

## APPENDIX A - Glossary

**Alluvial valley** – a valley that contains a river flowing in channels composed of materials eroded and deposited by the river itself. The channel is mobile and is able to change its size, shape, bed elevation, and course in response to a change of flow regime.

**Aquatic ecosystem** - Any water-based ecosystem, such as a stream, pond, lake or ocean.

**Aquifer** - Porous, water-saturated layers of sand, gravel, or bedrock that can yield an economically significant amount of water.

**Community** - Populations of all species living and interacting in an area at a particular time.

**Competition** - Two or more individual organisms of a single species (intraspecific competition), or two or more individuals of different species (interspecific competition), attempting to use the same scarce resources in the same ecosystem.

**Connectivity** - A standard by which is measured the ability of a system or species to interact, move, migrate, or otherwise attain connection in order to reproduce, seek food, shelter, or an environment to achieve persistence or sustainability.

**Dissolved oxygen (DO)** - Amount of oxygen gas dissolved in a given volume of water at a particular temperature and pressure, often expressed as a concentration in parts of oxygen per million parts of water.

**Distinct population segment (DPS)** - Distinct vertebrate population segments of a species, discrete in having separable or isolated physiological, ecological, or behavioral characteristics.

**Ecosystem** – Community of different species interacting with one another and with the chemical and physical factors making up its nonliving environment.

**Endangered Species Act (ESA)**– This 1973 legislation and its subsequent amendments to provide protection for species and their habitats. The ESA defines three crucial categories: "endangered," "threatened" species, and "critical habitat." Subspecies of plants and animals and distinct population segments can also qualify for protection.

**Fluvial** – Of or related to living in a stream or a river.

**Hybrid** - Offspring produced by crossing two individuals of unlike genetic constitution.

**Hydrologic cycle** - Biogeochemical cycle that collects, purifies, and distributes the earth's fixed supply of water from the environment, to living organisms, and back to the environment.

**Lacustrine** – Of, related to, or growing in a lake.

**Lahontan cutthroat trout** - *Oncorhynchus clarki henshawi* an inland subspecies of cutthroat trout endemic to the physiographic Lahontan basin of northern Nevada, eastern California, and southern Oregon.

**Metapopulation** - Fish population defined by its expansive presence in accessible habitat whereby its needs for sustainability are met through diversity of habitats, corridors for movement, and interconnection.

**NEPA, National Environmental Policy Act** – Legislation passed in 1969, that identified a national policy to "use all practicable means" to minimize environmental impact of federal actions. The Act specifically requires decisions regarding all federally controlled or subsidized projects, such as highways, dams, airports, etc., to outline possible adverse impacts in an environmental

impact statement. (EIS) NEPA also established the Council on Environmental Quality in the executive branch, which develops and recommends new environmental policies to the President.

**Networked Population** – a naturally dispersed population linked through the stream network so that no matter where or when a portion of a population is lost or reduced, individuals from other locations in a stream system can repopulate an impacted area.

**Non-point source pollution** – Pollution to water, land, or air coming from non-specific sites, such as vehicle exhaust, toxic run-off from mining, pesticide use by agriculture, or excretions of livestock.

**Phylogeny** - The lines of descent in evolutionary development of any plant or animal species.

**Pleistocene** – Of the first geologic epoch of the Quaternary Period, characterized by a series of advancing and retreating continental glaciers in the Northern Hemisphere and the development of modern humans and toolmaking cultures.

**Population** – The total of interbreeding organisms that represents a level of organization at which speciation occurs.

**Population viability analysis** – Scientific methodology for identifying the size of a population of species necessary to sustain it.

**Recovery** – Improvement in the status of listed species to the point at which listing is no longer appropriate under the criteria set out in section 4 (a)(1) of the Endangered Species Act." [50CFR 402.02]

**Refugia** – Habitat used by species for protection; places that help reduce environmental stress or that contain optimum conditions for persistence of a species.

**Self-sustaining** – Naturally, self-reproducing.

**Species** - A naturally existing population of similar organisms that usually interbreed only among themselves, and are given a unique Latin binomial name to distinguish them from all other creatures.

**Source or point source pollution** – Easily discernible source of pollution, such as specific industrial drainage pipes or incinerators.

**Stakeholder** – Any individual, group, organization, or professional representative who has an interest in the management of a system.

**Subspecies** - Any natural subdivision of a species that exhibits small, but persistent, morphological variations from other subdivisions of the same species living in different geographical regions or times: the subspecies name is usually the third term in a trinomial species name.

**Total dissolved solids (TDS)** – A water quality term to describe solids dissolved in water, which is made up of various amounts of positive and negatively charged elements (ions).

**Total maximum daily load (TMDL)** – A water quality term to describe the total amount of a chemical constituent that can be added to a water body before it goes over the limit of what can be assimilated.

## **APPENDIX B – ACCRONYMS**

**BIA** – Bureau of Indian Affairs  
**BLM** – Bureau of Land Management  
**BOR** – Bureau of Reclamation  
**BRD** – Biological Resource Division  
**CDFG** – California Department of Fish and Game  
**cfs** – cubic feet per second  
**DO** – Dissolved oxygen  
**DPS** – Distinct Population Segment  
**EA** – Environmental Assessment  
**EIS** – Environmental Impact Statement  
**EMI** – Ecosystems Management International, Inc.  
**ESA** – Endangered Species Act  
**GIS** – Geographic Information Systems  
**HCP** – Habitat Conservation Plan  
**LCT** – Lahontan cutthroat trout  
**Mg/L** – Milligrams per liter  
**MOG** – Management Oversight Group  
**NDOW** – Nevada Division of Wildlife  
**NEPA** – National Environmental Policy Act  
**PLPT** – Pyramid Lake Paiute Tribe  
**PVA** – Population Viability Analysis  
**SPPC** – Sierra Pacific Power Company  
**TDS** – Total Dissolved Solids  
**TMDL** – Total Maximum Daily Load  
**TRIT** – Truckee River Recovery Implementation Team  
**USACOE** – United States Army Corps of Engineers  
**USFS** – United States Forest Service  
**USFWS** – United State Fish and Wildlife Service  
**USGS** – United States Geological Survey

## APPENDIX C – TRUCKEE RIVER OBSTACLES

Obstacle	Distance Upstream From Pyramid Lake	Type of Obstacle	Obstacle To Fish Migration		Proposed Corrective Measures	
			Adult	Young	Adult	Young
Marble Bluff Dam	4 miles	Concrete Dam >10 ft	Yes	Yes	Operate Year Around	Operate Year Around
Numana Dam and Pyramid Indian Diversion	12.5 miles	Concrete Dam >10 ft	Yes	Yes	Ladders	Screens
Olinghouse 3 Pump	21.5 miles	Pump & Rock Dam	?	?	?	?
Gardella Ditch Diversion (S-S Ranch)	21.75 miles	Rock Dam	?	Yes	?	Screens
Ceresola Ditch Diversion (Fellnagle)	27 miles	Rock Dam	?	Yes	?	Screens
Olinghouse 1 Pump	27.8 miles	Rock Dam and Pump	?	?	?	?
Proctor Ditch Diversion	28.5 miles	Rock Dam	?	Yes	?	Screens
Pierson Ditch Diversion	30.5 miles	Rock Dam	?	Yes	?	Screens
Herman Ditch Diversion	31.5 miles	Rock Dam	?	Yes	?	Screens
Gregory Ditch Diversion	34.5 miles	Rock Dam	?	Yes	?	Screens
Outlet Ditch Christensen Ranch Pond	36 miles	Return Ditch	Yes	No	Fish barrier	
Washburn Ditch Diversion	36.5 miles	Rock Dam and return canal	?	Yes	?	Screens
Derby Dam and Truckee Canal	39.5 miles	Concrete dam	Yes	Yes	Ladders	Screens

Obstacle	Distance Upstream From Pyramid Lake	Type of Obstacle	Obstacle To Fish Migration		Proposed Corrective Measures	
			Adult	Young	Adult	Young
Tracy Powerplant	44 miles	Rock Dam and Pumps	?	Yes	Eliminate thermal barrier ?	Screens
Hill Ditch Diversion	47 miles	Rock Dam	?	Yes	?	Screens
McCarran Ditch Diversion	51.5 miles	Rock Dam	?	Yes	?	Screens
Lagomarsino Noce Ditch Diversion	56.75 miles	Rock Dam	?	Yes	?	Screens
North Truckee Drain confluence	58.6 miles	Return ditch	?	?	Fish barrier	?
Pioneer Ditch Diversion	63.50 miles	Rock Dam	?	Yes	?	Screens
Eastman Ditch Diversion Box ~ 300 feet downstream from Glendale St. Bridge (river right)	64 miles	Rock Dam	?	Yes	?	Screens
North Truckee & Sessions (Glendale) Ditch Diversion	64	Rock Dam	?	Yes	?	Screens
Cochran Ditch Diversion	66 miles	Concrete Dam < 6 ft.	No	Yes	?	Screens
Wingfield Park Dams	66.25 miles	Concrete Dam < 6 ft.	?	No	Ladder modification?	?
Idelwild Pond Return Drain	66.5 miles	Return ditch	No	?	Fish barrier	Screens
Idelwild Pond Diversion	67.25 miles	Rock Dam	No	?	?	Screens
Chalk Bluff Pump Station	69.8 miles	Concrete Dam < 6 ft.	?	?	Structure should be tested	Structure should be tested
Orr Ditch Diversion	70 miles	Rock Dam	No	Yes		Screens

Obstacle	Distance Upstream From Pyramid Lake	Type of Obstacle	Obstacle To Fish Migration		Proposed Corrective Measures	
Southside Ditch Diversion	71 miles	Concrete Dam <6 ft (inactive)	No	No		
Lake Ditch Diversion	71.50 miles	Rock Dam	No	Yes		Screens
Last Chance Ditch Diversion	73 miles	Rock Dam	No	Yes		Screens
Washoe Power Diversion & Highland Ditch	76 miles	Concrete Dam > 10 ft.	Yes	Yes	Ladder Modification	Screens
Verdi Power Diversion & Coldron Ditch	80.5 miles	Concrete Dam > 10 ft.	Yes	Yes	?	Screens
Steamboat Ditch Diversion	83.5 miles	Rock Dam	No	Yes		Screens
Fleish Power Diversion	86 miles	Concrete Dam > 10 ft.	Yes	Yes	Ladder modification	Screens
Farad Power Diversion	88.5 miles	Concrete dam < 6ft (to be constructed)	?	?	?	?
Lake Tahoe Dam	121.1 miles	Concrete Dam > 10 ft.	Yes	Yes	?	?

## **APPENDIX D - STAKEHOLDER ROLE AND REVIEW: IMPLEMENTATION OF SHORT-TERM ACTIONS**

The Short-Term actions are a set of tasks that the TRIT and the MOG have identified as being environmentally necessary to move towards recovery of the LCT in the Truckee River basin. The short-term tasks are anticipated to be initiated over the first five years of the recovery effort.

The development of the short-term actions has been done under the direction of the Endangered Species Act and the Recovery Plan (FWS 1995). The Recovery Plan calls for the identification of specific actions that are determined to be necessary to move towards recovery of the LCT. Recovery plans or species management plans do not require NEPA documentation prior to finalization and are not required including economic analysis.

Short-term actions will require a review to determine what level of administrative environmental compliance will be required prior to implementation. Determination of the level of environmental compliance required for each short-term action will be based on:

- Existing California and Nevada state environmental laws, management actions and planning guidelines
- Existing Tribal planning and fishery management actions
- National Environmental Policy Act (NEPA)
- Other Federal and State laws

A series of four steps are outlined to identify what options exist for stakeholders to engage in the annual planning process for implementation of short-term actions. It is anticipated that the recovery process will follow these steps:

- Develop an Annual Work Plan with recommendations for action
  - Action: Identify specific actions to be completed
  - Action: Identify the appropriate lead agency or group
  - Action: Prioritize the proposed actions
  - Action: Perform technical review of the study plans and data management requirements
  - Action: Hold public stakeholder meetings to discuss and refine annual work plan
- Present the Annual Work Plan to the MOG for concurrence and approval
  - Action: Guide the development of the annual short-term actions
  - Action: Discuss with MOG comments and suggestions identified by stakeholders
  - Action: Approve proposed short-term actions
  - Action: Identify level of environmental compliance
- Prioritize the work tasks and implement actions to accomplish the short term action

- Action: Develop appropriate environmental compliance process
- Action: Develop Requests for Proposals and/or review proposals submitted by researchers
- Action: Respond to stakeholder technical concerns stated at the public meetings
- Review results and provide feedback through Adaptive Management Program
  - Action: Perform annual review of the short-term actions
  - Action: Determine appropriate level of response
  - Action: Perform peer review on study reports

## **Stakeholder Participation and Recommendations**

### **Background**

Beginning in the spring of 2000 and continuing through 2002, EMI facilitated a series of stakeholder meetings to discuss the TRIT planning process and recommendations. Stakeholder participation included identification of pertinent groups and individuals, interviews with key watershed groups, distribution of supporting documentation, and facilitation of discussions related to TRIT plan development. Information was gathered from state, tribal, and local government officials, stakeholders, watershed groups, and the general public.

### **Process**

Eleven public stakeholder meetings were held to solicit input on the TRIT plan and process from May 24, 2000 to November 13, 2002. These meetings were held on Saturdays and/or in the evenings at various locations in Truckee, California, Reno and Fernley, Nevada, to accommodate public participation.

A Truckee River LCT science meeting was held on July 29, 2000 and organized to allow stakeholders to meet with researchers and develop a better understanding of the scientific and technical issues. Scientists presented an array of information, including: Genetics, LCT life history, fisheries management and distribution of fishes in the Truckee River Basin, habitat studies and restoration, and the development of short-term actions for the TRIT plan.

## Discussion Points

Discussions with stakeholders evolved as the TRIT plan was being developed. Initially the discussions focused on issues related to:

- Lahontan cutthroat trout recovery as related to requirements of the Endangered Species Act. Specific concerns were related to Section 4d rule.
- Determination of the differences between recovery and restoration.
- Impacts on existing recreational salmonid fisheries
- Development of a consistent mission statement
- Genetics and hybridization
- Role of the draft USFWS Intercross Policy for fish management
- The role and quality of science
- Management action coordination between Nevada, California and the USFWS
- Timetable for completion of the TRIT plan and its relationship to ongoing Truckee River Operation Agreement negotiations.
- Definition of what it will take to delist LCT

The public stakeholder process was augmented by the development of a TRIT website where technical and background information and reports were posted. Continued education and coordination through utilizing these tools resulted in improved information transfer and an evolution of discussion and coordination.

Stakeholder meetings held in late 2002 focused on specific components of the draft TRIT plan. Points of interest included:

- Review and discussion of LCT Recovery Criteria
- Review and discussion of short-term actions
- Discussion of site specific actions and response
- Discussion of the implementation of Adaptive Management and continued stakeholder involvement
- Water management of the Truckee River specifically Stampede Reservoir
- Management coordination between the endangered cui-ui and LCT
- Water quality in the lower Truckee River and its impact on LCT fishery
- Inclusion of recreational fisheries into short-term actions for the Truckee River system
- Role of the Pyramid Lake Paiute Tribe and recovery of LCT
- Need for coordination on tributary habitat restoration and monitoring

- Role of genetics to identify appropriate sources of LCT for reintroduction and potential hybridization monitoring.
- Development of Habitat Conservation Plans and strategies to protect private landowners
- Prioritizing stream reaches for management
- Ensuring scientific peer review of all produced reports and documents

## **Recommendations**

Recommendations were formulated from public comments *not otherwise addressed in this report* in sections on genetics, short-term actions and timelines, and the adoption of principles of adaptive management.

The recommendations outlined are assimilated from comments made at the stakeholder meetings.

