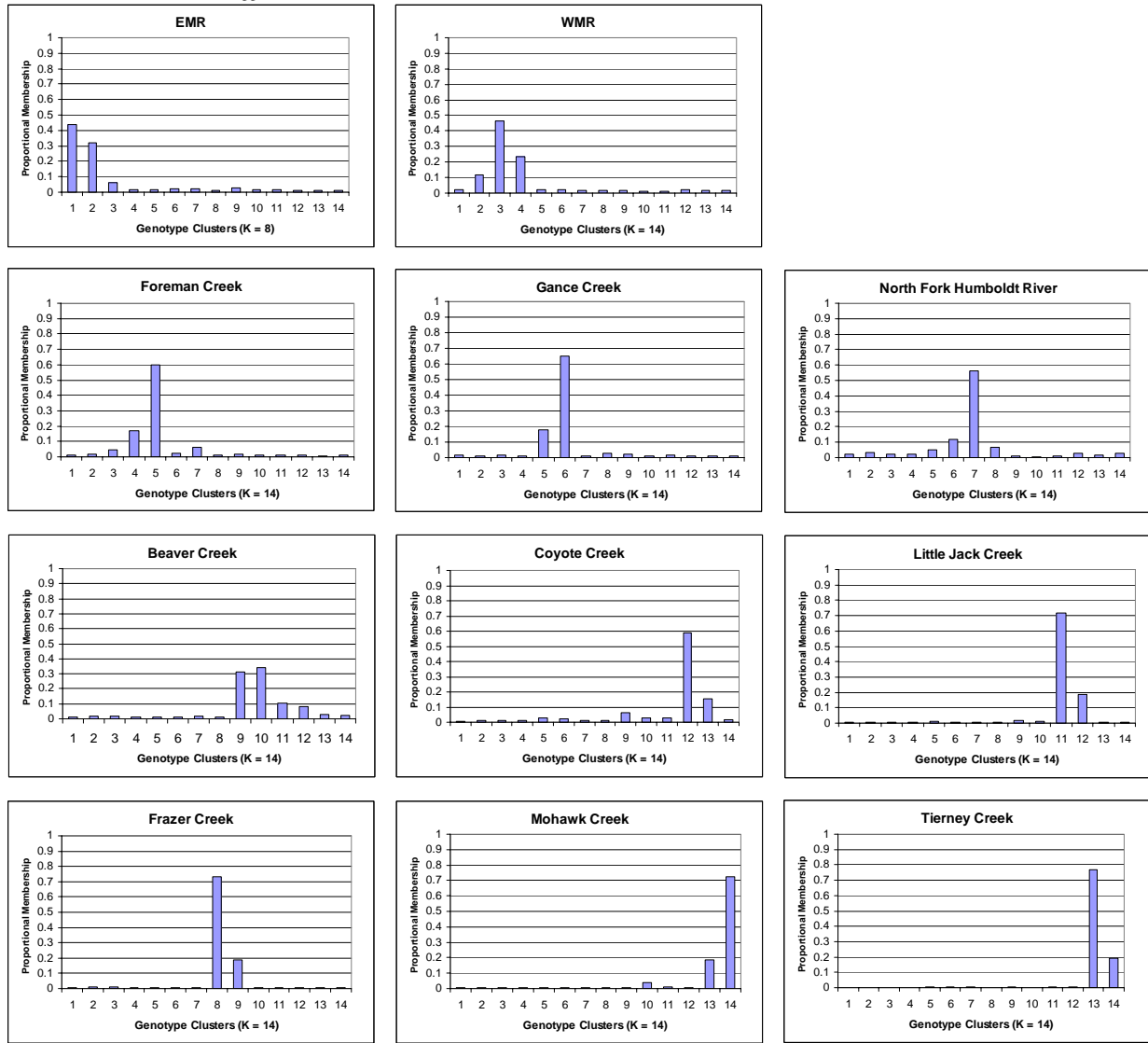
Figure 23. Results of STRUCTURE analysis for main stem Humboldt and Reese river populations, revealing multiple genotype clusters per population and overlap in cluster membership within and among subbasins. Fourteen genotype clusters had the highest LnP(D) = -124.3, *P* = 1.

<u>Little Humboldt River Subbasin</u>. Abel and Indian creek LCT populations are very small and

isolated (Figure 24). LCT occupy ~2 km of habitat in Abel Creek and are being pushed into the

headwater reaches by nonnative brook trout encroachments, which have in recent years breached

what had been a barrier to upstream movement. Indian Creek has < 2 km of occupied habitat and a concomitant small population size (see Figure 24). These populations although geographically proximate, show significant genetic differentiation from each other ($F_{ST} = 0.397$, $P = 0.000549$) and all other Humboldt River populations (Table 7). Abel and Indian creek populations are also significantly bottlenecked, but under IAM only (Wilcoxon Sign Rank test; Abel, $P = 0.005$; Indian, $P = 0.003$) with average heterozygosities lower than all other Humboldt River populations sampled (Abel, $\overline{H} = 0.4435$; Indian, $\overline{H} = 0.526$) with the exception of Mohawk and Tierney Creeks.

Table 7. Pairwise F_{ST} estimates between Abel and Indian Creeks and the Marys River, North Fork Humboldt River, Maggie Creek, Rock Creek and Reese River subbasins. *P*-values were obtained after 1820 permutations. Indicative adjusted nominal level (5%) for multiple comparisons is 0.000549.

	Abel	Indian
Marys River		
EMR	0.272	0.226
WMR	0.279	0.193
North Fork Humboldt River		
Foreman	0.317	0.222
Gance	0.289	0.213
NFH	0.263	0.184
Rock Creek		
Frazer	0.329	0.219
Maggie Creek		
Little Jack	0.340	0.217
Coyote	0.266	0.216
Beaver	0.273	0.191
Reese River		
Mohawk	0.527	0.552
Tierney	0.484	0.506
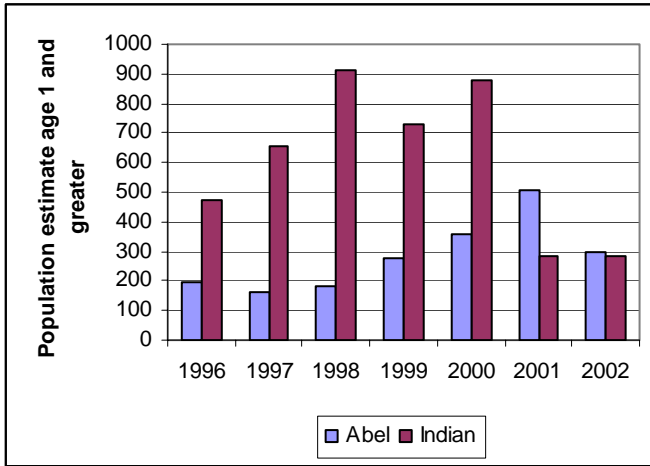
Figure 24. LCT population size estimates for occupied reaches in Abel and Indian creeks (1996-2002) for fish ≥ 1 year (Peacock, *unpublished data*).

Bayesian genotype cluster analysis revealed additional structure within and between Abel and Indian creek populations. Four genotype clusters were identified, with one cluster in Abel Creek and three distinct clusters in Indian Creek [average LnP(D) = -1127; *P* = 0.993, Figure 25]. No overlap was evident in cluster membership between these two populations (Figure 26). Ninety-nine percent of Abel Creek fish assigned to cluster 1, while 50.8% of Indian Creek individual assigned to cluster 2, 26.6% to cluster 3, 22.1% to cluster 4 and with < 0.5% assigning to cluster 1.
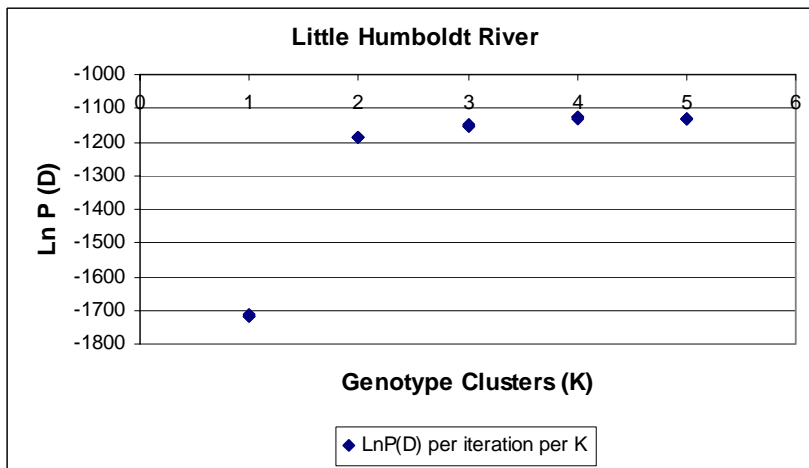
Figure 25. Results of STRUCTURE analysis for the LCT populations in the Little Humboldt River showing the LnP(D), for each iteration (blue diamonds) per genotype cluster (*K*) designated (1-5 clusters modeled). The best fit of the data is four clusters [LnP(D) = - 1127.8, *P* = 0.993 (see text)].
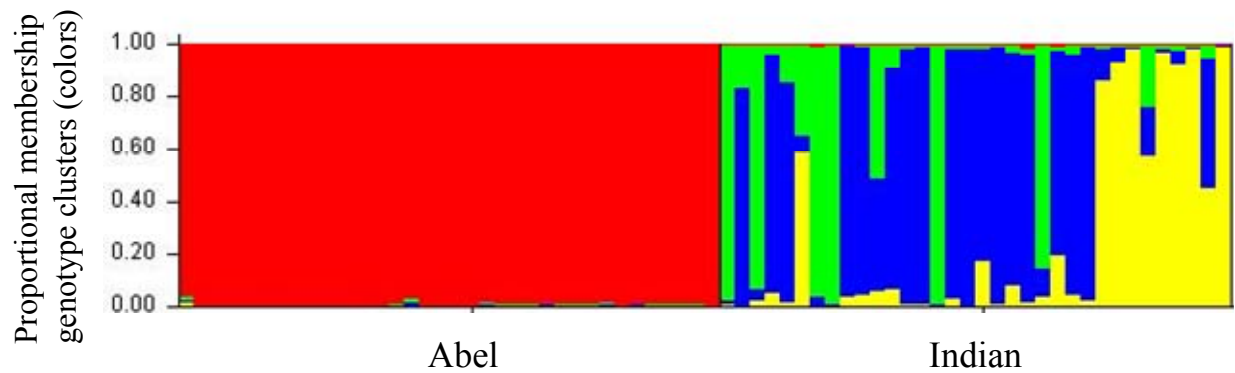
Figure 26. Results of STRUCTURE analysis showing proportional membership of each individual sampled in 4 genotype clusters. LCT in Abel Creek form a distinct genotype cluster with no overlapping membership among Indian and Abel creek LCT.

Northwestern DPS. Currently only 15% of the streams in the Quinn River drainage are occupied by Lahontan cutthroat trout, and most of these habitats are isolated headwater reaches above barriers (Sevon *et al.* 1999). Streams sampled for this study (Washburn, Crowley, 3 Mile and McDermitt creeks) historically flowed into the main stem Quinn River, though none do so today (Figure 27). The streams in the McDermitt Creek drainage remain interconnected, but non-native salmonids threaten the integrity of this LCT population network (Peacock and Kirchoff 2004). Riser, upper McDermitt, lower Sage, and Indian creeks contain introgressed (Lahontan cutthroat trout x rainbow trout) populations (Williams *et al.* 1998; Peacock and Kirchoff 2004). Line Canyon Creek currently, with ~3.5 km of habitat above a natural barrier, has the only non-hybridized population of LCT in the McDermitt Creek system.

Line Canyon, Washburn, and Crowley creeks have some of the lowest population estimates of all sampled streams contain native LCT populations sampled, given the size of occupied habitat (Figure 28). However, despite Line Canyon Creek's small population size, average

heterozygosity ($\overline{H} = 0.619$) and average allelic richness ($R_S = 4.67$) were high compared to

Washburn ($\overline{H} = 0.391$, $R_S = 3.2899$) and Crowley ($\overline{H} = 0.3489$, $R_S = 3.0699$) creeks and the

much larger 3 Mile Creek population ($\overline{H} = 0.4518$, $R_S = 3.3126$). All populations were

significantly differentiated from one another genetically ($P = 0.0083$, P-values obtained after 120

permutations) with pairwise F_{ST} estimates suggesting both significant contemporary and

historical isolation among these populations (Table 8). LCT populations in Washburn and Line

Canyon creeks were shown to be genetically bottlenecked under IAM only (Wilcoxon Sign Rank

test, $P = 0.00488$, 0.00928 respectively). Crowley and 3 Mile were bottlenecked under all

mutation models tested (IAM, TPM and SMM; Wilcoxon Sign Rank test, $P \leq 0.05$).

Figure 27. The Quinn River basin in northeastern Lahontan Basin shown in light shading on smaller inset map of the entire Lahontan basin. The larger map shows the McDermitt Creek drainage, Washburn, Crowley and 3 Mile creeks (Figure reprinted from Peacock and Kirchoff (2004) *Transactions of the American Fisheries Society* 133:309-325).

