Exploring Reintroduction of Lahontan Cutthroat Trout in a Headwater Stream

By

JONATHAN EDWARD STEAD
B.S. (University of California, San Diego) 1998
M.S. (University of California, Davis) 2007

THESIS

Submitted in partial satisfaction of the requirements for the degree of

MASTER OF SCIENCE

in

Ecology

in the

OFFICE OF GRADUATE STUDIES

of the

UNIVERSITY OF CALIFORNIA

DAVIS

Approved:

_____________________________________

_____________________________________

_____________________________________  

Committee in Charge

2007
Acknowledgements

This research was funded and assisted by the U.S. Fish and Wildlife Service, and benefited from the cooperation and assistance of the California Department of Fish and Game and the U.S. Forest Service. U.S. Fish and Wildlife personnel from Lahontan National Fish Hatchery deserve recognition; Jay Bigelow was forthcoming with information and fish, and Al Duncan transported fish to Sagehen Creek. The cooperation of Kimberly True and Lyn Rosten from the California-Nevada Fish Health Lab was appreciated. From the California Department of Fish and Game, Joe Ferreira and Don Paganelli transported fish to Sagehen Creek, Joe Maret paid a special visit to help diagnose an infection, and Mark Adkison and John Hiscox assisted with collections of fish from Austin Meadow. Roger Bloom and the Wild Trout crew helped collect fish for the disease survey at Sagehen Creek, and Bill Somer’s assistance in coordinating with various members of the Department was invaluable. Dan Schultz and Deborah Urich from the U.S. Forest Service’s Sierraville Ranger District loaned me their time and/or equipment. Permits were provided by the California Department of Fish and Game and the U.C. Davis Institutional Animal Care and Use Committee.

Many individuals provided field assistance, and here I will try to name those who made major contributions. Volunteers Cameron Zuber, Anna Senecal, and Jim Plehn cannot be thanked enough, and Mr. Zuber’s exceptional work ethic will undoubtedly reward him in the future. I was fortunate to employ several hard working field assistants, namely Stephanie Mehalick, Dan Ryan, and Lyla Hunt. Having the full support of Mr. Ryan’s family during a moment of crisis will not be forgotten. Peter Graf provided
equipment for and assistance with radio transmitter installations. Moyle lab members and associates, including Patrick Crain, Filipe Ribeiro, Joe Sullivan, Sabra Purdy, Brett Baker, and Morgan King, were there when I needed them. Jenella Loye provided protocol development expertise, and Charlotte Cox and Leonie Newhouse diligently recorded data when extra hands were required. Sagehen Creek Field Station personnel, particularly Jeff Brown, were generous with facilities and equipment. Joaquin Feliciano, Albert Parkins, and Mike Johnson’s lab provided field gear. A number of anglers assisted with hook-and-line collections of fishes from Sagehen Creek. Without help from these people and others I could never have accomplished what I did.

No less important was intellectual assistance that I received from a variety of sources. Neil Willits provided valuable statistical advice. Several helpful reviews were provided by my thesis committee, Peter Moyle, Sharon Lawler, and Virginia Boucher, and Josh Israel reviewed the strain evaluation work. I appreciate the thoughtful guidance provided by my major professor, Peter Moyle, the no-nonsense attitude of my “boss,” Virginia Boucher, which kept me sane when nothing made sense, and the financial support that each of them facilitated. I appreciate discussion with Patrick Crain, Robert Schroeter, and Joseph Cech. I also thank Robert Schroeter for providing access to unpublished data.
# Table of Contents

Acknowledgements ........................................................................................................... ii

Introduction .......................................................................................................................... 1
  Study Site ......................................................................................................................... 2
  References ....................................................................................................................... 4
  Figures............................................................................................................................... 5

Chapter One - Comparative Response of Two Hatchery Strains of Lahontan Cutthroat Trout to Experimental Stream Reintroduction ............................................................. 6
  Introduction ................................................................................................................... 6
  Methods ........................................................................................................................ 11
    2005 Young-of-year Strain Evaluation ..................................................................... 11
    2006 Yearling Strain Evaluation .............................................................................. 15
  Results .......................................................................................................................... 21
    2005 Young-of-Year Strain Evaluation ..................................................................... 21
    2006 Yearling Strain Evaluation .............................................................................. 23
  Discussion ..................................................................................................................... 26
  References .................................................................................................................... 38
  Tables ............................................................................................................................. 44
  Figures............................................................................................................................... 45

Chapter Two - Occurrence of *Renibacterium salmoninarum* in Three Species of Wild Trout in Sagehen Creek, California ........................................................................... 53
  Introduction ................................................................................................................. 53
  Methods ........................................................................................................................ 55
  Results .......................................................................................................................... 58
  Discussion ..................................................................................................................... 59
  References .................................................................................................................... 65
  Figures............................................................................................................................... 67

Chapter Three - Movement of Hatchery Reared Lahontan Cutthroat Trout and Wild Brook Trout in a Small Headwater Stream ........................................................................... 68
  Introduction ................................................................................................................... 68
  Methods ........................................................................................................................ 70
  Results .......................................................................................................................... 73
  Discussion ..................................................................................................................... 74
  References .................................................................................................................... 78
  Tables ............................................................................................................................. 80
  Figures............................................................................................................................... 81
Introduction

Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*) is endemic to the Lahontan Basin of northeastern California, northern Nevada, and southern Oregon. Over the past 150 years, the Lahontan cutthroat trout (LCT) has disappeared from the vast majority of its range as a result of anthropogenic biotic and abiotic perturbations. Listed as endangered under the U.S. Endangered Species Act in 1970 (Federal Register Vol. 35, p. 16047), its status was changed to threatened in 1975 to facilitate management (Federal Register Vol. 40, p. 29864).

One primary management strategy in the conservation of declining cutthroat trout subspecies is reestablishment of extirpated populations through translocations within their historic range (Harig et al. 2000). Stated recovery goals of the U.S. Fish and Wildlife Service include stocking LCT in headwater reaches to promote a transition in the fish community in support of native species, and reintroduction and maintenance of isolated populations that can serve as genetic repositories, reducing the likelihood of extinction due to catastrophic events (Coffin and Cowan 1994). The effectiveness of fish translocations, however, is limited by a lack of data on factors that lead to success (Hendrickson and Brooks 1991; Harig et al. 2000). Some earlier reintroductions of LCT in the Truckee River basin, for example in Martis Creek Lake, have failed (Moyle and Vondracek 1985).

The goal of this current research has been to investigate strategies for reestablishing Lahontan cutthroat trout in small streams. The original focus was on comparing the response of available hatchery propagated strains to reintroduction through
(1) a series of experiments in which the biological response of the different strains, such as survival and growth, could be compared directly, and (2) a radio-telemetry study where the movement patterns of multiple strains could be compared and evaluated. Due to factors outside of my control, primarily the detection of pathogens at the hatcheries where the various strains are cultivated, the fish that I had intended to use in the study were not always available as planned. This resulted in substantial inconvenience and delays, as well as a new interest in fish disease, and I opportunistically used time that had been intended for other work to conduct a disease study.

In Chapter One I describe the results of two separate strain evaluation experiments, one conducted in 2005 and the other in 2006. It appears as though the Independence strain responded more favorably than the Pilot Peak strain to the reintroductions, although the results do not conclusively support the use of one strain over others. In Chapter Two I describe an investigation into the incidence and prevalence of disease in the wild trout at my study site, an investigation which revealed that *Renibacterium salmoninarum*, the causative agent of bacterial kidney disease, is common there. Only one Lahontan cutthroat trout strain was available for the radio-telemetry study described in Chapter Three, so for the sake of comparison I included wild brook trout. Independence strain LCT tended to remain in the vicinity of their release sites, without exhibiting a tendency to move in a primarily up- or down-stream direction.

**Study Site**

All field studies were conducted at Sagehen Creek, located on the eastern slope of the Sierra Nevada Mountains approximately 12 km north of Truckee, in Nevada County,
California (Figure 1). A small, spring-fed stream originating from snowmelt at about 2,530 m above sea level, Sagehen Creek meanders through approximately 10 km of forest and meadow before reaching Stampede Reservoir at about 1,780 m above sea level. Prior to the filling of Stampede Reservoir in 1969 the creek flowed directly into the Little Truckee River, which feeds the Truckee River (draining Lake Tahoe) and terminates in Pyramid Lake, Washoe County, Nevada. Flows in Sagehen Creek are seasonally dynamic; average discharge (1956-2005) is 0.35 m$^3$·sec$^{-1}$, with base flows in September of 0.06-0.08 m$^3$·sec$^{-1}$, and peak flows in winter or spring typically two orders of magnitude greater (USGS 2005).