1. **Economics** - Acquire the services of specialists on environmental and social economics. Of specific interest is determining the cost (both beneficial and detrimental) to local Truckee River communities as a result of LCT recovery. Offer these services to locations where citizens have expressed a strong opposition to LCT recovery because of its perceived threat to socio-economic stability. Specific effort should be made to document the economic impact of managing the Truckee River fishery for LCT recovery.
2. **Building Relationships** - USFWS personnel and representatives from other affiliated organizations need to visit communities and stakeholders regularly to develop and maintain ongoing relationships with landowners and business people. There is a general mistrust of the Endangered Species Act and its application.
3. **Easing the Process**- USFWS personnel should work closely with both recreational fishing groups and water users to inform, counsel, and ease the burden of paperwork necessary for filing Safe Harbor Agreements, Habitat Conservation Plans, or other programs that may assist in implementing LCT recovery efforts.
4. **Auxiliary Funding and the Creating of Partnerships** - USFWS has access to auxiliary funding available in the form of grants, which can help citizens become involved in volunteer efforts to restore and enhance riverine systems. Work with citizens and other agencies to foster efforts promoting habitat health, showing how such efforts can specifically benefit communities economically. Of specific interest is

the development of a working relationship with the Sage Hen Creek, University of CA research station.

5. **Communications** - USFWS and CDFG could improve relationships with citizens in Truckee River communities by visiting them regularly and maintaining open communication. It is essential that the FWS keep the public informed regarding progress and activities related to LCT recovery.

Stakeholders want to understand the scientific logic that is driving management actions. It was requested that the FWS and the States provide timely interim reports via the web to stakeholders. This should include annual fishery surveys, habitat assessments and information on watershed perturbations such as fires, floods and water quality concerns.

6. **Water management and conservation** – Transparency of information regarding water quantity and use is paramount to the health of all systems in the Truckee River basin. Of specific interest is developing the ability to forecast and guide management of water in the Truckee River system in both the summer and winter. It is unclear to most citizens of the decision-making process, which is used to allocate water for the Truckee River system.
7. **Increase Qualitative Analysis (Social History)** - Conduct personal interviews with people who recall socio-environmental conditions over the past half-century. With several interviews, the cross-referencing of information could become an important source for data.
8. **Develop Improved Data Base of Existing and Historic Information** - Important information is being developed by Pyramid Lake Paiute Tribe, historians, Truckee River watershed groups, fishing groups, academic institutions, the Forest Service, the States of CA and NV and other land and resource management entities. This information should be integrated together and used to assist in the development of integrated LCT recovery plans.
9. **Provide guidance to entities on habitat restoration in Truckee River tributaries for LCT recovery** - Fishing groups and citizens want instructions and direction on the restoration of aquatic habitat for LCT in tributaries of the Truckee River. Develop Adopt-a-Stream program with local fishing and environmental groups.

Explain via the web and future stakeholder meetings the logic and background information on why the LCT was listed as endangered in 1970. The social and administrative history of this decision should be developed for history and future management understanding.

Genetic History and Implications for Management and Recovery of  
Lahontan Cutthroat Trout (*Oncorhynchus clarki henshawi*) Populations

**DRAFT REPORT**

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## INTRODUCTION

Molecular genetic data have become a standard tool for understanding the evolutionary history and relationships among species (Avice 1994; Hillis et al. 1996). These data often permit a level of resolution typically unavailable from morphological and ecological data that generally define more broad, overall species characteristics (Gall and Loudenslager 1981; Avice 1994; Hillis et al. 1996). Recent advances in high-resolution molecular markers have increased the use of genetic data to address the evolutionary history of populations at finer spatial and temporal scales, e.g., individual drainages, that other methods cannot. Examples of emerging applications include the definition of conservation units (see Nielsen 1995), and use of genetic data to complement inferences about ecological patterns and processes (e.g., Milligan et al. 1994; Moritz 1994; Avice 1994; Dunham et al. 1999; Sunnock 2000; Peacock and Ray 2001). Often, particularly in the case of finer-scale applications, the interpretation of genetic patterns may be confounded by unknown historical or contemporary events (e.g., historical patterns of hybridization or colonization events and contemporary habitat fragmentation and hatchery supplementation). Patterns of genetic variability observed at fine scales typically do not point toward a single, unequivocal answer about the history of a population, but they do limit the possibilities (Slatkin 1993; Ray 2001). Inferences about evolutionary history and ecological patterns must integrate all available information to provide a more robust understanding of a species' biology for application in conservation efforts (Dowling et al. 1992; Moritz 1994; Dunham et al. 1999).

Although genetic data are powerful tools in constructing phylogenetic trees, patterns of relatedness are necessarily inferred. The strength of this inference depends upon an accurate interpretation of genetic patterns. Genetic differences between individuals within and among populations, subspecies and species represents the accumulation of genetic changes over time and thus reflect long-term demographic and ecological patterns. The interaction between demographic and ecological variables can create a specific genetic signature, although genetic results in some instances can describe multiple demographic and ecological scenarios (Wright 1940; Richards and Leberg 1996). However, because we can rarely measure infrequent events that may have profound impacts on the genetic structure of populations, contemporary ecological and demographic dynamics alone do not necessarily reveal long-term (historical) patterns that shape phylogenetic relationships. Data collected on ecological and demographic processes in extant populations can be used to test genetic hypotheses and strengthen inference from genetic data. Combining demographic, ecological and genetic data sets adds a temporal perspective unavailable from any single data set. **Genetic data should, therefore, be interpreted in combination with all available taxonomic and ecological information** (Dowling and Brown 1989; Dowling et al. 1992; Moritz 1994; Dunham et al. 1999).

In this report, we review genetic information in the context of what is known about the morphology, ecology, life history and zoogeography of Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*, LCT) to provide a brief synthesis of what is known about the biology of this

threatened subspecies, and implications for recovery in the Truckee River basin. The information in this report is intended as a guide for development of the recovery objectives for LCT in the Truckee basin. Specifically, we address whether certain LCT strains are appropriate for use in recovery activities in the Pyramid Lake, Truckee River and Lake Tahoe system.

In 1996, U.S. Fish and Wildlife Service contracted Dr. Jennifer Nielsen, Hopkins Marine Station, Stanford University, to evaluate transplanted out-of-basin populations thought to be the original Pyramid Lake strain of LCT. The primary goal of this analysis was to determine probable origin of these fish using microsatellite genetic markers (Dunham *et al.* 1999; Nielsen 2000). Microsatellites are state-of-the-art genetic tools used to address within-species, population-level questions. Composed of tandemly repeated DNA sequences found in non-coding regions of the nuclear genome, microsatellites are among the most highly variable genetic markers available (Jarne and Lagoda 1996). The Dunham and Nielsen genetic studies were designed to examine relationships among populations within the western Lahontan basin, in the context of relationships among populations throughout the entire Lahontan basin. The primary goal was to resolve relationships among populations that the less variable protein and mitochondrial DNA markers were unable to clarify.

The genetics section of the Truckee River Recovery and Implementation plan has two primary goals. The first is to review genetic studies of LCT and summarize the current understanding of the evolutionary relationships among populations throughout the Lahontan basin. The second is to evaluate transplanted populations of LCT thought to be the original Pyramid Lake strain within the framework of this evolutionary history.

## MAJOR ISSUES REGARDING GENETICS AND RECOVERY OF LCT IN THE TRUCKEE RIVER BASIN

### **Reintroductions**

At the time the 1995 recovery plan for LCT was finalized, it was estimated that less than 0.2% of lacustrine (lake) habitat and about 2.2% of stream habitats in the Truckee River basin were occupied by Lahontan cutthroat trout (Coffin and Cowan 1995). The only known surviving indigenous population (indigenous = derived from genetic ancestors that evolved in the Truckee River basin) in the basin resides in Independence Lake, and the main inlet tributary (Independence Creek). This population is very small and isolated (Coffin and Cowan 1995), and natural production cannot sustain reintroductions needed for recovery efforts throughout the basin. In addition to this population, there are several out-of-basin populations of LCT that likely originated via translocation from fish indigenous to the Truckee River basin. These include stream-living populations in the Pilot Peak Mountains (Morrison Creek) of Utah; the Desatoya Mountains (Edwards and Willow Creeks) of Nevada, and Yuba River basin (Macklin Creek) of California. The Macklin Creek population is believed to have originated via a transfer of fish from Lake Tahoe in the early 1900s (E. Gerstung, California Department of Fish and Game,

personal communication). There are no reliable records linking the other populations to a likely source, but Hickman and Behnke (1979) suggested morphological resemblances indicate a “probable Pyramid Lake” origin for the population in Morrison Creek. The current stocks of LCT propagated for sport fisheries and recovery efforts in the Truckee River basin are a genetic mixture of primarily non-indigenous sources. Because indigenous LCT are nearly extinct in the Truckee River basin, reintroductions are necessary for recovery of viable, self-sustaining populations. Given that sufficient ecological conditions are available, reintroductions must address the following genetic issues:

*Hatchery propagation versus wild sources for reintroductions.* As indicated above, potential sources of LCT for reintroductions in the Truckee River basin are very reduced in numbers or distribution. Removal of fish for reintroductions may therefore pose significant risks to the source populations. Furthermore, it may also be possible the source populations cannot provide sufficient numbers of fish to be useful for reintroductions. In any case, there is a considerable amount of uncertainty and potential risk involved with direct use of fish from wild sources.

Hatchery propagation can provide a viable opportunity for recovery, if adequate measures are taken to ensure that hatchery broodstocks are representative of wild sources (see Allendorf and Ryman 1987; Lande and Barrowclough 1988; Campton 1995; NRC 1996; Kapuscinski 1997; Reisenbichler 1997; Waples 1999; Lichatowich 1999). There are at least five important issues. **First**, all potential sources representing indigenous genetic material should be considered for use in development of broodstocks for reintroductions. As described directly above and below, translocated and wild sources of LCT are currently represented by small, isolated populations. **Second**, there should be enough founders (breeding adults) in each broodstock to represent the population from which they were drawn. **Third**, when mating individuals in the brood stock, appropriate breeding protocols should be used to minimize inbreeding and maximize genetically effective population size. This will minimize potentially deleterious effects of inbreeding and loss of genetic variation. **Fourth**, efforts should be made to minimize selection for traits that are advantageous in the hatchery, but potentially disadvantageous in the wild. Hatchery environments are dramatically different from the wild, and holding fish under unnatural conditions for any period of time may unintentionally lead to artificial selection. The primary goal of captive propagation is to support reintroductions and promote establishment of natural reproduction. Ideally, hatchery supplementation should be phased out in as short a time as possible once self-sustaining representatives of each broodstock are established. **Fifth**, there should be adequate resources for routine genetic monitoring and assessment to ensure the above goals are met. Routine monitoring is an often-ignored, but critical aspect of hatchery propagation. Other aspects of hatchery management, such as water quality maintenance, disease management, etc., must be evaluated in the context of genetic goals. The specific guidelines for hatchery management practices to maintain the genetic integrity of LCT in the Truckee River basin must be outlined in a separate effort.

*Selection of broodstock for hatchery propagation.* The genetic integrity (e.g., amount of variation, hybridization) of the known indigenous population of LCT in the Truckee River basin (Independence Lake), must be assessed, along with genetic affinities of potential candidate populations for reintroductions (e.g., Edwards and Willow Creeks; Morrison Creek; Macklin Creek; and existing broodstocks). Efforts should be made to ensure that all potential source populations of LCT are accounted for. Review of fishery inventory data for the Truckee River basin should be conducted to determine if there are opportunities for additional surveys to locate indigenous populations of Lahontan cutthroat trout. Once a determination of candidate broodstocks is complete, it will be necessary develop a rationale for allocating recovery efforts among the different candidates. For example, how much hatchery space should each candidate receive? Are some candidates more or less suited for hatchery propagation? Which candidates appear to most closely represent the genetic legacy of indigenous LCT in the Truckee River basin?

*ESUs and local adaptation.* A primary goal of the Endangered Species Act is to preserve genetic variability within and between species (Waples 1995). The National Marine Fisheries Service (NMFS, Waples 1991a) developed an “evolutionarily significant unit” (ESU) policy to clarify “distinct vertebrate population” language in the Endangered Species Act (ESA; Waples 1995). The ESU and DPS concepts describe a population or group of populations that (1) are substantially reproductively isolated (e.g., geographically isolated) from other conspecific population units and (2) represents an important component in the evolutionary legacy of the species (Waples 1991b). These criteria have been adopted by NMFS to identify and guide conservation of salmonid species by addressing questions of genetic and therefore possibly adaptive differences among populations. If populations are genetically divergent, they may be under different environmental selection pressures and *possibly* on different evolutionary trajectories. For example, differences in morphological and life history traits (body size, spawning time, spawning age, dispersal time and dispersal age) may reflect adaptation to local conditions (e.g., Taylor 1991; Healey and Prince 1998). Life history and ecological data can be coupled with genetic data for more comprehensive insights into possible adaptive genetic differences among populations. The ESU approach has been used by NMFS to evaluate, among others, listing petitions for a number of salmonid species (McElhany et al. 2000; <http://www2.nwfsc.noaa.gov:8000>).

There is good evidence to suggest the Truckee River basin population of LCT is a distinct vertebrate population segment, as defined by the ESU policy (Waples 1991). The Truckee River basin is a hydrologically closed system, and thus populations of LCT are reproductively isolated from populations in other basins (e.g., Carson and Walker). This, along with genetic evidence, suggests that indigenous LCT in or from the Truckee basin represent a unique population (or former population). The current recovery plan for LCT (Coffin and Cowan 1995) recognizes three distinct population segments, including a group representing the Carson, Walker, and

Truckee River basins. The lumping of these three basins into a single group was based on evidence indicating the populations were hydrologically isolated only about 10,000 years ago from the rest of the Lahontan basin. Given the dramatic degree of divergence observed within other species of salmonids over similar time frames (e.g., Taylor et al. 1996; Gislason et al. 1999), we suspect important evolutionary differences exist among LCT indigenous to the Carson, Walker, and Truckee River basins.

There is some question of local adaptation within the Truckee basin. Many salmonid species are thought to exhibit local adaptation on a very fine spatial scale (Allendorf and Leary 1988). Significant genetic differences among populations can suggest local adaptation and evolutionary divergence. However, local adaptation is difficult to demonstrate in extant wild populations and is complicated by the fact that genetic differentiation among populations may be the result of metapopulation dynamics and/or genetic drift and not natural selection.

Indirect evidence suggests there may have been a genetic and adaptive differentiation among original Pyramid Lake trout and other western Lahontan basin lacustrine populations (Ellstrand 1992; Rank 1992; Ford 2000; Imsland 2000). For example, Behnke (1992) believed that LCT in Pyramid Lake were locally adapted piscivores. The genetic basis for these traits is not known. LCT presumably from the original Pyramid Lake population have survived, however, for many decades in radically different environments, such as Donner (Morrison) Creek (Hickman and Behnke 1979). The lacustrine population of LCT in Walker Lake was extirpated when the lake naturally desiccated 4500-5500 and again 2000-3000 years before present (Grayson 1987), yet fish persisted within the river, and subsequently recolonized the lake to form a highly productive fishery. In short, there is little evidence to indicate that local adaptation ever existed, or if it did, what the specific nature of locally adaptation was. Using the terminology of Rieman and Dunham (2000), LCT may have a flexible or “facultative” life history. Because there are so many characteristics and conditions that may indicate or lead to local adaptation, it is essentially an “irrefutable hypothesis.” However, given the massive ecological alterations that have occurred to the Truckee River basin over the past century, it makes little sense to debate the issue of local adaptation and regardless of local adaptation arguments, if the progenitors of the transplanted populations (Macklin, Edwards and Pilot Peak) were derived from the Pyramid Lake strain, these populations *may* represent evolutionarily distinct lineages native to the Truckee River drainage. In terms of restoring the evolutionary legacy of LCT in the Truckee basin, the best strategy is to provide maximum representation of remaining indigenous genetic variation, including translocated populations.

### **The problem with hybrids**

In terms of genetics, the largest obstacle to long-term recovery of naturally reproducing, viable populations of LCT in the Truckee River basin is the issue of hybridization with nonnative rainbow trout (*Oncorhynchus mykiss*). Rainbow and LCT are closely related species that readily interbreed. Although no longer stocked extensively throughout the Lahontan basin, rainbow

trout continue to be stocked annually into the Truckee river by Nevada Division of Wildlife (NDOW) to support a popular sport fishery. In addition to the annually stocked fish, a naturally reproducing population of rainbow trout is thought to occur in the Truckee River. Hybridization could compromise efforts to establish a naturally reproducing population of LCT in the Truckee drainage. Control of populations of nonnative fishes is difficult and can be prone to reversal by accidental or purposeful stocking of nonnatives after initial removal efforts. Given that in many western waters there is either active introgression or introgression potential, the role of hybrids in recovery of salmonids is a pertinent issue, but one that is very much open to debate.