Additional population genetic structure was evident from the Bayesian cluster analysis. Eight

distinct genotype clusters were identified with little to no overlap in cluster membership among

individuals from separate streams (Figure 28). LCT in Washburn and Crowley creek populations

assigned primarily to single genotype clusters whose membership is confined primarily to those

streams [clusters 5 (94.6%) and 8 (86.9%)]. Line Canyon and 3 Mile creeks had two distinct

genotype clusters each (Line, 6&7; 3 Mile, 2&3). Cluster 4 was found in all populations but with

< 4% proportional membership in any single population. Indian Creek in the Little Humboldt

River subbasin was included for an out-of-basin comparison. Indian Creek individuals assigned

primarily to cluster 1 (96%), which had < 0.5% assigned membership in the Quinn River

populations.

Table 8 Pairwise F_{ST} estimates between Washburn, Crowley, 3 Mile and Line Canyon creeks in the Quinn River basin. *P*-values were obtained after 120 permutations. Indicative adjusted nominal level (5%) for multiple comparisons is 0.008).

	Washburn	Crowley	3 Mile
Crowley	0.3690		
3 Mile	0.4418	0.4407	
Line Canyon	0.1902	0.3106	0.2765

The Willow-Whitehorse LCT found in the Coyote Lakes Basin of Southeastern Oregon, are

distinct genetically from all other LCT populations. Pairwise F_{ST} estimates range from 0.1303 –

0.5865 with all but one estimate above 0.15 (indicating *great* genetic differentiation), and the

majority (64%) above 0.25 indicating *very great* differentiation. The lowest pairwise F_{ST}

estimates were with the North Fork Humboldt River tributaries. All pairwise estimates with the

Quinn River LCT populations were > 0.25.
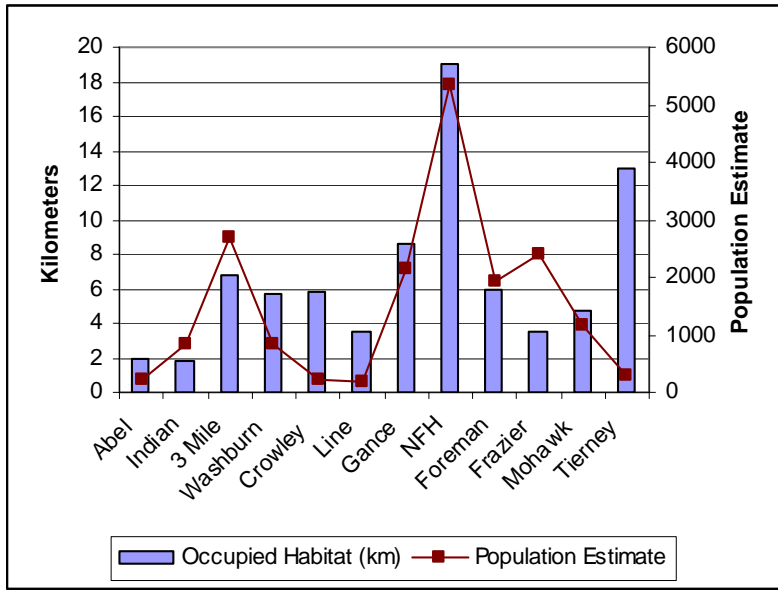
Figure 28. Kilometers of occupied habitat (blue histogram) and LCT population estimates (dark squares) from a subset of populations included in a long term LCT population viability study (Peacock, *unpublished data*).
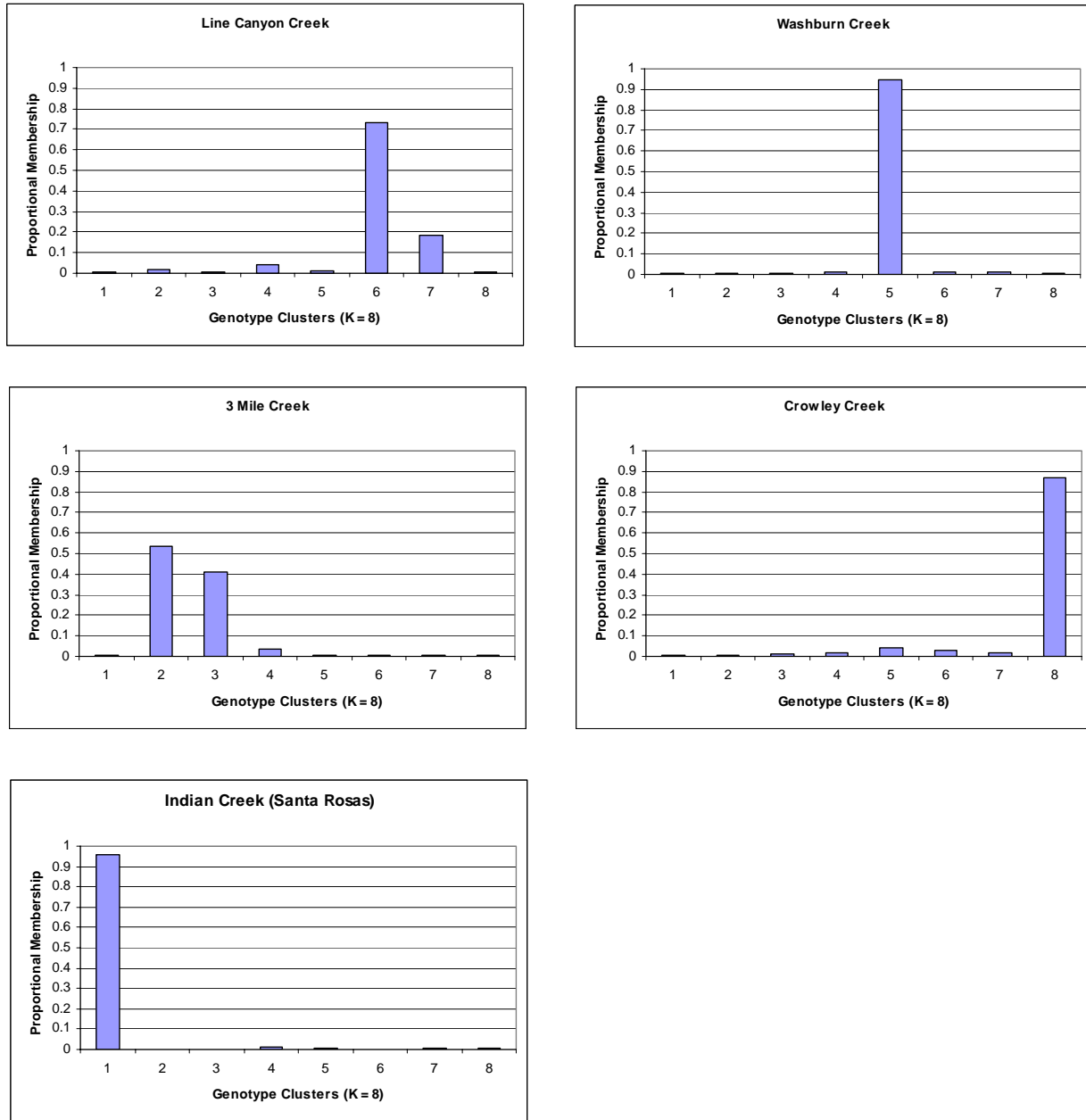
Figure 29. Eight genotype clusters were identified for the four populations sampled from the Quinn River basin (Line Canyon, 3 Mile, Washburn and Crowley creeks) and one population from the Little Humboldt River subbasin (Indian Creek). Each panel represents the average proportional membership of each population in each of the eight genotype clusters [LnP(D) = -2843.07, $P = 1.0$).

Western DPS.

Truckee River basin**.** There are no extant fluvial LCT populations native to Truckee River watershed. Independence Lake is the only extant native in-basin LCT population. STRUCTURE analysis revealed two genotype clusters within the Independence Lake population [LnP(D) - 11143.14, $P = 0.999$].

Carson River basin. Three of the four native extant fluvial LCT populations in the Carson River basin (East Carson River, Murray and Poison Flat creeks) were sampled for this study in addition to three out-of-basin populations of Carson River origin (Pacific Valley River, Milk Ranch and Marshall Canyon creeks in the Mokelumne River drainage, California). The out-of-basin populations have low average heterozygosities ($\overline{H} = 0.264, 0.157, 0.228$ respectively), low allelic richness (**Appendix 1**) and are significantly bottlenecked under all mutation models tested (Wilcoxon Sign Rank test; Pacific $P = 0.0005$, Marshall $P \leq 0.001$, Milk Ranch $P \leq 0.005$). These data suggest small founder populations. Average heterozygosity of the extant in-basin fluvial Carson basin populations is higher ($\overline{H} = 0.535, 0.502, 0.51$) and comparable to other native and naturally occurring fluvial populations in the Humboldt River basin. East Carson River and Murray Creek show evidence of genetic bottlenecks under IAM and TPM (Wilcoxon Sign Rank test; East Carson $P \leq 0.04$, Murray $P \leq 0.009$), and Poison Flat Creek under all three mutation models ($P \leq 0.005$). All Carson River populations were significantly differentiated from each other (Table 9). Pairwise F_{ST} estimates among the extant in-basin fluvial Carson River populations suggest moderate levels of genetic differentiation, whereas all pairwise F_{ST} estimates among the out-of-basin populations suggest these are highly differentiated gene pools.

Population level phylogenetic analysis shows the extant Carson River populations forming a

single clade distinct from the out-of-basin populations, which cluster into a separate clade

(Figure 30). The low bootstrap support for most tree nodes within each clade (except East

Carson River and Murray Creek) likely reflects the low levels of genic diversity in these

populations as a result of small founder populations, which can result in a loss of phylogenetic

signal. If the Carson River populations are modeled as two distinct gene pools in STRUCTURE the

extant native Carson River basin populations form one genotype cluster and the out-of-basin

populations form another. Although two genotype clusters is not the best fit for the data, the two

cluster analysis shows the out-of-basin populations are more similar to each other than to the

extant fluvial Carson River basin populations. The best fit of the data is $K = 9$ [Figure 31;

average LnP(D) = -2646.07, $P = 1$]. The East Carson River and Murray Creek populations in the

Carson River basin show significant within stream genetic structure. Multiple genotype clusters

were evident within these LCT populations (Figure 32).
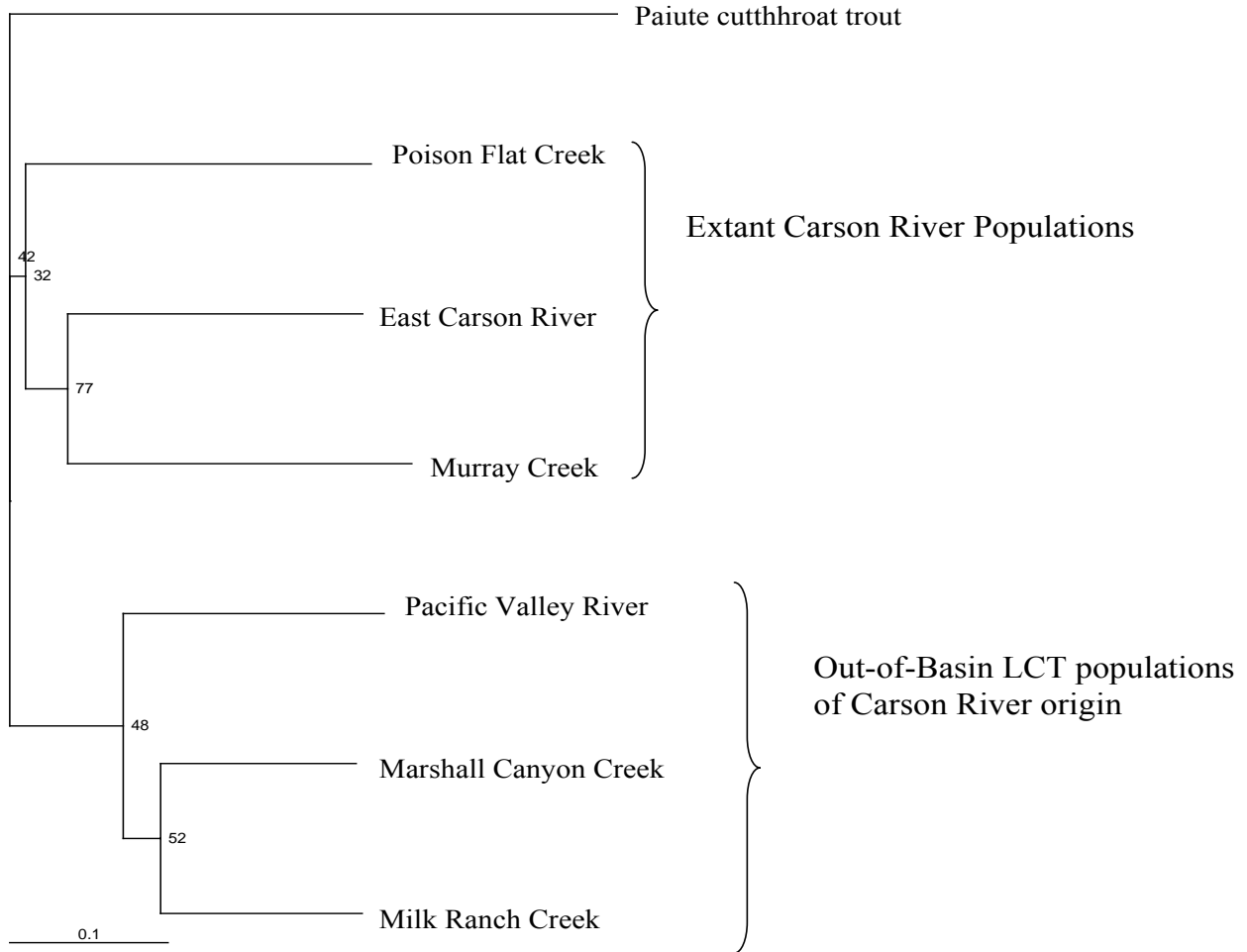
Figure 30. Phylogenetic analysis of Carson River LCT populations using a Cavalli-Sforza genetic distance measure and a neighboring-joining tree with Paiute cutthroat trout as outgroup. 2000 iterations were conducted in the program POPULATIONS (version 1.2.26) with bootstrap values indicated at the tree nodes. Populations group weakly into two separate clades, extant in-basin populations (East Carson River, Murray and Poison Flat creeks) and out-of-basin populations derived from Carson River LCT (Pacific Valley River, Marshall Canyon and Milk Ranch creeks). Scale represents genetic distance.

