

# EXHIBIT 95

Short-Term Action Plan  
for Lahontan Cutthroat Trout  
(*Oncorhynchus clarki henshawi*)  
in the Walker River Basin



Photo by M. Sevon



Developed by Walker River Basin Recovery Implementation Team  
for U.S. Fish and Wildlife Service, Reno, Nevada  
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## **I. INTRODUCTION**

Lahontan cutthroat trout (LCT) (*Oncorhynchus clarki henshawi*) was listed as an endangered species in 1970 (Federal Register Vol. 35, p.13520). In 1975, pursuant to the Endangered Species Act of 1973 as amended (ESA), LCT was reclassified as threatened to facilitate management and to allow for regulated angling (Federal Register Vol. 40, p.29864). In 1995, the U.S. Fish and Wildlife Service (FWS) released its recovery plan for LCT, encompassing six river basins within LCT historic range, including the Walker River basin. The Lahontan Cutthroat Trout Recovery Plan (1995) identified development of ecosystem plans for LCT in the Truckee and Walker River Basins. This Short-Term Action Plan (Action Plan) for the Walker River Basin represents a three-year planning effort to develop the “ecosystem” based plan identified in the 1995 Recovery Plan. The Action Plan identifies short-term activities or research that will further our understanding of the conservation needs of LCT specific to the Walker River basin and utilizes adaptive management to refine the long-term recovery strategy.

The 1970 Federal Register notice identified two primary listing factors that related directly to LCT: Present or threatened destruction, modification, or curtailment of habitat or range; Natural or manmade factors affecting the species continued existence. Three additional ESA listing factors that were considered in the reclassification of LCT and not addressed as having a direct impact were: Over-utilization of the species for commercial, scientific, or education purposes; Disease or predation; Inadequacy of existing regulations.

The Recovery Plan (USFWS 1995) specified five additional conditions contributing to decline and affecting the potential for recovery of LCT in the Walker River basin: reduction and alteration of stream flow and discharge; alteration of stream channels and morphology; degradation of water quality; reduction of Walker Lake elevation and concentration of chemical components; introductions of non-native fish species.

This Action Plan and the tasks identified herein are intended to eliminate or minimize the threats that impacted LCT and through continued implementation of this process ensure the long-term persistence of the species.

## **II. THE PLANNING PROCESS**

To address the complexity of issues related to recovery of LCT, FWS determined that basin-specific interagency and interdisciplinary teams, as

well as public stakeholder participation, would be beneficial for developing LCT recovery efforts. In 1998, FWS organized a Management Oversight Group (MOG) to address LCT recovery range wide. In 1999, the Walker River Basin Recovery Implementation Team (WRIT) was organized to develop a strategy for LCT restoration and recovery efforts in the Walker River basin. Public stakeholder involvement began in 2000. As a result of these efforts a short-term action plan was developed to assist in recovery of the species.

**Figure 1. Entities Represented on the WRIT**

- Bureau of Land Management (BLM)
- California Department of Fish and Game (CDFG)
- University of Nevada at Reno (UNR)
- U.S. Bureau of Reclamation (USBOR)
- U.S. Fish and Wildlife Service (USFWS)
- U.S. Forest Service (USFS)
- U.S. Geological Survey, Biological Resources Division (USGS)
- Walker River Paiute Tribe
- Bureau of Indian Affairs (BIA)

**Additional Entities who provided input to the WRIT Process**

- Trout Unlimited
- Walker River Irrigation District (WRID)
- Mono County

USFWS guidelines require that recovery plans incorporate scientific methods and analyses that are subject to review. Therefore, members of the WRIT have technical experience variously associated with fishery biology, geomorphology, hydrology, restoration ecology, population viability analysis, and genetics and are familiar with resources of the Walker River basin. Through a collaborative effort spanning over three years, WRIT developed short-term actions they believe are necessary to develop information on lacustrine and fluvial LCT life history requirements and address threats to the species persistence.

During plan formulation, the list of short-term actions being considered by WRIT was presented twice to public stakeholders. Several issues were identified by the public as important: instream flow requirements for fish and recreation; fish management; recreational fishery impacts; habitat restoration; water management; economic impacts to local communities; land management along the riparian zone; water quality; and the genetic basis for LCT recovery. Recommendations from the public have been considered in the design of short-term actions.

The recovery of LCT will be a long-term effort and require coordination among the United States, the States of Nevada and California, tribes, and the public. Priority will be given to partnerships that maximize the potential for recovery and avoid adverse impacts to existing recreational and ecological resources. This initial short-term strategy is focused on gathering information about habitat requirements and initiating or completing demonstration projects and research that will further our collective understanding of the opportunities for restoring a viable naturally reproducing lacustrine LCT population in Walker Lake and protecting extant riverine LCT populations within the Walker River basin.

Development of a comprehensive recovery effort for Walker River basin LCT was based on the following assumptions:

- The Walker River basin watershed is significantly fragmented due to water and human development.
- Historic LCT distribution and utilization of the entire Walker River basin has been, and continues to be severely compromised.
- Recovery of LCT will be a long-term effort that will require monitoring, review and evaluation.
- The water quality and quantity, especially temperature, significantly limits the habitat for LCT in portions of the Walker River system.
- Five reintroduced headwater populations exist in the Walker River basin that are the result of tasks identified and implemented under the 1995 Recovery Plan.
- Habitat degradation and fragmentation in the Walker River basin currently limits the potential success for recovery of LCT.
- Non-native salmonid fisheries are an important recreational use of the Walker River system.
- Historically LCT in the Walker River basin functioned as a networked population where different life stages and year classes of fish utilized different portions of the river system and repopulation of depleted areas occurred from other locations in the river system.

The State, Federal and Tribal organizations provide the primary vehicles for implementing tasks identified in the plan. The State, Federal and Tribal organizations will, to the extent possible, collaborate and integrate their efforts. Entities will share technical data and recommendations for action. In addition, stakeholder meetings will be coordinated for periodic review of the short-term tasks and accomplishments, providing insight and suggestions on local and regional opportunities, and assisting in the review and refinement of the annual work plans.

## **Recovery Goals, Criteria and Timeline**

The objective of the 1995 plan is to delist LCT from the List of Threatened and Endangered Wildlife and Plants. The following criteria were recommended by WRIT as being necessary to assist in the recovery of LCT in the Western Distinct Population Segment (DPS). These recovery criteria may be periodically revised through an adaptive management program as new information collected through implementation of identified short-term recovery actions is acquired.

### **Recovery Criteria**

1. A self-sustaining, networked LCT population composed of wild, indigenous strains, established in interconnected habitat, i.e., in streams, lakes, mainstem and tributaries of the Walker River basin.
2. Connectivity exists between suitable spawning and rearing habitats to support natural reproduction and recruitment, to restore self-sustaining lacustrine LCT in lakes, mainstem and tributaries of the Walker River basin.
3. A self-sustaining lacustrine population is naturally reproducing with an age class structure consisting of at least four year classes, a stable or increasing population size supported by documented reproduction and recruitment. These conditions must be demonstrated to have been met for a minimum period of 20 years.
4. Water is obtained through water right purchases or other means to protect and secure a stable Walker Lake ecosystem and meet life history and habitat requirements of LCT.
5. A flow regime for the mainstem Walker River is implemented which facilitates LCT migration, life history and habitat requirements.
6. A commitment is secured from respective responsible entities to operate and maintain reservoirs and fish passage facilities within the basin in a manner that facilitates migration and reproductive behavior of LCT.
7. Threats to LCT and its habitat have been reduced or modified to a point where they no longer represent a threat of extinction or irreversible population decline.

### **Adaptive Management**

*Adaptive management is an approach and process that incorporates monitoring, research and evaluation to allow projects and activities, including projects designed to produce environmental benefits, to go forward in the face of some uncertainty regarding consequences (Holling, 1978; Walters, 1986).*

The recovery of LCT will be accomplished in small, definable steps. In view of the uncertainty of setting a definitive long-term recovery strategy for LCT, the MOG and WRIT agreed to adopt an adaptive management approach.

The impact of the short-term actions is scientifically evaluated on a periodic basis, with subsequent decisions and actions taken as necessary to achieve the objectives. The successful application of an adaptive management program will be promoted by stakeholder participation. Additionally, an adaptive management program utilizes science, management and stakeholder coordination to accomplish overall program objectives.

General features of adaptive management are:

- Development of clear, measurable objectives for recovery actions that relate directly to the risk, uncertainty, or the problem being addressed;
- Selection of indicators of success, failure, or general performance that are practical to use and capable of signaling change at a level needed to meet recovery objectives;
- A clear assignment of responsibility for responses when triggers, thresholds, or standards are exceeded, as demonstrated through monitoring;
- A fair, objective, and well understood program for collecting, managing, and interpreting information for monitoring and research projects; and,
- Provisions to deal with expected disputes over interpretation of information.

A structured and documented review of the short-term actions and the study results will be integrated into the recovery process. Short-term actions will be implemented through a cooperative approach that utilizes existing agency expertise and capability. WRIT will provide the primary technical expertise with individual actions coordinated through the appropriate agency, Tribe or group. FWS will retain the primary responsibility initially for information and data consolidation and management. As capability is developed by the cooperating agencies, this effort may be transferred to them.

Management actions that will assist with recovery of the ecosystem upon which the lacustrine LCT depends include: improving instream water quality; proposed modifications or removal of barriers that impede fish movement within the basin; the potential to increase water flow to Walker Lake; habitat improvements with restoration of natural riparian communities; potential to manage for wild populations of lacustrine strains

believed to be indigenous to their respective basins; and society's desire to preserve and restore the natural character and function of river and lake systems.

The short-term tasks outlined in this plan for the recovery of LCT in the Walker River basin were developed on an approach that focuses on three components:

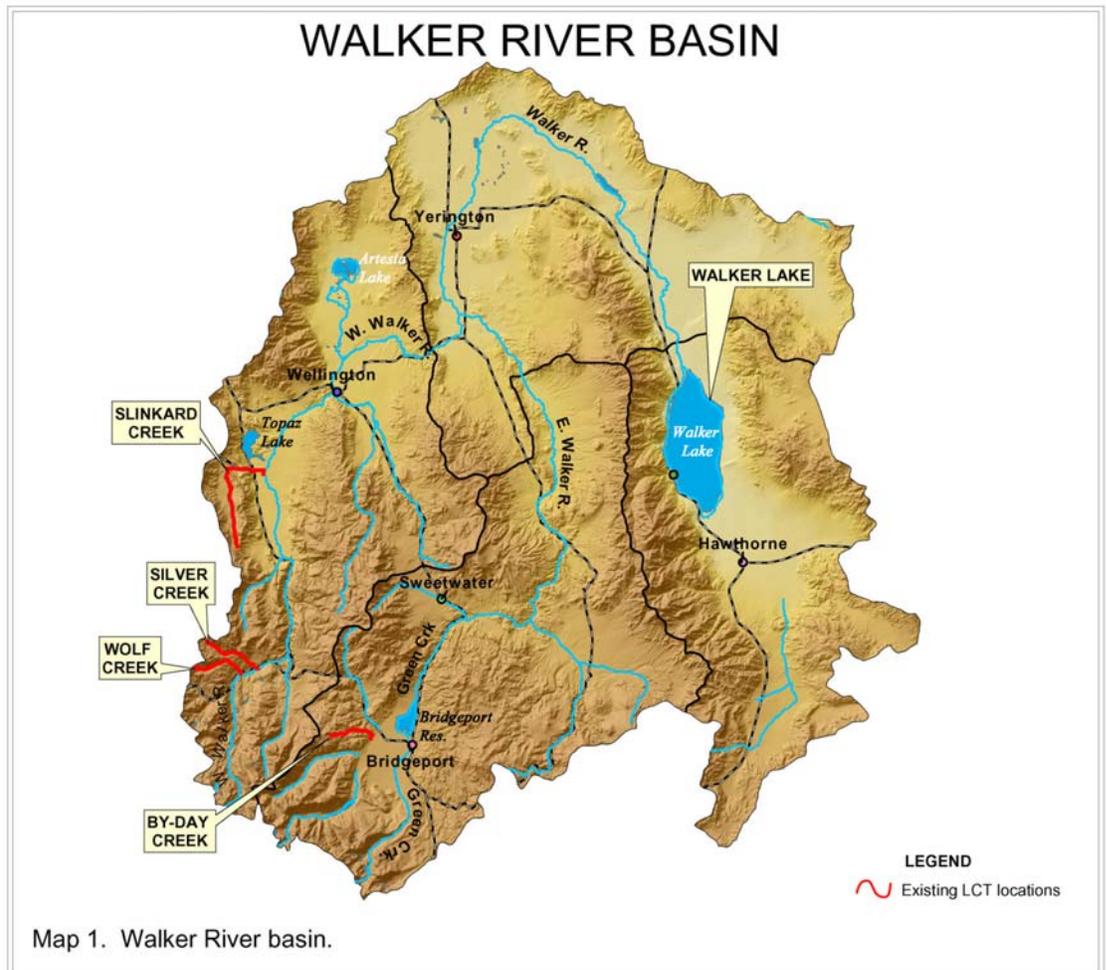
1. Developing a thorough understanding of the issues and management of the Walker River basin.
2. Gaining critical information for refining a future recovery strategy for LCT in the Walker River basin.
3. Implementing a scientifically based Adaptive Management Program that allows researchers and managers to gain insight from each short-term action so that future decisions can be based on credible science, a logical process, and includes stakeholder involvement.