Hybridization can represent a significant threat to the conservation of native taxa (Leary *et al.* 1987; Spruell *et al.* 2000; Utter 2000). An intercross or hybridization event is defined as mating between individuals of different species that produces viable offspring. Heterospecific hybridization may lead to extinction by outbreeding depression or genetic assimilation (Ellstrand 1992). Outbreeding depression is the breakup co-adapted gene complexes that have evolved in species in response to particular environments (Dobzhansky 1948; Shields 1983). This can disrupt formation of species specific developmental, physiological and behavioral traits resulting in loss of reproductive fitness and local adaptations (Leary 2000). Genetic assimilation is the gradual replacement of native species genome with that of the nonnative taxon. Closely related species and their potential hybrids pose particularly difficult problems in conservation of native species when ESUs contain few pure populations of the native species as in the Truckee River basin.

Removal or minimization of interaction potential between rainbow and LCT with barrier placement has been the most common approach to preserving unique Lahontan cutthroat populations. However, isolation and fragmentation of populations greatly increases extinction risk (Dunham *et al.* 1997; Dunham and Rieman 1999; Ray *et al.* 2000). The incidence of hybridization in populations of other cutthroat trout subspecies that coexist with rainbow trout is highly variable, for example coastal cutthroat trout and rainbow trout are known to naturally hybridize in parts of their range and not others (e.g., Hawkins 1997; Weigel *et al.* 2000; Allendorf *et al.* 2001). A similar pattern also holds for LCT (Gall and Loudenslager 1981). In the 1970s, rainbow trout were repeatedly stocked in large numbers in eastern basin streams including Gance Creek and Three Mile Creek in the Humboldt and Quinn River basins, but no extant populations of rainbow now exist here. Whereas in other streams, e.g., Sage and Indian Creeks in the McDermitt system of Quinn River basin, hybridization represents a significant threat to native fish populations (Peacock and Briggs 2001). Thus, it is not inevitable that hybridization will be a problem if rainbow trout cannot be removed from the Truckee River basin. However, where and how rainbow and cutthroat trout coexist will be an important to assess defining hybridization risk within the Truckee basin and throughout the range of LCT.

U.S. Fish and Wildlife Service and NMFS have recently issued a joint intercross policy, which

although pending, can provide guidance on dealing with intercross issues in the Lahontan basin. The proposed policy was developed to address diverse hybridization issues while remaining consistent with the ESA mandates (Fed. Reg. 61:4710-4713). Under the proposed policy interbred populations consisting of hybrids and their descendants could be protected under the ESA if in general they, “(1) exhibit the morphological, physiological, behavioral, ecological, genetic, or other measurable traits that characterize the listed species, (2) more closely resemble the listed species than intermediates between the listed species and other species, and (3) have a defined goal in the recovery of the listed species.” Specific situations in which intercross populations would be considered for ESA protection include, “(1) taxonomically recognized species of natural hybrid origin (i.e. not a result of anthropogenic factors) that are threatened or endangered; (2) intercross progeny deliberately produced as apart of an approved recovery and genetic management plan to compensate for loss of genetic viability in a highly endangered species (e.g. Florida panther), or (3) intercross progeny or populations representing significant, unique, or essential portions of the genetic resource of the listed species.” Number three is the only specific situation applicable to LCT populations. Using ESU language, introgressed populations that contain “an important component in the evolutionary legacy” of the listed species could, therefore, be protected under the ESA. Choosing a specific percentage of hybridization to apply in all situations is certainly more unrealistic given limitations of genetic markers to detect hybridization gradients and consideration of unique ESU/DPS factors.

LCT from Macklin, Morrison and Edwards Creeks represent a potentially important part of the evolutionary history of the Truckee river basin. Reintroduction of these fish into the Pyramid Lake, Truckee River and Lake Tahoe interconnected system will expose them to potential hybridization with the extant rainbow trout population in the Truckee River. In general, because hybridization has resulted in extinction of many taxa, policies should be designed to reduce anthropogenic hybridization (Allendorf et al. 2001). Hybrid taxa resulting from anthropogenic causes should be protected only in exceptional circumstances (see Intercross policy above). Elimination of hybridization potential should be the overall goal in the Truckee basin and passive or active means to control hybridization should be applied as needed (e.g., Montana Bull Trout Scientific Group, 1996). This means cessation of planting of rainbow in the Truckee basin and assessment of the extent of hybridization between naturalized rainbow and LCT. Genetic monitoring of introgression will therefore be essential.

### **Adaptive management**

It is clear that recovery of LCT in the Truckee River basin must face a large degree of uncertainty. Examples include uncertainty regarding selection of appropriate broodstock, survival and reproduction of reintroduced fish, and hybridization. Furthermore, there are a variety of management alternatives available to address the issues associated with genetics and recovery of Lahontan cutthroat trout. Effective management is possible, providing some basic guidelines are followed: consider a range of alternatives and favor actions that are robust to uncertainties; favor actions that are informative; probe and experiment; monitor results; update

assessment and modify policy accordingly, and favor actions that are reversible (Ludwig et al. 1993). A key to success in the face of uncertainties will be “learning as we go” through adaptive management experiments. Adaptive management is an intuitively pleasing concept, but it is seldom implemented effectively by management agencies (Walters 1997). Careful collaboration between agencies and academic institutions, along with external peer review should ensure that “adaptive management” activities genuinely work to advance recovery of Lahontan cutthroat trout.

## HISTORICAL BACKGROUND

### **Pleistocene distribution**

LCT is one of approximately 14 allopatrically distributed subspecies of cutthroat trout (*Oncorhynchus clarki*; Behnke 1992). This subspecies dates back at least 30,000 years (Behnke 1972; Trotter 1987), and perhaps back to the Pliocene geological epoch (~2.5 - 4.5 million years before present; Taylor and Smith 1981). Genetic differentiation among cutthroat trout subspecies is most pronounced among Lahontan, Westslope (*Oncorhynchus clarki lewisi*) and coastal (*O. clarki clarki*) subspecies (Leary et al. 1987). These subspecies are also more genetically similar to rainbow trout (*Oncorhynchus mykiss*) than they are to the other cutthroat trout subspecies.

LCT is endemic to the Lahontan basin of northeastern California, southwestern Oregon and northern California (Figure 1). This subspecies evolved in pluvial Lake Lahontan and associated satellite basins in the north-central Great Basin province of western North America (Figure 2; Behnke and Zarn 1976). At that time, LCT had access to myriad stream and large lake habitats within the basin. The high stand of Lake Lahontan occurred about 14,000 years ago, when the lake itself covered approximately 22,100 km<sup>2</sup> in a drainage basin of about 117,000 km<sup>2</sup> (LaRivers 1962; Thompson et al. 1986). Following its high stand, Lake Lahontan rapidly desiccated to near present day levels about 8,000 years ago (Figure 3; Benson and Thompson 1987). LCT, therefore, have a long history in both fluvial and lacustrine habitats in the Great Basin.

Two major river systems in the eastern basin, the Humboldt and Reese rivers, were connected to pluvial Lake Lahontan, but were never inundated by the lake (see Figure 3). Morphological and genetic data suggest that cutthroat trout may have diverged into a western (ostensibly lacustrine) and eastern (fluvial) form prior to the dry-down of pluvial Lake Lahontan (Behnke 1992; Williams et al. 1992; Williams et al. 1998). Observed genetic differentiation within the Lahontan Basin was therefore possibly initiated early in the Pleistocene (~ 1 million years ago; Gall and Loudenslager 1981). As a result, cutthroat trout in the eastern basin may represent a separate subspecies, the Humboldt cutthroat (*Oncorhynchus clarki spp.*), specifically adapted to a fluvial life history. Subspecific distinction has not been formally accepted, however.

### **Modern distribution**

As pluvial lakes rapidly desiccated some 8,000 to 10,000 years ago, populations of cutthroat trout

in the eastern Lahontan basin became physically isolated from those in the western basin. As the drying trend advanced, populations were further isolated into basins and subbasins within this larger eastern and western split.

The western Lahontan basin retained remnants of pluvial Lake Lahontan (Pyramid, Independence, Summit and Walker lakes). Although the three major river basins that contain LCT in the western Lahontan basin (Carson, Walker and Truckee rivers) were never inundated by Lake Lahontan, these stream systems, which originate in the eastern Sierra Nevada mountains, do drain into lacustrine habitats that are remnants of the pluvial lake. The east and west forks of Walker River flow into Walker Lake. Lake Tahoe is the source for the Truckee River which flows into Pyramid Lake. Mahogany Creek drains into Summit Lake. Walker, Pyramid and Summit are terminal lakes (with no outlet), supporting highly alkaline and nitrogen-limited ecosystems. The stream drainages provided spawning habitat and undoubtedly formed networked systems with the lakes that supported all life stages.

The remaining major drainage in the western Lahontan basin is the Quinn River/Black Rock Desert basin located in the north-central portion of the western basin. The Quinn River basin was inundated by Lake Lahontan. In the post-lake period, this system had as many as 46 streams occupied by LCT but now has only 11 extant populations (Coffin and Cowan 1995). Summit Lake, north of the Black Rock Desert, was formed by a landslide approximately 12,500 years ago and was subsequently isolated, along with associated streams, from the rest of the western basin drainages.

North of the Quinn River basin in Oregon, the Coyote Lake basin contains Coyote Lake, small ephemeral lake, and the Willow and Whitehorse stream systems. Though now physically separated from the Quinn River basin, the Coyote Lake and Quinn River populations were probably connected during the Pleistocene. The Quinn River/Black Rock Desert and Coyote Lake basin populations are currently isolated from the remainder of the western basin populations.

In the eastern Lahontan basin, the Humboldt River basin has had LCT populations in at least 10 of its major subbasins historically. These subbasins include Marys River, areas of the East Humboldt River, North and South Forks of Humboldt River, Little Humboldt River, Reese River Maggie Creek, Pine Creek and Rock Creek. The Humboldt River basin supports the largest number of extant fluvial LCT populations native to the Lahontan basin. There were no lacustrine populations in the eastern basin after the desiccation of Lake Lahontan (Coffin and Cowan 1995).

### **Recent population trends**

In the last 150 years, LCT has been virtually eliminated from the western Lahontan basin and currently persists in only about 10% of their original habitat in the eastern Lahontan basin. Loss

of cutthroat populations has been attributed to habitat fragmentation, loss and degradation, overexploitation, competitive interactions and introgression with nonnative salmonid species (Gerstung 1988; Coffin and Cowan 1995; Dunham *et al.* 1997, 1999). Most remaining *naturally reproducing* populations persist in small, isolated stream habitats that were formerly part of large, interconnected lake and/or stream networks. Many popular fisheries in the western basin, including Pyramid and Walker lakes are currently supported exclusively by hatchery reproduction. The Heenan Lake population was originally created by stocking. Two strains of LCT are present in the reservoir, the Heenan strain derived from West Carson river fish introgressed with Rainbow trout and the Independence strain derived from Independence Lake LCT. This population is currently maintained by rearing fish propagated from egg and sperm collected from the Independence strain spawners exclusively. There is a small population of naturally reproducing fish derived from the West Carson river/Rainbow trout hybrid swarm.

### **Western Lahontan Basin**

Naturally reproducing populations of LCT historically occupied several major lacustrine systems in the western Lahontan basin (Figure 4). These include Lake Tahoe and associated lakes (e.g., Fallen Leaf and Cascade Lakes); Pyramid, Winnemucca, Donner, and Independence lakes in the Truckee River basin; Walker and Twin lakes in the Walker River basin; and Summit Lake in the Quinn River/Black Rock Desert DPS (LaRivers 1962). Naturally reproducing populations now persist only in Independence and Summit lakes (Coffin and Cowan 1995).

Pyramid Lake is the only western basin lake that has contained water continuously since the Pleistocene (Hubbs and Miller 1948). The strain of trout that was endemic to Pyramid Lake had persisted in a continuous lake environment for at least 50,000 to 100,000 years prior to extirpation in the 1940s (Behnke 1992). This extirpation represented the first change in the fish fauna of Pyramid Lake since the Pleistocene (and possibly the Pliocene), the most enduring fish fauna in the Lahontan basin (Hickman and Behnke 1979). The Pyramid Lake strain of LCT was considered the largest native trout in western North America (Behnke 1992). Major changes in the lake, including dramatic decrease in lake levels, with accompanying increases in total dissolved solids (Dickerson and Vinyard 1999), may have significantly constrained the productivity of the fishery the last 60 years (Dunham 1996). Genetic differences between the current and historical LCT strains in Pyramid Lake could preclude the current fishery from achieving productivity similar to the original native strain. Potential overstocking of hatchery fish into the lake ecosystem may also be affecting productivity of the existing fishery.

Ideally, recovery of a naturally reproducing LCT population in the Pyramid Lake ecosystem would involve use of the original strain of cutthroat trout from this system. In the first half of the 20<sup>th</sup> century, prior to the development of LCT hatchery stocks, fish from Pyramid Lake were the only stock used for augmentation and *de novo* creation LCT populations throughout the Lahontan basin (Hickman and Behnke 1979). Records on specific location and success of these transplants were, however, not generally kept (Nevada Division of Wildlife records). Genetic data indicate

these transplants were largely unsuccessful. Genotypes typical of western Lahontan basin populations, which should resemble the extinct Pyramid Lake population are uncommon to nonexistent in eastern Lahontan basin populations (Gall and Loudenslager 1981, Williams et al. 1992, 1998, Dunham et al. 1999, Nielsen 2000). There are, however, three LCT populations that were transplanted into out-of-basin and/or fishless streams prior to the 1940s that may represent the Pyramid Lake strain originally found in Pyramid Lake, Lake Tahoe and the Truckee river. Trout from Nevada Fish Commission were sent to Wendover, Nevada in the early part of the century and stocked into the fishless Morrison Creek, Pilot Peak drainage, Utah (Hickman and Behnke 1979). Hickman and Behnke (1979) used the pseudonym “Donner Creek” to protect the actual locality of the unique fish population. In the original analysis, meristic and morphological data supported a western Lahontan basin origin for these cutthroat trout populations and Hickman and Behnke (1979) suggested Donner Creek fish could be the original Pyramid Lake strain. Anecdotal information and stocking records (California Fish and Game) for one population (Macklin Creek, Yuba River drainage) suggests a Lake Tahoe origin. The source of cutthroat trout in Edwards Creek in the Desatoya Mountains in central Nevada, is less certain. Morphologically and meristically the fish in Edwards Creek group with western basin and may have been transplanted originally from the Truckee basin, possibly Pyramid Lake (M. Sevon, Nevada Division of Wildlife, personal communication). Documentation of the origin of known or suspected transplants of unknown origin could play a key role in rebuilding populations previously extirpated.

## NATURAL HISTORY

### **Cutthroat trout in a desert environment**

Despite the loss of habitat that accompanied the dry-down of Lake Lahontan, 8-10,000 years ago, and subsequent isolation of some drainages, LCT populations persisted in large, interconnected aquatic ecosystems. These systems were either lacustrine habitats with tributary streams or large stream networks consisting of a mainstem river and smaller tributary streams. In the early part of the 1900s these large networks were fragmented by water diversions, barriers and loss of habitat throughout the basin (Figure 5). Most LCT streams today are isolated. The LCT populations in the lake systems of western Lahontan basin (except Independence Lake) are maintained by hatchery production as barriers prevent spawning in river habitat. Historically, lacustrine habitats may have acted as refugia during brief periods when connected stream habitat was either unsuitable or unavailable, but intact fluvial habitats have always been essential for reproduction. A possible example of natural extirpation of a lacustrine population of LCT is Eagle Lake, California. Behnke (1992) speculated that the long-term desiccation of a key spawning tributary led to extirpation of cutthroat trout in Eagle Lake. Examples of human-caused extirpations of lacustrine LCT from loss of fluvial spawning habitat include loss of naturally spawning populations in Pyramid and Walker Lakes (LaRivers 1962). Cutthroat trout in large, interconnected systems can have both migratory and nonmigratory (resident) life history strategies (Young 1995; Northcote 1997; Gresswell 1997; Rieman and

Dunham 2000). Resident fish live and spawn within a single stream whereas migratory fish spawn in their natal stream but live elsewhere in the interconnected system (Dunham and Vinyard 1996). Life history strategies may not have a genetic basis per se. Resident fish, however, are typically smaller-sized individuals. Life history strategy may depend upon a combination of fish size (which does have a genetic component) and size frequency within the population. Multiple life histories can enhance population persistence by spreading individuals (and associated risks) among different habitats, and can enhance productivity by allowing individuals to exploit a broader range of habitats (Rieman and Dunham 2000). Connectivity may also enhance population persistence by allowing dispersal or “straying” among populations, a prerequisite for metapopulation dynamics (McElhany *et al.* 2000; Rieman and Dunham 2000; Ray *et al.* 2000). Genetic data from the Marys River system (Elko County, Nevada) suggests both migratory and resident life histories are present within this large interconnected system (Neville, unpublished data).