As with many areas in its historic range, LCT is presumed to have disappeared from Sagehen Creek (ca. 1900) at the same time that the area was logged and grazed heavily. In the early 20th century non-native trout were introduced and sport angling began in earnest, and by the mid-twentieth century the creek was being stocked with large numbers of introduced trout on a regular basis. It is certain that LCT was extirpated from Sagehen Creek prior to 1952, when the first systematic sampling by researchers occurred (Flittner et al. 2006). The current research was centered around a reach (elevation 1,950 m) that supports an abundance of invertebrates, native Paiute sculpin (Cottus beldingii), and naturalized populations of brook (Salvelinus fontinalis), brown (Salmo trutta), and rainbow trout (Oncorhynchus mykiss). The entire Sagehen Creek basin is currently protected by the U.S. Forest Service as an Experimental Forest and recent large-scale disturbance has been minimal.
References


Figures

Figure 1. Map of the Truckee River basin identifying features mentioned in the text.

1-Martis Creek
2-Independence Lake
3-Sagehen Creek
4-Little Truckee River
5-Stampede Reservoir
Chapter One

Comparative Response of Two Hatchery Strains of Lahontan Cutthroat Trout to Experimental Stream Reintroduction

Introduction

The purpose of this research is to evaluate the performance of two strains of Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*) that are being used to reestablish populations of this threatened species. Based on morphological, genetic, and ecological differences the U.S. Fish and Wildlife Service divided Lahontan cutthroat trout (LCT) into three distinct population segments (Peacock and Kirchoff 2004) and the Western Distinct Population Segment includes LCT in the Truckee, Walker and Carson River basins (TRIT 2003). In the western part of its range LCT now occupies less than three percent of historically occupied habitat (Coffin and Cowan 1994). In the Truckee River basin LCT occupies only 2.2 percent of historic stream habitat, and even less of its historic lake habitat (Figure 1.1). The only native population of LCT remaining in the Truckee River basin occurs in Independence Lake, Nevada County, California and its tributary, Independence Creek (Gerstung 1988). Other populations in the basin are the result of reestablishment efforts, and as is the case throughout its range, these few LCT populations are typically found in small headwater creeks where they are isolated from non-native trout (Moyle 2002). Potential causes for their widespread extirpation have been examined in some detail, and non-native trout are generally considered to be a
major cause of the decline of LCT (Griffith 1988; Behnke 1992; Schroeter 1998; Dunham et al. 2002; Peacock and Kirchoff 2004).

Although isolation from invasive species in headwater streams can be an effective short term conservation measure, demographic and environmental stochasticity inherent in small, isolated populations puts them at risk of extinction (Lande 1993). Based on a study of microsatellite allelic diversity, LCT populations generally show signs of genetic isolation and tend to have small effective population sizes (Nielsen and Sage 2002). More recent work has demonstrated that isolated populations of LCT can experience fairly rapid genetic differentiation and loss of genetic diversity, and that connectivity may be a key factor in population persistence (Neville et al. 2006). Salmonid population sizes necessary for long term viability may be as large as 2,500 (Allendorf et al. 1997), and for cutthroat trout to self-sustain a population of this size in a small headwater stream could require 8-25 km of stream habitat (Hilderbrand and Kershner 2000). There are few Lahontan cutthroat trout populations of this size anywhere, and none in the Truckee River basin. In addition to demographic effects, headwater populations with no natural source for recolonization are vulnerable to extinction as a result of severe flooding and drought (Dunham et al. 1997). With so few populations remaining, many of which exist above barriers where they are unlikely to persist indefinitely (Hilderbrand and Kershner 2000), intensive management of existing populations and establishment of additional populations is necessary to prevent extirpation of LCT from the Truckee River basin, and at larger scales, from extinction range-wide.

A tenet of conservation biology is that the source of individuals used in translocations should be as genetically similar to the native population as possible, and
this often means from as close geographically as possible (Campbell 1980). Additionally, reintroduction via translocation of a small number of individuals can result in founder effects and genetic bottlenecks (Maudet et al. 2002), decreasing individual fitness and population persistence (Saccheri et al. 1998; Westemeier et al. 1998). Multiple translocations, however, may mimic gene flow and improve the success of reintroductions (Swanson et al. 2006).

With these genetic concerns in mind, reestablishment objectives are severely hindered by a lack of numerically and demographically robust populations that are unaffected by hybridization and/or population bottlenecks, from which individuals for translocation can be obtained. This is particularly true in the Truckee River basin, where only one native population remains extant, in Independence Lake, Nevada County, California. Fortunately, federal and state agencies have initiated hatchery propagation of two genetically distinct strains of LCT from the Truckee River basin. Although there are concerns that hatcheries may select for fish not as fit for survival in the wild (Allendorf and Ryman 1987; Tave 1993; Cross 2000), if properly managed these hatchery strains can be a significant resource for LCT conservation.

Stockwell and Leberg (2002) point out that while translocation research has largely emphasized genetics, genetic data are best interpreted in an ecological context. Local adaptation to different environmental conditions among salmonid populations (Hemmingsen et al. 1986), even within the same watershed (Donaghy and Verspoor 1997), has been demonstrated. Distinct life-history traits and other adaptations may contribute significantly to the success of reintroductions (Reznick et al. 2004). If environmental or ecological conditions are more similar between the recipient site and a
more distant source than a nearby source, then fish from the distant source could respond more favorably to translocation. Simply put, environmental aspects of the donor and recipient habitat are likely to influence the success of translocations. These considerations may be particularly important when considering reintroductions of LCT in the western part of its range, as the fairly complex genetic structure of LCT populations is influenced both by landscape attributes and life history patterns (Neville et al. 2006).

Some early workers believed there were two species of Lahontan cutthroat trout in the Truckee River basin, while others recognized two distinct spawning runs in the Truckee River but considered them only one species (Behnke 1992). The belief that there were two species may have been based on morphological differences among different age classes but the degree of differentiation that existed among historic Truckee River basin LCT populations has never been fully resolved. Morphological and ecological differences can still be found among populations of LCT and some workers allude to differences between lacustrine and fluvial populations (e.g. Gerstung 1988). It is unclear to what degree observed phenotypic differences have a genetic basis, although Behnke (1992) cautions against assigning a genetic basis to subtle ecological differences in LCT populations. It is also unclear whether the hatchery strains being propagated represent the full range of historic phenotypes and if there is a genetic basis to possible phenotypic differences between strains.

Two hatchery propagated strains of LCT are available for reestablishment efforts, Pilot Peak and Independence. Both were originally derived from the Truckee River basin, although the history of the Pilot Peak strain is less certain. Discovered by fish biologists in 1977 in the Pilot Peak drainage in Utah (outside the Lahontan basin), Pilot
Peak fish are presumed to be pure Pyramid Lake strain LCT because they were in the Pilot Peak drainage prior to 1952 (according to local accounts) and the only LCT used for propagation prior to 1950 were from Pyramid Lake (Hickman and Behnke 1979; Behnke 1992). An analysis of meristic characters also indicates that the Pilot Peak fish are probably original Pyramid Lake strain LCT (Hickman and Behnke 1979). Eggs of 35 family groups from the Pilot Peak fish in Utah were used by the U.S. Fish and Wildlife Service to create a broodstock at Lahontan National Fish Hatchery during 2000-2003, and periodic collections of eggs and/or milt from wild fish in the Pilot Peak drainage are used to augment the hatchery broodstock line (Jay Bigelow, Lahontan National Fish Hatchery, personal communication). The Independence strain is derived from about 5,000 one-year-old LCT collected by the California Department of Fish and Game from Independence Lake and planted into Heenan Lake, Alpine County, California in 1975 (Sommer 2000). Marked, spawning fish in Heenan Creek (tributary to Heenan Lake) are used as a broodstock source and fish are raised in Hot Creek Hatchery, Mono County, California. Some hatchery reared fish are marked and stocked back into Heenan Lake to maintain the broodstock. Both Pilot Peak and Independence strain LCT have been used for various translocations since the initiation of hatchery propagation.

The current successful hatchery propagation of these two strains makes them an abundant and valuable resource for conservation of LCT in the western part of its range, but predicting how translocated populations will respond to novel ecological conditions is extremely difficult (Stockwell and Leberg 2002). As resource managers attempt to reestablish populations by reintroducing LCT within its historic range, more information regarding possible ecological differences between the Pilot Peak and Independence
hatchery propagated strains is needed to improve the effectiveness of these efforts. Both strains are from Truckee River basin stock, but local adaptation or genetic differences between the two strains may create differences in their suitability for reintroduction into different habitats.