### **III. OVERVIEW OF THE WALKER RIVER BASIN**

The Walker River basin comprises an area of approximately 4,050 square miles from the headwaters of the eastern Sierra Nevada to its terminus at Walker Lake (Map 1). The basin has been subjected to extensive human impacts from land and water development, population growth and recreation. These impacts have altered the physical and biological integrity of the Walker River basin causing water quality degradation, habitat fragmentation, geomorphic instability, and have resulted in a decline of native fish populations.

The surface flows of the Walker River basin are determined by (1) the amount of water available in the headwaters of the East and West Forks of the Walker River, (2) storage and managed releases from three major and several smaller reservoirs, and (3) diversion of surface water and groundwater (well) pumping.

The Walker River extends approximately 160 miles from the headwaters to the terminus at Walker Lake (Map 1), a terminal lake system. The basin is characterized by alpine lakes, high, moderate, and low gradient streams, and a desert terminal lake. The Walker River exhibits extremes in hydrologic conditions, typical of Great Basin rivers, from nearly dry during drought periods to high water from flood events.



### ***West Walker River***

West Walker River originates in Kirkwood and Tower lakes (Map 1), below Hawksbeak, Ehrnbeck and Tower peaks, in California. Flowing north and dropping more than 4,000 feet in elevation over 14 miles, it enters Leavitt Meadows, where Leavitt Creek, which drains Leavitt Lake, joins the West Walker. From Leavitt Meadows the river flows east by northeast, entering Pickle Meadows, where it accumulates waters from Poore, Wolf, Little Wolf, Cloudburst, and Silver creeks. West Walker River then flows east, joining the Little Walker River at Highway 395 and turning north for ten miles, flowing through a narrow canyon to Antelope Valley. In this stretch, the West Walker accumulates water from at least six additional tributaries, including Grouse, Deep, and Slinkard creeks. Prior to the West Walker joining with East Walker River the majority of the flow is diverted into a canal leading to Topaz Reservoir. Topaz Dam and

Reservoir were constructed in 1922 and modified in 1937 to support irrigation downstream. When the West Walker enters Nevada, it flows generally northeast into Hoyer Canyon and Smith Valley and finally Wilson Canyon where the river enters Mason Valley.

### ***East Walker River***

East Walker River accumulates waters from Virginia, Robinson, Buckeye, Swauger, Green, and Summer creeks all of which are upstream of Bridgeport Valley. In Bridgeport Valley, Bridgeport Dam and Reservoir were built and began storing water in 1923. Downstream from the reservoir, the East Walker flows for approximately seven miles before it enters Nevada in the southern portion of Mason Valley where it flows generally northwest for seven miles before joining with the West Walker River to create the mainstem Walker River.

### ***Walker River***

Walker River generally flows north through Mason Valley until reaching the valley's northern end near Wabuska, Nevada. Here the river changes course, turning eastward to southeast where it enters the Walker River Paiute Indian Reservation before entering Weber Reservoir, created by Weber Dam (completed in 1935). The reservoir is located approximately 4 miles upstream of Schurz, Nevada and 16 miles upstream of Walker Lake. The river then flows generally south through alluvial flats before entering Walker Lake.

### ***Walker Lake***

Walker Lake is the terminus of the Walker River and is geographically situated between the Wassuk Range to the west and the Gillis Range to the east. Walker Lake is the remnant and southernmost arm of Pleistocene Lake Lahontan. The shorelines formed by Lake Lahontan extend up to an elevation of 4370 feet and are readily visible today (Adams 1997).

Based on water and sediment samples collected from the bottom of Walker Lake (Benson 1988) and the surrounding exposed lakeshore, a history of past lake levels was reconstructed, and subsequently conclusions regarding Walker Lake's hydrology and climate can be scientifically inferred. Based on sediment samples collected by the USGS during the 1970's and 1980's the following timeline of events can be made:

- Walker Lake was low or periodically dry during the period of 13,000 to approximately 4,800 years before present (BP).

- Walker Lake basin filled again beginning about 4,700 years ago.
- Walker Lake remained high for approximately 2,000 years (until about 2,000 years ago).
- From 2,000 to 1,000 years ago, Walker Lake declined in elevation and was dry for approximately 300 years.
- Approximately 1,000 years ago Walker Lake began to increase in elevation again.

Beginning in the 1800's, explorers, ranchers, and settlers began to keep records of the level of Walker Lake. U.S. Geological Survey (USGS) first began to measure the water level of Walker Lake in 1908. Records show that Walker Lake elevation and volume generally declined during 1882 - 1995 (Figure 2), and has continued to decline beyond the 1995 elevation.

### **Human Influence on the Walker River Basin**

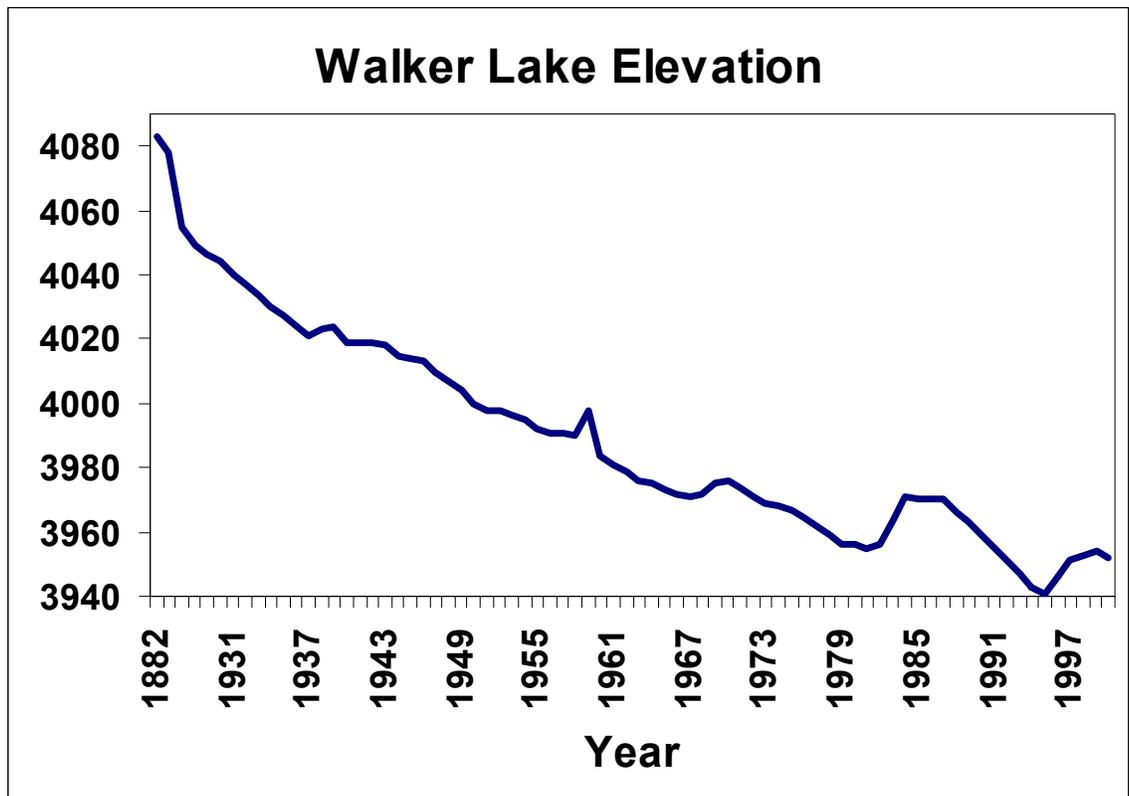
The Walker River basin has been inhabited by humans for at least 11,000 years. Archeological research and the oral histories of the Paiute, Shoshone, and Washoe Tribes indicate that the people in the Walker River basin depended on aquatic and riparian life in the Walker River and Walker Lake for sustenance (Houghton 1994).

With the discovery of gold in the California Territory in 1848, accelerated settlement of the Great Basin began. Between 1855 and 1862, settlers immigrated to Smith, Antelope, and Mason valleys. Agriculture and ranching began to divert and utilize the water of the Walker River during this period.

The first measurements of Walker River flow were documented in June 1881 by I.C. Russell. He recorded Walker River flow at 400 cubic feet per second (cfs) approximately 3 miles upstream of Walker Lake (Russell 1885). This is a measurement equaling approximately 290,000 acre-feet (a/f) annually (Russell 1885, Nevada Division of Water Planning 2001). Information gathered by Russell is often referenced for baseline evaluation today.

In 1882, he measured Walker Lake's surface elevation to be 4,080 feet, MSL (mean sea level), with a maximum length of 25.6 miles, width of approximately 5 miles, and surface area of about 95 square miles. The lake's depth was assessed to be 224 feet maximum, with volume estimated at about nine million a/f. Total dissolved solids (TDS) was

Figure 2. Walker Lake 1882-2000



Source: Reproduced from Nevada Division of Wildlife

estimated at 2,560 milligrams per liter (mg/l) (Russell 1885, Nevada Division of Water Planning 2001a).

Records from mid 19<sup>th</sup> century indicate an abundance of LCT in Walker Lake, with reports of 20 pound, three-foot long LCT being caught. Other reports show that their numbers were declining. Several articles attributed LCT decline to commercial trade and diversion dams preventing or restricting LCT from migrating upstream to tributaries to spawn (McQuivey 1996).

With the 20<sup>th</sup> century came increased demand on Walker River water as rapid growth of mining and agriculture continued. In 1909, an estimated 58,000 acres of land were under irrigation in the basin and by 1919, irrigated acreage in the basin had increased to 103,000 acres (Nevada Division of Water Planning 2001b).

In 1919, Walker River Irrigation District (WRID) was formed, which provided the financial ability for water users in Nevada to construct Topaz and Bridgeport reservoirs. These two California reservoirs have a

combined storage capacity of 107,400 acre-feet (af) (Public Resource Associates 1994). Bridgeport Dam restricted access of LCT to spawning habitat in East Walker River and upstream tributaries. Water depletions and diversion dams on the West Walker limited LCT access to upstream areas. In 1929, the Yerington weir was constructed on the Walker River which thereafter prevented fish access to both East and West Walker River.

During 1882 – 1929, there was a steady decline in Walker Lake elevation. In 1929, Walker Lake volume was 43.4 percent less than that measured in 1882 (Public Resource Associates 1994, Nevada Division Water Planning 2001).

Weber Dam construction was completed in 1937 by Bureau of Indian Affairs (BIA) to assist with Tribal agricultural irrigation. Design storage capacity of Weber Reservoir was 12,500 acre-feet. The dam created an additional migration barrier to LCT.

The cumulative effects of agricultural diversions are reduction in flow and a decline in water quality in the river (e.g., high water temperature and low dissolved oxygen) and Walker Lake (high total dissolved solids (TDS)). In 1963, TDS in Walker Lake was 8,440 mg/l, and lake volume was 70% less than 1882; the introduced Sacramento perch (*Archoplites interruptus*) population disappeared in that year (Cooper and Koch 1984). In summary, the historic uses of water in the basin have contributed to declining water quantity, quality, and fragmentation of the Walker River basin.

#### **IV. EXISTING ECOSYSTEM CONDITIONS IN THE WALKER RIVER BASIN**

Regulated flow in the Walker River basin has disrupted the channel forming processes that create and maintain river and stream habitats. Portions of the Walker River seasonally dry due to agricultural diversions. Other areas in the river seasonally become braided and shallow due to alterations of the channel forming processes and reduction or elimination of the riparian vegetation. Channelization and bank armoring further degrade riverine habitats by modifying and simplifying many reaches of the Walker River. The combined effects of these actions result in a loss of habitat diversity required by native fish and insect species (Mooney 1983; Gerstung 1988; Hicks et al. 1991; Behnke 1992; Church 1995).

Degradation of native riparian communities, associated with altered hydrology and land use practices, has added to the loss of channel diversity and habitat complexity (Kondolf et al. 1987; Stromberg and Patten

1990). Healthy, intact riparian zones provide hydraulic diversity, add structural complexity, buffer the energy of runoff events and erosive forces, moderate temperatures, and provide a source of nutrients (USFS 1989). Riparian zones are especially important as a source of organic matter in the form of woody debris (Triska 1984). The woody debris helps control the amount and quality of pool habitat.

Irrigation diversions, dams, berms and levees have been constructed throughout the Walker River basin. Many of these structures fragment the river basin and act as barriers to fish migration, limiting the ability of migrating adults, juveniles and fry to migrate to required life history habitats (Deacon and Minckley 1974; Behnke 1992). Certain barriers are complete obstructions to upstream immigration, while others may be partial barriers. When access is limited, fish may spawn in and utilize sub-optimal habitats. Out-migrating fry and juveniles may be injured or killed during downstream migration and passage over obstructions.

### **Basin Hydrology and Water Quality**

Limited data exist on water quality and hydrologic relationships in the basin. As human development increased, the management of the Walker River changed. Today there are increased demands for water resources in Walker River basin. Prior to the development of the diversions and storage facilities in Walker River basin, the natural hydrologic regime of the basin reflected regional climate and runoff patterns. Typically summer and fall periods are dry with occasional summer thunderstorms impacting local areas. Winter high flow conditions occur with rain on snow events and may result in localized and sometimes basin wide flooding. Spring flows are typically high due to snow melt run-off.

Water quality issues of concern are temperature, dissolved oxygen, and TDS. Water diversions and irrigation return flows have contributed to water quality deterioration, specifically, warm summer temperatures, low dissolved oxygen related to high biological oxygen demand, and high TDS. Today the complexity of water management and infrastructure in the Walker River basin poses substantial challenges to recovery of LCT (Figure 3, USGS 1998).