In the western Lahontan Basin, the two remaining lacustrine systems that support naturally reproducing populations of LCT (Summit and Independence lakes), are presumed to adopt both migrant and resident life histories, similar to other salmonid species in lacustrine systems. Today LCT also inhabit many streams that rarely or never connect with river habitats, here LCT populations are constrained to the resident life-history, where they cannot escape local risks. Across the eastern Lahontan basin, presence of LCT in local stream habitats is strongly tied to habitat size (Dunham *et al.*, in press). This pattern suggests that populations constrained to smaller habitats are at higher risk of extirpation, and populations in larger habitats somehow avoid risks, perhaps through metapopulation dynamics (Dunham and Rieman 1998; Ray *et al.* 2000).

### **Metapopulation dynamics**

LCT invokes the theory of metapopulation dynamics (Coffin and Cowan 1995; Dunham *et al.* 1997; Rieman and Dunham 2000). Metapopulation theory applies to discrete and independent populations that persist through an extinction/recolonization dynamic, whereby populations that go extinct are recolonized by individuals from extant populations (Levins 1969, 1970; Hanski and Gilpin 1997). In order for metapopulation dynamics to effectively extend the persistence of a population network, populations must fluctuate independently, so that when one population is small or extinct, another is large enough to provide rescue or colonists. Population asynchrony can be achieved only if two conditions are met: (1) populations experience sufficiently independent environments, and (2) populations exchange very few individuals per generation. Independent environments are necessary for generating asynchrony in population fluctuations, and low interpopulation exchange is necessary for maintaining this asynchrony.

In a strict sense, salmonid population dynamics do not fit metapopulation theory. First, tributaries and mainstem rivers and/or lakes within interconnected systems are not discrete habitat patches. Second, all or a large fraction of individuals regularly migrate between the far-

flung habitats available in any interconnected system. The vagility of these fish reduces the potential for population subdivision. Third, migrating individuals from separate natal tributaries often share a common habitat as adults. Environmental fluctuations in the shared habitat affect all adults similarly, synchronizing (to some extent) the dynamics of all populations that use the shared habitat. Finally, the longevity of salmonids, combined with the fact that individuals of different age classes occur in different habitats, both reduce the potential for complete extinction of local populations. Thus, the salmonid life-history spreads the risk of each *single* population over space and time. Metapopulation theory deals only with the spread of risk among *multiple* populations.

Yet there is potential for metapopulation dynamics at some scale in these aquatic systems. The mechanisms for population subdivision in this vagile trout include (a) inherent homing behaviors and (b) the ephemeral nature of aquatic habitat connectivity in a desert environment. The homing behavior of spawners allows asynchrony among natal environments to affect asynchrony among populations. Although the survival and growth of adults from different populations may be synchronized in a common habitat, adult fertility and the survival of younger classes are affected by the natal environment. If natal environments differ among populations, there is potential for asynchrony among populations. Homing behavior guarantees that this asynchrony is perpetuated across generations. Discontinuities in the aquatic habitat can also reduce population synchrony by reducing interpopulation exchange. In desert environments, especially in areas managed for multiple use, there are several sources of disruption in aquatic habitat connectivity, including: (a) occasional, seasonal or permanent dessication of watercourses due to natural causes (e.g., precipitation cycles) or anthropogenic causes (e.g., de-watering, tamarisk invasion, livestock damage to the water channel or vegetation cover); (b) regions of high water-temperature due to natural or anthropogenic effects on channel condition or vegetation cover; (c) regions dominated by exotic fauna that exploit, exclude or interbreed with LCT; or (d) mechanical barriers to movement, such as natural waterfalls or water diversion facilities (even minimal dams can form complete barriers along the diminutive streams in this arid landscape). Thus, the homing behavior of LCT, combined with variation between natal environments and multiple opportunities for natural or anthropogenic disruption of habitat connectivity, creates the potential for population asynchrony and metapopulation dynamics.

In these arid environments, LCT persistence may require *both* the spreading of risk among age classes within a population (age-structured dynamics) and the spreading of risk among populations (metapopulation dynamics). Age-structured dynamics may allow LCT to survive impacts that affect regions smaller than the normal reach of a population, while metapopulation dynamics allow LCT to survive impacts that affect regions smaller than the maximum dispersal distance of an adult individual. The difference between the ‘normal’ and ‘maximum’ scales of adult movement will determine the extent to which metapopulation dynamics can enhance LCT persistence. Another important determinant of the potential for metapopulation dynamics is access to multiple habitats. The more habitats a population (or population network) has access to,

the less vulnerable the population should be to local habitat degradation or local catastrophe. The fact that many (30 or more) local populations of LCT in the eastern Lahontan basin have declined to undetectable levels in recent years (Elliott *et al.* 1997) suggests

that these fish no longer have access to the multiple habitats they may need for survival (Dunham *et al.* 1997, 1999, in press).

Further evidence of the relevance of habitat connectivity is emerging from research on LCT populations in the Marys River basin. Age-structured data from several different streams in this basin suggest that fish of different ages use different portions of the habitat. Therefore, different age classes may have different habitat requirements. Models developed for these populations also predict that isolated populations, are more vulnerable to extinction under current or foreseeable environmental conditions (Peacock *et al.* 1999; Ray *et al.* 2000). These models predict that while populations within individual streams are vulnerable to local extinction, the population network as a whole is persistent. The mechanisms responsible for persistence in this network are (a) population dynamics that are independent and often uncorrelated among streams, perhaps due to environmental distinctions among streams, and (b) density-dependent movement of some age classes between streams. The general lesson drawn from this modeling work to date is that age-structured movement patterns within interconnected waters can facilitate persistence fluvial LCT populations, despite periodic local extinctions (Ray *et al.* 2000). Therefore, maintaining connectivity and habitat diversity in stream systems may be as crucial to the persistence of fluvial LCT as maintaining connectivity between spawning and lake habitats is for the persistence of lacustrine LCT.

## GENETIC ANALYSES

### **Genetic data - what it can tell you**

Implicit in genetic data is the genetic history (gene genealogy) of individuals and thus the populations they comprise (Slatkin 1985; Slatkin and Maddison 1990; Avise 1994; Moritz and Hillis 1996). This history encompasses not only contemporary processes but also long-term patterns of population increases and decreases due to death, reproduction and movement (dispersal and/or migration) of individuals among populations (Slatkin 1985, 1987; Hedrick 2000). The historical relationships among populations, subspecies and species can be reconstructed as a phylogeny (phylo=historical, geny=genes) of contemporary individuals. The genetic similarities and the differences among individuals and among populations provide the information used to reconstruct phylogenetic (historical) relationships. The phylogenetic distance between groups of individuals reflect both the time since their separation and the events that have occurred since separation (e.g., changes in group size). Populations are commonly connected by small amounts of dispersal, so detecting their genetic differences requires analysis of highly variable genetic markers—markers that accumulate mutations more rapidly than weak migration can homogenize these differences among populations (Wright 1969). Genetic data are typically

highly variable and often exceed variation found in morphological characters. As a result, genetic data have been routinely used to distinguish among populations, subspecies and species for the past 30 years (Lewontin and Hubby 1966; Avise 1994; Weir 1996).

The genetic marker and method of analysis proposed for a study must be appropriately matched (Moritz and Hillis 1996; Parker et al. 1998; Hedrick 1999; Sunnucks 2000; Figure 6). Thus when choosing a genetic marker system to address a particular question it is critical to consider: (1) the evolutionary time frame of the question being asked, (2) the rate and mode (e.g., neutrality vs. selection) of evolution of the genetic marker, and (3) mode of inheritance (e.g., maternal, biparental) and expression (dominant, codominant). The rate of evolution of the marker will have direct bearing on the amount of genetic variation [e.g., heterozygosity ( $H$ )] found in population(s). The greater the amount of heterozygosity within and between populations the greater the chance of detecting differences if they exist. However, if a genetic marker evolves at a very fast rate, it is an inappropriate marker to resolve very old phylogenetic relationships (e.g., > 10 million years). The fast rate of evolution will erase the phylogenetic history that you are trying to reconstruct; in other words, the genetic divergence among populations results in virtually no shared alleles. Conversely, genetic markers with slow rates of evolution are inappropriate markers to resolve relationships among more recently isolated populations or recently diverged subspecies or species (e.g., 10,000-250,000 years). When dealing with questions of contemporary gene flow, population isolation, and recent speciation events, a highly variable marker with a fast rate of evolution can increase resolution significantly.

*Genetic markers.* There are three general classes of genetic markers that are routinely used in population genetic and phylogenetic studies: (1) allozymes, (2) mitochondrial and chloroplast DNA, and (3) nuclear DNA (for a general review see Parker et al. 1998). These classes of markers differ in their molecular structure, mutation rate, and function and thus utility in population genetic studies (Table 1; Hillis et al. 1996; Sunnucks 2000). Allozymes, mitochondrial DNA and a specific class of nuclear markers (microsatellites) will be reviewed here. These markers were chosen because they have been used in the study of LCT population structure and hybridization.

*Allozymes.* Allozymes are allelic variants of proteins that are the product of genes (DNA sequences) at a particular location (locus) along a segment of DNA (Avise 1994; Hedrick 2000). Proteins play a vital biochemical role, catalyzing chemical reactions and forming structural components in the body. Analysis of allelic protein variation via starch gel electrophoresis by Lewontin and Hubby (1966) and Harris (1966) was a landmark development in population and evolutionary genetics and marked the beginning of the field of modern molecular genetics. Proteins used in starch gel electrophoresis are isolated from various animal (and plant) tissues. The variation in allozymes is the result of physical differences in protein structure that can be ultimately traced back to mutations or 'substitutions' in the DNA sequence (sequence of base

pairs) which codes for the string of amino acids that make up the protein. Not all substitutions in a coding sequence result in amino acid substitutions, and not all differences in the amino acid composition of a protein can be assessed through protein electrophoresis. The result is that there are relatively few variants (alleles) per protein coding gene (locus) (Hartl and Clark 1997). Allozymes have been used extensively in population biology. They are assumed to be selectively neutral but there is evidence for selection at some protein coding loci (see Parker *et al.* 1998). Because of possible selective constraints on loci, and indirect inference of allozyme variants, the degree of polymorphism at allozyme loci can vary tremendously within and across taxa (Parker *et al.* 1998). Therefore it is difficult to define a set time frame in which allozyme data can resolve phylogenetic relationships.

*Mitochondrial DNA.* Animal mitochondrial DNA (mtDNA) is a closed, circular molecule found in the mitochondrion, a cellular organelle involved in cellular respiration. Mitochondrial DNA codes for approximately 37 genes whose protein products mediate cellular respiration. The mtDNA molecule is a single molecule that is inherited maternally (through the egg). Unlike the paired DNA molecules in the nuclear genotype, the mitochondrial ‘haplotype’ does not undergo sexual recombination. MtDNA can be isolated from either tissue or blood. Variation in mtDNA is assessed at the sequence level, because examining the protein products of these genes cannot necessarily assess ‘point’ mutations (substitution of one DNA base pair for another). There are few ‘noncoding’ regions (regions that do not code for a gene product) in the mtDNA sequence. Thus, selective pressures may reduce the rate of accumulation of point mutations in this portion of the genome. However, partially due to lack of recombination and low efficiency of DNA repair mechanisms, mtDNA evolves at a rate faster than single-copy genes in nuclear DNA, which makes this molecule extremely useful for phylogenetic analyses. MtDNA variation can resolve relationships of species that have diverged as long as 8-10 million years before present (Hartl and Clark 1997). As species begin to diverge, the number of substitutions accumulate most rapidly in the noncoding regions of the mtDNA. As differences between two sequences increase, two factors reduce the rate of sequence divergence: the number of shared (identical) base pairs declines, and the average selection pressure on the remaining shared base pairs increases. After about 8-10 million years, sequence divergence is too slow to allow sufficient resolution of divergence times. Thus mtDNA is not appropriate for reconstruction of relationships among populations, subspecies and species that diverged >10 million years ago (Hartl and Clark 1997).

*Microsatellites.* Microsatellites are one of a class of highly variable, noncoding (selectively neutral) genetic markers called VNTRs (variable-number-tandem-repeats) that are found dispersed throughout the nuclear genome (Jeffreys 1985; Tautz 1993; Sunnucks 2000). Unlike allozyme or non-PCR (polymerase chain reaction = the amplification of DNA sequences using polymerase enzymes) based mtDNA methods, these markers can be assayed using non-lethal fin clips and archived scale samples, facilitating retrospective analyses and the study of depleted populations. A number of microsatellite markers are commonly used in molecular population biology, and the choice of a particular marker depends upon the question being asked (Parker *et*

al. 1998; Spruell *et al.* 2000; Sunnucks 2000).

Microsatellite markers are routinely used to examine population-level questions such as gene flow and genetic differentiation among populations (e.g., common toad, *Bufo bufo*, Scribner *et al.* 1994, Hitchings and Beebee 1998; rattlesnake spp., Gibbs *et al.* 1997; large mouse-eared bat, Petri *et al.* 1997; ant spp., Chapuisat *et al.* 1997; pikas, *Ochotona princeps*, Peacock 1997 and Peacock and Smith 1997a, b; brown trout, *Salmo trutta*, Estoup *et al.* 1998; coastal cutthroat trout *Oncorhynchus clarki clarki*, Wenberg *et al.* 1998; bull trout, *Salvelinus confluentus*, Spruell *et al.* 1999). These are co-dominant markers composed of simple sequence motifs of two to four DNA bases that can be repeated up to ~100 times at a locus. Microsatellites are among the fastest evolving genetic markers, with  $10^{-3}$ -  $10^{-4}$  mutations/generation (Goldstein *et al.* 1995). The extensive variation at these loci is largely due to their selective neutrality and mode of evolution. The amount of genetic variation found at these loci has increased the power to resolve relationships between individuals, as well as between populations and closely related species. Because individual loci are identifiable, variation at microsatellite loci can be analyzed using standard statistical models of gene flow (Wright 1969; Weir and Cockerham 1984). Recently, gene flow analyses have benefitted from statistical models developed specifically for microsatellites (Goldstein *et al.* 1995; Slatkin 1995; Michalakis and Excoffier 1996; analysis software GENEPOP, Raymond and Rousset 1995; FSTAT, Goudet 1995).

Microsatellites have been useful in constructing within-species, population-level phylogenies (McConnell *et al.* 1997; Rowe *et al.* 1998; Petren *et al.* 1999) and phylogenies of closely related species (Pepin *et al.* 1995; Primmer *et al.* 1996; Takezaki and Nei 1996; Goldstein and Pollock 1997). Bowcock *et al.* (1994) used microsatellites to construct a phylogeny of human populations with divergence times of >200,000 years. This phylogenetic tree reflected the geographic origin of the individuals with remarkable accuracy. The reliability of microsatellite markers to reconstruct historical relationships among populations is particularly relevant to the question being asked here, namely, what is the origin of founders for the populations of putative Pyramid Lake fish? The evolutionary rates of microsatellite markers fit within the estimated timescale of divergence of populations within the Lahontan basin (mid-late Pleistocene) and are thus well suited to reconstructing population-level phylogenetic relationships, especially for populations within the western Lahontan basin where most divergence has occurred post dry down of pluvial Lake Lahontan (~8,000-10,000 before present).