As far as I know, ecological differences between strains of Lahontan cutthroat trout have never been investigated experimentally. Klieman et al. (1994) recommend that reintroduction programs include a preparatory reintroduction and monitoring period and argue the benefits of an experimental approach. The advantages of an experimental approach to fish translocations have been discussed elsewhere (e.g., Stockwell and Leberg 2002). Here I present the results of two field experiments designed to compare the short-term performance of Pilot Peak and Independence strain LCT reintroduced to Sagehen Creek, a small headwater stream in the Truckee River basin. The first experiment was conducted with young-of-year (age zero) hatchery fish in 2005, and the second experiment was conducted with yearling (age one) hatchery fish in 2006. As an additional point of reference I included in the second experiment a small number of LCT translocated from a wild population of LCT already habituated to a small Sierra Nevada headwater stream. My purpose was to determine which strain was most likely to be successful when planted into small streams as part of restoration efforts.

Methods

2005 Young-of-year Strain Evaluation

In this experiment I evaluated young-of-year Pilot Peak and Independence strain Lahontan cutthroat trout. Independence LCT were spawned on June 1, 2005 and raised
by the California Department of Fish and Game at Hot Creek Hatchery, Mono County, California. Pilot Peak LCT were spawned during spring 2005 by the U.S. Fish and Wildlife Service at Lahontan National Fish Hatchery in Gardnerville, Nevada. Spawning occurred from late February through April, with a peak in late March. An undifferentiated sample of both strains was netted from hatchery raceways and transported to Sagehen Creek on September 3, 2005.

I paired six fish enclosures side by side in Sagehen Creek, to create three replicates, and stocked one cage in each replicate with Independence fish and the other with Pilot Peak fish. The enclosures were constructed of a wooden frame, 0.9 m long, 0.6 m wide, and 0.76 m tall, and bound by 3.175 mm plastic netting on all sides but the top (Figure 1.2). I placed the replicates at three locations along a 310 m (linear) reach roughly in the middle of Sagehen Creek basin (elevation 1,950 m), spanning Sagehen Creek Field Station. Selected non-randomly, I chose locations for each replicate that provided pool habitat and I adjusted their positions as necessary to minimize differences in depth, flow, and shading between enclosures within each pair. A layer of substrate from the stream placed in each enclosure provided cover for the study fish and habitat for prey organisms. Bird netting covering the top of each enclosure minimized the risk of predation, which may have otherwise been unnaturally high, as the sides of the enclosures could have provided convenient perch for avian and mammalian predators.

On September 7, 2005 I stocked the enclosures. I briefly sedated the fish prior to weighing (wet weight, ± 0.1 g) and measuring (total length, ± 1 mm) them, in order to improve the accuracy of the measurements and minimize stress due to handling. I stocked five Pilot Peak LCT into one enclosure, and then stocked the other enclosure in
that replicate with Independence LCT until the cumulative biomass in the Independence enclosure was nearly equal to the biomass in its paired Pilot Peak enclosure. I repeated this procedure at each replicate. Following stocking the enclosures were not disturbed, with the exception of once daily brushing to keep them free of debris, until I collected the study fish on November 8, 2005, two months later. At that time each fish was briefly sedated, weighed, measured, and released into Sagehen Creek following recovery from the procedure.

Environmental data collected at each enclosure immediately prior to initiating the experiment and immediately following its termination included maximum depth, water velocity at 60% depth in the center of the enclosure, and water velocity at 60% depth one meter up and downstream from the enclosure. Temperature at each replicate was recorded every half hour with a HOBO H8 temperature data logger (Onset Inc.) stationed between the two enclosures.

I evaluated initial length difference between strains using a two-tailed, unpaired t-test. To check for possible differences by strain in environmental conditions among enclosures, including water velocity, maximum depth, and total biomass, I used two-tailed, paired t-tests. To investigate factors associated with recapture of individual fish at the end of the experiment I defined two groups, recaptured fish and not-recaptured fish. I used a two-by-two Chi Square contingency table to test whether probability of recapture was dependent on strain and an unpaired, two-tailed t-test to evaluate possible size differences between recaptured and not-recaptured fish.

I compared body condition between strains before and after the experiment, as well as body condition between recaptured and not-recaptured fish, using analysis of
covariance. I compared initial body condition between strains by modeling initial weight with strain as the independent factor and initial length as the covariate. This analysis compares least squares means, where the dependent variable, in this case weight, is adjusted to the geometric mean of the covariate prior to comparison. This type of model can be thought of as a comparison of length-adjusted weights, an indicator of body condition. Although Fulton’s condition factor (see Nielsen and Johnson 1983) has frequently been used to make body condition comparisons among fish of different sizes, statistical analyses on such ratios are less sensitive to treatment effects than analysis of covariance, and can lead to incorrect conclusions (Packard and Boardman 1988). The model used to test for differences in condition of recaptured and not-recaptured fish differed only in that recapture status was substituted for strain as the class evaluated. I compared final body condition between strains in a similar model, with final weight as the dependent variable, strain and location (replicate) as independent factors, and final length as the covariate.

I also defined two new variables for each enclosure; change in mean weight (final mean weight minus initial mean weight) and change in mean length (final mean length minus initial mean length) over the course of the experiment. I used mean values for each enclosure because these small fish were not individually marked. I evaluated differences in change in weight and length by strain using two-tailed, paired t-tests.

Analyses of covariance were conducted on log transformed weight and length data, in order to meet the assumptions of parametric analysis and were run initially in SAS v. 9.1 (SAS Institute Inc.) with a test of all pair-wise interactions, in order to verify the assumption of homogeneity of regression slopes among classes. Insignificant
interactions were removed from the models. Other analyses were performed using SAS or JMPIN v. 4.0.4 (SAS Institute Inc.). I verified assumptions of normality with the Shapiro-Wilk test for normality of residuals, and assumptions of homogeneity of variance with Levene’s test. Significance of statistical tests was judged at $\alpha = 0.05$. A sequential Bonferroni correction (Rice 1989) was applied to alpha levels used to judge the significance of tests for differences between strains in initial condition, final condition, change in weight, and change in length, as these response variables were correlated. Power analyses were conducted using publicly available software for power and sample size computation (Lenth 2006).

2006 Yearling Strain Evaluation

Lahontan cutthroat trout strains evaluated in the yearling strain evaluation were Pilot Peak and Independence, as well as wild LCT collected from Austin Meadow Creek. The same cohort of both Independence and Pilot Peak strains used in the 2005 young-of-year evaluation described above were used in 2006, but the individuals used in 2006 were reared for roughly 10 additional months prior to arrival at Sagehen Creek. For Independence fish additional rearing continued at Hot Creek Hatchery, but Pilot Peak fish used in the 2006 experiment were transferred from the Lahontan National Fish Hatchery to net pens in June Lake, Mono County, California on September 1, 2005, and reared there for 9.5 months. Both strains were fed commercial trout pellets. An undifferentiated sample of Pilot Peak LCT were netted from pens in June Lake and transported to Sagehen Creek on July 14, 2006, and yearling Independence LCT were netted from hatchery raceways at Hot Creek Hatchery and transported to Sagehen Creek on July 11,
2006. In order to minimize differences in body condition and habituate the fish to Sagehen Creek, both hatchery strains were held in net pens in Sagehen Creek and fed to satiation on commercial trout pellets twice daily for approximately one month prior to beginning the experiment.

Austin Meadow LCT were collected from Austin Meadow Creek, Nevada County, California, at approximately 2,075 m above sea level. This stream is tributary to North Fork Creek, which feeds Middle Yuba River and is outside the Lahontan Basin. Devoid of fish prior to 1970, the California Department of Fish and Game translocated 88 LCT from Macklin Creek to Austin Meadow during 1970 and 1971 (Somer 2000). Macklin Creek, also outside the Lahontan Basin, contains a naturalized population of LCT believed to be derived from somewhere in the Truckee River basin (Gerstung 1986; Somer 2000), a scenario supported by a microsatellite DNA study that associates Macklin Creek fish with Pilot Peak wild and hatchery fish (Nielsen and Sage 2002). Wild Austin Meadow strain LCT were collected by backpack electrofisher and transported to Sagehen Creek on August 9, 2006, where they were temporarily held in net pens for four days prior to initiating the experiment.

I constructed 18 temporary fish barriers which enclosed nine reaches of Sagehen Creek, spread out over 1.7 linear km, each approximately 30 stream-meters long (measured along the thalweg). The reaches were centered on Sagehen Creek Field Station, roughly in the center of the Sagehen Creek watershed. Study reaches were selected non-randomly, to ensure the presence of suitable habitat and to minimize habitat differences among reaches. Each study reach contained a relatively deep pool, with riffles upstream and down, and was located at least 30 stream-meters from the next study
reach. Study reaches were bound, upstream and down, by a fish barrier that spanned the creek, constructed primarily of 1.27 cm mesh hardware cloth (Figure 1.3a). The bottom of the hardware cloth was folded flush with the creek, padded with seine netting to fill any gaps in the contact zone, secured to the creek bed, and buried under a shallow layer of natural substrate. A sheet of seine net was stretched across the creek, above the hardware cloth and out of the water, to prevent fish from jumping the barrier. Upon completion the barriers caused only minimal disturbance to stream flow where they contacted the creek (Figure 1.3b), and daily cleaning ensured that they did not prevent drift or debris of a size likely to be consumed by the study fish from flowing through.