Table 9. Pairwise F_{ST} estimates between populations of Carson River origin. Indicative adjusted nominal level (5%) for multiple comparisons, $P = 0.0033$, obtained after 300 permutations.

	E Carson	Murray	Poison	Pacific	Marshall
Murray	0.1362				
Poison	0.1457	0.1771			
Pacific	0.3261	0.3977	0.2936		
Marshall	0.4127	0.4511	0.3780	0.4837	
Milk Ranch	0.3103	0.3530	0.3258	0.4270	0.3519
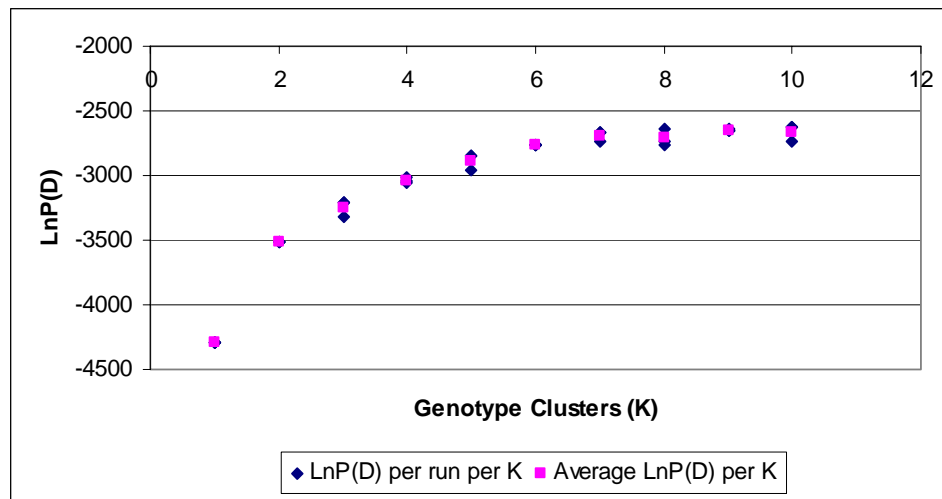
Figure 31. Results of STRUCTURE analysis for the Carson River populations showing the LnP(D), for each iteration (blue diamonds) and average (pink squares) per genotype cluster (*K*) designated (1-10 clusters modeled). The best fit of the data is nine clusters [LnP(D) =,-2646.07, $P = 0.999$; (see text)].
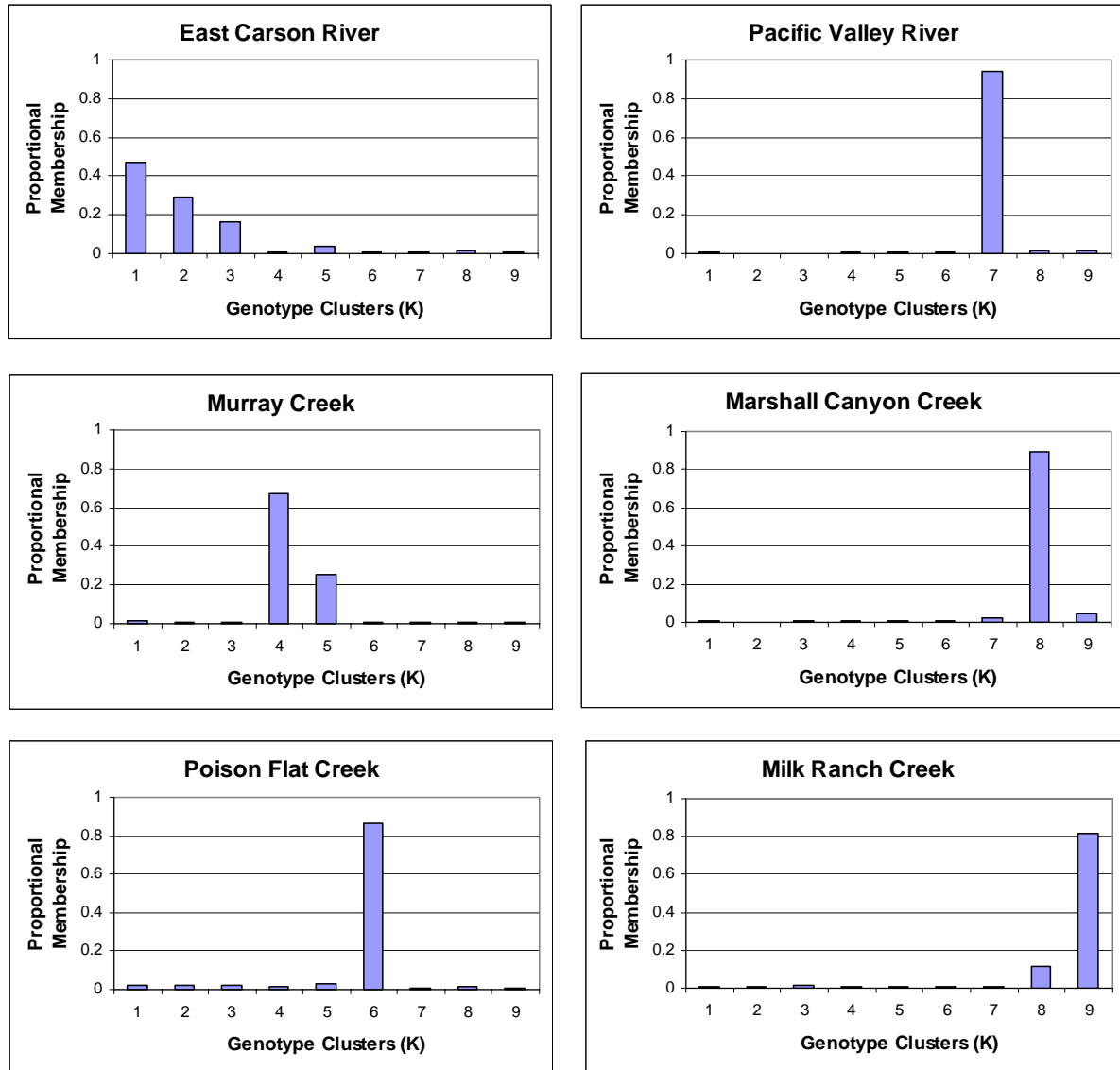
Figure 32. Nine genotype clusters were identified for the six Carson River basin populations sampled. Each panel represents the average proportional membership of each population in each of the eight genotype clusters.

Walker River basin. All Walker basin fluvial LCT populations were effectively extirpated in the early 20th century. By the early 1900's a single small population of LCT was found in By-Day Creek where LCT occupy ≈ 3 km of stream habitat. The population is small and has low average heterozygosity ($\overline{H}$ = 0.309) and allelic richness (**Appendix 1**). Stocking records suggest that

LCT from By-Day Creek were subsequently planted into Slinkard Creek and from Slinkard into

Wolf, Mill and Silver Creeks. The populations established from By-Day also have low

heterozygosities ($\bar{H}$ = 0.324, 0.314, 0.276, and 0.352) and allelic richness. All populations are

fixed for a single allele at OCH 16 (216), which has 23 alleles overall that have been identified

throughout the range of LCT populations sampled.

STRUCTURE analysis revealed three genotype clusters among the four Walker basin populations

[LnP(D) = -1205.03, P = 1.0; Figure 33]. Contrary to stocking records the Wolf, Mill and Silver

creek populations do not appear to be derived from the Slinkard Creek population. LCT in By-

Day, Wolf, Mill and Silver have proportional membership in two primary genotype clusters (red

and blue, Figure 34), whereas Slinkard Creek individuals are assigned to a third cluster (green)

that has very little representation in the other four populations.
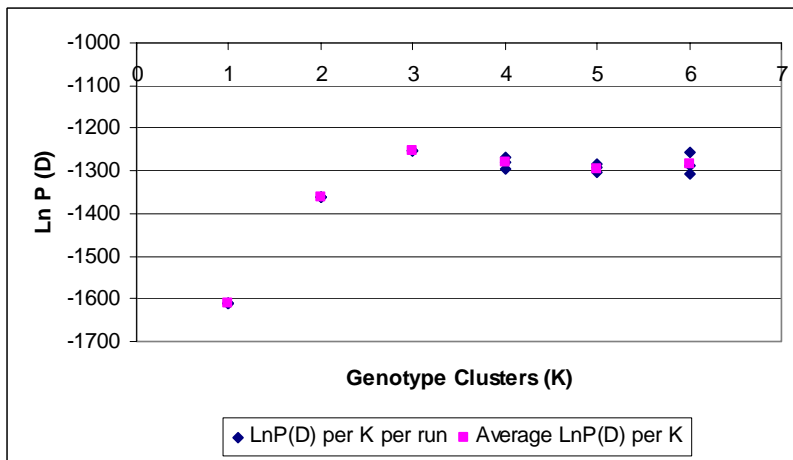
Figure 33. Results of STRUCTURE analysis for the Walker River populations showing the LnP(D), for each iteration (blue diamonds) and average (pink squares) per genotype cluster (*K*) designated (1-6 clusters modeled). The best fit of the data is three clusters, which has the highest LnP(D) = -1252.03, P = 1; K = 2, P = $1.99521e^{-48}$; K = 4, P = $4.79196e^{-13}$ (see text).
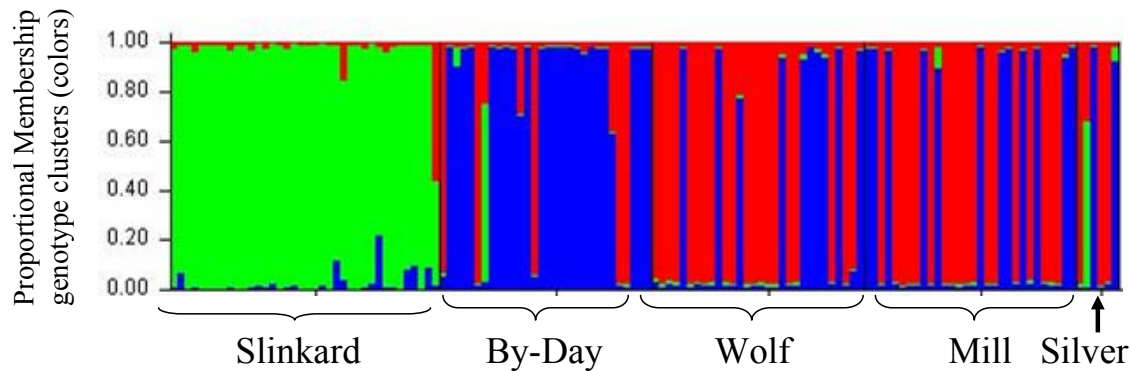
Figure 34. Results of STRUCTURE analysis for Walker basin populations showing proportional membership (Y axis) of each individual sampled in each of three genotype clusters. Individuals in Slinkard Creek form a distinct genotype cluster from By-Day, Wolf, Mill and Silver creeks.

These data suggest that the Slinkard Creek population was either founded with a small number of fish non-randomly sampled from By-Day or Slinkard Creek has suffered significant bottleneck event(s) in which genetic diversity was lost. All populations are bottlenecked under all three mutation models ($0.002 \geq P \geq 0.0009$). The pattern of proportional membership in the three genotype clusters, however, suggests that Slinkard was founded from a non-random sample from By-Day and that Wolf, Mill and Silver creeks were founded directly from By-Day LCT and not from LCT in the Slinkard Creek population.