### ***West Walker River***

For the period 1939 through 1993 the average annual flow was approximately 185,000 af downstream from the confluence of Little Walker

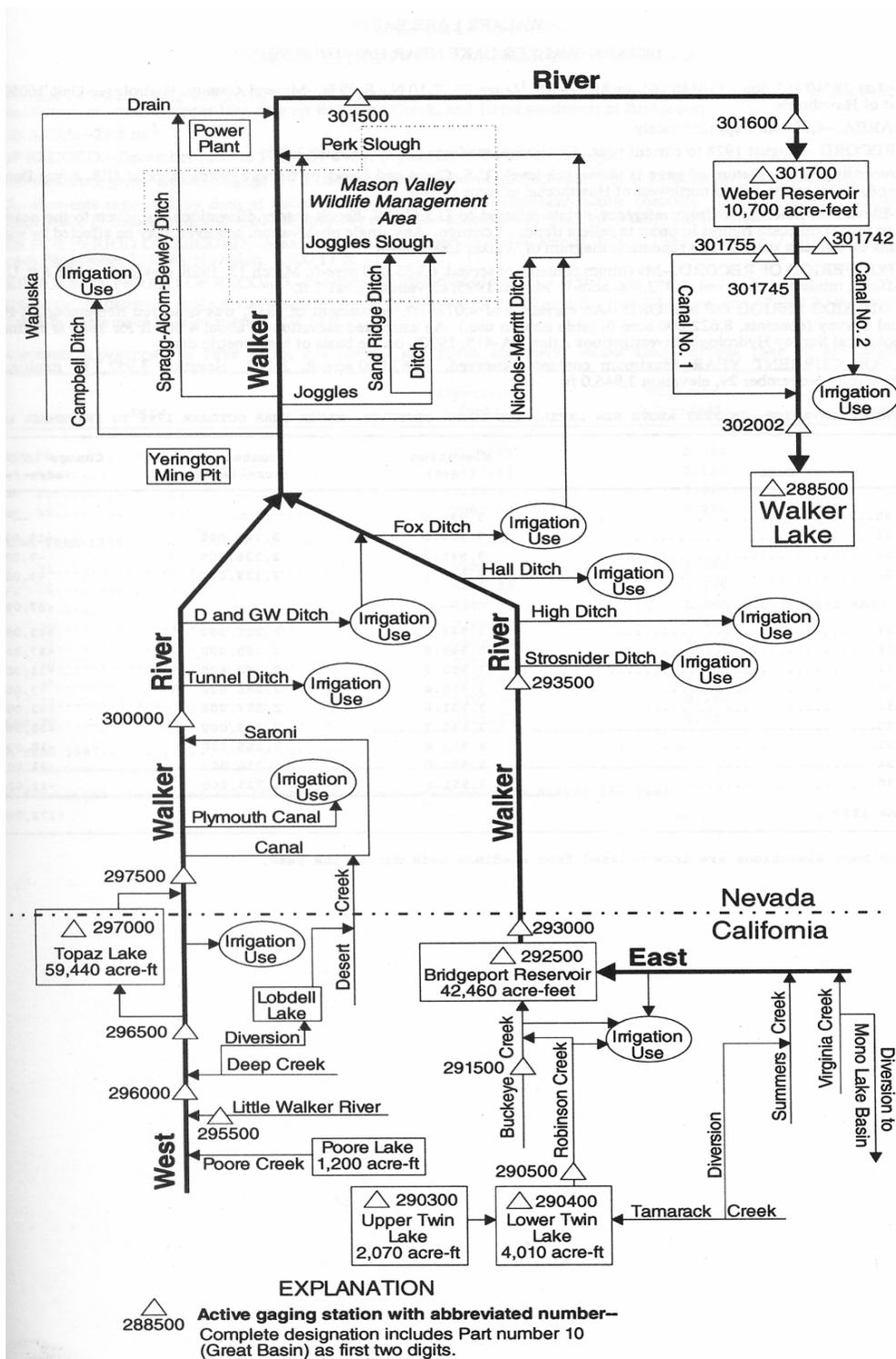


Figure 3. Walker River Hydrologic System Produced from USGS 1998

River (Thomas 1995). For the same period where the river flows northward into Antelope Valley the average annual flow was approximately 195,000 af (Thomas 1995).

Below Topaz Reservoir the average annual flow was 180,000 acre-feet (period of record 1939 – 1993). All diversions made to Topaz Reservoir are used for irrigated agriculture within the Walker River basin. Irrigation return flows and flood flows are discharged to the Alkali (Artesia) Lake Wildlife Management Area with return flows to the West Walker River.

In general, TDS is below the 500-ppm maximum limit for uses of water supply, irrigation and livestock set by the Nevada Bureau of Health Protection Services (Thodal and Tuttle 1996). TDS levels vary with seasonal stream flow volumes and return flows from irrigation.

Water temperature and dissolved oxygen exhibit seasonal variability. Annual temperature in the headwater areas and the West Walker River vary from 32 ° F to as high as 75 ° F in the downstream area (Horton 1996). Dissolved oxygen levels, which are impacted by temperature, flow volume and plant growth ranges between 5.2 and 13.65 ppm (Koch et al. 1979; Humberstone 1999). Cool water aquatic life generally does best between 7 and 9 ppm of dissolved oxygen.

### ***East Walker River***

The average annual combined flow of the collection tributaries into Bridgeport Reservoir for the period 1939 through 1993 was 132,000 af (Thomas 1995). For the same period, the average annual discharge from Bridgeport Dam to East Walker River was 107,000 af. Considerable variability in flow occurs in response to agricultural demands.

In general, the water quality of the East Walker River meets or exceeds the State of Nevada's agricultural and water supply standard for TDS, dissolved oxygen, nutrients and temperature (Thodal and Tuttle 1996). In 1988, a release of sediment-laden water from Bridgeport Reservoir resulted in reduced water quality, inadequate over-wintering habitat, and a fish and invertebrate kill (Nevada Division of Water Planning 2001). On December 31, 2000, a heating oil spill occurred on the East Walker River below Bridgeport Dam, the effects are still being investigated.

### ***Walker River***

Between 1939 and 1993, the combined average annual flow into Mason Valley was 233,000 acre-feet (Thomas 1995). The inflow of water varies

annually due to upstream watershed conditions and seasonally due to upstream reservoir releases. The average annual flow at Wabuska (Parker gage) for the period of 1939 through 1993 was approximately 128,000 af (Thomas 1995).

The water quality of Walker River represents the combined influence of the East and West forks, irrigation return flow and natural runoff. Point and non-point sources of pollutants may impact the Walker River basin. Point sources of pollutants include discharges from wastewater treatment plants and irrigation return flows.

### ***Walker Lake***

Walker Lake is currently approximately 13 miles long, 5 miles wide, with a maximum depth of 90 feet deep; this volume is approximately 50 percent smaller than it was in 1882. Flows from the Walker River, occasional runoff from the Gillis Range and the northern portion of the Wassuk Range, and direct precipitation provide the only inflow to Walker Lake. The Hawthorne Army Depot captures some of the runoff from the Wassuk Range (Humbstone 1999).

Walker Lake is a biologically productive, nitrogen-limited terminal lake, and classified as a monomictic lake; it turns over once annually, typically in the fall (Beutel and Horne 1997). During the summer Walker Lake normally stratifies into three distinct layers:

- Epilimnion – upper layer of the lake, which may have surface water temperatures exceeding 20°C, the thermal threshold for LCT survival.
- Hypolimnion – the lower layer of the lake, which has lower dissolved oxygen, cooler temperatures, increased levels of hydrogen sulfide and ammonia. Higher levels of hydrogen sulfide and ammonia combined with lower levels of dissolved oxygen restrict the use of this area by LCT.
- Metalimnion – The transition area between the top (epilimnion) and the bottom layer (hypolimnion) of Walker Lake. This layer provides suitable temperatures and dissolved oxygen for LCT during the summer months. As water temperature rises and dissolved oxygen concentration decreases during summer, the metalimnion becomes smaller and restricts the amount of area in which LCT can survive. Beutel and Horne (1997) referred to this condition as the temperature-oxygen squeeze.

As a result of irrigation demand and drier than normal years since the 1997 flood, Walker Lake elevation and therefore its ecological condition

continues to decline. As Walker Lake continues to decrease in elevation, the combined effects of increased TDS and alkalinity will lead to osmoregulatory problems for aquatic organisms. Osmoregulatory stress directly affects kidney function, gill hyperplasia, gill cell function, and blood congestion in the kidneys (Sevon 1988 ). TDS values for 1999 (11,295 mg/l) and 2000 (11,500 mg/l) reflect the inflow of the 1997 flood which provided a pulse of water to Walker Lake. TDS concentration has increased from a recorded 2,500 mg/L in 1882, to 14,600 mg/L in 2003 (Nevada Division of Water Planning 2001a). Research indicates the lake will no longer support a viable LCT fishery, if TDS reaches or exceeds 16,000 mg/L, (Dickerson and Vinyard 1999a; Sevon 1995).

Water temperature in the lake continues to be a challenge. The upper thermal limits for fluvial LCT ranges from 22 ° C to 24 ° C as experienced in laboratory studies (Dickerson and Vinyard 1999b; Dunham et al. 1999; Meeuwig 2000; Dunham et al. 2002). Desert Research Institute (DRI) work has indicated a lethal temperature range of between 18° and 20° C for LCT in Walker Lake water, although Sollberger (2000) has found evidence of LCT surviving 22° to 24° C for short periods of time in the lake. Higher temperatures decrease the maximum amount of oxygen that can be dissolved in water, leading to oxygen stress if the water is receiving high loads of organic matter (Moore 1989; Michaud 1991).

The lake is nitrogen limited, which is typical of a Great Basin terminal lake. Blooms of blue-green algae, *Nodularia spumigena*, are associated with low levels of inorganic nitrogen (Horne 1994). This algal species comprises 97 percent of the total phytoplankton biomass found in the lake (Horne 1994). Its presence promotes warming of the surface waters and a decrease in light penetration, which is essential to the growth of other phytoplankton and zooplankton species (Horne 1994). Decomposition of algal cells during the summer creates oxygen depletions in the hypolimnion of Walker Lake, thus trout are unable to remain near the bottom of Walker Lake where water temperatures are more conducive to survival. Oxygen depletion restricts the production of invertebrates on which forage fish and young LCT feed (USFWS 1995).

### **Riparian Ecosystem**

Functional riparian zones are important to stream systems, providing bank stability, wildlife habitat, nutrient cycling to the stream, lowered water temperatures, and a reduction in the colonization potential of non-native species such as tamarisk (Cleavy et al. 1997; Schade and Fisher 1997; Kennedy and Merenlender 2000; Waite and Carpenter 2000; Dent et al. 2001; Poole and Berman 2001; McArthur and Richardson 2002;

Schade et al. 2002). Tall whitetop, an invasive species which out-competes native riparian plants, currently presents a problem especially in disturbed areas on the East Fork of the Walker River. Two other invasive species, purple loosestrife and Eurasian watermilfoil, are establishing themselves in Walker River basin and have the potential to clog wetlands, waterways, and overtake riparian areas if left unchecked (Eisworth et al. 2000). Human impacts to the Walker River basin are potential increases in sediment pulses due to watershed disruption and reduced input of large woody debris. Woody debris in streams increases the amount and quality of pool habitat, increases sediment storage, improves nutrient cycling and provides refugia from predators and high flow events (Robison and Beschta 1990; Triska 1984; Klotz 1997).

Before the construction of dams and diversions, over-bank flooding was more frequent, providing riparian seed dispersal and conditions necessary for seed germination. Much of the Walker River's historic flood plain has been converted to agriculture often utilizing the prime riparian habitat. The resulting river channel has limited riparian and aquatic cover, reduced channel complexity and limited habitat to sustain a self supporting LCT population (Hickman and Raleigh 1982).

Channel incision along the lower Walker River affected riparian communities as historic flood plains became disconnected from the river. Terraces formed as a result of channel incision, which ultimately restricts natural riparian processes and stream channel complexity. Existing mature cottonwoods remaining on the terraces are presently able to reach the water table, whereas in other desert systems channel incision has resulted in death of mature cottonwood forests (Bovee et al. 2002). Regeneration of young cottonwoods will not occur without the return of ecosystem-dependent floods (Cordes et al. 1997; Rood and Mahoney 2000; Bovee et al. 2002; Otis Bay Riverine Consultants 2002). Loss of the cottonwood canopy in the Walker River basin has led to higher stream temperatures due to a loss of shading along the watercourse (Stromberg and Patten 1990).

## **V. INSTREAM FLOW NEEDS TO SUPPORT ECOSYSTEM PROCESSES**

Species native to the Lahontan basin waters have been exposed to flow regimes that varied temporally, both seasonally and across years over their evolutionary past. As a result, native biota, such as fish, invertebrates, amphibians, and riparian plants, are adapted to such variation in flow regimes that date back to at least the Pleistocene and probably the Pliocene. In fact, important processes responsible for sustaining native species, for example the recruitment of riparian

vegetation, may even depend on the river's natural variability in flows. Recent evidence suggests that artificially constant flow regimes favor exotic species, such as salt cedar (*Tamarix ramossissima*), over native species that are tolerant of greater fluctuations in instream flows, such as Fremont cottonwood (*Populus fremontii*) (Stromberg and Patten 1990). Thus, to sustain and perpetuate the native aquatic and riparian ecosystem, a managed flow regime would ideally mimic natural variation in streamflow, seasonally and across years, as closely as possible.