*Phylogenetic analysis.* Analysis of genetic data to determine phylogenetic and therefore historical relationships is based upon explicit criteria developed from a large body of theoretical and empirical literature (Moritz and Hillis 1996; Swofford *et al.* 1996; Luikart and England 1999; Avise 2000). Methods include mathematical algorithms, which incorporate estimates of DNA mutation rates. However, because genetic markers used to infer phylogeny represent only a fraction of the genome, and certain demographic processes cannot be inferred from genetic data, construction of phylogenies is an estimation procedure (Swofford *et al.* 1996). General

assumptions of phylogenetic reconstruction include Mendelian inheritance of genes and independence among genetic loci, i.e., changes at one locus (gene) do not influence the probability of change at another locus. There are a number of different approaches that are commonly used to estimate phylogenetic relationships, e.g., parsimony, maximum likelihood and cluster analysis (Hillis et al. 1996; Swofford et al 1996; Luikart and England 1999). Each of these methods incorporates different assumptions and criteria for establishing relationships. Which method represents the best approach to phylogenetic reconstruction is currently a hotly debated topic in the scientific literature (Lyons-Weiler and Hoelzer, 1999; Milinkovitch and Lyons-Weiler 1998). The accuracy of phylogenetic analyses continues to improve through development of new methods for mathematical analysis and phylogenetic hypothesis testing (see Hillis 1995, Kuhner et al. 1998).

Phylogenetic analysis uses similarities in allele frequencies among populations to create phylogenetic trees. Allele frequencies at all loci are determined per population, and all pairwise comparisons are made among populations. Assuming isolation-by-distance, geographically proximate populations should show greatest genetic similarity. Genetic similarity among proximate populations may be due to current gene flow, or common ancestry (if movement among populations is no longer possible as a result of barriers). If genetic analyses do not reveal this general pattern, then other models must be invoked to explain the patterns observed. Populations that are at least semi-isolated (receiving little gene flow) *and small* are more susceptible to random genetic drift (Hartl and Clark 1997). Genetic drift can result in genetic changes that erase evidence of recent gene flow or common ancestry. Small populations are also susceptible to genetic bottlenecks, random reductions in population size and genetic variation, that make reconstruction of historical relationships somewhat problematic (Richards and LeBerg 1996). Thus, the potential resolution of phylogenetic analysis is reduced by drift and bottlenecks, and reduced further by use of genetic markers with low variability.

### **Assessing Differentiation among Lahontan cutthroat trout populations**

*Phenotypic Classifications: Morphological and Meristic data.* Morphological (shape, size) and meristic (countable) characters have both a heritable (genetic) and nonheritable (environmentally influenced) component. Natural selection and evolutionary history can shape morphological characters, but differences (or lack thereof) among populations, subspecies or species may also be influenced or determined by the environment. With the advent of genetic methods, taxonomic classification based solely upon morphological and meristic differences has become rare. Instead, these data are used in conjunction with genetic data to strengthen taxonomic inference (DeMarais et al. 1992, DeMarais et al.1993).

All cutthroat trout subspecies are similar morphologically, but differ in some meristic characters. A principal components analysis conducted on a suite of body characters and growth patterns showed that all cutthroat trout subspecies exhibit similar patterns of growth and overall body

shape (Gall and Loudenslager 1981). Systematic variation in meristic characters (pectoral and pelvic fin rays, branchiostegal rays, gill rakers, lateral series scales, and scales above the lateral line) differentiated two broad groups of LCT populations. The first group included populations native to the Walker and Truckee River drainages in western Lahontan basin, the Humboldt and Reese River drainages in the eastern Lahontan basin and Morrison Creek, a transplanted population in the Pilot Peak drainage in Utah. Morrison Creek fish are meristically most similar to native Walker basin and Independence lake populations. The second group consisted of all remaining eastern Lahontan basin populations (Gall and Loudenslager 1981). Because morphological and meristic characters can be influenced by the environment, variation in these characters may not have a genetic basis, and these characters do not necessarily provide information on genetic and evolutionary relationships (Gall and Loudenslager 1981). However, when combined with genetic data, morphological and meristic data can provide information on important environmental effects on phenotype, as discussed below.

*Allozyme data.* Limitations of phenotypic characters led to protein electrophoretic studies undertaken in the 1970s and 1980s. Protein markers (allozymes) were the most variable genetic markers available to address population genetic differentiation at this time. Allozyme data have been used to test for geographical patterns within and among inland cutthroat subspecies, and between cutthroat and closely related rainbow trout (*Oncorhynchus mykiss*) (Loudenslager and Gall 1980, Gall and Loudenslager 1981, Bartley et al. 1987, Leary et al. 1987, Xu 1988, Mirman et al. 1992, Bartley and Gall 1993).

On average, LCT populations have low levels of allozyme variability (11-35 loci, avg. alleles per locus = 2,  $\overline{H} = 0.039$ , N = 24 populations (Loudenslager and Gall 1980). Using *F*-statistics, we can test for genetic differentiation between pairs of populations. Using *G*-statistics, we can measure average genetic differentiation among groups of populations (Hartl and Clark 1997). Statistical analyses of allozyme data indicate that Lahontan basin populations tend to be genetically isolated, and have undergone extensive genetic subdivision since the end of the pluvial period (~10,000,  $G_{ST} = 0.445$  on a scale of 0-1, Loudenslager and Gall 1980). Allozyme data support earlier conclusions drawn from meristic data, that the Walker, East Carson, Truckee and Humboldt drainages are genetically distinct from other populations in the eastern Lahontan basin (Gall and Loudenslager 1981). Gall and Loudenslager (1981) referred to the populations in these drainages as separate ‘microgeographical races.’ The Reese river system in the central portion of eastern Lahontan basin was another distinct group of populations, genetically differentiated from the other drainages in both the eastern and western Lahontan basin (Loudenslager and Gall 1980; Gall and Loudenslager 1981; Xu 1988).

Allozyme data support a Lahontan basin origin for the Morrison Creek population. Genotypes in the Morrison Creek population clustered with other LCT populations and not with the Bonneville cutthroat populations within the Bonneville basin where Morrison Creek is located (Gall and Loudenslager 1981). However, refinement of the relationship between Morrison Creek fish and

other LCT populations proved difficult with allozyme data alone. Although allozyme data revealed substantial intra-subspecific divergence within the Lahontan basin, limited genetic variation precluded a more fine-scale population-level phylogenetic analysis of western basin populations (Bartley *et al.* 1987; Leary *et al.* 1987; Xu 1988). To some extent, failure to refine allozyme relationships between populations may have been due to the fact that these analyses included only a few populations from each drainage (Walker, East Carson, Truckee and Humboldt drainages).

Gall and Loudenslager's (1981) analysis of strains used for hatchery stocks, including LCT from Heenan, Walker, Independence and Summit lakes, reveal hybridization with rainbow trout in the Heenan stock only. All available pure LCT broodstocks were genetically diverse, except for Summit Lake, which was highly invariant. Because Gall and Loudenslager (1981) suggested that local, indigenous populations of LCT may each represent a 'microgeographic race', use of local (and perhaps locally adapted) fish in restoration activities was recommended over use of hatchery fish from genetically distinct portions of the Lahontan basin (Gall and Loudenslager 1981; also see Allendorf and Leary 1988; Allendorf and Waples 1995).

At larger scales, genetic differentiation is assured due to 'isolation-by-distance' (Wright *ref.*); i.e., individuals separated by larger distances seldom mate. Physical isolation and genetic differentiation at smaller scales can result from drift due to recent habitat loss and fragmentation (Dunham *et al.* 1997), or from strong differential selection (local adaptation). Local adaptation could partially explain the widespread failure of historical transplants of 'black-spotted' trout (possibly Pyramid-strain LCT; Coffin and Cowan 1995). However, transplants of cutthroat trout are frequently unsuccessful within formerly occupied habitat due primarily to restricted habitat size and presence of nonnatives (Harig 2000). It is worth noting that transplants of nonnative trout are often very successful (Fuller *et al.* 1999), so local adaptation is but one of many important issues in population recovery.

The results of Gall and Loudenslager's allozyme study (1981) are consistent with the pattern of habitat fragmentation and isolation of local populations in the basin (Dunham *et al.* 1997, 1999, *in press*). A lack of concordance between genetic relationships among populations, defined using genetic identity measures (Nei 1973), and specific geographic location (Loudenslager and Gall 1980, Gall and Loudenslager 1981, Xu 1988) suggest population isolation, small population size and low levels of within-population genetic variability.

*Mitochondrial DNA data.* In the 1980s, techniques to isolate and analyze mtDNA were developed and this genetic marker came into wide usage (Brown and Wright 1979; Brown *et al.* 1979; Dowling and Brown 1989; Moritz 1994). The faster rate of evolution and thus greater accumulation of genetic variation gave mtDNA an advantage over allozyme data in resolving questions of genetic and historical relatedness. MtDNA restriction-fragment-length-polymorphism (RFLP) analysis was used to examine the systematic and phylogenetic status of

naturally occurring cutthroat trout populations in Nevada (Williams *et al.* 1992, 1998). Phylogenetic trees were created using genetic distance matrices and either the neighbor-joining algorithm of Saitou and Nei (1987), the least-squares method of Fitch and Margoliash (1967).

MtDNA data suggest that cutthroat and rainbow trout, two closely related species in the *Oncorhynchus* genus, speciated roughly two million years ago (Williams *et al.* 1998). Genetic divergence and subspeciation events within the cutthroat group are thought to have occurred during the late Pleistocene, with much of the population level divergence having occurred since the end of the last glacial interval. Divergence among cutthroat trout populations within the Lahontan basin has occurred since subspeciation, and therefore is quite recent evolutionarily (Loudenslager and Gall 1980; Williams *et al.* 1998). As a result most of the significant genetic divergence and evolutionary events within the inland basins have occurred well within the last million years, and likely within the last 100,000 years (Williams *et al.* 1992, 1998).

There is very little mtDNA variation within populations found in the Lahontan basin. Individual LCT populations tend to have a single mtDNA RFLP variant or haplotype (Williams 1992, 1998). This pattern is thought to be typical of genetically pure wild trout populations (Billington and Herbert 1991). Inland trout populations in the Great Basin tend to be small, and genetic coalescence to a single mtDNA haplotype is a natural outcome of continually small population size over time. Multiple mtDNA haplotypes in small isolated populations would suggest either a recent reduction in population size (meaning genetic coalescence has not taken place yet), or introduced haplotypes (via introduced fish). The lack of mtDNA haplotype diversity within populations within the Lahontan basin suggests that recent stocking efforts have not enhanced breeding populations. Allozyme data show the same pattern. If Pyramid Lake fish bred successfully throughout the Lahontan basin, we would expect to find western-basin mtDNA haplotypes present in the eastern basin and multiple haplotypes within at least some populations.

Williams *et al.* (1992) analyzed 16 LCT populations from the Humboldt, Quinn, Truckee, Carson and Walker River drainages. Reese River, the only other major drainage in the Lahontan basin that supports LCT, was not included in this study. A second study (Williams *et al.* 1998) analyzed only samples from western-basin drainages; Quinn River, Summit Lake, Edwards Creek and the Willow/Whitehorse population in southern Oregon. MtDNA sequence divergence (0.13%) identified a clear genetic separation between eastern- and western-basin populations. A single, distinct haplotype predominates in each basin (Williams *et al.* 1992, 1998). The predominant eastern-basin mtDNA haplotype was not found in any western-basin populations, and only two fish from Humboldt River populations carried a western-basin haplotype. The Quinn River drainage was genetically distinct from other western populations and from the Humboldt River populations (Shiozawa and Evans 1997; Williams *et al.* 1998). The Quinn River populations have unique restriction sites that separate these populations from all other LCT (Williams *et al.* 1998). The sequence divergence between Humboldt River populations and western-basin populations was comparable to divergence between recognized subspecies, e.g., Yellowstone and Northern

Bonneville (0.32%), Colorado and Southern Bonneville (0.29%), Paiute and Lahontan (same mtDNA haplotype, Williams *et al.* 1998). These data support ESU designation for populations in the western basin, the Humboldt River and Quinn River drainages.

In an attempt to increase resolution of phylogenetic analyses using mtDNA, Nielsen (2000) sequenced a 198 base-pair segment of the mtDNA d-loop (a highly variable, noncoding region). Although there was clear separation between LCT and coastal cutthroat trout subspecies there were no appreciable sequence differences among LCT populations within the basin (Nielsen 2000). This result suggested that further resolution of population level differences would have to be undertaken with a more variable genetic marker.

The lack of mtDNA haplotype variation within populations and regional fixation of single or few mtDNA haplotypes can be explained by metapopulation dynamics, where populations within basins operate as isolated metapopulations in which extinction-recolonization dynamics have winnowed the number of haplotypes down to one per basin (Hedrick & Gilpin 1997). This hypothesis is supported by ecological data that suggest LCT populations have experienced reductions in population size or local extinction due to droughts, floods and other environmental impacts (Dunham and Vinyard 1996 Dunham *et al.* 1997). Repeated bottlenecks in population size, due to losses of subpopulations within large systems, most likely have resulted in genetic coalescence to single mtDNA haplotypes. Time to fixation in a metapopulation (where local populations fluctuate by definition) is determined by the scale of local extinctions, where large scale (large geographical area) extinctions bring fixation much faster than small-scale, independent extinctions (Ray 2000).

*Microsatellite data.* Limited sampling of populations throughout the basin precluded a range-wide, population-level phylogenetic analysis under previous genetic studies. As a result, the existing genetic data could not be used to address genetic relatedness among fish from Macklin, Morrison and Edwards creeks and populations within the Lahontan basin. A separate study was undertaken to specifically address Macklin, Morrison and Edwards creek fish in the context of population-level phylogenetic relationships throughout the range of LCT (Dunham *et al.* 1998; Nielsen 2000).

The rate of evolution of microsatellites makes these appropriate markers to address divergence times on the order of those within the Lahontan basin (<100,000 years). Primers for eight highly polymorphic microsatellite loci (average alleles per locus = 19.6, range 8-36) developed from closely related salmonid species (*Oncorhynchus nerki*, *O. mykiss*, *O. tshawytscha*, *Salvelinus fontinalis*, *Salmo salar*) were used to construct a phylogenetic tree for ten populations from the Truckee, Walker, Carson and Humboldt river drainages and Macklin, Morrison and Edwards creeks (Table 2). Samples from Paiute trout, Westslope and Coastal cutthroat subspecies were used as ‘outgroups’ (taxa assumed to be more distantly related than the focal taxa; Swofford *et al.* 1996). Two of the ten populations were hatchery fish from the Pyramid Lake Lahontan National

Fish Hatchery and Pilot Peak Lahontan Fish Hatchery. The Pyramid Lake hatchery propagates stock were derived from Independence strain from Heenan Lake, native Walker lake strain (now extirpated), and Independence, and Summit lake populations. Hatchery fish currently stocked in Pyramid Lake are taken exclusively natural spawners from the lake. The Pilot Peak hatchery consists of stock developed from the Morrison Creek population, which may have derived from the extirpated Pyramid Lake strain.

A genetic distance matrix (summarizing genetic distances between all population pairs) was calculated using an approach developed by Goldstein *et al.* (1995) for use with microsatellite loci (Dunham *et al.* 1998, Nielsen 2000). This method assumes a strict single-step mutation model ( $\pm$  one repeat unit) for each microsatellite locus (Estoup *et al.* 1995; Rousset 1996). Microsatellite data were used to generate an unrooted, consensus, neighbor-joining tree (Saitou and Nei 1987). Unrooted refers to a method of phylogenetic tree construction which does not reference a common ancestor. Random bootstrap replications (1000 replications) of neighbor-joining trees were used to assess the reproducibility of the relationships among populations in the final consensus tree (Nielsen 2000). The bootstrap procedure involves randomly drawing a subset of the original data (with replacement) and estimating a phylogenetic tree (Hartl and Clark 1997). Also measured were the geographic distance and the genetic differentiation ( $F_{ST}$ ) between each pair of populations. These measures of physical and genetic distance were compared to evaluate relative historical influence of gene flow and genetic drift on the non-hatchery populations in the analysis (Nielsen 2000).

As with allozyme data, results of regional  $F_{ST}$  pairwise comparisons using microsatellite data showed a lack of concordance between geographic distance and genetic distance for the natural populations. Again, this lack of concordance could result from metapopulation dynamics and coalescence. This scenario are supported by ecological data which suggest that populations within basins tend to be isolated and frequently experience reductions in population size due to highly variable environmental perturbations (Dunham and Vinyard 1996).