After completion of the barriers and prior to initiation of the experiment I removed all non-native trout present within the study reaches with a backpack electrofisher. Native Paiute sculpin were returned to the reaches from which they were collected. Total water volume was calculated for each reach from a series of 10 equally spaced depth transects, with five depth measurements per transect. Study reaches were stocked according to volume, with equal numbers of both hatchery strains, plus two or three Austin Meadow fish, for a total wet weight density of $40 \text{ g}\cdot\text{m}^{-3}$. The stocking density of $40 \text{ g}\cdot\text{m}^{-3}$ was based upon existing data from backpack electrofishing surveys of non-native trout in Sagehen Creek conducted in September 2001 (mean density = 50.3 g·m⁻³) (Peter Moyle and Virginia Boucher unpublished data), and from backpack electrofishing surveys of LCT in Gance and Frazer creeks in Nevada conducted in 1998 (mean density = 51.4 g·m⁻³) (Robert Schroeter unpublished data). I adjusted the density of roughly 50 g·m⁻³ from the existing data down to 40 g·m⁻³ for three reasons; (1) LCT often exist at lower densities than non-native trout (Schroeter 1998), (2) the smaller
Nevada streams provide greater visual buffering between fish than Sagehen Creek (R. Schroeter personal communication), potentially resulting in higher salmonid densities (Chapman 1966), and (3) because the competitive advantage of larger fish due to the establishment of size dominance hierarchies (Newman 1956; Chapman 1962) would likely be less pronounced at lower densities (Gurevitch et al. 1992).

On August 14, 2006 I began randomly netting LCT from their holding pens in Sagehen Creek and stocking them into the study reaches. I used the formula,

\[ V_R \cdot (40 \text{ g} \cdot \text{m}^{-3}) = T_{AM} + n(M_{PP}) + n(M_{IN}), \]

solved for \( n \), as a guide for reaching the target density of 40 g·m⁻³, where \( V_R \) was the volume (m³) of the reach, \( T_{AM} \) was the total weight (g) of the two or three Austin Meadow fish placed into the reach (Austin Meadow fish were stocked first), \( M_{PP} \) was the average weight (g) of the Pilot Peak fish, \( M_{IN} \) was the average weight (g) of the Independence fish, and \( n \) was the number of individuals of each hatchery strain to be stocked into the reach. Fish were collected from the holding pens, sedated, fitted with two 1×2.5 mm medical grade elastomer alpha-numeric visual implant tags (VI tags; Northwest Marine Technologies, Inc.), weighed (wet weight, ± 0.1 g), and measured (standard length, ± 1 mm). One VI tag was inserted into adipose tissue behind each eye, allowing for long-term recognition of individuals. Fish were allowed roughly 1 hour to recover in an aerated cooler before being stocked into study reaches. Stocking was completed on August 16, 2006. Study fish were collected from the study reaches with a backpack electrofisher approximately 82 days later, on November 4 and 5, 2006. Study
fish were then sedated, identified based on VI tags, re-weighed, re-measured, and released back into Sagehen Creek following recovery from the procedure.

Depths associated with volume calculations described above were used to determine the average depth of each study reach, and additional habitat data were collected on August 6, 2006, with the exception of velocities, which were measured on August 24, 25, and 26. For each study reach I visually identified the dominant three substrate size categories present (seven categories were utilized), estimated the percentage of each reach that would be shaded when the sun was directly overhead, and counted all pieces of totally or partially submerged large woody debris (≥10 cm diameter and ≥ 50 cm length). I also estimated the percentage of each reach containing riffle, run, and pool habitat, following definitions in Overton et al. (1997). I recorded whether a reach contained any undercut banks (undercut ≥ 30 cm deep), and whether it contained substantially undercut banks (undercut ≥ 60 cm for a combined length of ≥ 100 cm). Average velocities were computed for each study reach based on a series of five transects, with five stations per transect, at which velocity was measured with an electromagnetic flow meter (Flo-Mate 2000, Marsh-McBirney, Inc.). Temperature in the deepest portion of each study reach was recorded hourly with a HOBO H8 temperature data logger (Onset, Inc.).

Initial differences in length among the three strains were evaluated using analysis of variance. Differences in initial body condition were investigated using analysis of covariance with initial weight as the dependent variable, strain as the independent factor, and initial length as the covariate. The model of final body condition included weight as the dependent variable, strain and location (stream reach) as independent factors, and
final length as the covariate. I investigated factors potentially associated with recapture of individual fish at the end of the experiment, including strain, location, and length, using multiple logistic regression. I defined two groups, recaptured fish and not-recaptured fish, and further assessed size differences between the two groups using an unpaired, two-tailed t-test. Next I used analysis of covariance to test for a difference in initial body condition between the two groups. Change in weight (final weight minus initial weight) and change in length (final length minus initial length) over the course of the experiment were calculated for each recaptured fish and used as the dependent variables in two separate analyses of covariance, with strain and location as independent factors and initial length as the covariate.

I conducted an exploratory analysis of habitat variation among reaches, in relation to inter-reach variation in fish performance, using a mixed model analysis of covariance. I modeled the change in fish weight as a function of strain (fixed effect) and location (random effect), with initial length as a covariate. I iterated the model one time for each habitat covariate, with the habitat covariate inserted into the model prior to the location effect, and evaluated the significance of the habitat effect based on a Type I mixed model test.

All data manipulations and analyses were conducted on log transformed weight and length data, in order to meet the assumptions of parametric analysis. Analyses of covariance were run initially in SAS v. 9.1 (SAS Institute Inc.) with a test of all pair-wise interactions, in order to verify the assumption of homogeneity of regression slopes among classes (strains, experimental reaches, and recaptured/not-recaptured fish). Insignificant interactions were removed from the models. Other analyses were performed either with
SAS or JMPIN v. 4.0.4 (SAS Institute Inc.). I verified assumptions of normality with the Shapiro-Wilk test for normality of residuals, and assumptions of homogeneity of variance with Levene’s test. Significance of statistical tests was judged at $\alpha = 0.05$. A sequential Bonferroni correction (Rice 1989) was applied to alpha levels used to judge the significance of effects in models of initial condition, final condition, change in weight, and change in length, as these response variables were correlated. Wherever more than two means were compared within a single model the reported P values were adjusted using the Tukey-Kramer adjustment for multiple means (a.k.a. Tukey highly significant difference test). Power analyses were conducted using publicly available software for power and sample size computation (Lenth 2006).

Results

2005 Young-of-Year Strain Evaluation

Pilot Peak fish had a higher length-adjusted mean weight, or body condition, than Independence fish at the beginning of the study (Figure 1.4a; $F_{1,76} = 20.50$, $P < 0.0001$). While the average body condition of Independence fish remained relatively constant over the duration of the experiment, the average body condition of Pilot Peak fish declined to a level near that of the Independence fish (Figure 1.4b; $F_{1,68} = 5.04$, $P = 0.0280^*$). There was no difference by strain in increment of length increase (Figure 1.5a; $t = 2.23$, df = 2, $P = 0.1553$). Independence fish generally gained more weight than Pilot Peak fish (Figure 1.5b; $t = 4.28$, df = 2, $P = 0.0504$), and Pilot Peak fish actually lost weight in replicate one.

* Asterisk indicates a P value that was not significant following the sequential Bonferroni correction.
Immediately prior to initiating the experiment the mean length of Pilot Peak fish was 70.5 mm and the mean length of Independence fish was 45.2 mm, a difference of 25.3 mm ($t = 22.768$, $df = 77$, $P < 0.0001$). Due to this size difference the equal biomass ratio of Pilot Peak to Independence fish stocked into each replicate was 5:21 in replicates one and two, and 5:22 in replicate three. Despite efforts to equalize biomass there was a trend towards greater biomass in the Pilot Peak enclosures, where average biomass was 1.9% greater than in Independence enclosures immediately following stocking ($t = 4$, $df = 2$, $P = 0.0572$). Because Independence fish gained more weight, the total biomass in the Independence enclosures at the end of the study was, on average, 17.1% greater than total biomass in the Pilot Peak enclosures ($t = 4.57$, $df = 2$, $P = 0.0448$), despite six missing Independence fish.

Of the 79 fish stocked into the enclosures, 73 were collected at the end of the experiment, and all six of the missing fish were Independence strain. The probability of recapture, however, was not associated with strain (Pearson $\chi^2 = 1.522$, $df = 1$, $P = 0.2173$); more Independence fish (9%) than Pilot Peak fish (0%) were not recaptured, but over four times more Independence fish were used in the experiment. There was virtually no size difference between recaptured (mean length = 50 mm) and not-recaptured (mean length = 47 mm) fish ($t = 0.84$, $df = 77$, $P = 0.4035$; Power ≈ 98%), nor was there any detectable difference in body condition, based on initial length-adjusted weights ($F_{1,76} = 0.6604$, $P = 0.4189$).