Through implementation of short-term tasks identified in this Action Plan, the WRIT anticipates the development of a flow prescription for the Walker River basin that is similar to the approach taken on the Truckee River. Namely, the method used by Otis Bay Ecological Consultants and the FWS to determine ecosystem flow requirements which contained several features: (1) it evaluates the entire range of natural flow conditions; (2) it integrates the needs of multiple biota such as fish, invertebrates, and riparian vegetation; and (3) it addresses the sediment transport processes that control channel geometry and perpetuate a dynamic riverine system. Flow regime recommendations derived from this methodology will mimic natural hydrologic patterns that sustain the riverine ecosystem and its native species.

## VI. LCT LIFE HISTORY CHARACTERISTICS

LCT populations historically persisted in large interconnected aquatic ecosystems throughout their range (Figure 4). These systems were either lacustrine habitats with tributary streams or large stream networks consisting of a river and tributaries. LCT can express both resident and migratory life histories such that resident forms use tributary habitats only and migratory forms use both river and/or lake habitats in addition to tributaries (Northcote 1992; Rieman and Dunham 1998; Neville-Arsenault 2003).



Figure 4. Lahontan cutthroat trout *Oncorhynchus clarki henshawi* Source: Laurie Moore

Fluvial LCT prefer cool streams characterized by pools in close proximity to cover and velocity breaks, vegetated stable stream banks, and riffle-run

areas which contain relatively silt-free, gravel substrate (USFWS 1995). LCT in fluvial habitats typically occupies rocky areas, deep pools, and areas near overhanging logs, shrubs, or banks.

Lacustrine LCT are adapted to a variety of lake habitats, from small alpine lakes to large desert terminal lakes (Moyle 2002). LCT can tolerate higher alkalinity and TDS than other non-anadromous salmonids (Young 1995). For this reason LCT has been stocked in saline-alkaline lakes in Nevada, Oregon, and Washington for recreational purposes (USFWS 1995).

Fluvial populations of LCT appear to be intolerant of competition or predation by non-native salmonids and rarely coexist with them (DeStaso and Rahel 1994; Schroeter 1998; Dunham et al. 2000). However, while there is limited understanding of non-native salmonid interactions with lacustrine LCT, there are examples of co-existence in lake environments, e.g., the Independence Lake population currently coexists with brook trout, brown trout, and kokanee (Lea 1968; LaRivers 1962). Other lacustrine cutthroat subspecies compete very well with non-native salmonids in prime lake habitats (Sigler and Sigler 1987, Young 1995).

Specific habitat requirements of LCT vary seasonally and with life stage. Like most cutthroat trout species, LCT are obligatory stream spawners which predominantly use tributary streams as spawning sites. Fish may exhibit three different strategies depending upon conditions, outmigration as fry, as juveniles or remain in the river as residents (Ray et al. 2000; Neville-Arsenault 2003). For fluvial LCT, spawning occurs from April through July, depending on stream elevation, stream discharge, and water temperature (USFWS 1995). Lacustrine LCT migrated from Walker Lake (January to April) and up Walker River basin tributaries to spawn in riffles or the downstream end of pools (USFWS 1995).

Historically, the lower Walker River may not have been used as the primary spawning and rearing habitat. Instead, the lower Walker River was likely used as a migratory corridor to the upper river and its tributaries. These habitats provided more suitable gradient, substrate size, water temperature, and flow regimes necessary to support reproduction.

Historically, LCT occurred throughout the Walker River drainage from the headwaters in California downstream to Walker Lake (LaRivers 1962; Gerstung 1988). It has been documented that LCT were found in Upper and Lower Twin Lakes and in tributaries above the present day Bridgeport Dam on the East Fork of the Walker River; in many tributaries in the upper sections of the West Fork of the Walker River (Becker pers. comm.) and seasonally downstream in the Walker River to Walker Lake.

Fluvial LCT fed primarily on aquatic insects, zooplankton and terrestrial forms of food. The lacustrine form of LCT utilized the habitat and food sources of Walker Lake, which included zooplankton and other fish species such as tui-chub. It is likely that a certain proportion of the hatched lacustrine form of LCT stayed in the tributaries and became acclimated to the local habitats and exhibited life history characteristics more typical of fluvial species.

LCT evolved in a range of habitat types including high elevation, cold water streams to warmer, more alkaline lake environments. Evidence from the contemporary dynamics of extant LCT populations suggests that localized, natural events historically caused the local extirpation of small populations of LCT. These events could include landslides, fires, runoff and/or development of natural barriers that restricted seasonal movements. LCT persistence is associated with their ability to maintain connectivity between populations, i.e. networked populations (Ray et al. 2000). A *networked* population is defined as an interconnected stream and/or lake system linked through migration or dispersal so individuals from other locations in the stream system can repopulate impacted areas (Ray et al. 2000). This ability to disperse and repopulate extirpated habitats allows populations to persist in environments that are highly variable in both time and space (Dunham et al. 1997; Rieman and Dunham 1998; Ray et al. 2000; Neville-Arsenault 2003). Periodic re-population by upstream or downstream sources enabled LCT to survive extreme circumstances and provided for genetic exchange (Neville-Arsenault 2003).

As populations become isolated due to physical and biological fragmentation, migration rates decrease, local extirpation may become permanent, and the entire population may move incrementally toward extinction (Ray 2001). Inherent in a networked population is movement among tributaries. As a result, this pattern may not necessitate re-establishment of separate populations in each tributary in the Walker River basin.

Because the Walker River basin has been altered removing important habitat elements that once supported LCT in the basin, more information is needed to characterize suitable habitat (river and lake) for all life stages to determine ecological requirements of a self-sustaining, interconnected network population of LCT. In the Walker River system, information on the thermal requirements of LCT is limited because the population was extirpated before basic ecological information was obtained. However, laboratory and field research show LCT can tolerate elevated water temperatures (Vigg and Koch 1980; Dickerson and Vinyard 1999;

Dunham et al. 2002). Upper thermal limits from laboratory studies and research conducted on stream populations ranges from 22°C to 24°C (Dickerson and Vinyard 1999; Dunham et al. 1999; Meeuwig 2000; Dunham et al. 2002). Other investigations previously conducted, ongoing, or in development within the Truckee River basin that may provide ecological insights to restore an interconnected, self-sustaining network of LCT populations in the Walker River Basin include: Development of specific ecosystem monitoring and inventory protocols to summarize and evaluate existing information and develop recommendations to improve data collection; effect of water quality on survival of LCT eggs in the Truckee River (Hoffman and Scopettone 1984); an assessment of nonpoint source pollution in the lower Truckee River (Lebo et al. 1994); introductions of LCT in selected reaches of the river to track their growth performance, movement and/or residency; perform a watershed assessment to identify water quality and migration barriers and connect access to desirable spawning and rearing habitat within the basin; and develop/implement hydrologic studies to evaluate site specific habitat improvement projects.

### **Non-Native Species**

Introductions of non-native fish into the Walker River system began in the 1800s, by private and state entities (USFWS 1995). The addition of non-native salmonid species has contributed to the decline of most if not all of the cutthroat trout subspecies including LCT. In aquatic ecosystems modified by human disturbance, non-native fish species often become dominant and out-compete native fish species (Deacon and Minckley 1974; Shepard et al. 1997; Brandenburg and Gido 1999; Schindler 2000; Knapp et al. 2001). At present, there are over 40 non-native fish species within LCT's historic range (Behnke 1992). Non-native salmonids have adverse effects on the distribution and abundance of native species in Sierra Nevada streams (Moyle and Vondracek 1985; Moyle and Williams 1990). The two most prevalent non-native salmonids are rainbow and brown trout, which are common in the East and West Forks of the Walker River. Brook trout and brown trout compete with cutthroat trout for space and resources (Gerstung 1988; Gresswell 1988; Griffith 1988; Fausch 1989; Hildebrand 1998; Schroeter 1998; Dunham et al. 1999). Rainbow trout, a closely related species, spawns at the same time and uses the same spawning habitat as LCT with which it interbreeds creating hybrids individuals. Carp and centrarchids are the most common introduced species in the lower Walker River. Non-native salmonid populations are augmented with hatchery-reared fish to enhance recreational fishing opportunities in California. In the East and West forks in Nevada the non-native salmonid fishery is maintained primarily by hatchery fish.

Despite coexistence of LCT and rainbow trout, hybridization is not well documented for lacustrine LCT, whereas hybridization between fluvial LCT and rainbow trout readily occurs. Hybridization, however, is a threat for lacustrine fish due to the requisite river habitat for spawning. Although the Independence Lake LCT persist despite the presence of brown and brook trout and kokanee and historic stockings of rainbow trout (Gerstung 1988; Lea 1968). Existing examples of coexistence provide opportunities to understand the mechanisms that preclude hybridization and competition and allow development of management tools for lacustrine LCT recovery.

### **LCT Genetics**

Recovery of self-sustaining LCT populations will ultimately involve habitat restoration, but success of these efforts may also depend upon re-establishing populations of strains native to each of the three distinct population segments defined for this subspecies. Early genetic analyses (Loudenslager and Gall 1980; Gall and Loudenslager 1981; Xu 1988) revealed significant differentiation among LCT in the Walker, Carson, Truckee, Reese and Humboldt River drainages. Genetic differences may be the result of adaptations to different habitat types e.g., lake versus river dominated ecosystems.

The use of genetic data to make informed decisions about which LCT strains to use in recovery of western DPS waters will depend upon a working knowledge of both the extent of population differentiation among basins and the hierarchical relationships among populations within basins.

Genetic data in recovery planning will be used to:

- (1) determine genetic relationships of populations within and among basins,
- (2) assess levels of genetic variation per population, and
- (3) compare levels of genetic variation among populations to help assess contemporary and past population dynamics and extinction risk.

### ***Background***

Phylogenetic analysis (phylo = historical, genetic = genes) is an analytical tool to determine evolutionary (or historical) relationships among populations, subspecies or species. This approach is based upon the general premise that the greater the number of genes individuals have in common the more closely related they are. An analogous human example would be individuals in a nuclear family are more genetically

similar to one another than to their first cousins, first cousins in turn are more genetically similar to each other than they are to their second cousins and so on.

The historical relationships among populations within species, or subspecies can therefore be reconstructed using the genes found in contemporary individuals, i.e., the longer the time since populations or species had a common ancestor the fewer genes they are likely to have in common. Thus it is both the genetic similarities and differences among individuals within populations and among populations that provides the information used to elucidate historical relationships

Genetic data are typically more useful for phylogenetic analysis than morphological characters because they tend to be more variable, i.e., there are more traits to compare among individuals. As a result, genetic data have been routinely used to distinguish among populations, subspecies and species for the past 40 years (Lewontin and Hibby 1996; Avise 1994; Weir 1996).

Over the past thirty years researchers at the University of California Davis, Brigham Young University, Clear Creek Genetics Laboratory (Boise, ID) University of Montana, Stanford University and the University of Nevada at Reno have conducted genetic analyses on Lahontan cutthroat trout populations throughout its range (Loudenslager and Gall 1980; Gall and Loudenslager 1981; Mirman et al. 1982; Leary et al. 1987; Williams et al. 1992; Dunham et al. 1998; Williams et al. 1998; Nielsen 2000; Nielsen and Sage 2002).

The University of Nevada at Reno (Dunham et al. 1998; Nielsen and Sage 2001; Peacock et al. 2001) recently compiled and evaluated all existing genetic studies on LCT. Studies conducted to date, have used one type of or a combination of three classes of genetic markers: (1) proteins (allozymes). (2) mitochondrial DNA (mtDNA), and (3) nuclear DNA (microsatellites) which provide information on LCT evolution at different spatial and temporal scales (Table 1, appendix D: full genetic summary).

### ***Historical and Contemporary Patterns***

Genetic data support the designation of three evolutionarily distinct groups of populations or evolutionarily distinct units (ESUs) within the historical range of LCT. These ESUs or distinct population segments (DPS) are: (1) the Humboldt River basin populations including the Reese River populations, (2) populations in the Quinn River basin and (3)

populations in the Truckee, Carson and Walker river drainages comprise the western basin DPS. Genetic data further delineate genetically distinct groups of populations between river drainages within these larger ESU designations, e.g., the Reese river populations are genetically distinct from the Humboldt populations. Populations within the Truckee, Carson and Walker river drainages are also distinct from each other and have been referred to as separate microgeographic races of LCT (Gall and

**Table 1. Classes of Genetic Markers**

**Classes of Genetic Markers**

- **Allozymes** – protein products of nuclear DNA sequences. Allozymes are widely used for phylogenetic analyses. Their use is limited to identification of significant differences between genetically different populations. Closely related populations exhibit low levels of allozyme variation.
- **Mitochondrial DNA** – is a maternally inherited single molecule, which is widely used for phylogenetic analyses at the population, subpopulation and species level. The level of resolution of the mitochondrial DNA differences between and among populations and species is dependent upon the level of genetic variation. Mitochondrial DNA exhibits a faster rate of evolution than allozyme markers.
- **Microsatellites** – nuclear non-coding DNA that is highly variable. Microsatellites exhibit the highest and fastest rate of evolution and therefore have the highest accumulation of variation within and among populations. Microsatellites are use for phylogenetic analyses at population, subspecies and closely related species levels. Microsatellites are useful markers for examining relationships among populations at small spatial scales such as may be found in geographically close basins.

Loudenslager 1981). Recovery activities e.g., transplantation of fish into recovered habitats should, if possible, involve fish native to the respective DPSs and individual drainages.

There are few natural populations of LCT remaining in the Walker River basin. However, original Walker River basin fish were found in By Day and O'Harrel creeks. The progeny of these fish have subsequently been transplanted into Slinkard, Murphy, Mill, Wolf and Bodie creeks. Because genetic analysis indicate that these fish represent the original Walker River basin LCT, they will form the basis for further development of the Walker River basin LCT to be used in recovery activities in this basin.