As expected, average heterozygosity for the ten microsatellite loci ( $\bar{H} = 0.41$ ) was much greater than average heterozygosity at allozyme loci ( $\bar{H} = 0.039$ ), since microsatellite markers have faster rates of evolution. There was a clear differentiation between LCT and other cutthroat trout subspecies (Figure 7). Coastal and Westslope subspecies appeared as outgroups in 79% and 99% of phylogenetic trees, respectively.  $F_{ST}$ , which ranges from 0 (identical) to 1 (fixed for different alleles), was 0.524 between Westslope and Lahontan subspecies, 0.488 between Coastal and Lahontan subspecies. Microsatellite data support a pattern of differentiation between eastern and western Lahontan basin populations (53% bootstrap value and  $F_{ST} = 0.496$ ). The  $F_{ST}$  between eastern and western populations was comparable to values calculated between distinct subspecies (see above).

Allozyme, mtDNA, and microsatellite data all reveal genetic population structure within the Lahontan basin and suggest a pattern of genetic structuring (Dunham *et al.* 1999; Nielsen 2000). Within the western Lahontan basin, microsatellite data indicate there are two main groups of populations (Figure 5; 55% bootstrap value): (1) Paiute cutthroat, Summit Lake, East Carson River and Pyramid Lake hatchery and (2) Macklin Creek, Morrison Creek, Edwards Creek and Pilot Peak hatchery. We should emphasize here, however, that sample sizes were very small for some populations, and single populations are used to represent entire basins or subspecies in the Nielsen (2000) report. Single populations represent Paiute cutthroat trout (Fourmile Creek) and LCT in the Walker basin (Slinkard Creek). By the early 1900s the only remaining naturally reproducing LCT population in the Walker basin was By-Day Creek, a small tributary of the East Walker River, which drains into Walker Lake. LCT from By-Day Creek were subsequently transplanted into Murphy, Mill, Slinkard and Bodie Creeks within the Walker River basin. Slinkard Creek is the largest and most robust extant Walker basin population.

More loci, samples and populations are needed to make a truly rigorous inference from the genetic data about the order of populations within these groupings and populations included within groups. All genetic data sets analyzed to date, however, suggest similar large geographic scale patterns of genetic relatedness.

The  $F_{ST}$  values calculated between Paiute cutthroat trout and western-basin LCT populations (0.667) and between Paiute and eastern-basin LCT (0.619) both indicate substantial genetic differentiation. However, at this point the pattern or structuring of this variability is uncertain. Paiute cutthroat trout may have diverged from Lahontan cutthroat prior to the eastern-western split in LCT genotypes (Nielsen 2000). Nielsen's (2000) phylogenetic analysis and Williams *et al.* (1992) mtDNA sequence divergence analyses suggest a close relationship between Paiute cutthroat trout and Summit Lake LCT. This conclusion is not supported by the  $F_{ST}$  analysis (Lahontan and Paiute cutthroat trout,  $F_{ST} = 0.667$ ). Because data were combined from all western-basin populations for the subspecies comparisons, the relationship between particular LCT populations and Paiute populations could not be determined from this analysis. The proximity of the geographical range of Paiute cutthroat and the Carson River drainage may explain the closer relationship between these populations suggested in the bootstrap analysis (see Figure 7). It is unclear at this point why the Summit Lake population and Paiute cutthroat, a separate species, cluster together. Again, more loci, larger sample sizes, and additional populations may help clarify these relationships.

The Pyramid Lake hatchery trout represent a mixed stock originating from western basin populations (Walker, Independence, and Summit lakes), which explains the genetic linkage between hatchery and western basin populations to Summit Lake and East Carson River populations. However, the percentage of bootstrapped trees that reproduce this particular relationship among Paiute, Summit Lake, East Carson River and Pyramid Lake hatchery samples is low (bootstrap values for each pairing are 46%, 32% and 24%, respectively). These low

bootstrap values suggest that these populations may be so closely related that the linkage order among them cannot be determined with any certainty. These populations grouped together in 55% of the 1000 bootstrapped trees, which suggests a non-spurious relationship, but this is also a relatively low bootstrap value. Again, more loci, larger sample sizes and additional populations could increase bootstrap values and clarify among-population relationships.

The relationship between Macklin Creek and Morrison Creek (Pilot Peak wild trout) in the second group is robust (74% bootstrap value). Founders for the Pilot Peak hatchery were drawn from Morrison Creek and the hatchery population clusters within this group. Edwards Creek, in the Desatoya Mountains, the remaining transplanted population of putative Truckee basin fish, is also in this group. The genetic clustering of these populations and the position of the group within the phylogeny indicates that these fish are likely western-basin LCT (i.e., they are linked to stocking from Lake Tahoe and the Truckee basin, Gerstung 1985). The stocking records for Macklin Creek provide additional evidence of a Lake Tahoe origin for Macklin Creek fish. The close relationship of Morrison Creek (Pilot Peak) and Macklin Creek supports a Truckee basin origin for Morrison Creek as well. The next most closely related population is Independence Lake, the only other Truckee River basin population included in the analysis (40% bootstrap value). The order of the rest of the populations in the phylogenetic tree fit with geographic location of these populations. The Walker River basin, the closest basin geographically to the Truckee River basin in the analysis, is represented by Slinkard Creek. The Slinkard Creek population clusters with the Independence strain in Heenan Lake which is derived from Independence Lake in the Truckee basin. West Marys River and Frazier Creek, eastern Lahontan basin; and other cutthroat trout subspecies, Westslope and Coastal cutthroat).

Genetic and ecological data suggest that Lahontan basin LCT populations have undergone genetic bottlenecks (reduction in population size) repeatedly throughout their history. In addition, small numbers of fish may have been used to stock the out-of-basin or fishless streams with putative Pyramid Lake fish. Small sample numbers from a larger population will represent only a subset of the genetic variation in the original (larger) population. This can influence the reconstruction of genetic relationships and population order in a phylogenetic tree. High bootstrap values represent unambiguous relationships. The nodes in the phylogenetic tree that separate important groups of LCT within the Lahontan basin have on average higher bootstrap values. Westslope and Coastal cutthroat subspecies are clearly differentiated from LCT. The differentiation between LCT in the eastern and western Lahontan basin is also robust (53% of trees exclude West Marys River and Frazier Creek samples from the cluster of western-basin samples). The western basin LCT populations all cluster (40%; Walker, Carson and Truckee basins).

The genetic (allozyme, mtDNA and microsatellites) and morphological data collectively suggest that fish transplanted into Macklin, Morrison and Edwards creeks derive from the western Lahontan basin populations. Discussion of whether the genetic composition of these populations represents the variation found in the original lacustrine strain has centered on maintenance of

lacustrine life history traits (e.g., large body size) in a fluvial environment. Unfortunately there is no way of knowing whether these populations have maintained adaptations to a lacustrine life-history, or even if lacustrine adaptations existed. Small population size, coupled with random genetic drift may result in loss of alleles for particular morphological and physiological traits (Nielsen 2000). Levels of heterozygosity for individuals populations would indicate whether recent genetic bottlenecks and loss of genetic variation had occurred. Populations will lose heterozygosity if they remain small for considerable periods of time (100s of generations). Loss of genetic variation could However average heterozygosity values were not reported for populations in Nielsen's study (2000). Additional genetic analyses of data used in Nielsen's (2000) phylogenetic study could be used to assess founder events, genetic bottlenecks, and population isolation, data which could be used to assess the likelihood of loss of traits due to loss of variation (Waser and Strobeck 1998; Luikart and Cornuet 1998, 1999; Luikart *et al.* 1999; Nielsen *et al.* 1998; Beerli and Felsenstein 2000).

### Summary

The isolation of populations, metapopulation dynamics and fluctuation in population size with the random fixation of alleles (allozyme, mtDNA and microsatellite loci) has led to significant genetic differentiation throughout the Lahontan basin. Morphological (Hickman and Behnke 1976), mtDNA and microsatellite data (Williams *et al.* 1992, 1998; Dunham *et al.* 1998; Nielsen 2000) support genetic divergence between eastern and western Lahontan basin cutthroat trout sometime during the Pleistocene. Genetic data (allozyme, mtDNA and microsatellites) further separate (1) Reese River populations from the rest of the populations in the eastern Humboldt drainage, (2) the Walker, East Carson, Truckee and Humboldt populations from each other and (3) the Quinn River drainage populations from all other LCT populations (Gall and Loudenslager 1981; Williams *et al.* 1992, 1998; Dunham *et al.* 1998; Nielsen 2000). Morphological and genetic data show that the transplanted populations of putative Truckee basin trout are likely of Lahontan basin origin. Phylogenetic analysis and stocking records of Macklin Creek further suggest that these populations are original Truckee basin fish. Gall and Loudenslager (1981) defined the Walker, Carson, Truckee and Humboldt drainages as potential microgeographic races of LCT and recommend that population isolation and local adaptation should therefore preclude using trout from one drainage for recovery activities in another (Gall and Loudenslager 1980; Allendorf and Leary 1988).

### HYBRIDIZATION

Major issues:

- Genetic markers (e.g., microsatellites, SNPs, SSRs, PINEs)
- Degree of hybridization
- Significance of hybrid populations in an ESU/DPS context
- Sampling bias (e.g., juveniles vs. adults; spatial-temporal dimension)
- Spatio-temporal patterns of hybridization (can we predict where hybridization will be an issue?)

- Consequences of hybridization (e.g., outbreeding depression, genetic swamping, hybrid zones)
- Effects on important phenotypic traits: e.g., physiology, growth, behavior, survival

The American Fisheries Society hosted two recent symposia on hybridization in fish (August 29 - September 2, 1999, Charlotte, North Carolina and May 31-June 1, 2000, Boise, Idaho). The latter of these symposia focused specifically on hybridization in cutthroat trout. The presentations given at these symposia represent the current state of knowledge and policy on hybridization for conservation and restoration of endangered fishes. These presentations are referenced extensively here.

Salmonid populations in the Truckee River basin are predominantly nonnative. Rainbow, brook (*Salvelinus fontinalis*), brown, and lake trout (*Salvelinus namaycush*), as well as kokanee salmon have been stocked into Truckee basin waters over the last century. Most of these species interact competitively with native LCT and are at least partially responsible for extirpation of the native strain that occupied the Truckee basin system. Kokanee and lake trout are particularly detrimental to lacustrine LCT populations. In lakes, kokanee successfully compete for zooplankton, a major LCT food source (Behnke 1992), and lake trout are efficient predators of cutthroat. There are few remaining pure LCT populations in the basin and, except for Independence lake, are primarily comprised of fish transplanted from LCT populations outside the Truckee basin (Coffin and Cowan 1995; Gerstung 1985, 1988).

Rainbow and LCT are close-related species that readily interbreed. Although no longer stocked extensively throughout the Lahontan basin, rainbow trout continue to be stocked annually into the Truckee River by Nevada Division of Wildlife (NDOW) to support a popular sport fishery. In addition to the annually stocked fish, a naturally reproducing population of rainbow trout is thought to occur in the Truckee river. Hybridization potential could compromise recovery efforts of a naturally reproducing population of *pure* LCT in the Truckee drainage. Removal of populations of nonnative fishes is difficult and can be prone to reversal by accidental or purposeful stocking of nonnatives after initial removal efforts. Given that in many western waters there is either active introgression or introgression potential, the role of hybrids in recovery of salmonids is a pertinent issue but one that is very much open to debate (Allendorf *et al.* 2001).

Before management decisions can be made concerning hybrid populations, the presence and extent of hybridization must be quantified. Interbred populations can show varying degrees of hybridization ranging along a continuum from one pure species to the other. For many species and especially salmonids, morphological traits are unreliable for hybrid identification (Leary *et al.* 1987). First generation (F-1) hybrids of salmonid fishes are often not morphologically intermediate between parental taxa. Furthermore with limited hybridization and only a small proportion of genes from the nonnative taxon present in a population, hybrid individuals may be morphologically indistinguishable from the genetically predominant taxon (Leary *et al.* 1987).

The extent of hybridization in these populations would thus be underestimated using morphological determination of hybrids. As with population structure studies, allozyme and mtDNA markers have been useful markers in hybridization studies (Gall and Loudenslager 1981; Leary *et al.* 1987; Williams *et al.* 1992, 1998; Bartley and Gall 1993). However, because genetic markers evolve at different rates the amount of genetic divergence between closely related species as measured by particular markers will differ. Slower evolving markers will show fewer differences between closely related species than faster evolving markers. If genetic markers are diagnostic, rate of evolution may not be a problem, however, the capacity to assign individuals to *particular hybrid lineages within complex hybrid populations* is limited by the sensitivity of diagnostic characters used, *i.e.*, variability of the genetic marker. For example, maternally inherited markers such as mtDNA are not useful in identifying extent of hybridization if matings are predominantly between nonnative males and native females. In this case mtDNA will not reveal any hybridization as the progeny of such crosses will receive their mothers' mtDNA genotype. Estimates of the frequency, history, and consequences of hybridization depend upon truly diagnostic traits (Williams and Currens 2000). Although molecular genetic markers provide powerful tools, detection and quantification of hybrids can be problematical in the absence of fixed allelic differences between native and introduced populations (Utter 2000). For hybridization studies genetic markers should therefore be evaluated in terms of diagnostic ability. Depending upon the question being asked in potentially hybrid or known hybrid populations, and importance of the population in an ESU context, certain markers may be better suited than others. There is now a diversity of genetic markers available for use in conservation and population biology (see table 1). Useful reviews on the appropriate use of recently developed markers have also been published (Hedrick and Miller 1992; Parker *et al.* 1998; Sunnucks 2000). Newly developed markers systems such as interspersed nuclear elements (PINEs and SSRs) have been shown to be particularly useful for hybridization studies in salmonids (Spruell *et al.* 2000; Ostberg and Rodriguez 2001). Simple sequence repeats (SSRs) have been developed specifically for use in rainbow-cutthroat trout hybridization studies (Ostberg and Rodriguez 2001). Recent studies show a bimodal distribution in allele size at three microsatellite loci that may make these loci particularly suitable to distinguish both presence and extent of rainbow-cutthroat hybridization in LCT populations (Nielsen 2000; Peacock and Briggs 2000). These loci have been used to identify the extent of hybrid populations in the McDermitt creek system of the Quinn River basin originally identified using mtDNA markers (Williams *et al.* 1992; Peacock and Briggs 2000). Ideally a number of markers should be used to test for and monitor the extent of hybridization in critically important populations (for examples of this approach see Forbes and Allendorf 1991a, b; Dowling and Childs 1992; Scribner *et al.* 1994; Baker *et al.* 1999; Baker and Johnson 2000; Allendorf *et al.* 2001).

Representative sampling of populations is also extremely important in determining extent of and direction of hybridization. Common biases include nonrandom choice of sampling locations, misidentification of species in the field, and sampling preference for juvenile or adult fish (Williams and Currens 2000). Sampling programs should be careful to include a representative

sample of the breeding adults in the population. Analysis of individuals by geographic location should be conducted to look for hybridization gradients. The composition of the adult population will indicate the extent and type of hybrid individuals in the breeding population (i.e., F-1 individuals, backcrosses, etc.). Representative sampling of juveniles will reveal trends in hybridization, biases in production and survivorship of hybrids versus the parent taxa as well as genetic composition of hybrid juveniles (i.e., F-1, backcrosses, etc.). Genetic composition of hybrids can reveal genetic swamping/genetic assimilation of one genome over another. These data can be particularly useful monitoring the progression or stasis of hybridization in populations.

Research on the spatial and temporal patterns of hybridization between LCT and rainbow trout throughout the Lahontan basin can be used to look for relationships between habitat conditions and co-existence of native and nonnative populations (Strange *et al.* 1992; Schroeter 1998). At least one population, Long Canyon creek, within the Humboldt basin, has co-existing rainbow and LCT populations (Gall and Loudenslager 1981). This population should be monitored using a suite of genetic markers to determine if these populations have remained distinct and, if so, why. Additional populations with coexisting rainbow and LCT populations should be examined to look for generalizable patterns. In hybridized populations land use activities that have reduced habitat quality may increase the success of nonnatives and hybrids over native taxa (Dunham *et al.* 2000; Williams and Currens 2000). As conditions in recovery streams are improved for native taxa genetic monitoring of populations can be used to look for decreases in hybridization and/or partitioning of habitat among species.