Major environmental factors showed little variation between Pilot Peak and Independence enclosures. Maximum depth ($t = 0.46$, $df = 5$, $P = 0.6651$) and water velocity ($t = 0.80$, $df = 17$, $P = 0.4366$) did not differ by strain. Mean temperature among
the replicates ranged from 5.8 to 5.9°C, variation less than the ±0.7°C accuracy of the
temperature loggers used to record the data. There was essentially no temperature
difference among replicates. The maximum temperature recorded was 11.8°C and the
minimum was 2.0°C.

2006 Yearling Strain Evaluation

Independence fish gained weight proportionally to their lengths but both Pilot
Peak and Austin Meadow strains lost weight (Figure 1.6a; $F_{2,124} = 13.37, P < 0.0001$).
Weight change was positive for Independence fish and negative for Pilot Peak fish ($P =
0.0009$ and $P = 0.0006$, respectively). Change in weight was also negative for Austin
Meadow fish (mean change $= -0.74$ g), but due to the smaller sample size the weight loss
measured for Austin Meadow fish was not significantly different from zero ($P = 0.0557$).
Weight change of the Independence strain was greater than both the Pilot Peak and
Austin Meadow strains ($P = 0.0002$ and 0.0003, respectively), but there was no difference
in the magnitude of weight loss between the Pilot Peak and Austin Meadow strains ($P =
0.9868$). Independence fish also grew more in length than the other two strains (Figure
1.6b; $F_{2,124} = 16.85, P < 0.0001$). All strains exhibited a positive change in length (all $P <
0.01$), but the increment of length increase for Independence fish was significantly greater
than that of Pilot Peak and Austin Meadow fish ($P = 0.0004$ and $P < 0.0001$,
respectively).

Body condition differed among strains when the experiment was initiated (Figure
1.6c; $F_{2,158} = 7.35, P = 0.0009$). Pilot Peak fish had a lower adjusted mean weight than
both Independence ($P = 0.0006$) and Austin Meadow ($P = 0.0344$) fish. Initial body
condition did not differ significantly between Independence and Austin Meadow strains (\(P = 0.7791\)). At the end of the experiment the relationship among body condition of the three strains had not changed (Figure 1.6d); final adjusted mean weight differed among strains (\(F_{2,158} = 5.01, P = 0.0081\)), the adjusted mean weight of Pilot Peak fish was significantly lower than both Independence (\(P = 0.0069\)) and Austin Meadow (\(P = 0.0468\)) fish, and there was no difference in final adjusted mean weight between the Independence and Austin Meadow strains (\(P = 0.9996\)). Although there was substantial size overlap between all strains at the beginning of the experiment (Table 1), Pilot Peak fish were an average of 48 mm larger than Independence fish and 44 mm larger than Austin Meadow fish (\(F_{2,159} = 84.47, P < 0.0001\)). Initial length variation between the Independence and Austin Meadow strains was not significant (\(P = 0.7994\)).

Size was the only variable evaluated by multiple logistic regression that differed between recaptured (n = 136) and not-recaptured (n = 26) fish (\(\chi^2 = 4.18, df = 1, P = 0.0409\)). Direct comparison of size between recaptured and not-recaptured fish confirmed that not-recaptured fish were an average of 28 mm smaller than recaptured fish, based on initial length measurements (Figure 1.7; \(t = 4.63, df = 160, P < 0.0001\)). Strain (\(\chi^2 = 1.38, df = 2, P = 0.5028\)) and location (\(\chi^2 = 6.53, df = 8, P = 0.5876\)) were not associated with probability of recapture. Body condition at the time of stocking was statistically equivalent between recaptured and not-recaptured fish (\(F_{1,159} = 0.4346, P = 0.5107\)). Based on the actual sample sizes of recaptured and not-recaptured fish, and a standard deviation equal to the standard error of length adjusted least squares means within each group times the square root of the corresponding sample size, the power of this analysis to detect a difference in condition similar to the difference between the two
hatchery strains was 95%. All recaptured fish appeared to be free of disease, based on visual inspection. The coloration of hatchery fish was more pronounced than it had been prior to stocking and fin injuries detectable prior to stocking were no longer visible. Of the 26 fish not recaptured, 2 were found dead during daily cleaning of the barriers and the other 24 were unaccounted for.

Location had a significant effect in the models of final weight ($F_{8,124} = 2.78, P = 0.0073$) and change in weight ($F_{8,124} = 12.27, P < 0.0001$), but not in the model of change in length ($F_{8,124} = 1.37, P = 0.2173$). There were no interactions between location and strain, meaning that the response of the different strains relative to each other was consistent in all of the study reaches, but all strains responded more favorable (in terms of weight gain) in some reaches than others. For example, the adjusted change in weight (values are least squares means and lack units) ranged from $-0.0168 \pm 0.0048$ SE to $0.0185 \pm 0.0055$ SE for the three strains, but weight change for the nine study reaches for all strains combined ranged from $-0.0416 \pm 0.0093$ SE to $0.0452 \pm 0.0072$ SE; variation in the growth response to the reintroduction was 2.5 times greater among reaches than among strains.

Mean water temperature recorded during the study period varied among reaches from $6.5^\circ C$ to $6.9^\circ C$, variation less than the $\pm 0.7^\circ C$ accuracy of the temperature loggers used to record the data. There was essentially no temperature difference among the study reaches. The maximum temperature recorded was $15.6^\circ C$ and the minimum was $0.3^\circ C$. Also, all reaches contained undercut banks (but not substantial undercut banks), so the influence of temperature and undercut banks on fish performance was not analyzed. Of the habitat covariates analyzed, substrate size was the best predictor of change in fish
weight (substrate size effect $F_{1,6.8} = 5.51, P = 0.0523$), although the effect was not statistically significant. None of the environmental data analyzed showed any significant effect on fish weight (all $P > 0.05$).

**Discussion**

Young-of-year Independence fish gained at least twice as much weight as Pilot Peak fish, for all replicates (Figure 1.5b). The change in length also was greater for young-of-year Independence fish, although just barely (Figure 1.5a). Differences in growth response were less significant statistically in the young-of-year evaluation than in the yearling evaluation, but it was conducted at a smaller scale, with smaller sample sizes, and less statistical power. Graphically, growth responses of young-of-year Independence and Pilot Peak fish, relative to each other, were similar in pattern to those observed in the yearling evaluation.

In the yearling experiment the performance of the Independence strain was again superior to that of the Pilot Peak strain. Independence fish gained more weight and grew more in length than the Pilot Peak and Austin Meadow strains (Figure 1.8). This second experiment incorporated a greatly improved experimental design which minimized cage effects and increased replication. The 2006 yearling experiment was conducted under nearly natural conditions, or as close to such as possible while still retaining the fish in a localized area from which they could easily be recaptured. Focusing on the more sophisticated yearling evaluation, I will refer to the young-of-year experiment throughout this discussion where those results might help interpret patterns observed.
The simultaneous increase in both length and weight of yearling Independence fish resulted in no detectable change in condition of Independence fish relative to the other two strains, both of which grew little in length and lost weight. The model of final body condition generated higher values than the model of initial condition (Figure 1.6c and d), but these values cannot be compared directly because they represent separately adjusted means. While I found no change in condition of the strains relative to each other, the final condition of each strain, relative to its initial condition, decreased during the study period. Across both experiments the only fish that gained weight rapidly enough to match their increase in length and maintain their body condition were young-of-year Independence fish (Figure 1.4a and b). A general decrease in body condition during the first few months following stocking is typical of hatchery trout released into small Sierra Nevada streams (Needham and Slater 1945; Reimers 1963).

Prior to initiating the yearling experiment I was concerned that the larger Pilot Peak fish might enjoy a competitive advantage so I stocked the experimental reaches at relatively low density to minimize effects of competition. The smaller Independence fish, however, appear to have responded more favorably to the reintroduction, despite any size-related competitive advantage the Pilot Peak fish may have had. Parallel size differences were present in the young-of-year fish evaluated, where the strains were housed in separate enclosures and inter-strain competition was not a factor. Selection by the relatively small mesh used in the young-of-year experiment for smaller prey, however, may have conferred an advantage to the smaller Independence fish, and this may be the most compelling reason to interpret results from the young-of-year experiment cautiously. Initial size differences in both experiments were due to
differences in elevation, water temperature, and spawning time of the broodstocks and it is not possible to obtain individuals of both strains from the hatcheries that are exactly the same age and size. While a comparison of such individuals would be ideal, the roughly three month age difference and initial size difference of hatchery strains used in this study represent real differences in the two strains that will likely apply to future translocations as long as the strains are propagated at the current facilities.