Large interconnected stream and/or stream and lake habitats are thought to be crucial to long-term population persistence of cutthroat trout populations in desert environments. Genetic and demographic data from LCT populations in the Humboldt DPS, other cutthroat trout subspecies and other inland trout species such as bull trout (Rieman and Dunham 1998; Ray et al. 2000) support this hypothesis. Most lacustrine LCT habitats are found in the western Lahontan basin drainages, e.g., Independence, Pyramid and Walker lakes. LCT historically occupied all of these lake habitats. Lake habitat is not sufficient, however, for recovery of naturally reproducing populations as river habitat is necessary for spawning and also provides habitat for younger aged fish, prior to migration back to lake habitat, and for fish that are resident in the river year round.

The large river systems in the eastern basin are comparable to the western lake and river systems, specifically, large mainstem rivers provide habitat and food resources analogous to the lake habitat for those large LCT that adopt a migratory life history. Data from contemporary studies as well as historical geological data (pre-European settlement) show that river and lake-habitats have periodically gone dry. The mainstem Mary's River in the Humboldt River system went dry during the drought period in the early 1990s and was re-colonized by fish during the post drought (Dunham and Vinyard 1996). Walker Lake has gone dry on at least three separate occasions during its history and has stayed dry ranging from 300-1000 years only to be re-colonized by fish from river habitat in each instance. Walker Lake dried up (1) 11,000 years before present and was rewetted at ~10,750 years; (2) 5,000 years before present and rewetted at 4,000 years and again at (3) 2,500 years before present and rewetted at 2,000.

During these periods fish found refugia in extant river habitats and re-invaded mainstem river and lake habitat when conditions were

appropriate. The LCT subspecies is thought to be at least 30,000 years old and may have evolved in the late Pliocene, which predates the drying episodes in the Walker River basin by as much as 2 million years. These data also show that fish have the ability to successfully re-invade lake habitats despite living in river environments for considerable periods of time. These data strongly suggest that fish presently confined to river habitat do have the ability to utilize lacustrine habitat. There is no evidence suggesting that present day fish which have been confined to headwater reaches of the Walker River basin for less than 50 years (a very short time period on an evolutionary timescale) have lost the ability to express both migratory (lake fish) and resident (river fish) life histories. The data from genetic and demographic studies suggest that long-term recovery will entail recreating complex interconnected habitats that permit expression of both migratory and resident life history strategies and provide the necessary habitat diversity for all age classes.

Decisions related to the determination of the appropriate strain or strains necessary to achieve recovery will be initially guided by the strategy outlined in the Recovery Plan (1995) to maximize genetic variation of the remaining stocks of LCT. The strategy states that any isolated population of fishes is a potentially unique gene pool with characteristics that may differ from all other populations, and whenever possible, genetic stocks should be maintained within their historic basin source. The Recovery Plan (1995) further states that recognition of the uniqueness of locally adapted LCT populations is recommended by many taxonomists and conservation biologists for restoration and future utilization of the resource.

## **VII. SHORT-TERM ACTION PLAN**

### **Short-Term Goals and Objectives**

The purpose of the Short-Term Action Plan is to identify and prioritize actions for implementation during the next five years (the first five years of the Short-Term Action Plan) to facilitate the restoration/recovery of naturally reproducing lacustrine LCT. The goal is to present a specific five-year action plan for restoration of the Walker River and Walker Lake ecosystem for recovery of LCT in conformance with the Recovery Plan (USFWS 1995).

Prioritization of recovery actions was central to the development of the Short-Term Action Plan. For example, the presence of fish passage barriers is a significant recovery issue fragmenting the ecosystem and acting as a constraint to recovery. While fish passage will be addressed

over time, certain recovery actions can be implemented immediately that will address habitat conditions and promote re-colonization of historic habitats. Proactive measures, including the use of hatcheries and streamside egg incubation facilities, will “jumpstart” the recolonization process.

Stocking of fluvial LCT in selected headwater reaches, as identified in the Recovery Plan, will be continued to promote a transition in the fish community in support of native fish species. As outlined in the Recovery Plan (USFWS 1995) and in the short-term action, it is proposed that certain tributaries will be managed exclusively for LCT. The sequencing and prioritization of actions promotes recovery progress while future activities that require additional data or commitments of resources are assessed. The process of recovery will be implemented and evaluated through an adaptive management program.

Development of the Short-Term Action Plan associated with the recovery of LCT in the Walker River basin were assessed by addressing each action with the following screening criteria.

*Each Short-Term Action should:*

- Address a specific factor identified as impacting the ability of LCT to sustain itself in the Walker River basin.
- Relate directly to the Recovery Goal and Recovery Criteria.
- Tie directly to a specific agency and/or Tribal entity management action.

The development of short-term actions required information and knowledge regarding the Walker River basin, understanding of the level and quality of the existing ecosystem information, and identification of technical and scientific areas of concern and opportunity. Once a baseline of information is determined, then development of specific short-term actions and a prioritization of those actions can occur.

**Table 2. Geographic Areas of Concern**

The Walker River basin was divided into four geographic sections based on specific geomorphic, hydrologic and management issues.

	<b>Basin/Watershed Area</b>	<b>Rationale</b>
I	<b>Headwaters</b>	
IA	West Walker River headwaters – upstream of Topaz reservoir	Headwater locations above the primary major barrier on the West Walker River

IB	East Walker River headwaters – upstream of Bridgeport Reservoir	Headwater locations above the primary barrier on the East Walker River
II	<b>West and East Forks of the Walker River above their confluence</b>	
IIA	West Walker River from Topaz Reservoir to the confluence with the East Fork of the Walker River	River corridor from the primary barrier to fish movement to the confluence with the East Walker
IIB	East Walker River from Bridgeport Reservoir to the confluence with the West Fork of the Walker River	River corridor from the primary barrier to fish movement to the confluence with the West Walker
III	<b>Confluence of the East and West Forks to Walker Lake</b>	Mainstem Walker River through the primary agricultural region
IV	<b>Walker Lake</b>	Lacustrine ecosystem

The WRIT focused initial efforts on developing a better understanding of primary sources of information and data that the various agencies, Tribes, and groups have on the Walker River basin. After a review of the existing information, the WRIT team identified five primary areas of technical and administrative concerns with which short-term tasks could be categorized.

**Table 3. Areas of Specific Technical Concern**

<i>Topic</i>	<i>Reference</i>	<i>Listing Factor</i>
General Issues	Applicable to all areas of technical concern	General concerns that support specific species responses
Genetics and Population dynamics	Strain issues Networked populations	Fish populations
Physical habitat and environment	Location, distribution, and access	Habitat loss
Biological and limnological (chemical) environment	Water quality, biological processes	Biological sustainability
Recreation	Fishing and water use	Habitat and people impacts

The WRIT focused on identifying specific actions that could address the following questions:

1. Does the short-term action address a specific threat or issue in the Walker River basin that led to the listing of LCT?
2. Does the short-term action address the goal of LCT recovery?
3. Can the short-term action be assessed against the criteria for recovery established by the WRIT?
4. Can the short-term action be accomplished in a timely and cost effective manner?
5. Are prerequisite studies required prior to implementation of the short-term action?

## Walker River Basin Short-Term Actions

The actual short-term tasks identified by the WRIT are a result of approximately two years of discussion, debate, evaluation and recommendation. The short-term tasks identified in the next five tables comprise the Short-Term Action Plan as part of the recovery effort for LCT in the Walker River basin. Five groups of short-term tasks are identified for the Walker River basin.

- Group A – General integrating issues
- Group B – Genetics and population dynamics
- Group C – Physical habitat and environment
- Group D – Biological and limnological (chemical)
- Group E – Recreational fisheries

Once the short-term tasks were identified, the WRIT determined the timeframe for each proposed short-term action. Each action was assigned a timeframe in terms of when in the process the individual action should be implemented. The assigned priorities are as follows: Year 1-3 high priority and need; Year 3-5 medium priority or need for prerequisite study to be completed; and year 5+ lower priority or action that could begin and/or continue beyond year 5 if conditions and information needs dictate.

Responsibility for implementing the specific actions has not been designated. This task will occur after the MOG reviews the recommendations and direction for implementation occurs. Five task groups reflecting the approach outlined above are presented in Tables 6 through 10. Items marked with a + are noted as extending beyond the initial five-year period.

**Table 4. Short-Term Tasks for Recovery Task Group A  
General Integrating Issues**

<b>TASK</b>	<b>TITLE</b>	<b>TIMELINE</b>	<b>RESPONSIBILITY</b>
<b>A1</b>	<b>Document existing data and the level of analysis required to make useable by the WRIT</b>	<b>HIGH Yrs 1-5</b>	<b>FWS data acquisition with handoff to other WRIT members</b>
A1a	Develop an integrated GIS-based data system and identify specific analytical tools for analysis	Yrs 1–5+	
A1b	Compile all fish management plans, regulations and data	Yrs 1-2	

A1c	Compile existing water management plans, policies, regulations and data	Yrs 1-2	
A1d	Compile existing habitat, data, and other land management plans	Yrs 1-2	
A1e	Compile existing multiple use and Tribal resource management plans as appropriate	Yrs 1-2	
A1f	Identify landowners who may be partners in LCT recovery efforts	Yrs 1-5+	
A1g	Identify and evaluate existing water quality, sediment and flow data	Yrs 1-5+	
<b>A2</b>	<b>Develop an education and outreach program for WRIT activities (would be coupled with MOG outreach program)</b>	<b>HIGH Yr 1</b>	<b>FWS initiate with handoff to CA, FS, and WRPT</b>
<b>A3</b>	<b>Continue to develop longer-term tasks for implementation of the WRIT plan and tie to adaptive management plan</b>	<b>MEDIUM Yrs 3-5</b>	<b>WRIT</b>
<b>A4</b>	<b>Develop monitoring plans for LCT recovery efforts with specific protocols. Link to adaptive management program (tie to specific B, C, D, and E tasks)</b>	<b>MEDIUM Yrs 3-5</b>	<b>Action agency</b>
<b>A5</b>	<b>Determine necessity and level of peer review necessary for tasks on a case-by-case basis</b>	<b>LOW Yrs 4-5</b>	<b>WRIT</b>

**Table 5. Short-Term Tasks for Recovery Task Group B  
Genetics and Population Dynamics**

<b>TASK</b>	<b>TITLE</b>	<b>TIMELINE</b>	<b>RESPONSIBILITY</b>
<b>B1</b>	<b>Identify native and non-native salmonid populations that are maintained by natural reproduction</b>	<b>HIGH Yrs 1-5</b>	<b>Appropriate entities with funding</b>
<b>B2</b>	<b>Identify the role of hatcheries in Walker River basin LCT recovery. Develop HET to coordinate remaining B2 tasks</b>	<b>HIGH Yrs 1-5</b>	<b>FWS initially to Hatchery Evaluation Team (HET)</b>
B2a	Organize a <i>hatchery evaluation team</i> to coordinate remainder of B2 tasks	Yr 2	FWS initially to HET
B2b	Develop/Implement hatchery management techniques and protocols for LCT propagation and broodstock development and maintenance	Yrs 2-5	
B2c	Develop/Implement production objectives for federal/state/tribal LCT hatcheries to assist in recovery program	Yrs 2-5	

B2d	Compile and evaluate stocking records for existing populations (LCT and other salmonids) or those planned for recovery actions	Yrs 2-5	
B2e	Determine what additional research will be required for growth and performance assessments	Yrs 2-5	
B2f	Identify locations and opportunities to improve LCT broodstock and propagation programs	Yrs 3-5	
<b>B3</b>	<b>Develop report on hybridization potential and technical studies needed to identify/characterize hybrids from other salmonid species.</b>	<b>LOW Yrs 4-5</b>	<b>FWS</b>
<b>B4</b>	<b>Complete genetic research and reports</b>	<b>HIGH Yrs 1-2</b>	<b>UNR with funding from others</b>
B4a	Develop recommendations for implementing and evaluating genetic management programs	Yr 2-5	
B4b	Determine which strains of LCT should be used in the Walker basin recovery efforts	Yrs1-2	Basinwide WRIT

**Table 6. Short-Term Tasks for Recovery Task Group C  
Physical Habitat and Environment**

<b>TASK</b>	<b>TITLE</b>	<b>TIMELINE</b>	<b>RESPONSIBILITY</b>
<b>C1</b>	<b>Develop and/or support a quarterly Water quality sampling and analysis Program for Walker River Basin including Walker Lake</b>	<b>MEDIUM Yrs 1-3</b>	<b>FWS with handoff to entities upon initiation</b>
C1a	Evaluate existing plans and protocols	Yr 1	
C1b	Identify cumulative, cause and effect relationships of point and non-point source pollutants	Yrs 1-2	
C1c	Recommendations for future monitoring	Yrs 2-3	
<b>C2</b>	<b>Identify and evaluate fish passage and existing barriers within the Walker River Basin</b>	<b>MEDIUM Yrs 3-5</b>	<b>FWS initially</b>
C2a	Recommend passage and barrier activities	Yrs 3-5	

<b>C3</b>	<b>Develop watershed analysis of the Physical components of the Walker River Basin</b>	<b>HIGH Yrs 1-5+</b>	<b>FWS initially</b>
C3a	Summarize and evaluate existing information	Yrs 1-3	
C3b	Prioritize river sections for assessment	Yrs 1-3	
C3c	Develop recommendations	Yr 3	
C3d	Develop watershed and regional partnerships	Yrs 3-5	
C3e	Evaluate cumulative, cause and effect relationships	Yrs 3-5	
C3f	Link to GIS data system	Yrs 1-5+	
<b>C4</b>	<b>Develop specific ecosystem monitoring and inventory protocols for future data collection and assessments</b>	<b>MEDIUM Yrs 3-5</b>	<b>WRIT with agency implementation</b>
C4a	Summarize existing information <ul style="list-style-type: none"> <li>· Biological</li> <li>· Physical</li> </ul>	Yrs 3-5	
C4b	Evaluate existing information	Yrs 3-5	
C4c	Develop recommendations for priority	Yrs 3-5	
C4d	Link to GIS data system	Yrs 1-5+	
<b>C5</b>	<b>Develop and implement hydrologic studies</b>	<b>HIGH Yrs 1-3</b>	<b>FWS</b>
C5a	Evaluate historical studies and determine what additional information and analysis necessary	Yrs 1-3	