Range-wide phylogenetic analyses do not show Walker basin populations clustering with other Western basin DPS populations or with other LCT populations from other basins for that matter. Instead, they cluster with high bootstrap support with rainbow trout (Figure 35). These data suggest either hybridization with rainbows or that the phylogenetic signal has been lost due to low levels of heterozygosity resulting from small population size, random genetic drift and/or repeated genetic bottlenecks. Pairwise F_{ST} estimates between Walker basin populations, other

Western Basin DPS populations and rainbow trout show Walker basin LCT populations to be

highly differentiated (Table 10). Wolf and Mill creeks show low to moderate differentiation from

By-Day creek (F_{ST} = 0.045, 0.106), whereas Slinkard is greatly differentiated from all other LCT

populations in the Western DPS including By-Day Creek (all $F_{ST} \geq 0.4$, with exception of

Independence, F_{ST} = 0.377), as well as rainbow trout (F_{ST} = 0.554).

Table 10. Pairwise F_{ST} estimates among extant native Western basin DPS populations, Truckee River basin historical museum samples and rainbow trout. All populations are significantly differentiated from each other. Pairwise F_{ST} estimates between Walker River populations are highlighted in red. *P*-values obtained after 1820 permutations. Indicative adjusted nominal level (5%) for multiple comparisons is P = 0.00055 (See table 1 for abbreviations)

	IND	TRM	CAR	MUC	POC	PAC	MIC	MAC	SLC	BDC	WOC	MILL
TRM	0.151											
CAR	0.189	0.211										
MUC	0.284	0.295	0.127									
POC	0.232	0.274	0.137	0.186								
PAC	0.340	0.406	0.223	0.387	0.298							
MIC	0.367	0.436	0.163	0.303	0.271	0.349						
MAC	0.403	0.486	0.262	0.365	0.264	0.305	0.385					
SLC	**0.377**	**0.421**	**0.494**	**0.593**	**0.509**	**0.678**	**0.684**	**0.701**				
BDC	**0.398**	**0.446**	**0.498**	**0.576**	**0.544**	**0.674**	**0.692**	**0.719**	**0.576**			
WOC	**0.389**	**0.432**	**0.485**	**0.557**	**0.527**	**0.656**	**0.674**	**0.702**	**0.554**	**0.045**		
MILL	**0.411**	**0.457**	**0.506**	**0.587**	**0.551**	**0.687**	**0.701**	**0.730**	**0.590**	**0.106**	**0.108**	
RBT	**0.320**	**0.340**	**0.394**	**0.404**	**0.413**	**0.546**	**0.578**	**0.619**	**0.553**	**0.554**	**0.536**	**0.568**
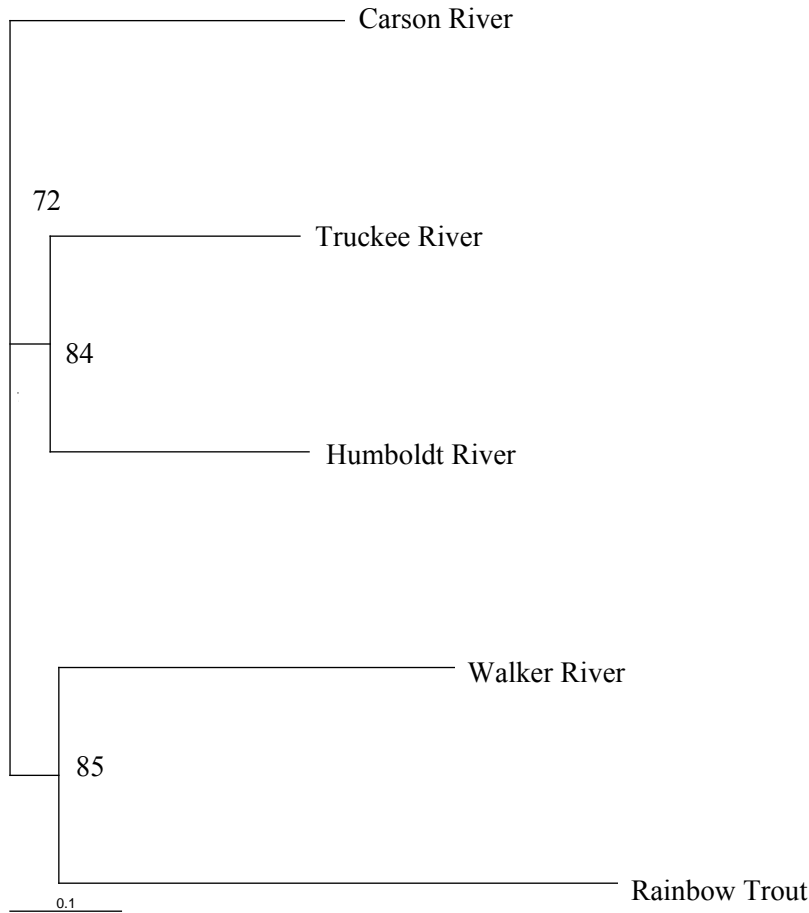
Figure 35. Phylogenetic analysis of Western basin and Humboldt River watersheds using a Cavalli-Sforza genetic distance measure and a neighboring-joining tree with rainbow trout as the outgroup. 2000 iterations were conducted in the program POPULATIONS (version 1.2.26) with bootstrap values indicated at the tree nodes. The Truckee, Carson and Humboldt rivers cluster with strong bootstrap support. The Walker River LCT populations do not cluster with other LCT populations but with Rainbow trout (see text). Scale represents genetic distance.

4) What is the likely origin of out-of-basin transplanted LCT populations based upon genetic comparison with extant LCT populations and museum preserved samples of LCT collected from 1872-1913 from the lower Truckee River and multiple locations in Lake Tahoe?

Hickman and Behnke (1979) suggested morphological resemblances indicate a "probable

Pyramid Lake" origin for the LCT population in Morrison Creek, Utah. Based upon this

analysis, LCT from Morrison Creek were used to start a brood stock for hatchery production of

this strain to be used in Truckee River basin LCT recovery activities. Morrison Creek LCT were

also subsequently planted into neighboring Bettridge Creek. Population level phylogenetic

analysis support a Truckee River basin origin for the Pilot Peak LCT (samples from Morrison,

Bettridge and Pilot Peak hatchery broodstock) which cluster with the Truckee River basin

museum samples with strong bootstrap support (**71%**, Figure 36). Independence Lake LCT,

however, do not cluster with Truckee River basin museum samples and are distal to all other

LCT populations of Western basin origin. Overall the population-level phylogeny for the

Western basin DPS remains very unresolved, despite a number unambiguous relationships:

Poison Flat Creek clusters with other Carson River populations with strong bootstrap support

(**80%,** Figure 36), Slinkard Creek clusters with the other Walker River populations (**92%**,), and

Pyramid Lake clusters with Summit Lake (**89%**), which reflects the Summit Lake origin of the

Pyramid Lake brood stock. Pyramid and Summit lake populations cluster weakly with Carson

River populations as Carson River LCT also contributed to the brook stock for the Pyramid Lake

population. Macklin Creek LCT (of putative Lake Tahoe origin), cluster with strong bootstrap

support (**89%**) with Willow-Whitehorse LCT populations in Oregon. The origin of Willow-

Whitehorse LCT, however, remains unresolved. O'Harrel Creek LCT (of putative Walker basin

origin) clusters weakly with the out-of-basin Carson River populations (29%), which suggests a

possible Carson River origin.

Because so few in situ populations exist in the Western basin DPS, establishing evolutionary

relationships at the population level is difficult. Also, given the complex population genetic

structure which characterizes the intact Humboldt River population networks (which probably
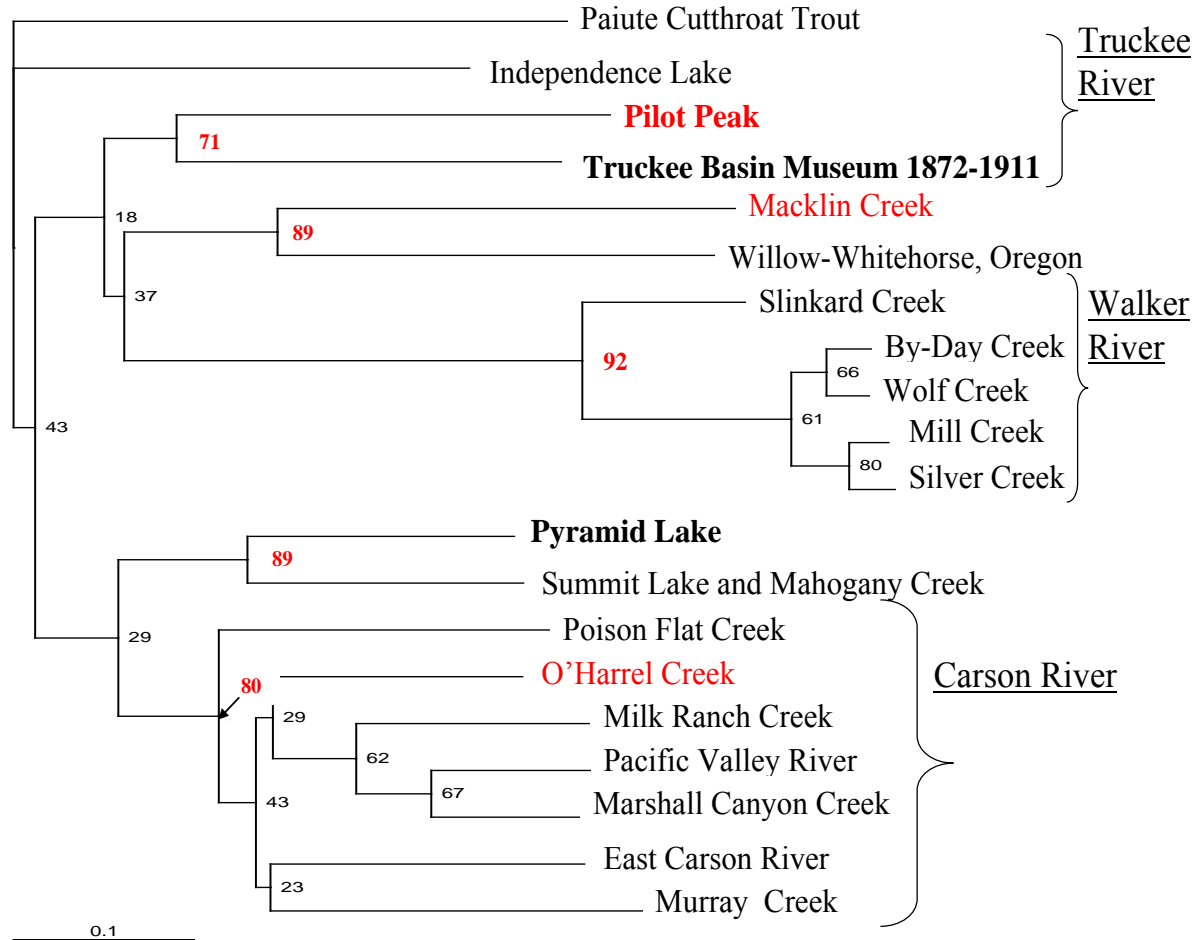
Figure 36. Phylogenetic analysis Western basin populations with putative or known origins using a Cavalli-Sforza genetic distance measure and a neighboring-joining tree with rainbow trout as the outgroup. 3000 iterations were conducted in the program POPULATIONS (version 1.2.26) with bootstrap values indicated at the tree nodes. Out-of-basin populations of putative Western basin origin are highlighted in red. Pilot Peak LCT (Bettridge, Morrison and Pilot Peak broodstock) cluster with Truckee River basin historical museum samples with strong bootstrap support (**71%**), Macklin Creek of putative Truckee basin origin cluster with Willow-Whitehorse LCT (**89%**) and O'Harrel Creek of putative Walker basin origin clusters weakly with out-of-basin Carson River populations. Pyramid and Summit Lakes cluster with strong bootstrap support (**89%**). Scale represents genetic distance.

reflects the historical norm for LCT throughout its range), unambiguously recreating the

historical relationships among populations in the Western basin that have been highly impacted

by anthropogenic disturbance is difficult. Extant Western basin populations and out-of-basin

populations of Western basin origin tend to be small, isolated, and show evidence of genetic

bottlenecks (see previous sections). Truckee River basin populations (Pilot Peak and

Independence Lake), however, do retain high levels of heterozygosity similar to historical levels

seen in the Truckee basin museum samples (Pilot Peak $\overline{H}$ = 0.487, Independence Lake $\overline{H}$ =

0.668, and Truckee basin museum $\overline{H}$ = 0.644), but as with populations in the Carson and Walker

basins, Truckee River populations show evidence of genetic bottlenecks (Wilcoxon Sign Rank

test: Bettridge Creek, IAM and TPM, P < 0.05; Morrison Creek, IAM P = 0.0009 and TPM P =

0.01; Pilot Peak broodstock, IAM P = 0.01, TPM P = 0.04; Independence Lake, IAM P = 0.002).