Differences between hatchery and wild fish likely affected their response to the reintroduction in the yearling evaluation. The small, cold, mountain stream from which Austin Meadow fish were collected yields slower growth than the hatchery environment, where water temperatures are higher. At Austin Meadow I previously determined the age of a 59 mm TL LCT as being one-year-old (J. Stead, unpublished data), considerably smaller than the one-year-old hatchery fish used in this experiment (Table 1). I also captured a mature Austin Meadow female that was 140 mm, and LCT typically mature between age two and four (Moyle 2002). Based on these observations I assume that some of the Austin Meadow fish used in the experiment were as much as two years older than the hatchery fish they were compared to, despite overlapping size ranges. Fish typically grow more rapidly early in life, until maturation, when increasing amounts of energy are diverted from growth of somatic tissue to growth of gonadal tissue (Moyle and Cech 2004). Also, Austin Meadow fish were collected by electrofishing four days prior to initiation of the experiment, and electrofishing negatively affects growth rates of cutthroat trout (Dwyer and White 1995; Dwyer et al. 2001). I would, therefore, expect the Austin Meadow fish to grow slower than the hatchery fish. I do not view this as a problem because the main objective of this study was to compare the two hatchery
strains. Furthermore, if wild fish were to be used for LCT reestablishment, then translocation of those fish would probably involve electrofishing, most likely of relatively stunted fish from a headwater stream. In that sense the comparison of these wild fish to the hatchery fish is a realistic one, in terms of evaluating short-term response to reintroduction, despite the underlying age and handling differences.

It is possible that the results were influenced by the fact that 16% of the fish were not recovered. The majority of the missing fish apparently escaped, however, rather than died. The body condition model for recaptured and not-recaptured fish was sufficiently powerful to detect a difference if one existed, suggesting it is not the case that the weakest fish perished. Preliminary trials indicated that the mesh, chosen to construct fish barriers because of its ability to pass drift and prey organisms, barely contained the smallest study fish. Daily cleaning of the barriers would have revealed fish that died in the study reaches and only two were found. Predation was also an unlikely source of mortality because large nonnative trout had been removed from the study reaches and 97% of fish eaten by garter snakes (Thamnophis spp.) at Sagehen Creek are less than 30 mm (White and Kolb 1974). The size range of the study fish was within the 100-300 mm size range of fish that osprey (Pandion haliaetus) prefer as prey (Table 1; Van Daele and Van Daele 1982; Edwards 1988) and osprey were present around Sagehen Creek during the study period, but there is no reason to suspect that osprey would selectively prey on the smaller study fish. Failure to recapture some of the smaller study fish therefore should not significantly influence interpretation of the results.

I do not know whether differences in initial body condition between the two hatchery strains influenced their response to the reintroduction. It has been shown that
inland cutthroat trout of a given length typically weigh more in lotic than lentic environments (Kruse and Hubert 1997), although the basis for this difference is not known. If the basis is genetic then the consistent relationship of body condition among strains before and after the yearling experiment (Figure 1.6c and d) could reflect adaptations to a given environment, manifested as differences in body form, because fishes with different body forms do not have comparable indices of body condition. Pilot Peak fish, however, were raised in a lentic environment (net pens) for ten months prior to arrival at Sagehen Creek. Perhaps hatchery raceways provided just enough similarity to a stream environment to affect the form of Independence fish, allowing them to grow better in the experimental reaches, assuming that the basis for the difference in body condition of lentic and lotic inland cutthroat trout is environmental. It seems unlikely, however, that small but significant differences in body condition would persist through the month of equal treatment and twice daily satiation feeding that both strains experienced prior to the experiment, and then through the entire study, if its basis was purely environmental. Interestingly, differences in appearance between the strains present when the fish arrived at Sagehen Creek were even more pronounced at the end of the study. Despite otherwise similar coloration among all strains, the Independence fish had markedly fewer spots on their ventral and ventro-lateral sides than the Pilot Peak and Austin Meadow fish, and were easily distinguishable based on that feature alone.

Observations of body condition in the young-of-year evaluation do little to clarify the true nature of differences in body condition observed in the yearling experiment. The body condition of young-of-year fish initially was greater in Pilot Peak fish (Figure 1.4a), the opposite of the consistent relationship between Pilot Peak and Independence fish in
the yearling experiment. This may have been due to hatchery conditions, as the young-of-year fish did not experience the month of equal treatment prior to initiating the experiment that the yearling hatchery fish did. During the young-of-year experiment the condition of Pilot Peak fish declined rapidly (Figure 1.4b), and had this brief experiment continued the final relationship of body condition between the two strains may have approached that observed throughout the yearling experiment. While the decline in condition of young-of-year Pilot Peak fish could represent a return from an inflated body condition, resulting from differences in hatchery rationing, to a more normal condition for the strain, it also could be a real indicator of poor adaptation to the recipient environment. Perhaps the more important observation is that young-of-year Independence fish maintained their body condition throughout the study, despite expectations that their condition would decline (Needham and Slater 1945; Reimers 1963).

The growth responses to the reintroduction of the two strains, particularly in the yearling evaluation, were sufficiently different to suggest superiority of the Independence strain despite uncertainty surrounding interpretation of their body condition. Based on five years of stocking experiments conducted with 23 groups of hatchery trout in a small Sierra Nevada stream, Reimers (1963) found that weight loss and a lack of significant growth in length during the first summer and fall occurred invariably. While the Pilot Peak strain responded to the reintroductions in a manner similar to the rainbow trout in Reimer’s experiments, Independence fish gained weight and grew significantly more in length than the other two strains.
The different growth response of the two hatchery strains may indicate that the Independence strain is better adapted to headwater streams like Sagehen Creek. The Independence strain originated from Independence Lake, located approximately 2.4 km north of Sagehen Creek (Figure 1.1). Prior to dam and reservoir construction Sagehen and Independence Creeks were adjacent tributaries of the Little Truckee River. While its Independence Lake origin and broodstock establishment in Heenan Lake suggests that the Independence strain is lacustrine-adapted, its degree of adaptation to lakes may be overestimated. Independence Lake is a reservoir with a modern capacity of 0.0216 km$^3$, but prior to construction of an outlet dam in 1939 its capacity was only 0.0037 km$^3$ (Berris et al. 1998). The pre-1939 “lake” consisted of two smaller water bodies that merged when the area behind the dam was flooded. It is hard to imagine that LCT occupying those modest waters evolved in isolation from LCT that inhabited the network of mountain streams and rivers connected to them by Independence Creek.

On the other hand, Pyramid Lake, from which the Pilot Peak strain is most likely derived, is a much larger natural lake with a capacity of 26.4 km$^3$ (Reuter et al. 1993). The world record sport-caught cutthroat trout from Pyramid Lake weighed 18,600 g, and even larger fish were reportedly caught by commercial and Paiute Tribe fishers (Wheeler 1967). Behnke (1992) speculates that the large size of historic Pyramid Lake LCT may have had a genetic basis. Pilot Peak strain fish resided in small streams in Utah since before 1950 but it is unknown whether rapid evolution demonstrated in other populations of introduced fishes (e.g. Stearns 1983; Reznick et al. 1990; Hendry et al. 1998; Kinnison et al. 1998) has occurred in response to the new environment. Nevertheless, the Pilot Peak strain is likely derived from original Pyramid Lake LCT, a stock of LCT that may
have represented an extreme form of the species. While the Pilot Peak strain has been targeted for use in recovery of LCT populations in the large interconnected Truckee River and Pyramid Lake system (TRIT 2003), one interpretation of our results is that the Independence strain is better adapted to small headwaters than the Pilot Peak strain.

Where sufficient genetic and ecological data are lacking to determine with certainty which strain is best suited for reestablishment, a tempting, yet controversial alternative would be to utilize multiple strains and allow selection to determine which characters are necessary for reestablishment. While typically discouraged (IUCN 1995), advantages of using multiple strains for reintroduction theoretically include the elimination of inbreeding depression and the enhancement of evolutionary potential (Frankel and Soule 1981). Although Leberg (1993) found little experimental evidence for a positive effect of founding populations from two different genetic strains on population size or growth, unusual combinations of alleles that confer no immediate evolutionary advantage may be uniquely suited for a future set of environmental conditions (Primack 1998). The advantages associated with mixing stocks may be particularly important if founder effects or hatchery propagation have reduced genetic diversity in a strain desirable for use in population reestablishment.

Intraspecific inter-population crosses, however, can lead to outbreeding depression in salmonids (Gharrett et al. 1999; Gilk et al. 2004), and in other fishes (Leberg 1993; Goldberg et al. 2005). Outbreeding depression, a general term for a variety of genetic phenomenon that can lead to reduced fitness following hybridization, can occur when hybridization produces an intermediate phenotype that is maladaptive in both of the contributing populations’ environments (Templeton 1986), and when
hybridization disrupts coadapted complexes of epistatic genes, genes that only function properly in combination (Dobzhansky 1950). The degree to which concerns about outbreeding depression should rule reintroduction of seriously threatened species is debatable, and Templeton (1986) argues that outbreeding depression is frequently transient and can be overcome by selection.