**Table 7. Short-Term Tasks for Recovery Task Group D  
Biological and Limnological**

<b>TASK</b>	<b>TITLE</b>	<b>TIMELINE</b>	<b>RESPONSIBILITY</b>
<b>D1</b>	<b>Identify where LCT existed in the past and what species assemblages exist there now</b>	<b>HIGH Yrs 1-2</b>	<b>FWS</b>
D1a	Review historic information and document LCT Specific information	Yrs 1-2	
D1b	Conduct oral history reviews with Tribal members, ranchers and fishermen	Yrs 1-2	

<b>D2</b>	<b>Develop, implement, and monitor a Wild LCT Management Plan that will not impact donor or newly established populations</b>	<b>HIGH Yrs 1-5</b>	<b>FWS initially with handoff to appropriate entities</b>
D2a	Monitor population abundance and variability	Yrs 1-5+	
D2b	Determine minimum number of fish and/or eggs from donor populations to establish populations required to support recovery	Yrs 2-3	
<b>D3</b>	<b>Evaluate creeks and tributaries that feed into Walker Lake for future use</b>	<b>LOW Yrs 4-5</b>	<b>FWS initially with handoff to appropriate entities</b>
D3a	Identify creeks and identify existing fish assemblages, habitat conditions and flow needs	Yrs 3-4	
D3b	Identify and improve, if lake level rises, connections of tributaries to Walker Lake	Yrs 4-5+	
D3c	Secure water rights for tributaries that are useable (if necessary)	Yrs 4-5+	
<b>D4</b>	<b>Develop specific fish distribution GIS overlays for both native and non-native fish</b>	<b>HIGH Yrs 1-3</b>	<b>FWS initially with handoff to appropriate entities</b>
D4a	Identify fish assemblages by reaches	Yr 1	
D4b	Identify fish densities/population structure	Yrs 1-2	
D4c	Document life history requirements for each species and determine biological overlap	Yrs 2-3	
D4d	Identify fish distribution patterns (by season)	Yrs 1-2	
<b>D5</b>	<b>Evaluate the extent of non-native fish survival in the Walker River basin and develop approaches to minimize the effects of non-native salmonid populations on LCT recovery</b>	<b>MEDIUM Yrs 3-5</b>	<b>FWS with handoff to research entities</b>
D5a	Identify and evaluate the potential impacts to LCT of self-sustaining non-native salmonid populations and recommend appropriate actions	Yrs 1-5+	
D5b	Develop and implement measures to reduce or eliminate impacts of non-native salmonid populations to extant or introduced LCT populations where appropriate	Yrs 1-5+	

<b>D6</b>	<b>Initiate habitat surveys to evaluate potential LCT introduction streams and validate against existing LCT inhabited streams</b>	<b>MEDIUM Yrs 3-5</b>	<b>WRIT develop process with handoff to agencies</b>
D6a	Complete C3 and C4 tasks	Yrs 1-3	
D6a	Implement physical and biological protocols. Concentrate on the interconnected, networked population approach outlined in genetics section	Yrs 3-5+	
<b>D7</b>	<b>Completion of the Rosaschi Ranch Management Plan</b>	<b>HIGH Yrs 1-2</b>	<b>Forest Service and FWS</b>

**Table 8. Short-Term Tasks for Recovery Task Group E  
Recreational Fisheries as Related to LCT Recovery**

<b>TASK</b>	<b>TITLE</b>	<b>TIMELINE</b>	<b>RESPONSIBILITY</b>
<b>E1</b>	<b>Evaluate the potential of LCT recovery as a recreational fishing opportunity</b>	<b>HIGH Yrs 1-5+</b>	<b>FWS initially with handoff to appropriate entities</b>
E1a	Summarize and evaluate existing information	Yrs 1-2	
E1b	Development recommendations for study and/or assessment	Yr 2	
E1c	Implement specific studies and/or actions as appropriate	Yrs 1-5+	
E1d	Develop marketing program for recreational LCT fishing opportunities	Yrs 1-5+	
<b>E2</b>	<b>Determine the interaction of LCT recovery on the Walker Lake recreational fisheries</b>	<b>LOW Yrs 4-5</b>	<b>FWS initially with handoff to appropriate entities</b>
E2a	Summarize and evaluate existing information	Yrs 4-5	
E2b	Develop recommendations for monitoring, study and/or assessment	Yrs 4-5+	
E2d	Implement monitoring, specific studies and/or actions as appropriate	Yrs 4-5+	

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## 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.

**Bacteria** - Prokaryotic, one-celled organisms. Some transmit diseases. Most act as decomposers and get the nutrient they need by breaking down complex organic compounds in the tissues of living or dead organisms into simpler inorganic nutrient compounds.

**Biological oxygen demand (BOD)** - Amount of dissolved oxygen needed by aerobic decomposers to break down the organic materials in a given volume of water at a certain temperature over a specified time period.

**Coevolution** - Evolution when two or more species interact and exert selective pressures on one another that can lead each species to undergo various adaptations.

**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) content ( level)** - 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.

**Eutrophication** - Physical, chemical, and biological changes that take place after a lake, an estuary, or a slow-flowing stream receives inputs of plant nutrients - mostly nitrates and phosphates - from natural erosion and runoff from the surrounding land basin.

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

**Genetic diversity** - Variability in the genetic makeup among individuals within a single species.

**Genotype** - The fundamental constitution of an organism in terms of its hereditary factors; a group of organisms each having the same hereditary characteristics.

**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.

**Phenotype** - Characteristics of an organism that result from both its heredity and its environment.

**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** - The viability and survivability of a species or distinct

population segment of a species over the course of many generations, sometimes measured at 95 percent change of persistence for at least 100 years.

**Species** - A naturally existing population of similar organisms that usually interbreed only among themselves, and are given a unique latinized (genus) 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.

**Total dissolved solids**– A reference to the salinity of water, which is made up of various amounts of positive and negative elements (in terms of chemical elements) affecting water quality.

**Total maximum daily load** -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**

### **Acronyms**

**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  
**DPS** – Distinct Population Segment  
**EA** – Environmental Assessment  
**EIS** – Environmental Impact Statement  
**ESA** – Endangered Species Act  
**GIS** – Geographic Information Systems  
**LCT** – Lahontan cutthroat trout  
**LGS** – Landmark Geographic Services  
**MOG** – Management Oversight Group  
**NDOW** – Nevada Division of Wildlife  
**NEPA** – National Environmental Policy Act  
**ODFW** – Oregon Department of Fish and Wildlife  
**PVA** – Population Viability Analysis  
**USFS** – United States Forest Service  
**USFWS** – United State Fish and Wildlife Service  
**USGS** – United States Geological Survey  
**WRPT** – Walker River Paiute Tribe

## **APPENDIX C**

### **Stakeholder Role and Review: Implementation of Short-term Actions**

The Short-Term actions are a set of tasks that the WRIT and the MOG have identified as being environmentally necessary to move towards recovery of the LCT in the Walker 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 to include 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**

Between October 2000 and March 2002, EMI compiled the following recommendations, which have been developed from interactions with public stakeholders pursuant to the recovery of the Lahontan cutthroat trout (LCT) in the Walker River basin. Information was gathered from meetings with city, county, tribal, and other government officials, formal stakeholder meetings, one science workshop, correspondence, and formal interviews.

A preliminary trip to the basin occurred in October 2000. In order to become familiar with fundamental social issues and concerns and to discern which communities would be interested in hosting stakeholder meetings, EMI representatives met with individuals and groups prior to holding formal public meetings. Yerington, Hawthorne, Gardnerville, and Reno, Nevada, Antelope Valley and Bridgeport, California, were visited.

Meetings with individuals and groups included Board of County Commissioners, Lyon County, Nevada; Roger Bezayiff, Walker River Water Master; Board of Water Commissioners; Keith Trout, Mason Valley News; Ken Spooner, Walker River Irrigation District (WRID); members of the (WRID); representatives of the Natural Heritage Center, Ronald Wolven, Chamber of Commerce, Hawthorne, Nevada; Lou Thompson, Walker Lake Working Group; City Councilman Ed Inwood of Bridgeport, California; President David Haight, Dynamic Action of Wells Group (DAWG); Rose Strickland, representative of the Sierra Club; Loretta Singletary, coordinator of the Walker River Basin Advisory Committee;

Sue Lynn, Consultant for Public Resource Associates; John Tracy, Ph.D., Desert Research Institute; Faith Bremmer, Reno Gazette.

Three series of formal public stakeholder meetings were held throughout the Walker River basin between February 2001 and March 2002: February 5-9 2001: Yerington, Hawthorne Nevada; Walker, Bridgeport, California; Reno, Nevada. June 18-22 2001: Yerington, Hawthorne, Nevada; Walker, Bridgeport, Bishop, California. March 4-8 2002: Yerington, Hawthorne, Nevada; Walker, Bridgeport, California; Reno, Nevada.

On Saturday March 31, 2001, a science workshop was held in Smith Valley at the request of public stakeholders. Speakers presented an array of requested topics, including: Regulatory Issues, Genetics, Lahontan Cutthroat Trout Distribution in the Walker River Basin, Habitat Studies and Restoration, and the development of short-term actions for the Recovery and Implementation Plan.

### **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. Recommendations also were developed from research and review of conclusions from other recent collaborative efforts. Citing of specific comments that support recommendations follow. A complete documentation of all public comments is located on the web: [www.walkerriverit.com](http://www.walkerriverit.com).

1. **Economics** - Economic studies of every community should be given high and immediate priority. These studies should not be dependent upon NEPA. Rather, the studies should be given status similar to that which the scientific processes and questions have received.
2. **Economics** - Acquire the services of specialists on community development and economic planning. Offer these services to communities wherein citizens have expressed a strong opposition to LCT recovery because of its perceived threat to socio-economic stability. Especially strong opposition exists in Walker, California, a community significantly impacted by the floods of 1997. The results of the flooding, at that time, may not have been adequately recognized. Some socio-economic circumstances may have worsened as a result, which may be a reason for the profound threat extant over ensuing recovery efforts. Active recognition of this community's economic needs could prove mutually beneficial to both the community and recovery efforts.

3. **Building Relationships** - Fish and Wildlife Service (FWS) personnel need to visit communities and stakeholders regularly to develop and maintain ongoing relationships with landowners and business people.
4. **Easing the Process**- FWS personnel should work closely with both the recreational market and agriculturalists to inform, counsel, and ease the burden of paperwork necessary for filing Safe Harbor Agreements, Habitat Conservation Plans, or other programs.
5. **Auxiliary Funding and the Creating of Partnerships** - FWS 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.
6. **Communications** - California Dept. of Fish and Game (CDFG) could improve relationships with citizens in headwater communities by visiting them regularly, maintaining open communication.
7. **Communications** - CDFG would benefit from publishing a chronological summary of the rationale and events related to the closure of Wolf and Slinkard Creeks. Though this occurred in the past and operations may be different now, public perception remains that the agency is closed and secretive. Open communications, beginning here, could build trust and eventually cooperation in future recovery endeavors.
8. **Water use studies and conservation** – Transparency of information regarding water quantity and use is paramount to the health of all systems in the Walker River basin, yet information is incomplete and hard to access. The Nevada Division of Water Resources has numerous water management planning efforts and modeling projects pending. They are intended to increase database information for better water management, but the latest studies have had a narrow focus. Additional work is needed to identify groundwater right location, rights, and uses supplemental to surface water. According to their report, *Walker River Basin Water Rights, Volume (2001)*, part of the Nevada Water Basin Information and Chronology Series:

“At this time, there is insufficient information available to estimate the number of acres currently serviced by surface water rights and supplemental groundwater. . . unfortunately there is insufficient information available to estimate the current supplemental/non-supplemental values [of groundwater rights].” (14-16)

Development of these databases and studies of the relationship of groundwater use to irrigable acreage and surface water rights are both needed. Aerial monitoring of water use in the Mason and

Smith Valleys, with comparisons of irrigated acreage to water rights, may reveal excessive water use, eventually leading to better water management practices and conservation.

9. **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.
10. **Collaboration.** The U.S. Forest Service (USFS) and Bridgeport Paiute Indian Colony should work together. The USFS could benefit by engaging the Bridgeport Paiute Indian Colony in stewardship of Rosaschi Ranch. This effort would not only relieve the USFS personnel from additional responsibilities, but also contribute to the Colony's efforts to address economic stability for a growing number of members. Additional funding for stewardship is likely through the Bureau of Indian Affairs and the Environmental Protection Agency (EPA). Such a stewardship program would also provide long-term educational opportunities for tribal members.

### **Citing Public Comments and Sources for Recommendations**

#### *Regarding economics and community planning First series of public meetings, February 2001*

- What will the effects of recovery and implementation be on people? So often people are not included in what seems to constitute an "ecosystem."
- Will recovery and implementation threaten or change our lifestyles? Is this being taken into consideration?
- Will this process affect the economy and our economic well-being? Will this effort help us or hurt us? Will you be looking at the economics? How do you weigh the economic questions of ranching, farming, fisheries, recreation, and potential losses or benefits?
- Can we get, in writing, that no closures, no impact to our livelihoods will occur?