The out-of-basin populations in Edwards and O'Harrel creek also show evidence of bottlenecks

(IAM and TPM P = 0.0009; IAM P = 0.0005, respectively), whereas Macklin Creek is not

bottlenecked and Willow-Whitehorse LCT are bottlenecked under IAM only (P = 0.04).

A watershed based phylogenetic analysis is more informative (Figure 37). The out-of-basin

populations (Macklin, Edwards, and O'Harrel creeks), which do not cluster definitively with any

single population in the population-level analysis, show clear relationships with individual

watersheds (Figure 37). Macklin Creek clusters with the Willow-Whitehorse watershed (88%

bootstrap support), O'Harrel Creek with Carson River (100% bootstrap support), and Edwards

Creek with the Reese River (82% bootstrap support). Pyramid and Summit lakes cluster with the

Carson River (93% bootstrap support), reflecting the mixed stock ancestry of the original

Pyramid Lake broodstock. All LCT populations with the exception of the Walker River

populations form a single evolutionary clade which then clusters with the Willow-Whitehorse-

Macklin Creek LCT under strong bootstrap support (74%).

The watershed phylogenetic tree suggests the following: (1) Willow-Whitehorse and Macklin

Creek LCT are distinct from all other LCT populations, (2) Paiute cutthroat trout are

evolutionarily closely related to LCT (and Carson River LCT in particular), and (3) Walker

River populations, through small population size, isolation and genetic bottlenecks and possible

hybridization with rainbow trout have lost their phylogenetic signal. Within the primary LCT

evolutionary clade, relationships among watersheds within and among DPSs remain unresolved,

which may reflect both the non-equilibrium population dynamics inherent in LCT populations as

well as loss of genetic signal through anthropogenic disturbance.

5) Is there historical evidence for population genetic structure among the now extinct LCT

populations from within the Lake Tahoe-Truckee River basin based upon genetic analyses

of museum preserved samples collected from multiple locations within Lake Tahoe and the

lower Truckee River?

High average heterozygosity ($\overline{H} = 0.644$) and allelic richness (Table 11) of the Truckee River

basin historical samples, as well as inclusion of multiple locations in Lake Tahoe and lower

Truckee River increases the probability that past population dynamics can be resolved. Indeed,

population genetic structure was evident within native Truckee River basin LCT both historical

and extant populations. Collection date for the historical, museum-preserved samples of the

75

LCT strain indigenous to the Lake Tahoe and the Truckee River watershed obtained for this

study ranged from 1872-1913. Despite this ~40 year collection period the population genetic

structure revealed with the Bayesian clustering analysis was consistent over time (Figure 38).

Three genotype clusters were identified as the best fit of the data [LnP(D) = -647.36, *P* = 0.999].

 Collection dates covered the entire range from 1872-1913 for the individuals assigned to the

"green" cluster. Two of these individual were collected in Cascade Creek located at the southern

end of Lake Tahoe. It is likely that all fish in the green cluster were collected from this general

area. Individuals assigned primarily to the "red" cluster appear to have been collected either in

Lake Tahoe near Mt. Tallac at the southern end of the Lake or from streams draining into Lake

Tahoe from Mt. Tallac. Individuals from the lower Truckee River and Pyramid Lake also assign

primarily to the red cluster. Specific collection location data within Lake Tahoe is unavailable

for the majority of individuals that assign primarily to the blue cluster. These data suggest: (1)

population genetic structure within the historic LCT population in Lake Tahoe and the Truckee

River watershed and (2) connectivity and gene flow among LCT populations found in Pyramid

Lake, Truckee River and Lake Tahoe.
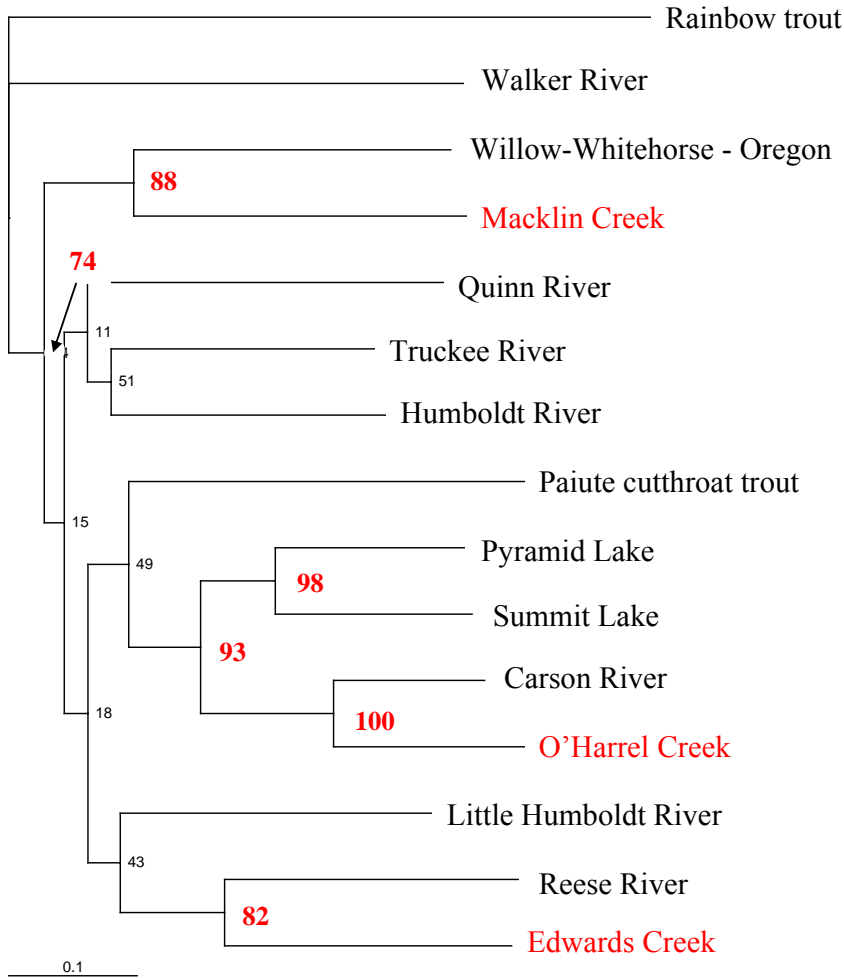
Figure 37. Phylogenetic analysis of LCT populations grouped by watershed and out-of-basin LCT populations of putative Western basin origin using a Cavalli-Sforza genetic distance measure and a neighboring-joining tree with Rainbow trout as outgroup. 3000 iterations were conducted in the program POPULATIONS (version 1.2.26) with bootstrap values indicated at the tree nodes. Macklin and Edwards creeks LCT were thought to be of Truckee River origin. Macklin clusters with Willow-Whitehorse populations (88%) in Coyote Lakes basin, Oregon. Edwards Creek LCT cluster with Reese River LCT populations (82%) and O'Harrel Creek of putative Walker basin origin clusters with Carson River LCT populations (100%). Walker River LCT populations do not cluster with any extant LCT populations. Scale represents genetic distance.

STRUCTURE analysis involving extant Western basin populations, populations of western basin

origin (Table 12), the historical Truckee basin samples, East Marys River, Summit Lake,

Willow-Whitehorse, and Paiute cutthroat trout as the outgroup resulted in 23 distinct genotype

clusters with little overlap in cluster membership among the Truckee, Carson and Walker

watersheds (Figure 39). Cluster membership overlap, however, is evident within the Truckee

River basin extant populations (Independence Lake and Pilot Peak) suggesting an historical

connection among these fishes within the basin. Multiple distinct genotype clusters within the

Independence Lake population as well as the historical Truckee River basin museum samples

suggests an historical pattern of complex population genetic structure within the Truckee River

watershed.
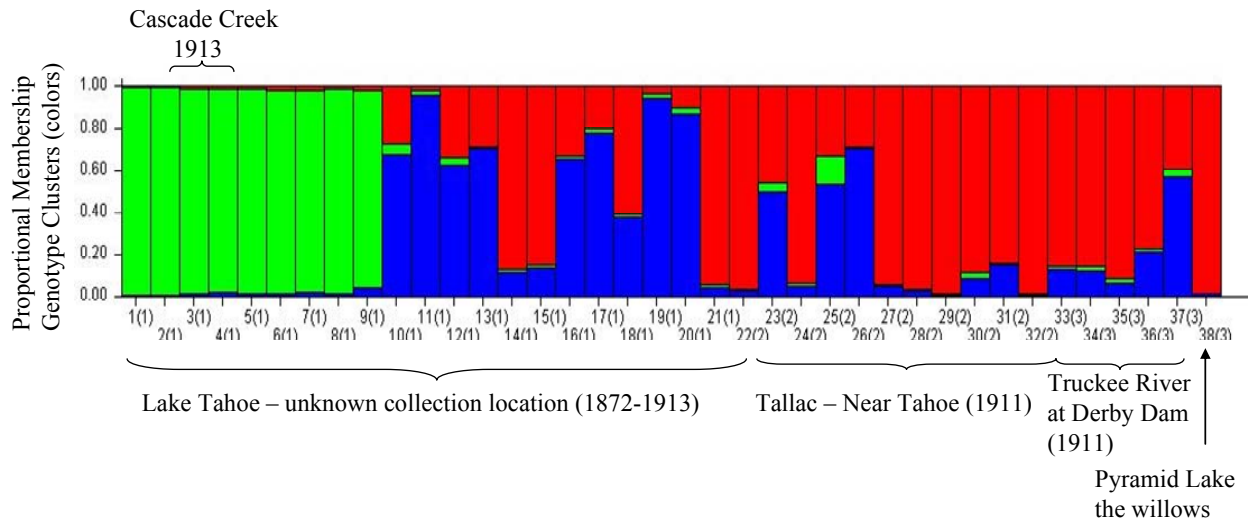
Figure 38. Results of STRUCTURE analysis for Truckee River basin historical samples (1982-1913) showing proportional membership (Y axis) of each individual LCT in each of three genotype clusters [LnP(D) = -647.36, $P = 0.999$; $K = 4$, 5 had little statistical support, $P = 3.440e^{-06}$ and $2.009e^{-208}$ respectively]. Geographic location of collection site/area for each individual fish is indicated on the X axis.

Table 11. Allelic richness per locus and population for populations in the Western basin DPS,

Summit Lake and Willow-Whitehorse LCT populations in the Northwestern basin DPS, East

Marys River (EMR) in the Eastern basin DPS and Paiute cutthroat trout. The bottom line

represents the allelic richness averaged over all loci per population.

Allelic Richness per locus and population
based on min. sample size of: 8 diploid individuals.

	Pilot	Independe	Museum	Macklin	Carson	Murray	Poison	Pacific	Milk		Marshall	Oharrel	Slinkard
OCH5	4.114	7.32	6.152	3.413	3.766	1.965	5.596	1.999	1.235		1.998	3.609	2.986
OCH6	2.972	3.728	5.505	2.934	4.579	3.765	1.918	1.466	1		2.041	3.244	2
OCH9	1.956	2.647	3.774	1.984	1.996	2.935	2	1.91	2.229		2	2	1.973
OCH10	1.095	2.081	1.585	1	1.39	1.698	1.379	1.466	1		1.195	1.708	1.216
OCH11	4.793	6.308	5.186	3.431	5.605	6.037	5.691	1.946	3.061		1.672	3.34	1.216
OCH15	3.763	8.804	7.252	4.22	4.174	3.814	3.548	2	1.862		2.16	2.566	2.192
OCH16	1.972	5.091	5.165	4.548	3.07	2.684	1.833	1	1.235		1.347	1.798	1
OCH17	4.152	7.889	7.277	5.584	5.297	3.092	3.823	2	2		1.493	1.821	1.966
	3.102125	5.4835	5.237	3.38925	3.734625	3.24875	3.2235	1.723375	1.70275		1.73825	2.51075	1.818625