## HATCHERIES

Major issues:

- When to use
- How to use - breeding protocols (maintaining outbred hatchery stocks) and genetic monitoring
- Concrete raceways vs. propagation in natural habitats
- Selection in captive environment
  - Growth, behavior, disease resistance

## RECOMMENDATIONS

### **General recommendations**

“The purpose of Act (Endangered Species Act) is to provide a means whereby the ecosystems upon which endangered species and threatened species depend may be conserved, to provide a program for the conservation of such... species, and to take such steps as may be appropriate...” (Kohm 1991). Data from studies at different spatial and temporal scales show that conservation of inland cutthroat trout species depends upon intact ecosystems and preservation of habitat diversity (Ray *et al.* 2000; Rieman and Dunham 2000). Diverse habitats help preserve life history

variability and long term evolutionary potential. In the words of the eminent 20<sup>th</sup> century ecologist, G. E. Hutchinson, *ecology is the theater and evolution is the play* (Hutchinson 1965).

Recovery of the Lahontan cutthroat trout subspecies ultimately depends upon restoring naturally reproducing populations across the subspecies range. The strain of LCT to use in recovery efforts should be determined from genetic and ecological data and made independently for each DPS.

#### Truckee River Basin

Based upon the current morphological and genetic evidence, the out-of-basin populations in Macklin Creek, Edwards Creek and Pilot Peak should be considered for recovery efforts in the Truckee basin and Pyramid Lake ecosystem. These populations may offer the best opportunity to recover evolutionarily significant aspects of the original Pyramid Lake LCT fishery. Analysis of archival samples of original Pyramid Lake fish may reveal similarity with transplanted populations reputed to descend from that strain. However, few archival samples of original Pyramid Lake fish have been located in museum collections. DNA extraction problems with preserved samples and small sample size of original Pyramid Lake fish may preclude a robust analysis.

Continuing research should be conducted to evaluate performance of these fish in lacustrine systems, e.g., survivorship and growth rates, as compared to existing lacustrine strains. However, **more importantly**, because the goal is to recover a naturally reproducing population within the Pyramid Lake ecosystem, these fish should be evaluated in regards to natural reproduction in the river, patterns of re-invasion of the system (reestablishment of population network), factors related to stocking success, and interaction with nonnatives. Genetic monitoring tools can be used to assess the success of different stocks in regard to survivorship, as well as rates and pattern of interspecific hybridization with naturalized and stocked rainbow trout. Genetic monitoring has the advantage of providing results quickly especially after fish have been re-established in Pyramid lake and the Truckee river.

#### Walker Basin

Additional genetic analysis should be conducted to identify appropriate LCT strain(s) and refine recovery strategies for the Walker basin. Few naturally reproducing LCT populations remain in the Walker River system. The cutthroat trout found in By-Day Creek are thought to be the only native population remaining in the basin. Individuals from this population have been successfully planted in other Walker basin streams where nonnative salmonids have been removed. At present this population and successful transplanted populations should be managed as broodstock. These populations should be regularly monitored for genetic variability.

#### Humboldt and Quinn River DPSs.

Ongoing genetic analyses (using more populations and/or more variable genetic markers) should be conducted to clarify ambiguities in the existing phylogenies. Because the Humboldt and Quinn

River systems are comprised of numerous and widely dispersed watersheds recovery strategies should be determined per watershed by the respective DPS teams.

### Specific recommendations

1. Macklin, Morrison and Edwards creek populations should be evaluated for use in recovery activities in Truckee system.

Justification:

- (a) best available data suggest these fish are from Truckee River system
    - morphological data
    - transplant records
    - microsatellite genetic analysis
  - (b) no evidence of introgression with either other cutthroat subspecies or rainbow trout
  - (c) important part of the evolutionary legacy of the species
2. Additional out-of-basin LCT populations should be investigated as potential broodstock for recovery activities in the western Lahontan basin. The Slinkard Creek population in the Walker River basin is currently the source of Lahontan cutthroat trout for recovery activities.
  3. Research Directions
    - (a) Expand genetic analyses to include additional loci, samples, and populations as top priority. Confirm phylogenetic pattern constructed with existing data and clarify it for other basins where recovery actions will focus next (e.g., Walker and Carson basins).
    - (b) Address specific questions about origin of transplanted populations.

Do these fish represent the genetic and morphological variation present in the pre-extirpation population? This cannot be determined absolutely. Even historical samples are not likely to capture what the population looked like genetically or morphologically pre-extirpation because there are so few samples relative to the historical population size. However, out-of-basin transplant populations can be characterized with regard to:

      - 1- founder effects - original transplant sizes
      - 2- bottlenecks - is there a genetic signature of recent population bottlenecks?
      - 3- effective population size ( $N_e$ ) for these populations – change this to have these populations lost more genetic diversity than you would expect due to small population size?
    - (c) Development of hatchery protocols to avoid mating of close relatives and maximization of  $N_e$  (e.g., equalize family size). Begin genetic “effectiveness” monitoring to ensure the hatchery population is retaining genetic variation.
    - (d) Develop hatchery stocking practices to avoid negative impacts on  $N_e$  of wild fish (e.g., minimize variance in family size).
    - (e) Evaluate success of stocking (e.g., do we need to stock specific sizes of fish, at specific

- times/places, do we need to acclimate fish prior to stocking?).
- (f) Develop off-site, quasi-natural locations for increasing numbers of broodstock without overwhelming current hatchery. Quasi-natural environments may increase capacity and reduce selection for “hatchery” characteristics that repeatedly show up in captivity. Waters such as Heenan and Marlette Lakes could be used as important rearing sources as they both already have LCT from other stocks.
  - (g) Develop faster and higher resolution genetic methods (e.g., SSRs, PINES) to track success of stocks of different genetic origin in the field and hatchery, and track hybridization with nonnative rainbow.
  - (h) Investigate species interactions (ecological and genetic) between rainbow and cutthroat trout. Do they segregate spatially, temporally, behaviorally? Is there selection against hybrids or evidence for outbreeding depression? These questions will help assess whether we need to actively manage to reduce hybridization.
  - (i) Field studies provide only circumstantial and weak evidence of local adaptation of various strains, due to confounding effects of prior rearing in hatchery, maternal effects, etc. Hatcheries could serve as controlled facilities for the classical “common garden” experiments to look at development of traits of different populations in a common environment. Key environmental variables include temperature and dissolved solids. Genetic differences can only be isolated using a common garden design. However, this would take about five years to complete at a minimum, given the generation time of LCT.

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Table 1. Attributes of markers commonly used in molecular population biology (from Sunnucks 2000)

	PCR assay	Single locus	Codominant	Allele genealogy feasible	Number of loci readily available	Connectibility of data among studies	Rapid transfer of new data	Overall variability
<b>Mitochondrial (and chloroplast)</b>								
Sequence	Yes	Yes	Yes <sup>c</sup>	Yes	Single	Direct	Yes	Low-high
RFLP	No, large	Yes	Yes <sup>c</sup>	Yes	Single	Direct	Yes	Low-moderate
<b>Multilocus nuclear</b>								
Mini- and/or microsatellites 'fingerprints'	No, large	No	No	No	Many	Limited	Yes	High
RAPD <sup>a</sup>	Yes	No	No	No	Many	Limited	Yes	High
AFLP <sup>a</sup>	Yes	No	No	No	Many	Limited	Yes	High
rDNA <sup>b</sup>	Yes	No	No	No	Few	Limited	Yes	Moderate-high
<b>Single-locus nuclear (single copy nuclear, scn)</b>								
Allozymes	No, protein	Yes	Yes	Rarely	Moderate	Direct	Yes	Low-moderate
Minisatellites	Few	Yes	Yes	Rarely	Moderate	Indirect <sup>d</sup>	Few	High

Microsatellites	Yes	yes	Yes	Yes	Many	Indirect <sup>d</sup>	Some	High
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Table 1  
continued

Anonymous scn	Yes	Yes	Yes	Yes	Many	Indirect <sup>d</sup>	No? <sup>e</sup>	Moderate? <sup>e</sup>
Specific scn	Yes	Yes	Yes	Yes	Moderate	Direct	Yes? <sup>e</sup>	Moderate? <sup>e</sup>
rDNA <sup>b</sup>	Yes	in effect	Yes	Yes	Few	Direct	Yes	Low-moderate

<sup>a</sup> Some RAPD (randomly amplified polymorphic DNA) and AFLP (amplified fragment length polymorphic DNA) bands can be converted to single-locus markers, in which case they behave like ‘anonymous scn’ or ‘specific scn’ categories

<sup>b</sup>rDNA consists of tandem arrays of a few regions. In some taxa the arrays are effectively identical and regions act as single loci, but in some taxa there can be many different sequences within individuals, in which case rDNA acts more like a multilocus system.

<sup>c</sup>mtDNA and chloroplast DNA are haploid and show one of a range of alternative positive states, in contrast to dominant markers that are either present or absent.

<sup>d</sup>Data from these markers are indirectly, but meaningfully, connectible given adequate models of molecular evolution.

<sup>e</sup>Insufficient research effort has been put into these markers

## FIGURES

Figure 1. Outline of the hydrographic Lahontan basin.

Figure 2. Pluvial Lake Lahontan (light gray shading) at high stand approximately 12,500 years before present. Modern day remnants of Lake Lahontan are indicated by in dark gray shading. Reese and Humboldt river systems in the eastern Lahontan basin were never inundated by ancient Lake Lahontan.

Figure 3. Post Pleistocene distribution of lake and river systems in the Lahontan basin (outlined). Map shows general distribution of Lahontan cutthroat trout pre-european settlement in the Lahontan basin (from Coffin and Cowan 1995).

Figure 4. Western Lahontan basin. Three river drainages are found in this basin: Truckee, Carson and Walker river systems.

Figure 5. Schematic of a metapopulation dynamics of an inland trout metapopulation (a) and effects of human disturbance (b). S1 and S2 represent resident stream subpopulations. S3 represents a migratory life history with fish moving throughout a larger portion of the interconnected system. S4 represents lacustrine fish who breed in stream habitat. Post human disturbance results in isolation for s1, s2 and s3 subpopulations. S4 is split into s4 and s5. S4 has limited access to spawning habitat and s5 is completely isolated from spawning habitat (from Campbell et al. 1999).

Figure 6. Spatial and temporal scales and questions for which classes of genetic markers are best suited.

Figure 7. Consensus neighbor-joining tree based on Goldstein et al. (1995)  $\delta\mu^2$  genetic distance estimated among populations of cutthroat trout. Bootstrap values (%) calculated from 1000 replicate trees are given at branch points (from Nielsen 2000).

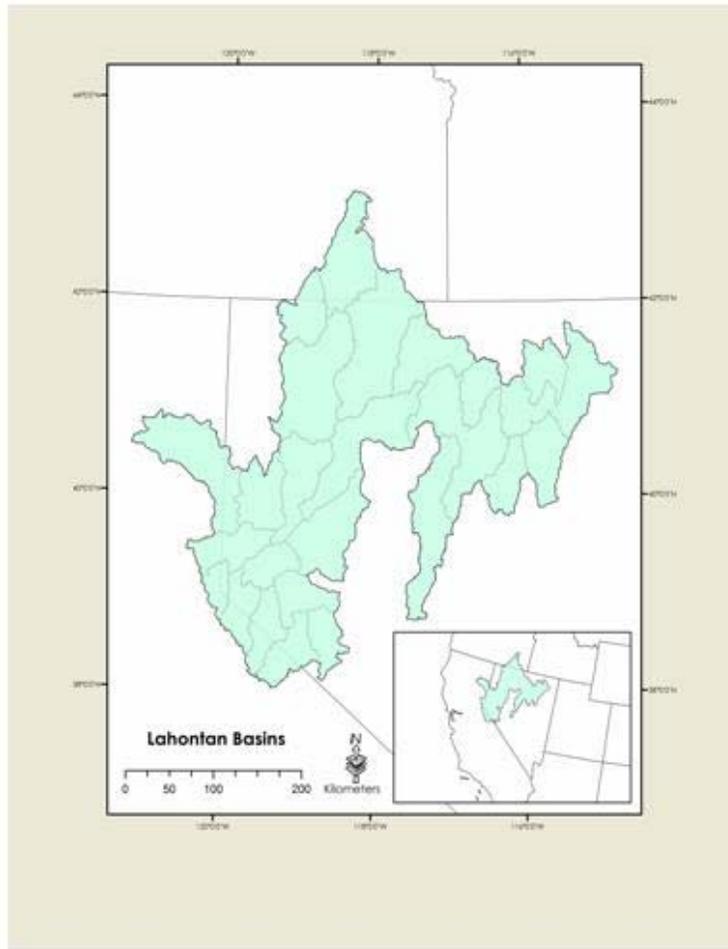


Figure 1.

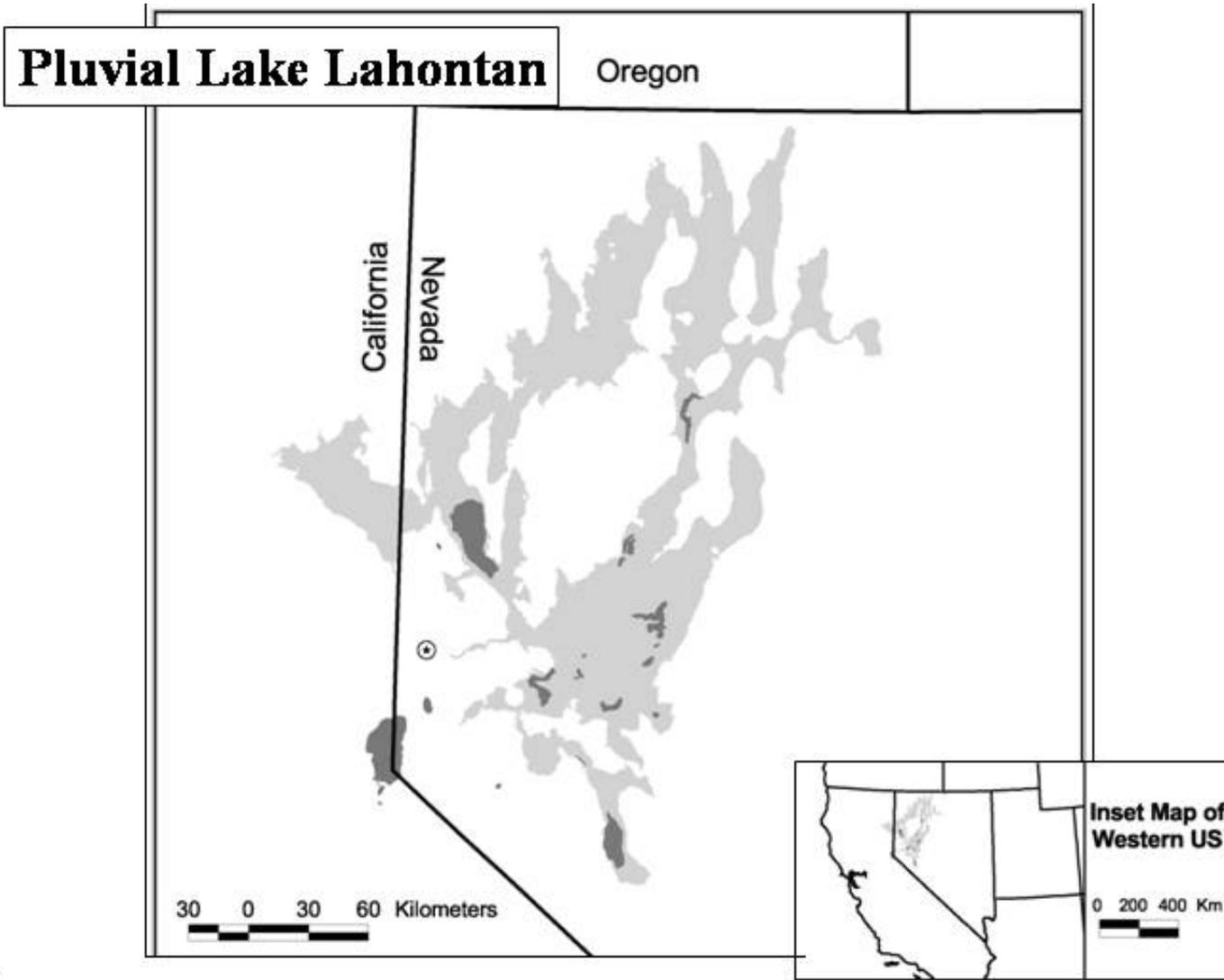


Figure 2.