#### *Second series of public meetings, June 2001*

- We're very concerned over economic impact to our communities. This is the one issue we're most concerned with.
- I've dealt with the NEPA process several times, but the management agencies do not have economists. At some point, a real economist needs to be pulled into this process.
- The Antelope Valley agricultural lifestyles are some of the most traditional in the country. Watch what you do with agriculture. This way of life is the least impacting, most

enhancing land use lifestyle you could put here. Anything but what they're (ag) doing would be higher impact.

- In other counties, the ag economics includes timber. Not here. Our numbers: 21 million in Mono County and 16 million in Inyo County, is all ag. There is little pesticide use. It's high pasture use, and flood irrigation here enhances wildlife. So, how much is that fish worth, if you hamper the ag business from operating as the trade-off?
- Alternative crops in Smith and Mason Valleys could be a possibility, but a cow/cafe operation isn't going to switch.

*Third series of public meetings, March 2002*

- What about the sports people. The recreational folks will suffer.
- With regard to closing streams: part of the biggest problem is that you're taking away the recreational money.
- Do any number of these actions and it will equal the closing down of our community.
- Has the WRIT considered the habitat they're attempting to use? West Walker is NOT the river is was five years ago.
- My major concern is the economic impact of this valley. This is my priority before the issue of the fish.
- Walker is barely subsisting. There has to be an economic consideration here.
- Fifty years ago, you could catch LCT. What's this now going to do to the economy?
- All these efforts to save a species. We're concerned about our economy. The community has got to be more important than a fish.
- I'm concerned that "self-sustaining" means death to this economy.
- West Walker: don't destroy a system just coming back.
- In Mono County, \$371 million in tourist dollars in 1999, and 60% of that is fish related. What is this effort going to do to these figures?
- Economic impact from Independence to Walker/Coleville. You've got to emphasize this.
- What if the impact of all your efforts is negative? What's the downside of all this? What would this area do if all your efforts don't work?
- Bridgeport Reservoir is for agriculture. It has a major fishery. You can't have competing interests. You can't manage recovery of a fish while doing management for agriculture up here. This effort will economically impact both recreations and agriculture.

- You're saying that agricultural purpose of the reservoir is now secondary to a fish? One fish is more important than these communities?

*Regarding the building of relationships, funding or the easing of process*

*First series of public meetings, February 2001*

- Where do we turn when the facilitators are gone?

*Second series of public meetings, June 2001*

- How is the public going to be involved in this process when facilitation is over?
- You should talk to businesses to individually survey them regarding impacts.
- Work with ranchers and farmers. For example, the government in California must pay ranchers and farmers for water diverted in Central CA.
- Why weren't you involved with 395 rebuilding? If you want cooperation with this community, help us restore West Walker River Canyon.
- We would appreciate a description of how FWS is augmenting their programs via private parties raising LCT.
- Rosaschi Ranch is currently a dismal failure, with noxious weeds. They are getting better, but fundamentally, for Feds to operate a ranch, it's not good.

*Third series of public meetings, March 2002*

- Rosaschi: Clean the ditch on Sweetwater side. Green it up. Wet it a couple of times a year. Allow for grazing.
- Rosaschi: This is a perfect example of giving something to someone who knows nothing. 20-23 tons of topsoil are lost a year out there, affecting the water quality.
- How could high school students be of use?
- Why isn't the Fish and Wildlife Service here tonight?
- I just want to know how you can make the fishing better for this community. I don't care what kind of fish it is.
- Oral Histories: Establish appointment with specific individual of the tribe, ranchers, and fishermen, and don't put this off. Some won't be around in five years.
- Keep Rosaschi Ranch in management plan *because* you're talking about ecosystem management, and it is located within the ecosystem.
- We need to include some plan for all riparian areas. Therefore, Rosaschi Ranch is valid to remain within the short-term actions.

*Regarding communication*

*First series of public meetings, February 2001*

- If you do get the LCT to survive, will sections of the river be closed? We already have concerns over closures. There are already limitations on fishing around here: Wolf Creek, for example. We don't get explanations or estimates of a specific time when this will be opened again

*Second series of public meetings, June 2001*

- This effort is going to take years. When you leave, where do we go for information and communication?
- CDFG: Releasing LCT? So, how are people protected for recreational use or from the killing of this fish?

*Third series of public meetings, March 2002*

- When are hatchery folks/scientists going to be here? These people aren't listening to us. We want to know how these actions may affect the economy.
- Somebody should have come here and told us how this might help our economy.
- Hard to be in favor of something when you don't know what it's going to be.
- There are 6,000 registered voters in Mono County. 1,000 live here. They've chosen to put the screws to us.
- CDFG: But are they going to plant LCT? What kind of communications are they going to establish with the public? Are they going to continue to plant rainbows?
- CDFG: What are they going to do in Virginia and Twin Lakes. It would be nice if they communicated with the public.
- What office/division/agency will be responsible for disseminating information regarding LCT/rainbow/planting recreational fisheries, numbers, etc?
- Can you say for sure that streams won't be closed? This is a major concern. You shut the streams down and you shut the communities down.
- I'm not confident that CDFG or FWS, or any entity will continue the communication efforts you've begun.
- The decision makers aren't here to hear the concerns and issues. We want to meet agency people.
- There needs to be ongoing communication with the stakeholders

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).

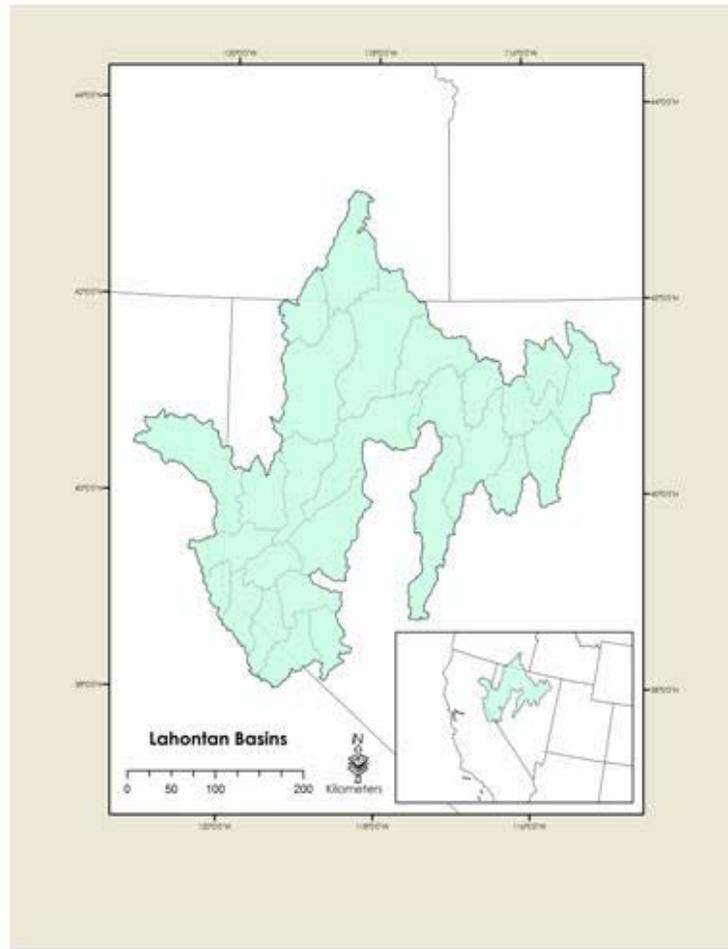


Figure 1.

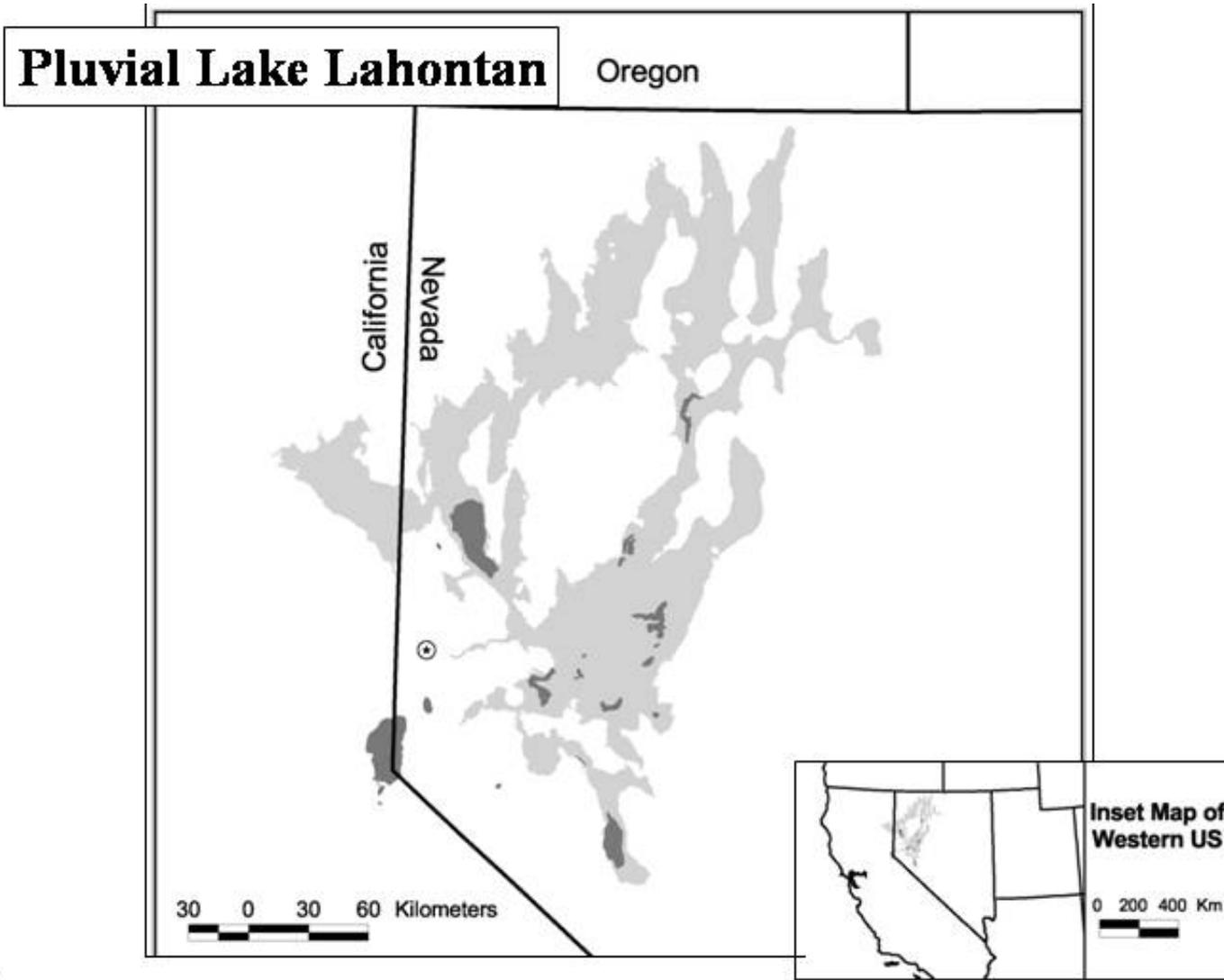


Figure 2.

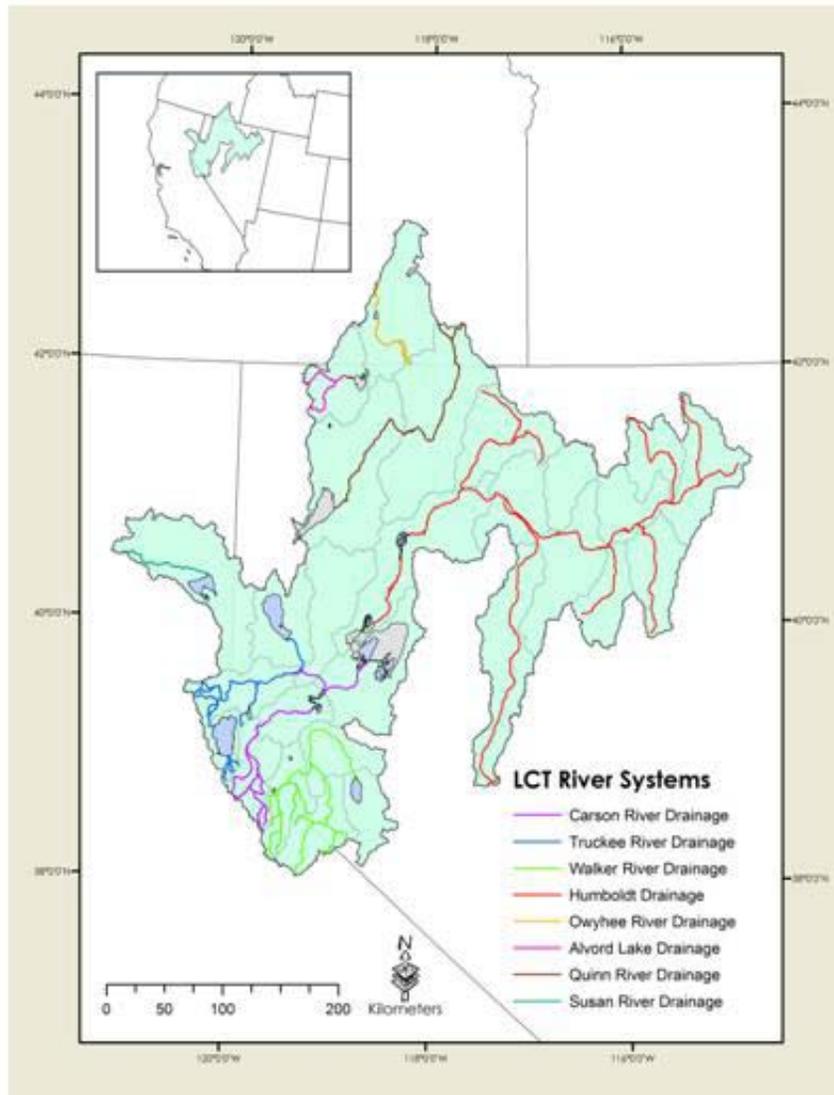


Figure 3.