While some of the strongest empirical evidence for outbreeding depression comes from recent fisheries literature, a review of translocation strategies for conserving evolutionary processes notes that one of the most commonly cited examples of translocation failure due to outbreeding depression was inadequately documented (Moritz 1999). Its failure may have been due to factors other than genetic processes, and this is only one of many points made in a well formulated argument that the theoretical and experimental evidence for inbreeding depression is much stronger than that for outbreeding depression. Conservation plans for LCT involving translocation should weigh the risks of inbreeding depression against those of outbreeding depression, and consider the pros and cons of single versus multiple stock translocations. Ecological information that can help gauge the likely response of specific LCT strains to reintroduction in different environments will help guide such efforts and remove some of the guesswork from reestablishment.

While much discussion has focused on genetic processes and how they relate to source determination, the effect of environment and location on the outcome of translocations should not be underestimated. Habitat quality is a major factor likely to influence the success of translocations (Griffith et al. 1989), and it may be that intraspecific stock differences are most likely to appear under stressful conditions.
(Stockwell and Leberg 2002). Leberg (1993) states that despite strict control in his experiment designed to test the importance of genetic variability to reintroductions, the environment may have had a greater effect on population performance than outbreeding and founder heterozygosity. He suggests that managing a population’s environment may be more important than managing its genetic background, and the results presented here warrant a similar note.

While there were clear differences in the change in weight among the strains evaluated, particularly in the yearling experiment, the magnitude and direction of the response for all strains was correlated across locations. The location effect on growth following the yearling reintroduction was stronger than the strain effect, indicating that environmental conditions in some of the reaches were better for LCT than others, regardless of strain, and despite our efforts to minimize differences among the reaches. Environmental data were collected at each reach but failed to indicate what it was that made one reach more suitable than another, because this experiment was not designed to evaluate the effects of environmental variability. Although the data did not allow for an analysis of the location effect, graphically it appears that there may have been a similar effect of location on growth in the young-of-year experiment (Figure 1.5b). Stated plainly, while genetic processes and ecological adaptations are important factors to any reintroduction, a reintroduction will only succeed in the long-term if environmental conditions are suitable. On the other hand, it may be that populations can be reestablished with a less than ideally suited strain if environmental conditions at the recipient site are favorable enough to overcome initial genetic or ecological deficiencies. If sufficient genetic variation is present then microevolutionary processes should be
sufficient to recreate specific adaptive phenotypes (Moritz 1999), even if outbreeding depression occurs in early generations (Templeton 1986).

This study represents a first attempt at evaluating ecological differences between two LCT strains propagated for use in reestablishment efforts. Although the yearling experiment allowed for a realistic evaluation of the strains’ response to reintroduction in a headwater stream, our approach was not without problems. Due to the level of effort required to create 18 temporary fish barriers that allow passage of prey items and to maintain them free of debris on a daily basis, I was unable to include experimental controls, such as reaches stocked with only one strain or the other. The ideal measure of response is fitness, which for a species with the generation time of LCT would take many years to evaluate. We will continue to monitor fish in Sagehen Creek on an annual basis, gaining more insight into growth, survival, and reproduction of the study fish and other LCT released there. Meanwhile, the growth response reported here is the best short-term indicator of what the longer-term response may be. The results presented here are not conclusive but they do indicate that the Independence strain may be better suited for reestablishment of LCT populations in small headwater streams than the Pilot Peak strain.

Meanwhile, I urge managers to keep sight of what it is we are trying to preserve. Like Templeton (1986), I believe the goal is to preserve the evolutionary lineage that is Lahontan cutthroat trout, as opposed to preserving every unique present-day category, or constellation of traits, in a static state. Given the current status of LCT populations, translocations will continue to be an essential tool in maintaining existing populations, reestablishing new populations, and ensuring that enough genetic variation is preserved to
maintain the potential for future evolutionary change. Translocations that involve mixing stocks should not be ruled out as a management option, and the power of selection to produce an appropriate phenotype, given enough variation to work with, should not be underestimated.
References


## Tables

Table 1. Statistics describing standard length (mm) of all study fish immediately prior to initiating the experiment.

<table>
<thead>
<tr>
<th>Strain (n)</th>
<th>Range</th>
<th>Mean ± 95% C.L.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Austin Meadow (21)</td>
<td>108-183</td>
<td>140.57 ± 10.55</td>
</tr>
<tr>
<td>Independence (72)</td>
<td>92-194</td>
<td>137.22 ± 4.89</td>
</tr>
<tr>
<td>Pilot Peak (69)</td>
<td>139-240</td>
<td>184.75 ± 5.52</td>
</tr>
</tbody>
</table>
Figures

Figure 1.1. Map of the Truckee River basin identifying features mentioned in the text.
Figure 1.2. Photograph of one of three paired replicates used to evaluate the response of young-of-year Pilot Peak and Independence strain LCT to reintroduction in Sagehen Creek.
Figure 1.3. Photographs showing (a) the gross design and layout of a temporary fish barrier used in the experiment, and (b) detail of the contact zone between a barrier and the creek.
Figure 1.4. Regression of weight on length for Independence and Pilot Peak strain LCT (a) before and (b) after the young-of-year strain evaluation experiment.
Figure 1.5. Bar graph showing the change in mean (a) length and (b) weight of Pilot Peak and Independence strain LCT over the course of the young-of-year strain evaluation experiment.
Figure 1.6. Graphs showing change in (a) weight and (b) length over the course of the experiment adjusted for initial length, (c) initial weight adjusted for initial length, and (d) final weight adjusted for final length, for Austin Meadow (AM), Independence (IN) and Pilot Peak (PP) strains. Squares are least squares means and bars are standard errors. Note that numeric Y axis values are relative only within an individual model, and should not be compared across panels.
Figure 1.7. Initial length frequencies of recaptured and not-recaptured study fish. Values shown on the x-axis are bin endpoints.
Figure 1.8. Illustration of the (1) initial and (2) final non-adjusted, mean log transformed length and weight of each strain evaluated in the reintroduction.
Chapter Two

Occurrence of *Renibacterium salmoninarum* in Three Species of Wild Trout in Sagehen Creek, California

**Introduction**

This research quantifies the prevalence of *Renibacterium salmoninarum* in naturalized populations of non-native trout, in order to gauge the risk to these populations from introducing hatchery-raised native trout that may carry the bacterium. *Renibacterium salmoninarum* is the causative agent of bacterial kidney disease, which can cause high mortalities in fish. Recent isolation of *R. salmoninarum* from Lahontan cutthroat trout (*Oncorhynchus clarki henshawi*) at hatcheries where this threatened species is propagated has had substantial effect on research and management activities. Activities that otherwise may have contributed to the species’ recovery have been postponed or abandoned because regulatory agencies typically restrict translocation of fishes from facilities where pathogens are detected. The basis for such regulations presumably are grounded in the rationale of protecting resident fishes from disease that may be introduced, or increased in prevalence, by translocation of infected fishes.

An obligate fish pathogen of wild and cultured fish, *R. salmoninarum* can be transmitted horizontally, by contact with an infected individual (Mitchum and Sherman 1981), and vertically, from parent to progeny (Evelyn et al. 1986). It primarily infects salmonids and is known from nearly all species of hatchery raised salmonids, from various continents (Klontz 1983). Recently, however, *R. salmoninarum* has been found
in a number of non-salmonid fishes as well (USFWS 2007). Bacterial kidney disease is typically chronic, although it can be acute, and asymptomatic *R. salmoninarum* infections are common in salmonids. It is one of the most prevalent diseases among hatchery reared salmonids and can cause high mortality of fishes in crowded hatchery and farm environments. For this reason many workers have investigated various techniques for detecting and managing bacterial kidney disease in a rearing environment and most of what is known is from studies of cultured fish.

Sagehen Creek is a stream in the Truckee River basin that supports naturalized populations of brook (*Salvelinus fontinalis*), brown (*Salmo trutta*), and rainbow trout (*Oncorhynchus mykiss*), although native Lahontan cutthroat trout (LCT) were presumably present historically. Fisheries research and management at Sagehen Creek are facilitated by the existence of a well developed research station and inclusion of the entire watershed in the recently designated Sagehen Experimental Forest. For these reasons Sagehen Creek has supported a number of recent LCT recovery activities, activities which have been reduced as a result of low level *R. salmoninarum* detections in hatchery fish. Essentially, experimental plants of LCT in the watershed from the hatcheries were halted indefinitely, as it was assumed that bacterial kidney disease from hatchery fish posed a threat to wild populations of the other trout species.