EMR	Pyramid	Summit	Willow	Paiute
5.635	6.589	7.045	3.861	3.324
4.199	3.145	2.855	3.457	2.058
2.222	3.38	3.39	2.533	1
1.911	1	1	1	1
7.264	7.393	6.65	4.549	2.991
6.081	6.901	6.216	9	2.784
5.442	5.742	2.841	3.972	1.354
5.037	7.494	4.159	5.449	1
4.723875	5.2055	4.2695	4.227625	1.938875
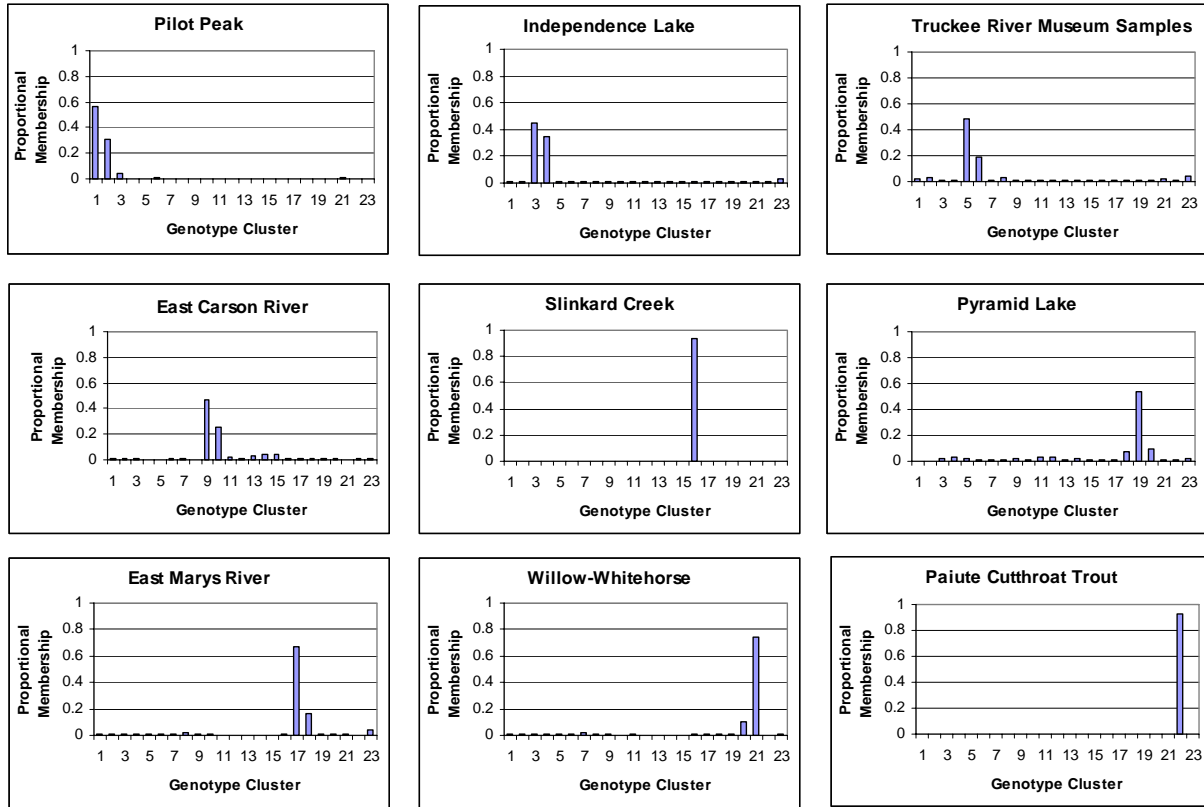
Figure 39. Results of STRUCTURE analysis showing proportional membership in each of 23 distinct genotype clusters identified for LCT populations in the Western basin DPS and East Marys River, Willow-Whitehorse, and Paiute cutthroat trout.

Table 12. Populations included in STRUCTURE analysis of Western basin DPS (See Figure 36).

Truckee River basin
       Pilot Peak (Bettridge, Morrison and Pilot Peak broodstock)
       Independence (Independence and Heenan Lakes)
       Truckee Basin Historical samples
       Pyramid Lake
Carson River
       East Carson River
       Murray Creek
       Poison Flat Creek
       Pacific Valley River
       Milk Ranch Creek
       Marshall Canyon Creek
       O'Harrel Creek
Walker River
       Slinkard
Humboldt River
       East Marys River
Summit Lake
Willow-Whitehorse
Paiute Cutthroat trout

DISCUSSION AND MANAGEMENT IMPLICATIONS

6) How can the level and pattern of genetic diversity within extant populations inform priority ranking for recovery activities?

Hierarchical genetic analysis of extant Lahontan cutthroat trout populations informs the recovery process by providing information on population dynamics at various spatial and temporal scales (Slatkin 1985; Dunham *et al.* 1998; Davies *et al.* 1999; Dunham *et al.* 1999b; Manel *et al.* 2003). **On a range wide basis, microsatellite data and phylogenetic analyses support the designation of three Distinct Population Segments for Lahontan cutthroat trout.** Significant

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genetic differentiation, in addition to morphological and meristic differences among LCT in each

respective DPS, suggest these populations form evolutionarily significant units replete with

adaptations specific to their native habitats (Waples 1995; Rosenfeld and Hatfield 2006). This

differentiation among DPSs warrants the development of recovery strategies on a DPS basis.

Historically, LCT were found in large interconnected stream and/or stream and lake systems

throughout their range (Coffin and Cowan 1995). Population dynamics in intact interconnected

stream systems provide our only insight into what is likely the historical norm for this

subspecies. Genetic patterns observed in these remaining interconnected stream systems can

provide a predictive framework with which to reconstruct population dynamics in now

fragmented watersheds throughout the range of LCT. Prior research on this subspecies indicates

that habitat quality (e.g., temperature; Dunham *et al*. 1999a) and diversity (e.g., habitats to

support all age classes and life history strategies; Ray *et al*. 2000; Neville *et al.* 2006) as well as

watershed area (Dunham *et al.* 1997; Dunham *et al.* 2002) are important factors in long term

persistence of LCT populations.

Metapopulation dynamics have been predicted for many inland salmonid species but few

definitive data have been collected to test this hypothesis (Rieman and Dunham 2000).

Independent extinction and colonization probabilities among demographically independent

subpopulations, represent two major assumptions of metapopulation theory (Hanski 1999).

Characterization of LCT population dynamics in intact, interconnected streams systems in the

eastern Lahontan basin (Marys River and Maggie Creek) using microsatellite genetic markers,

reveals population genetic structure at multiple spatial scales within watersheds (this study,

Neville *et al.* 2006). Patterns of genetic variation in these watersheds further indicate complex

dynamics involving habitat diversity and emerging life history strategies (Neville *et al.* 2006).

The observed population genetic structure, together with observed extirpation and

recolonization events, and evidence of genetic bottlenecks among interconnected tributary

subpopulations within the Marys River and Maggie Creek basins, support a

metapopulation (or more generally a networked population) dynamic for LCT (Ray *et al.*

2000; Rieman and Dunham 2000; Neville *et al.* 2006). Such dynamics can be viewed as a

natural risk spreading strategy for salmonids (Cooper and Mangel 1999) in highly variable

environments such as desert aquatic ecosystems. Indeed, many inland salmonid species exhibit

complex population dynamics on varied landscapes (Gresswell *et al.* 1994; Rieman and

McIntyre 1995; Dunham and Rieman 1999; Rieman and Dunham 2000; Harig and Fausch 2002;

Cegelski *et al.* 2006).

Similar patterns of population genetic structure as seen in LCT populations found in

interconnected habitat are observed in the isolated tributaries of the North Fork Humboldt River,

which were still connected in the early 20th century. Pairwise F_{ST} and Bayesian genotype cluster

analyses of tributary populations both within and among watersheds that drain directly into the

main stem Humboldt River (Marys River, North Fork Humboldt River, Maggie and Frazer

creeks) suggest low to moderate levels of genetic differentiation and a geographic pattern of

isolation-by-distance among watersheds. Populations in tributaries which drain into the Reese

and Little Humboldt rivers are more geographically isolated from the main stem Humboldt

River, and pairwise F_{ST} analysis shows these populations to be greatly differentiated from each

other ($F_{ST} > 0.25$) and from the other Humboldt River populations. These data also support a

pattern of isolation-by-distance within the larger Humboldt River drainage as well as

contemporary isolation of populations within the Reese and Little Humboldt rivers. Genotype

clustering analysis revealed population genetic structure within some of these isolated tributary

populations (Indian, Mohawk and Tierney creeks). These data suggest intact habitat diversity

sufficient to support multiple distinct genotype clusters. However, small population size,

evidence of genetic bottlenecks and physical isolation undoubtedly increases extirpation risk for

these populations that have no opportunities for natural recolonization.

Long-term recovery activities of LCT populations in the Eastern basin DPS should involve

habitat recovery – in terms of both habitat quality and interconnectedness in order to

facilitate restoration of the historic population dynamic in these watersheds. Since genetic

patterns reveal long-term movement dynamics among LCT populations across a large and varied

aquatic landscape, achieving long term persistence of LCT populations in small isolated habitats

is highly unlikely, especially given recent extirpations of isolated populations in both the

Humboldt and Quinn River basins (Elliot *et al*. 1997; Sevon *et al.* 1999). Essentially all historic

populations found in the southern Santa Rosa Range, western Montana Mountains, Jackson

Mountains, Calico Mountains and Granite Range have been lost due to fragmentation and

subsequent isolation.

LCT populations in the Quinn River basin are small, isolated and few in number. Of the 11

extant LCT populations listed in the LCT Recovery Plan (Coffin and Cowan 1995), eight are

identified as having the best potential for recovery. In terms of genetic resources, of these eight it

has been determined that Sage and Indian creek populations in the McDermitt Creek drainage

are introgressed with rainbow trout (Peacock and Kirchoff 2004), Riser and Corral creeks were

established with Line Canyon Creek LCT, and Washburn and Crowley creeks have very small

populations, no within tributary population genetic structure, and very low levels of

heterozygosity. Pairwise F_{ST} analysis shows that the pure LCT populations sampled (Line

Canyon, 3 Mile, Washburn and Crowley creeks) are *greatly* differentiated from each other ($F_{ST} \geq$

0.19). Line Canyon and 3 Mile creek LCT populations have the highest levels of heterozygosity

among the Quinn River populations sampled, and 3 Mile has the largest population size. Both 3

Mile and Line Canyon creek populations also have genetic structure with multiple genotype

clusters identified per population. F_{ST} analysis suggests the pure LCT populations sampled are

contemporaneously very disjunct and have been isolated for a considerable period. It is unlikely

that gene flow among these populations was ever very frequent, given the current F_{ST} estimates,

even when the streams were connected via the Quinn River. However, small population size,

repeated genetic bottlenecks and current population isolation can inflate estimates of genetic

differentiation due to the resulting non-equilibrium dynamics (Whitlock and McCauley 1990).

Even so, intermittent gene flow is important in maintenance of genetic variation and long-term

population persistence (Slatkin 1985; Fahrig and Paloheimo 1988; Peacock 1997; Peacock and

Smith 1997). If dispersal corridors are present, recolonization and long-term metapopulation

dynamics are possible. The data clearly suggest that the temporal scale of such a dynamic in the

Quinn River basin functioned over a much longer time frame historically than that seen in the

Humboldt River.

The northwestern DPS team has initiated recovery activities in the interconnected McDermitt Creek stream system which includes Line Canyon Creek. This has involved assessing levels of hybridization and building barriers to prevent further encroachment of non-native rainbow trout, with the ultimate aim of removing non-native or hybridized populations, reconnecting streams, and either transplanting or allowing natural colonization of pure LCT from Line Canyon and Riser and Corral creeks, to facilitate emergence of a metapopulation or networked population dynamic. **There is little opportunity to provide dispersal corridors among 3 Mile, Washburn and Crowley creeks at present; however, increases in both the quality of existing habitat and kilometers of occupiable habitat should be the priority. Preventing further losses of genetic variation should be of the highest priority. These populations should be monitored to assess additional losses of genetic variation in the short term as well as increases in population size and emergence of population genetic structure with increases in habitat quantity and quality in the long term.**

The Willow-Whitehorse LCT populations are of unknown genetic origin. Phylogenetic analysis shows they are distinct genetically from all other LCT populations. Willow-Whitehorse populations are moderately differentiated from Foreman Creek in the Humboldt River, but greatly differentiated from Foreman creek neighboring populations, Gance Creek and North Fork Humboldt, and significantly differentiated from all Quinn River populations. Given unknown founder population size, and ongoing effects of population isolation of the majority of extant

LCT populations, it is not possible with this analysis to establish the origin of Willow-

Whitehorse LCT, but it is unlikely they are of Quinn River origin.

The status of native LCT in the Western basin DPS is the most tenuous. There are no extant

fluvial populations of LCT native to the Truckee River basin. A population was founded with

Independence Lake LCT in the upper Truckee River, but non-native brook trout threaten long

term persistence of this population. Independence Lake and the out-of-basin Pilot Peak strain are

the *only* extant native Truckee basin LCT. **Pilot Peak LCT have the strongest phylogenetic**

relationship to historical, museum preserved LCT of known Lake-Tahoe-Truckee basin

origin prior to extirpation in the 1940s. The Independence Lake LCT *do not* show a strong

phylogenetic relationship with either Pilot Peak or Truckee River basin historical samples or any

other western basin populations. However, the population-level phylogenetic tree of western

basin LCT populations is largely unresolved. This is likely the result of a combination of factors

including historic genetic differentiation within basins, contemporary loss of most populations in

these basins, isolation of existing populations and the concomitant loss of genetic diversity

through small population size and potentially multiple genetic bottleneck events.

The Carson River has currently five extant fluvial populations within the basin (Coffin and

Cowan 1995) and a number of out-of-basin populations of Carson River origin. All populations

are significantly differentiated from one another and the out-of-basin populations have very low

levels of heterozygosity. Heterozygosities of native populations within the Carson basin,

however, are high and comparable to larger Eastern basin populations. Habitat restoration that

expands the kilometers of occupiable habitat in extant in-basin stream populations (East Carson

River, Murray and Poison Flat creeks) would facilitate population expansion and maintenance of

the genetic variation present in these populations. Securing additional habitat should be the long

term objective for this basin with the aim of *creating* a networked stream system(s).