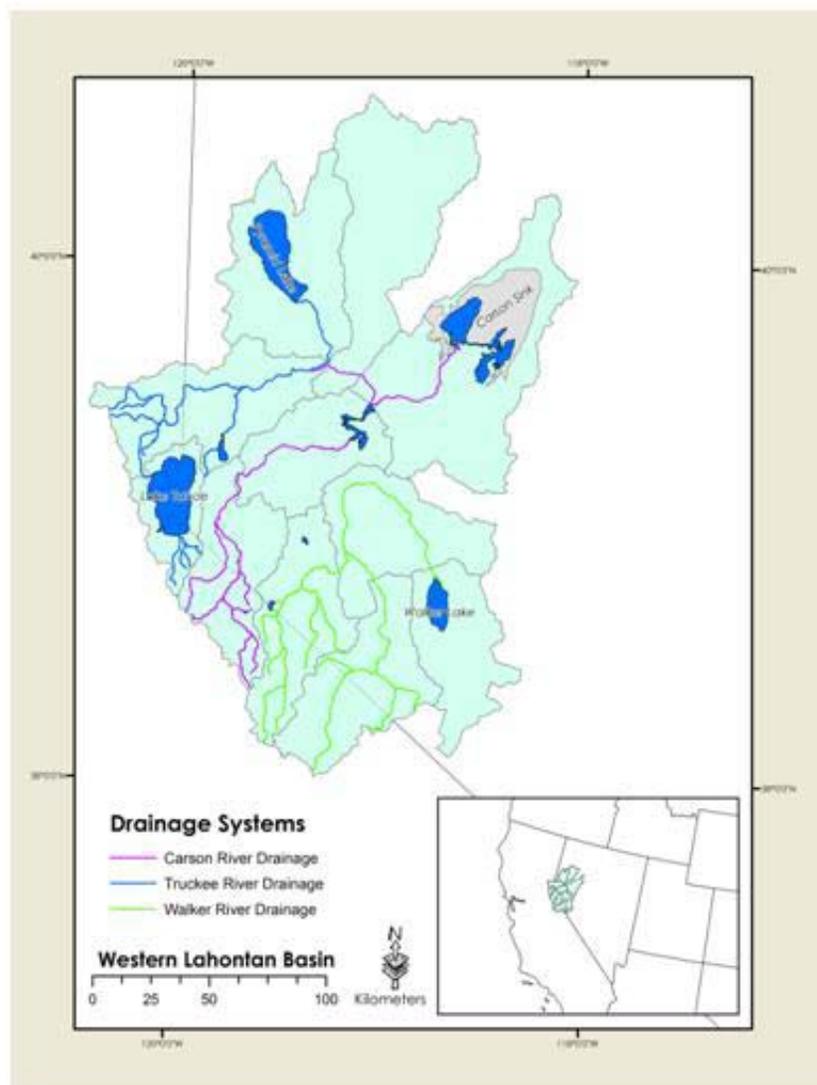


Figure 4.

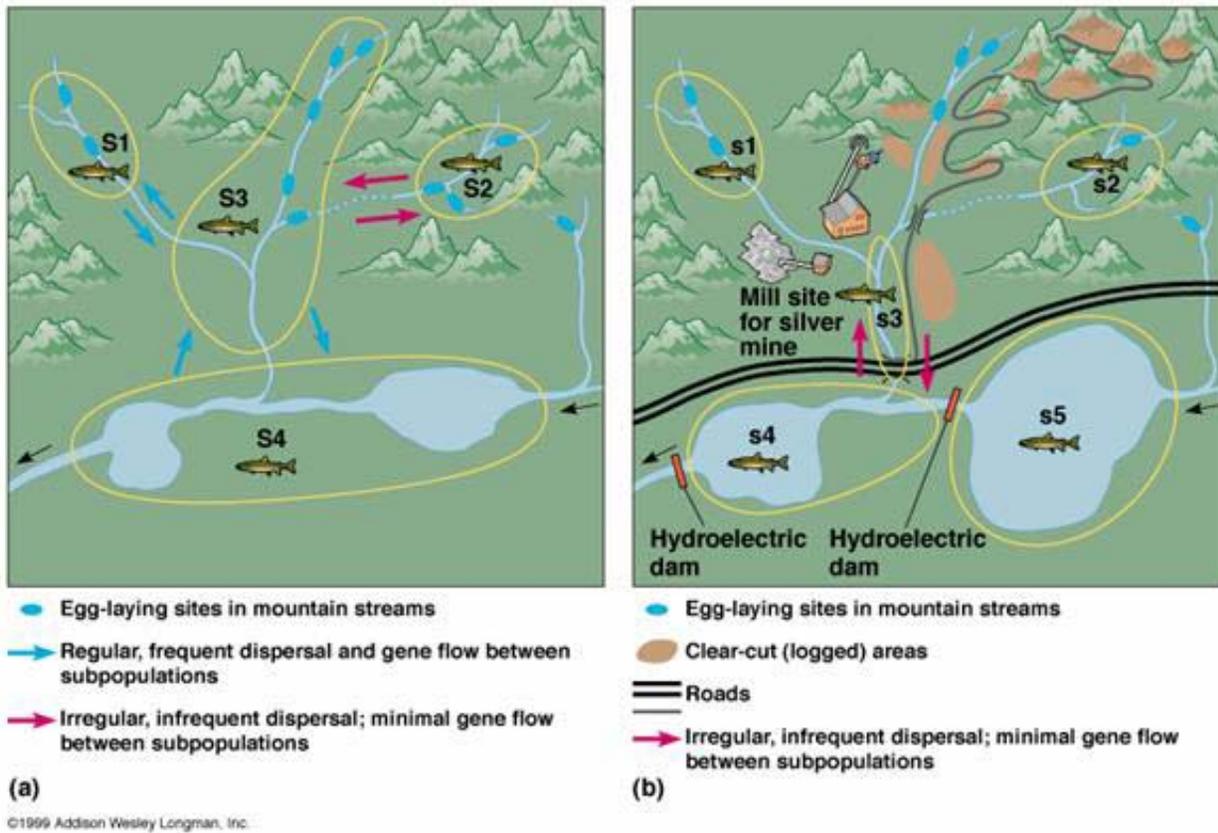


Figure 5.

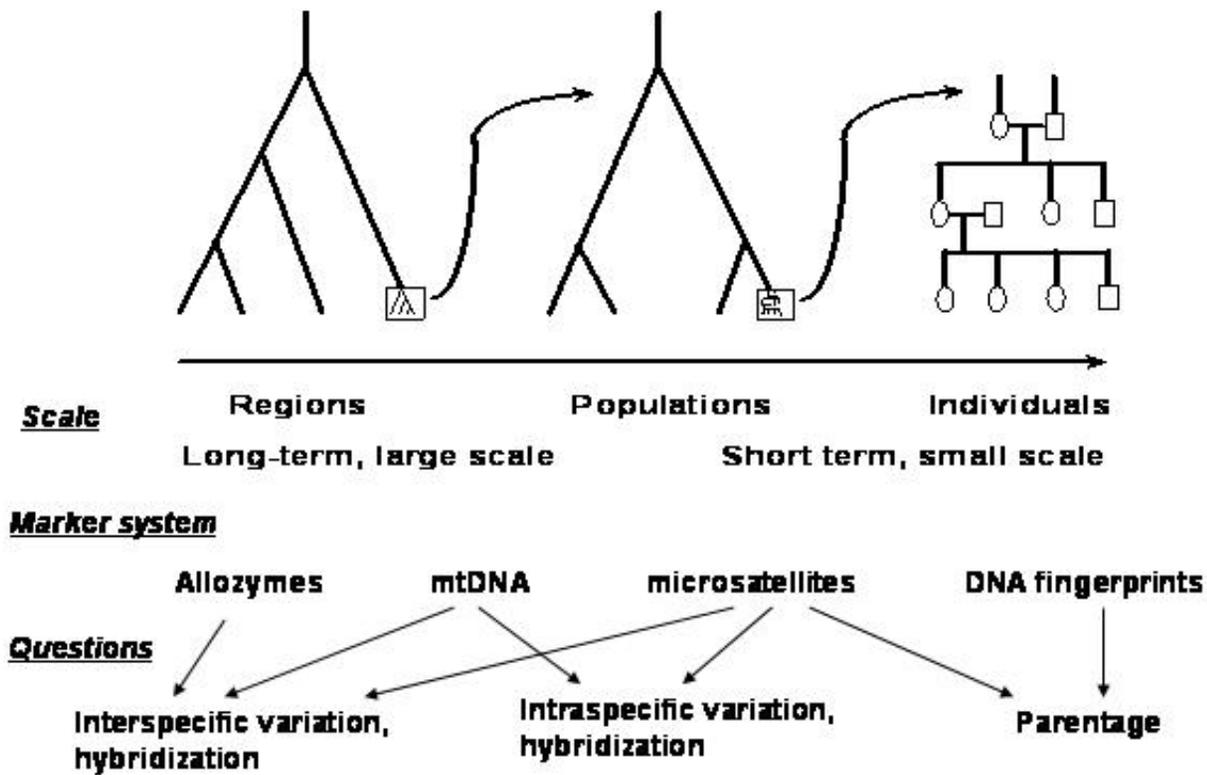


Figure 6.

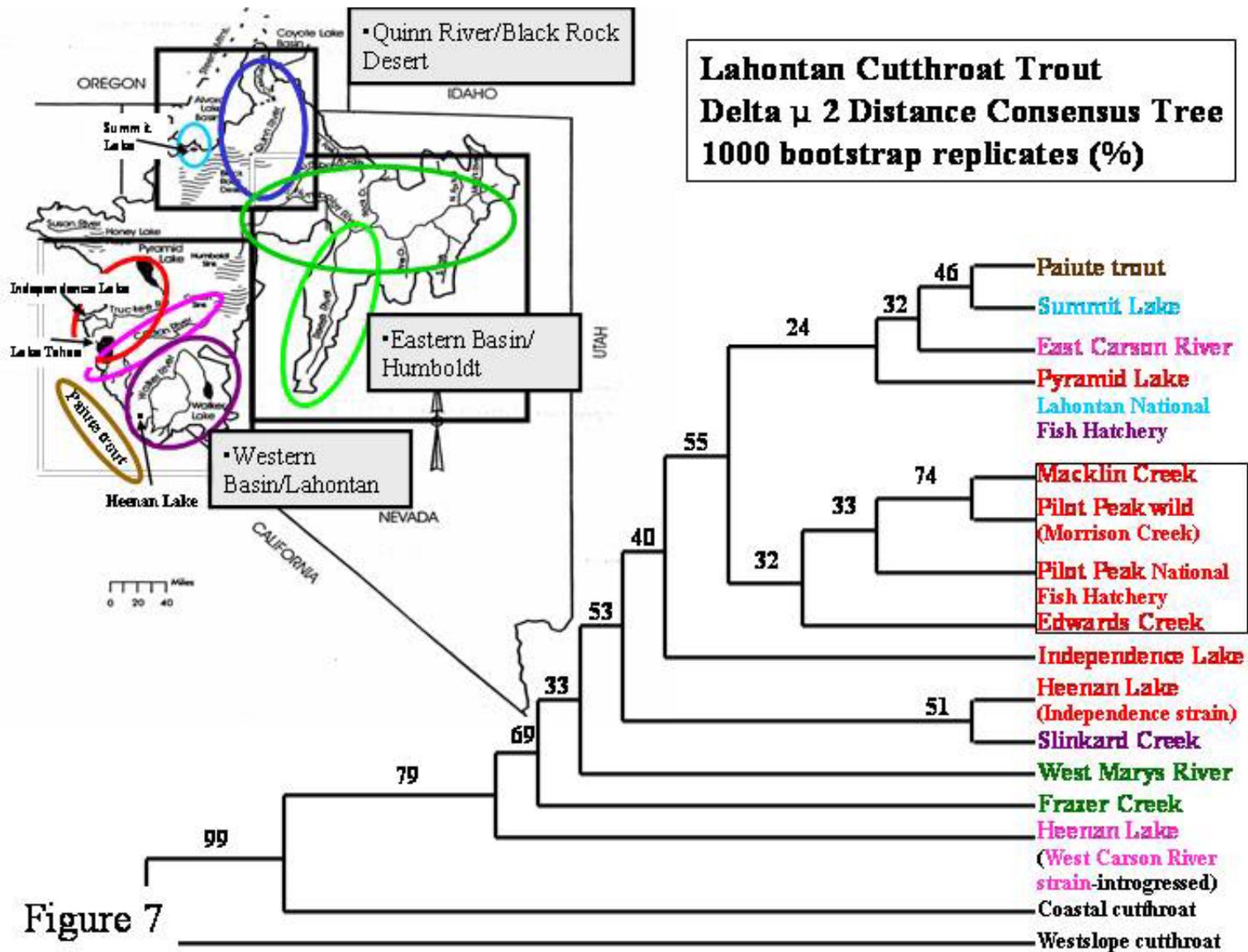


Figure 7