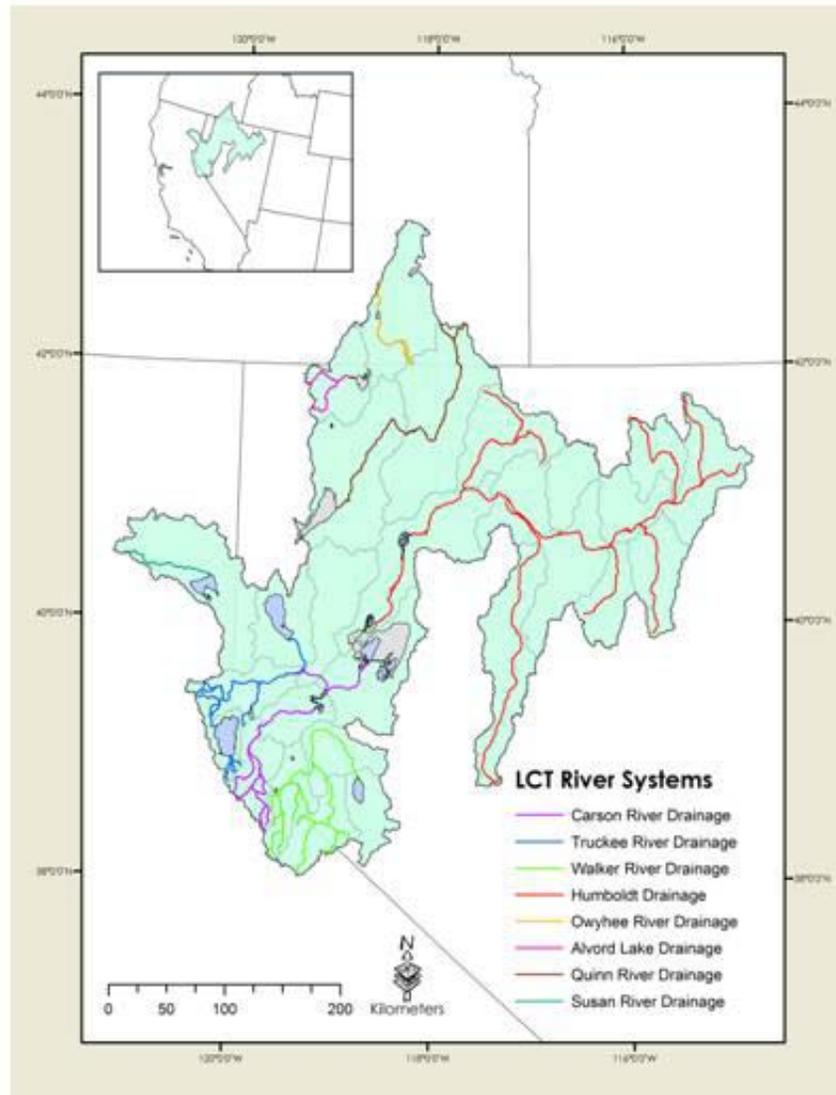


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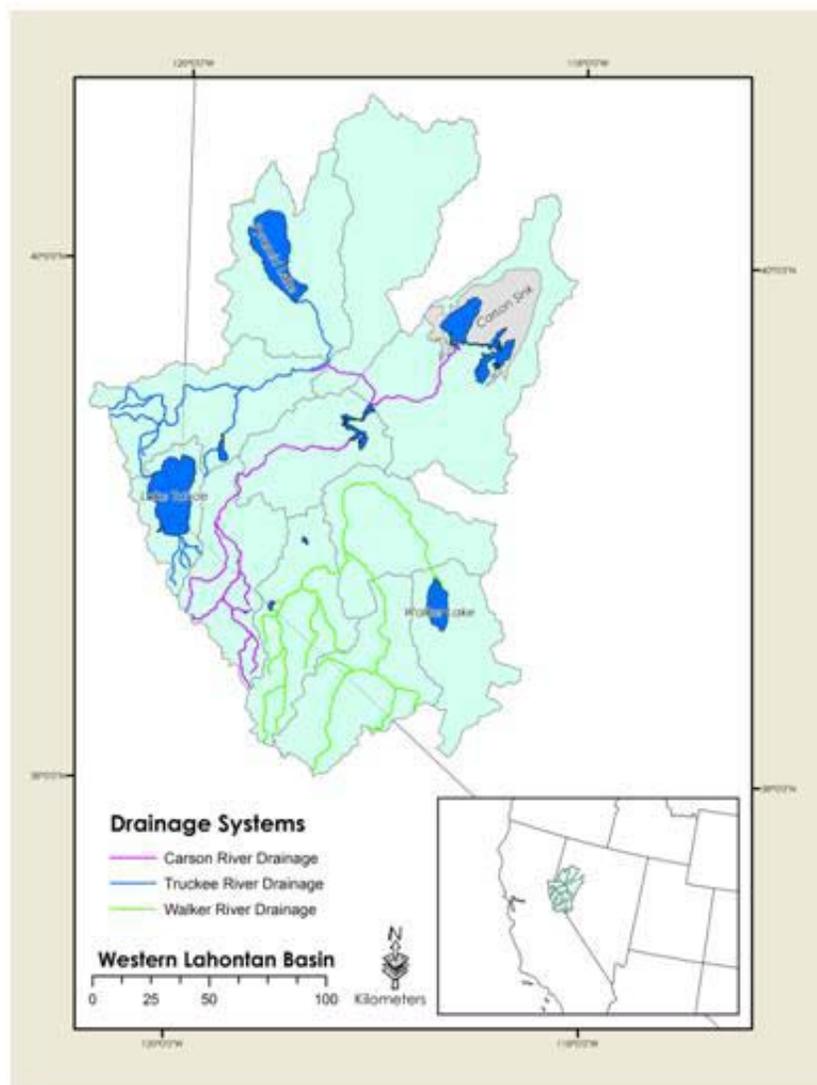


Figure 4.

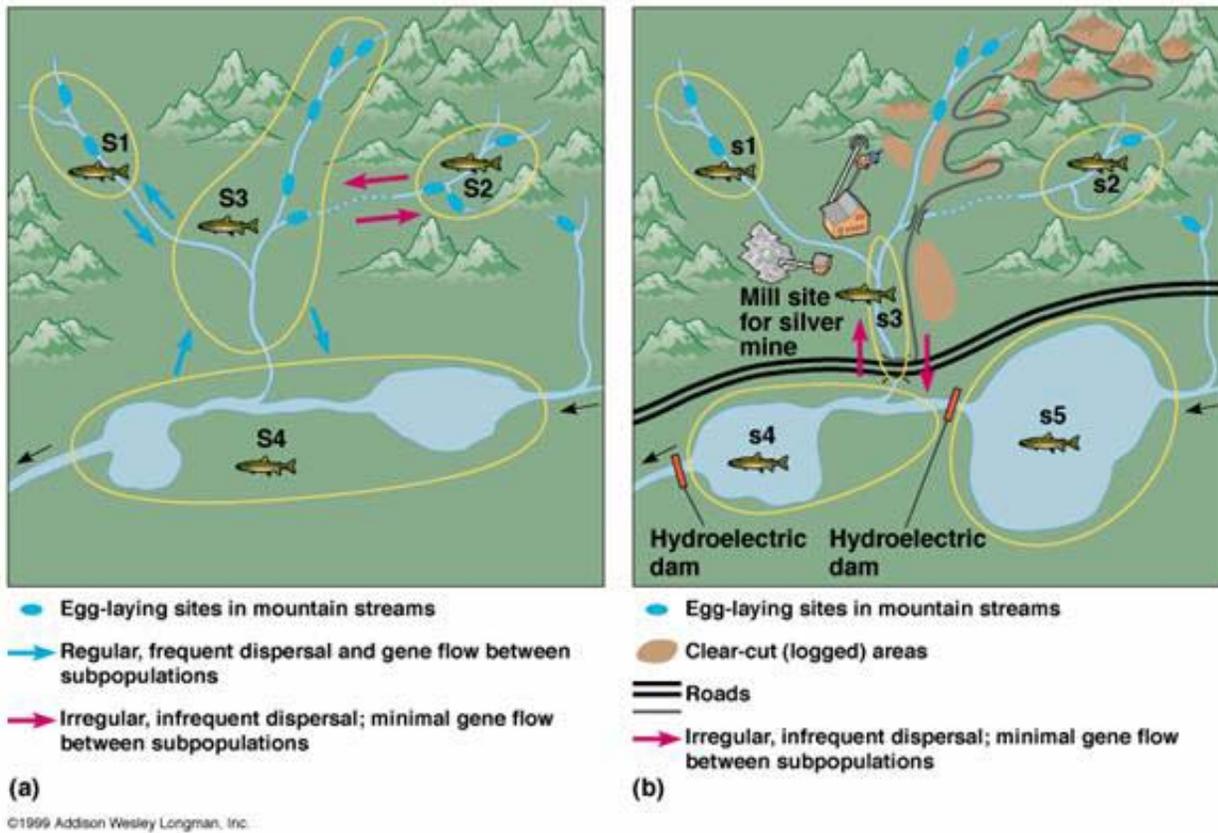


Figure 5.

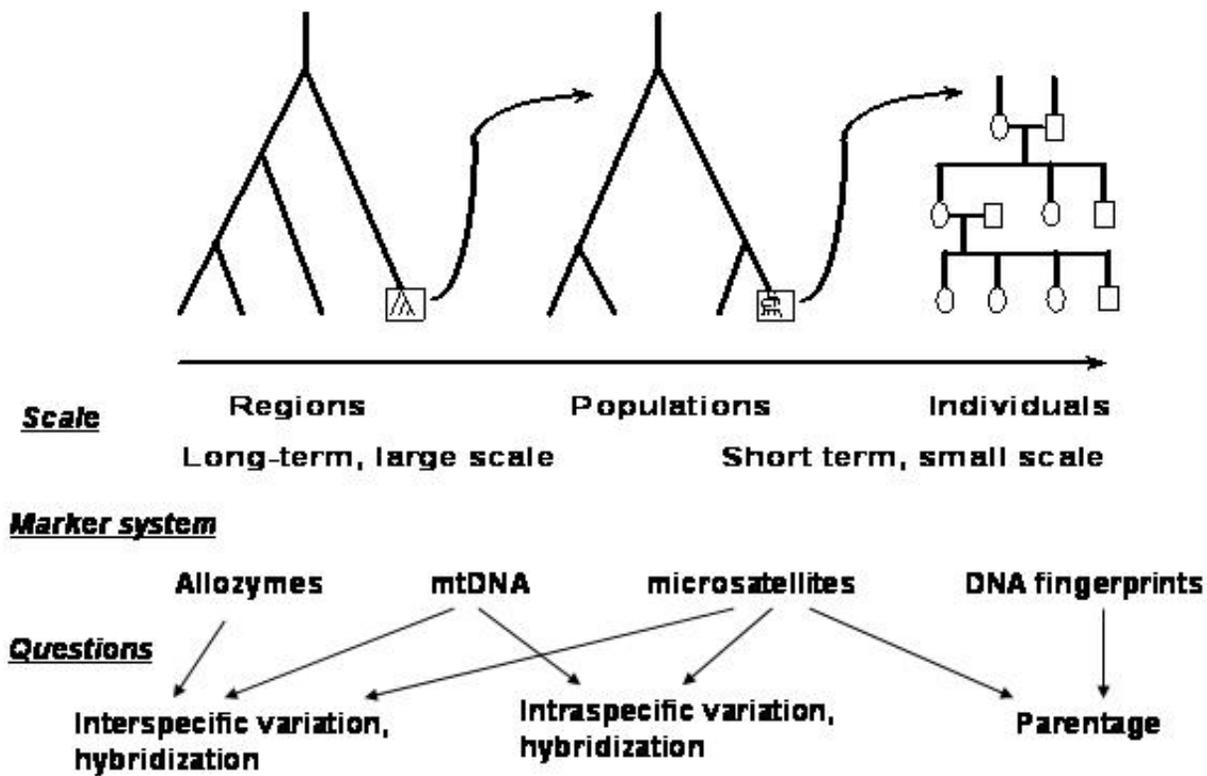


Figure 6.

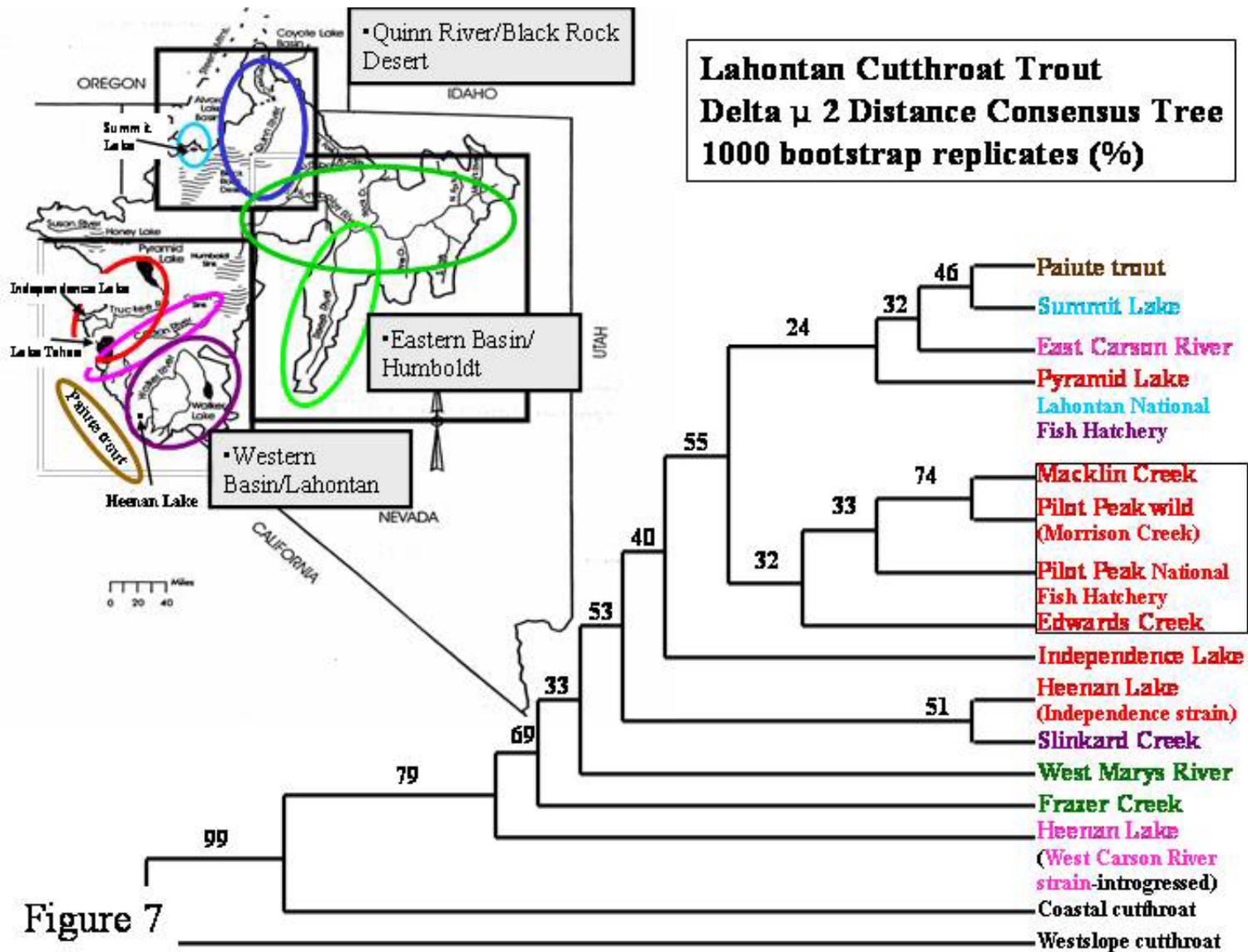


Figure 7