Extant Walker basin populations were founded from LCT in By-Day Creek, a small, isolated

population of unknown origin. It is unknown if By-Day Creek is a remnant LCT population from

when the basin was largely occupied or if LCT were planted in these waters sometime in the 20th

century from other extant Walker basin populations. The current fluvial LCT populations in the

Walker River basin are genetically very depauperate. Loss of genetic diversity through repeated

founder events and/or genetic bottlenecks has resulted in low phylogenetic signal and an

inability to reconstruct the evolutionary history of these populations not only with regard to other

LCT populations in the Western basin DPS but throughout the extant range. **Low levels of**

genetic diversity and inability to identify the evolutionary origin of these fish precludes

creating a broodstock from these populations for recovery activities in the Walker basin.

Where possible, restoration efforts should focus on expansion of stream habitat for these

populations to prevent further losses of genetic variation. As the gene pool in the source

population, By-Day Creek has low levels of heterozygosity and moving these fish to additional

isolated locations could lead to additional losses of genetic variation through founder effects.

Slinkard Creek shows evidence of non-random sampling of individuals from By-Day Creek to

create a founder population.

In accordance with the DPS/ESU approach restoration and/or the re-creation of self-

sustaining populations should involve LCT native to their respective DPS and their subbasin if possible. In the case of the Walker River basin in the Western basin DPS there is no appropriate native stock of LCT available to re-establish a native fishery that would likely exhibit attributes of the historic population. The Truckee and Walker river basins are unique among basins within the historic range of LCT due to the large lacustrine habitats found in these watersheds. The populations that were native to the these lakes have been extirpated for the past ~70 years and habitat degradation, non-native salmonids and water diversions make reestablishing naturally reproducing populations of native fish challenging. Phylogenetic analysis identified Pilot Peak LCT as Truckee River basin in origin. Bayesian genotype clustering analysis of Truckee River basin museum samples is illustrative, as genetic structure is evident within these historic samples collected from Lake Tahoe, lower Truckee River and Pyramid Lake. However, gene flow between fish collected in the Truckee River at Derby Dam and Lake Tahoe was also evident with shared membership in distinct genotype clusters. **This historic signature of population genetic structure suggests a networked population dynamic similar to that seen in the large fluvial systems extant in the Eastern basin DPS.**

Pilot Peak LCT have high levels of heterozgosity and allelic richness and retain the genetic signature of their source population. As such this strain likely retains any adaptations specific to lacustrine life history and represents the best chance for recreating native networked populations within the Lake Tahoe-Truckee River and Walker River watersheds in Western basin DPS.

RECOMMENDATIONS

- Restoration activities range-wide should focus on recovery of interconnected stream networks in each DPS.

- Due to genetic distinctiveness of LCT populations within each of the major river systems recovery activities should use fish native to each DPS.

- Pilot Peak hatchery stock represents the historical gene pool of extirpated LCT from the Lake Tahoe and Truckee River watersheds. These fish should be used in recovery/restoration activities within this watershed to: (1) preserve the genetic legacy of the western basin DPS LCT populations and (2) maximize the probability of successful reintroductions by employing the strain native to these waters.

- The extant Walker River basin populations are all derived from a single small isolated population, By-Day Creek. As a result of this small founder population, which has very low levels of heterozygosity, all of these populations are genetically depauperate. Therefore it is not advisable to create a hatchery broodstock with this strain for recovery purposes.

- The extant populations found within the Carson River watershed have significantly higher levels of genetic variability than the outplanted populations in the Mokelumne River in California. Therefore the in situ populations should be used for recovery activities in the expansion of occupied habitat in the Carson River watershed.

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Appendix 1. Summary of genetic variation per locus and population including number of individuals sampled per stream/lake (N), number of alleles sampled per population (A), allelic richness per population (R_S), expected and observed gene diversity (H_E, H_O), and inbreeding coefficient, F_{IS}. The indicative adjusted nominal level (5%) P value for F_{IS} was 0.0015 based on 7600 randomizations. Population abbreviations as per Table 1.

(A) Western Basin DPS

		Truckee River Basin				Carson River Basin						Walker River Basin				
		IND	HEL	TRM	PL	CAR	MUC	POC	PAC	MAC	MIC	SLC	BDC	WOC	MILL	SILV
	N	21	47	37	34	42	20	40	30	41	36	38	27	30	30	30
OCH 5	A	12	14	12	9	5	2	6	2	2	2	3	3	3	3	3
	R_S	10.18	9.231	9.318	7.798	4.396	2	5.984	2	2	1.998	2.682	1.984	2.554	2.461	2.714
	H_E	0.834	0.847	0.747	0.853	0.550	0.261	0.825	0.429	0.413	0.029	0.652	0.434	0.608	0.556	0.607
	H_O	0.800	0.888	0.774	0.753	0.733	0.524	0.300	0.891	0.333	0.425	0.647	0.481	0.700	0.592	0.750
	F_{IS}	0.041	-0.049	-0.037	0.141	0.048	-0.152	-0.080	0.223	-0.038	0	0.008	-0.108	-0.151	-0.065	-0.286
OCH 6	A	6	6	11	4	6	5	2	2	4	1	2	2	2	2	2
	R_S	5.577	4.265	8.230	3.452	5.706	4.800	1.999	1.844	2.876	1	1.965	1.975	1.972	1.826	1.964
	H_E	0.654	0.613	0.708	0.496	0.645	0.661	0.227	0.066	0.151	0	0.496	0.507	0.502	0.374	0.428
	H_O	0.600	0.543	0.667	0.515	0.675	0.700	0.257	0.067	0.079	0	0.484	0.500	0.567	0.276	0.500
	F_{IS}	0.082	0.113	0.059	-0.038	-0.047	-0.060	-0.133	-0.018	0.479	NA	0.023	0.013	-0.128	0.263	-0.200
OCH 9	A	2	5	5	4	2	3	2	2	2	3	2	3	2	2	4
	R_S	2.000	3.476	4.794	3.705	2	3	2	1.999	2	2.514	1.712	2.131	1.755	1.655	2.8
	H_E	0.452	0.488	0.558	0.642	0.399	0.609	0.507	0.217	0.498	0.503	0.300	0.503	0.326	0.266	0.533
	H_O	0.571	0.488	0.607	0.515	0.244	0.300	0.461	0.172	0.595	0.575	0.305	0.464	0.267	0.310	0.400
	F_{IS}	-0.263	-0.003	-0.089	0.197	0.389	0.508	0.089	0.205	-0.196	-0.078	-0.019	0.076	0.183	-0.167	0.273
OCH 10	A	2	3	2	1	3	2	2	2	2	1	2	1	1	1	1
	R_S	1.996	2.545	1.921	1	1.878	2	1.726	1.844	1.439	1	1.081	1	1	1	1
	H_E	0.176	0.239	0.091	0	0.048	0.108	0.052	0.066	0.024	0	0.027	0	0	0	0
	H_O	0.190	0.222	0.093	0	0.049	0.111	0.053	0.067	0.024	0	0.027	0	0	0	0
	F_{IS}	-0.081	0.070	-0.033	NA	-0.006	-0.030	-0.014	-0.018	0.000	NA	0.000	NA	NA	NA	NA
OCH 11	A	9	10	7	12	7	7	8	2	2	4	2	1	1	1	1
	R_S	8.117	7.640	7.000	9.449	6.793	6.991	7.121	2	1.95	3.717	1.081	1	1	1	1

	H_E	0.826	0.745	0.623	0.855	0.754	0.786	0.792	0.249	0.116	0.536	0.027	0	0	0	0
	H_O	0.631	0.413	0.278	0.806	0.809	0.950	0.943	0.286	0.121	0.606	0.027	0	0	0	0
	F_{IS}	0.235	0.446	0.554	0.057	-0.073	-0.209	-0.190	-0.149	-0.053	-0.067	0.000	NA	NA	NA	NA
OCH 13	A	5	10	NA	11	5	5	5	3	2	3	3	3	3	2	2
	R_S	5.000	8.968	NA	9.319	4.065	5	4.927	3	1.88	2.504	2.004	1.729	2.067	1.351	1.970
	H_E	0.669	0.855	NA	0.866	0.444	0.803	0.685	0.642	0.082	0.187	0.470	0.279	0.520	0.126	0.484
	H_O	0.500	0.808	NA	0.500	0.365	0.737	0.645	0.321	0.028	0.086	0.540	0.321	0.433	0.133	0.333
	F_{IS}	0.203	0.054	NA	0.423	0.176	0.082	0.058	0.499	0.660	0.541	-0.149	-0.152	0.166	-0.055	0.333
OCH 14	A	10	12	NA	14	4	7	5	1	1	4	4	4	4	4	4
	R_S	9.470	9.318	NA	10.23	4.065	5	4.927	3	1.88	2.504	2.701	2.426	2.621	2.663	2.969
	H_E	0.881	0.868	NA	0.835	0.631	0.773	0.732	0	0	0.357	0.636	0.576	0.613	0.620	0.681
	H_O	0.736	0.851	NA	0.870	0.714	0.750	0.714	0	0	0.323	0.567	0.586	0.533	0.620	0.833
	F_{IS}	0.117	0.020	NA	-0.042	-0.132	-0.021	0.025	NA	NA	0.094	0.107	-0.017	0.129	-0.001	-0.250
OCH 15	A	10	14	14	12	5	5	5	2	4	2	3	2	2	2	2
	R_S	9.609	11.404	11.232	9.238	4.856	4.892	4.407	2	3.065	1.997	1.825	1.685	1.64	1.942	2
	H_E	0.807	0.913	0.801	0.825	0.632	0.466	0.492	0.468	0.166	0.187	0.342	0.285	0.261	0.472	0.533
	H_O	0.631	0.893	0.759	0.857	0.642	0.350	0.483	0.560	0.175	0.206	0.368	0.185	0.167	0.400	0.667
	F_{IS}	0.217	0.022	0.053	-0.038	-0.042	0.249	0.016	-0.196	-0.054	-0.100	-0.078	0.350	0.361	0.152	-0.333
OCH 16	A	6	9	7	8	5	4	2	1	2	2	1	1	1	1	1
	R_S	5.678	6.049	6.225	6.851	4.024	3.8	1.993	1	1.676	1.529	1	1	1	1	1
	H_E	0.654	0.588	0.738	0.779	0.471	0.274	0.173	0	0.048	0.029	0	0	0	0	0
	H_O	0.700	0.600	0.833	0.741	0.487	0.250	0.189	0	0	0.029	0	0	0	0	0
	F_{IS}	-0.070	-0.021	-0.129	0.048	-0.034	0.087	-0.091	NA	1	0	NA	NA	NA	NA	NA
OCH 17	A	8	14	10	10	7	5	5	2	2	2	2	2	2	2	2
	R_S	7.559	10.367	8.983	8.983	6.448	4.785	4.471	2	1.839	2	1.689	1.976	1.735	1.785	1.773
	H_E	0.820	0.885	0.884	0.874	0.779	0.280	0.615	0.506	0.073	0.451	0.288	0.506	0.314	0.345	0.303
	H_O	0.947	0.915	0.500	0.906	0.833	0.200	0.361	0.667	0.075	0.470	0.236	0.667	0.241	0.367	0.333
	F_{IS}	-0.155	-0.034	0.435	-0.036	-0.069	0.286	0.413	-0.318	-0.026	-0.076	0.178	-0.318	0.231	-0.063	-0.111

(B) Eastern Basin DPS

Humboldt River Basin

		Marys River		North Fork Humboldt River			Rock Creek	Maggie Creek			Little Humboldt		Reese River	
		EMR	WMR	FOC	GAC	NFH	FRC	LJC	COC	BVC	ABC	INC	MHK	TIC
	N	36	48	24	26	48	54	39	54	55	36	33	36	30
OCH 5	A	8	9	11	10	12	6	5	12	12	3	6	2	2
	R_S	7.068	8.263	10.631	9.729	10.205	4.543	4.752	10.052	8.784	3	5.791	2	2
	H_E	0.807	0.804	0.896	0.854	0.886	0.582	0.721	0.859	0.826	0.544	0.643	0.236	0.282
	H_O	0.777	0.674	0.791	0.917	0.937	0.509	0.631	0.529	0.745	0.514	0.656	0.200	0.333
	F_{IS}	0.036	0.162	0.116	-0.073	-0.059	0.126	0.124	0.384	0.098	0.054	-0.020	0.151	-0.184
OCH 6	A	6	6	6	6	6	6	3	5	7	3	4	1	5
	R_S	5.079	5.197	5.861	5.785	5.356	5.251	3	4.162	6.297	2.543	3.458	1	4.371
	H_E	0.699	0.747	0.739	0.773	0.737	0.730	0.574	0.646	0.730	0.468	0.190	0	0.326
	H_O	0.571	0.723	0.773	0.792	0.812	0.473	0.474	0.755	0.681	0.343	0.133	0	0.300
	F_{IS}	0.185	0.031	-0.045	-0.025	-0.103	0.352	0.175	-0.170	0.066	0.268	0.299	NA	0.081
OCH 9	A	3	4	2	2	3	3	2	3	5	3	4	1	4
	R_S	2.528	3.93	2	2	2.404	2.352	2	2.969	3.93	2.543	3.611	1	3.462
	H_E	0.518	0.652	0.451	0.471	0.481	0.483	0.499	0.564	0.617	0.330	0.587	0	0.376
	H_O	0.555	0.638	0.583	0.577	0.362	0.444	0.631	0.600	0.629	0.314	0.452	0	0.461
	F_{IS}	-0.073	0.022	-0.293	-0.225	0.249	0.079	-0.265	-0.064	-0.021	0.048	0.230	NA	-0.227
OCH 10	A	2	3	2	1	3	1	1	2	5	1	1	1	3
	R_S	1.999	2.791	1.792	1	2.861	1	1	1.365	3.591	1	1	1	2.77
	H_E	0.221	0.263	0.042	0	0.198	0	0	0.019	0.295	0	0	0	0.134
	H_O	0.250	0.297	0.042	0	0.213	0	0	0.019	0.333	0	0	0	0.068
	F_{IS}	-0.129	-0.131	0.000	NA	-0.076	NA	NA	0	-0.131	NA	NA	NA	0.486
OCH 11	A	11	10	13	9	11	7	8	10	7	4	5	2	1
	R_S	9.658	8.378	12.39	8.635	10.131	6.433	7.302	8.361	6.692	3.618	4.885	1.854	1
	H_E	0.852	0.797	0.892	0.839	0.859	0.682	0.828	0.852	0.830	0.572	0.765	0.063	0
	H_O	0.833	0.723	0.913	0.808	0.846	0.653	0.888	0.800	0.634	0.517	0.689	0.064	0
	F_{IS}	0.021	0.093	-0.023	0.038	0.015	0.042	-0.073	0.061	0.236	0.096	0.099	-0.017	NA

OCH 13	A	10	10	8	10	13	6	6	11	14	3	7	5	1
	R_S	8.715	7.885	8	9.452	10.31	4.798	5.017	9.21	11.37	2.839	6.31	4.503	1
	H_E	0.802	0.819	0.791	0.866	0.845	0.650	0.701	0.882	0.871	0.535	0.818	0.491	0
	H_O	0.500	0.667	0.474	0.800	0.809	0.691	0.783	0.792	0.781	0.593	0.724	0.424	0
	F_{IS}	0.377	0.186	0.401	0.076	0.042	-0.063	-0.118	0.101	0.103	-0.109	0.114	0.136	NA
OCH 14	A	8	9	10	11	12	8	8	9	10	3	6	2	2
	R_S	7.479	7.672	9.64	10.393	10.074	6.500	7.424	8.057	8.906	2.528	5.725	1.613	1.916
	H_E	0.824	0.802	0.852	0.838	0.873	0.788	0.834	0.830	0.854	0.507	0.734	0.032	0.073
	H_O	0.942	0.829	0.826	0.869	0.804	0.704	0.842	0.811	0.700	0.333	0.647	0.032	0.074
	F_{IS}	-0.144	-0.035	0.030	-0.038	0.079	0.106	-0.009	0.022	0.180	0.342	0.118	0	-0.020
OCH 15	A	9	14	9	8	14	7	8	12	12	3	5	1	4
	R_S	7.831	11.001	8.621	7.328	12.84	5.667	7.937	9.16	10.622	2.997	4.400	1	3.999
	H_E	0.799	0.887	0.848	0.726	0.912	0.748	0.866	0.843	0.878	0.593	0.683	0	0.724
	H_O	0.828	0.818	0.913	0.792	0.952	0.888	0.852	0.773	0.944	0.600	0.727	0	0.586
	F_{IS}	-0.037	0.078	-0.077	-0.090	-0.044	-0.189	0.015	0.082	-0.075	-0.011	-0.065	NA	0.190
OCH 16	A	6	9	8	7	7	5	7	9	12	3	4	1	1
	R_S	5.98	7.493	7.575	6.772	6.936	4.73	6.229	7.168	10.163	2.847	3.467	1	1
	H_E	0.789	0.708	0.737	0.789	0.820	0.498	0.788	0.792	0.828	0.210	0.333	0	0
	H_O	0.742	0.723	0.708	0.782	0.913	0.500	0.757	0.812	0.851	0.129	0.290	0	0
	F_{IS}	0.059	-0.022	0.039	0.008	-0.113	0.014	0.040	-0.025	-0.028	0.386	0.129	NA	NA
OCH 17	A	9	10	7	8	11	11	5	9	9	3	7	2	3
	R_S	7.019	7.931	6.792	7.504	10.586	7.507	4.398	8.347	8.764	3	5.523	2	2.655
	H_E	0.672	0.797	0.837	0.786	0.891	0.757	0.559	0.825	0.865	0.676	0.505	0.506	0.457
	H_O	0.777	0.659	0.792	0.750	0.738	0.648	0.515	0.928	0.847	0.676	0.454	0.366	0.586
	F_{IS}	-0.157	0.172	0.054	0.046	0.172	0.143	0.079	0.073	0.020	0	0.100	0.275	-0.283

C) Northwestern DPS

(D) Out-of-Basin LCT populations of unknown origin

		WAC	CRC	3MI	LIC	SUL
	N	25	15	46	28	47
OCH 5	A	3	4	2	7	11
	R_S	3	3.254	2	6.825	10.823
	H_E	0.573	0.143	0.468	0.819	0.857
	H_O	0.478	0.058	0.454	0.846	0.872
	F_{IS}	0.166	0.588	0.029	-0.033	-0.018
OCH 6	A	2	2	4	5	4
	R_S	2	2	3.442	5	3.915
	H_E	0.231	0.254	0.438	0.772	0.573
	H_O	0.260	0.294	0.477	0.808	0.404
	F_{IS}	-0.128	-0.158	-0.089	-0.046	0.294
OCH 9	A	2	3	2	2	4
	R_S	2	2.992	2	2	4
	H_E	0.492	0.306	0.438	0.497	0.617
	H_O	0.4	0.094	0.553	0.520	0.638
	F_{IS}	0.186	0.694	-0.263	-0.047	-0.035
OCH 10	A	1	1	3	2	1
	R_S	1	1	2.188	1.815	1
	H_E	0	0	0.063	0.037	0
	H_O	0	0	0.064	0.037	0
	F_{IS}	NA	NA	-0.015	0	NA
OCH 11	A	4	4	2	6	11
	R_S	3.987	4	2	5.743	11
	H_E	0.573	0.566	0.468	0.777	0.732
	H_O	0.76	0.454	0.340	0.714	0.745
	F_{IS}	-0.326	0.197	0.273	0.081	-0.017

		Putative Truckee River					Walker River
		BEC	MOC	PPH	MAK	EDC	OHC
	N	30	31	26	40	33	42
	A	4	5	5	5	4	6
	R_S	3.996	4.894	4.713	4.353	4	4.752
	H_E	0.688	0.593	0.632	0.615	0.657	0.563
	H_O	0.714	0.645	0.708	0.684	0.714	0.500
	F_{IS}	-0.037	-0.088	-0.120	-0.112	-0.087	0.112
	A	3	3	3	3	2	5
	R_S	3	2.998	2.984	3	2	4.514
	H_E	0.646	0.616	0.571	0.617	0.048	0.362
	H_O	0.815	0.567	0.583	0.694	0.048	0.414
	F_{IS}	-0.261	0.079	-0.022	-0.125	0	-0.147
	A	2	2	2	2	2	2
	R_S	1.556	2	2	2	2	2
	H_E	0.037	0.420	0.305	0.325	0.206	0.493
	H_O	0.037	0.322	0.272	0.103	0.227	0.404
	F_{IS}	0	0.233	0.106	0.671	-0.105	0.179
	A	1	2	1	1	1	3
	R_S	1	1.500	1	1	1	2.530
	H_E	0	0.033	0	0	0	0.092
	H_O	0	0.033	0	0	0	0.970
	F_{IS}	NA	0	NA	NA	NA	-0.026
	A	6	6	5	4	1	4
	R_S	5.935	4.890	4.692	3.955	1	3.753
	H_E	0.726	0.616	0.659	0.550	0	0.680
	H_O	0.714	0.600	0.600	0.242	0	0.642
	F_{IS}	0.015	0.026	0.090	0.559	NA	0.055

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OCH 13					
A	2	4	4	7	12
R_S	2	3.993	3.556	6.783	11.82
H_E	0.296	0.566	0.417	0.792	0.783
H_O	0.174	0.571	0.500	0.750	0.894
F_{IS}	0.412	-0.009	-0.197	0.053	-0.141

OCH 14					
A	8	5	6	5	9
R_S	7.999	4.988	5.442	4.814	8.811
H_E	0.868	0.763	0.761	0.637	0.609
H_O	0.739	0.448	0.34	0.481	0.702
F_{IS}	0.148	0.4124	0.5518	0.244	-0.153

OCH 15					
A	8	4	9	5	10
R_S	7.913	3.647	7.015	5.000	9.954
H_E	0.783	0.627	0.748	0.794	0.734
H_O	0.869	0.294	0.533	0.678	0.659
F_{IS}	-0.111	0.531	0.436	0.145	0.103

OCH 16					
A	2	3	3	5	6
R_S	2	2.960	2.488	4.743	5.801
H_E	0.091	0.207	0.220	0.446	0.546
H_O	0	0.167	0.200	0.500	0.574
F_{IS}	1	0.195	0.090	-0.12	-0.053

OCH 17					
A	1	2	3	4	7
R_S	1	1.112	1.991	2.347	6.911
H_E	0	0.057	0.497	0.622	0.638
H_O	0	0	0.532	0.643	0.574
F_{IS}	NA	1	-0.069	-0.034	0.102

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OCH 13						
A	4	4	3	5	1	5
R_S	3.986	3.998	3	3.953	1	4.135
H_E	0.656	0.718	0.674	0.516	0	0.563
H_O	0.5	0.419	0.565	0.4	0	0.432
F_{IS}	0.238	0.416	0.161	0.176	NA	0.235

OCH 14						
A	4	4	4	6	2	7
R_S	3.517	3.652	3.682	5.766	2	6.332
H_E	0.627	0.577	0.666	0.771	0.5	0.701
H_O	0.345	0.391	0.500	0.657	1	0.629
F_{IS}	0.45	0.322	0.249	0.147	-1	0.104

OCH 15						
A	4	6	3	6	3	5
R_S	3.522	5.301	3	5.570	2.994	3.615
H_E	0.588	0.707	0.560	0.569	0.411	0.328
H_O	0.815	0.565	0.533	0.605	0.521	0.308
F_{IS}	-0.387	0.200	0.047	-0.063	-0.269	0.062

OCH 16						
A	2	2	2	5	1	3
R_S	2.000	1.999	2	4.985	1	2.478
H_E	0.38	0.275	0.263	0.723	0	0.121
H_O	0.433	0.258	0.304	0.710	0	0.025
F_{IS}	-0.139	0.063	-0.158	0.017	NA	0.794

OCH 17						
A	3	5	3	7	2	5
R_S	2.894	4.869	2.682	6.673	2	3.154
H_E	0.541	0.756	0.394	0.807	0.500	0.101
H_O	0.034	0.433	0.409	0.775	1	0.051
F_{IS}	0.936	0.427	-0.038	0.04	-1	0.493

(E) Paiute cutthroat trout and Rainbow trout

		PAIUTE	RBT				PAIUTE	RBT
	N	48	6					
OCH 5	A	5	4	OCH 13	A	5	16	
	R_S	4.745	2.402		R_S	5	3.651	
	H_E	0.642	0.633		H_E	0.759	0.939	
	H_O	0.617	0.667		H_O	0.613	0.847	
	F_{IS}	0.039	-0.053		F_{IS}	0.194	0.09	
OCH 6	A	3	14	OCH 14	A	2	NA	
	R_S	2.872	3.399		R_S	2	NA	
	H_E	0.228	0.889		H_E	0.285	NA	
	H_O	0.17	0.967		H_O	0.297	NA	
	F_{IS}	0.254	-0.087		F_{IS}	-0.044	NA	
OCH 9	A	1	9	OCH 15	A	5	12	
	R_S	1	3.189		R_S	4.928	3.238	
	H_E	0	0.851		H_E	0.286	0.858	
	H_O	0	0.7		H_O	0.272	0.722	
	F_{IS}	NA	0.177		F_{IS}	0.047	0.1	
OCH 10	A	1	3	OCH 16	A	2	17	
	R_S	1	1.354		R_S	2	3.636	
	H_E	0	0.175		H_E	0.049	0.942	
	H_O	0	0.185		H_O	0	0.714	
	F_{IS}	NA	-0.057		F_{IS}	1	0.241	
OCH 11	A	3	1	OCH 17	A	1	10	
	R_S	3	1		R_S	1	3.18	
	H_E	0.663	0		H_E	0	0.841	
	H_O	0.325	0		H_O	0	0.454	
	F_{IS}	0.509	NA		F_{IS}	NA	0.466	