To evaluate the potential for hatchery LCT to affect wild non-native trout populations, I conducted a survey of *R. salmoninarum* in Sagehen Creek. The purpose of the study was to determine whether bacterial kidney disease or *R. salmoninarum* is present in resident trout, in order to evaluate the potential for translocated trout to impact
the health of resident fishes. Resident trout were also tested for *Myxobolus cerebralis*, the parasite that causes whirling disease in salmonids.

**Methods**

I collected wild brook, brown, and rainbow trout from Sagehen Creek in 2005 and 2006. Most fishes were collected with a backpack electrofisher, although a small number of brown and rainbow trout were collected with hook and line. All brook trout were collected on July 5 and 6, 2005, from both upper and lower reaches of Sagehen Creek. Brown and rainbow trout were collected in July, August, and September 2005, and in September 2006, from upper, middle, and lower reaches of Sagehen Creek. All fishes were transferred to net pens in Sagehen Creek immediately after capture where I kept them for up to four days prior to performing dissections.

Following euthanasia, I inspected each fish for gross internal and external signs of disease and removed its kidney for laboratory examination. I cut the abdominal wall from the pectoral fin to the vent, avoiding the intestinal tract, and removed all organs (except the kidney) from the body cavity. Next I removed the swim bladder membrane to expose the kidney. Using a new pair of sterile forceps I scored the kidney along its edges and removed it from the body cavity, avoiding contamination with fecal material potentially present around the vent. Each kidney was stored separately in a plastic tube on ice, until it was placed in a freezer. Beginning in August 2005 I also removed the heads of brown and rainbow trout, following kidney extraction, so they could be tested for *Myxobolus cerebralis*. I placed the heads into plastic bags, similarly storing them on ice until placed in a freezer.
I sent all of the frozen heads and kidney tissue to the U.S. Fish and Wildlife Service’s California Nevada Fish Health Center in Anderson, California for laboratory testing. Kidney tissue was first tested by enzyme linked immunosorbent assay (ELISA) for the presence of the p57 protein, an antigen of *Renibacterium salmoninarum*. The tissue was diluted at a 1:8 ratio with phosphate buffer saline (Tween 20, ICI Americas, Inc.), homogenized, and then separated by centrifugation. These samples were assayed for *R. salmoninarum* antigen using *R. salmoninarum* specific antibodies (Kirkegaard and Perry Laboratories, Inc.) at concentrations of 1:1,000 (unlabeled coating antibody) and 1:1,500 (secondary horse radish peroxidase labeled antibody) dilutions.

The threshold for ELISA positive samples was an optical density (absorbance measured at 405 nm) greater than two standard deviations above the negative reference control density of 0.077. The negative reference control was based on pooled fall-run Chinook salmon (*Oncorhynchus tshawytscha*) kidney tissue tested both at the Nevada Fish Health Center and the U.S. Geological Survey’s Western Fisheries Research Center in Seattle, Washington, using both ELISA and quantitative polymerase chain reaction (QPCR). The negative tissue value of 0.077 optical density units is approximately the same value as is produced with a blank test of the buffer alone, indicating that p57 levels in negative tissue are below that which ELISA is able to detect. Samples for which the measured optical density was $\geq 0.077$ were considered potentially positive for *R. salmoninarum* antigen, and samples with an optical density less than 0.077 were considered negative.

All brook trout samples were processed at one time, and the three samples with the greatest optical density values by ELISA were tested corroboratively to confirm the
presence of specific *R. salmoninarum* DNA by QPCR. Brown and rainbow trout 2005 and 2006 samples were processed separately, so the three ELISA positive samples with the highest optical density values from each year were tested with QPCR, for both species, utilizing oligonucleotide primers and a TaqMan fluorogenic probe specific for the 57 kDa protein of *R. salmoninarum* (Chase et al. 1998). Total DNA was extracted from the kidney tissue (Qiagen DNAeasy Kit), and 5 µl of extracted DNA were added to 25 µl of *R. salmoninarum* mastermix containing PCR buffers, primers, probe, nucleotides, and molecular grade water. Samples crossing the assay threshold after 40 rounds of amplification were analyzed with sequence detection software (7300 Real-Time PCR System, Applied Biosystems), and the test results of each sample were visually verified.

I used analysis of covariance to determine whether there were differences in antigen levels between years or among species, by modeling antigen optical density units as a function of species and year. I ran this analysis first with a test of the pair-wise interaction, in order to verify the assumption of homogeneity of regression slopes among classes, and again without the insignificant interaction. Least squares means among species were compared using the Tukey-Kramer adjustment for multiple means (a.k.a. Tukey highly significant difference test). To evaluate whether there were differences in the proportion of trout with any detectable antigen (regardless of level), both among species and between years, I assigned two levels to the dependent variable, antigen status, which included positive (optical density values ≥ 0.077) and negative. With these variables and logistic regression I investigated differences in the frequency of antigen detection among species and between years. Statistical analyses were performed using
JMPIN v. 4.0.4 (SAS Institute Inc.), and significance of all statistical tests was judged at $\alpha = 0.05$.

Fish were screened for *Myxobolus cerebralis* by pepsin-trypsin digest of cranial elements (Thoesen 1994). Samples were pooled by species in groups of two to seven fish, heated in a 60°C water bath for 60 minutes to remove soft tissue from the bone and cartilage, and ground in a blender. Next the samples were placed in a pepsin solution (20 ml·g\(^{-1}\)), incubated at 37°C for 40-60 minutes, and centrifuged. After the supernatant was removed and the pellet was digested in a trypsin solution (20 ml·g\(^{-1}\)), the samples were incubated for 30 minutes on a rocker plate. Larger bone fragments were filtered from the samples and the samples were centrifuged again to concentrate spores if they were present and examined with phase contrast microscopy at 200-400 times magnification.

**Results**

I collected 60 brook trout, 54 rainbow trout, and 49 brown trout from Sagehen Creek over the two years of the study, all of which were tested for *R. salmoninarum*. The bacterium was present in some individuals of all three species tested. Over 74% of fish tested by ELISA in 2005 and 2006 were potentially positive for *R. salmoninarum* antigen. One of three brook trout, one of three brown trout, and two of three rainbow trout were confirmed positive by QPCR for specific *R. salmoninarum* DNA in 2005, but none of the three brown or two rainbow trout tested corroboratively by QPCR in 2006 showed active infections. Only two rainbow trout were tested corroboratively in 2006 because only two had optical density values above the 0.077 threshold. There were no brook trout collected in 2006.
Antigen levels differed among species ($F_{2,159} = 18.94, P < 0.0001$), but not between years ($F_{1,159} = 0.27, P = 0.61$). Brown trout had higher antigen levels than brook and rainbow trout (Figure 2.1; both $P < 0.05$), but brook and rainbow trout had similar antigen levels ($P > 0.05$). There was no detectable difference among species in the proportion of individuals ELISA positive for *R. salmoninarum* antigen when the level of antigen was disregarded, for the 2005 data alone ($\chi^2 = 0.91, df = 2, P = 0.6355$), and for the 2005 and 2006 data combined (model $\chi^2 = 27.82, df = 2, P < 0.0001$; species effect $\chi^2 = 3.17, df = 1, P = 0.0748$). Antigens were detected in a higher proportion of fish in 2005 (88%) than in 2006 (43%) (model $\chi^2 = 27.82, df = 2, P < 0.0001$; year effect $\chi^2 = 21.63, df = 1, P = 0.0000$).

In 2005 29 rainbow and 27 brown trout were tested for *M. cerebralis*, and another 12 rainbow and 18 brown trout were tested in 2006. All 86 of the trout tested were negative for *M. cerebralis*.

**Discussion**

This study has demonstrated that *R. salmoninarum* is present in wild trout resident in Sagehen Creek, a stream that had not been stocked for over fifty years when this study was initiated. Apparently *M. cerebralis*, the causative agent of whirling disease, is not present. The p57 protein detected by the ELISA test is a cellular metabolite and cell wall component of *Renibacterium salmoninarum*. It is a persistent protein, and can remain in the kidney long after the bacterial infection has been cleared by the fish’s immune system. Detection of specific *R. salmoninarum* DNA by QPCR, on the other hand, indicates the presence of viable *R. salmoninarum* cells in the tissue sample, or an active
infection. The large proportion of trout that tested positive for the p57 antigen suggests that the majority of trout in Sagehen Creek were exposed to *R. salmoninarum* prior to 2005 (Figure 2.1). Only a small proportion of fish were tested for active infections using QPCR. Those tested had the highest antigen optical density values and *R. salmoninarum* DNA was only detected in 4 of the 14 tested. This could indicate that active infections were present only in a minority of trout in the creek when the collections were made, assuming there is a correlation between antigen levels and the presence of an active infection.

Subtle differences may exist in the susceptibility of brook, brown, and rainbow trout to *R. salmoninarum*. Mitchum et al. (1979) studied the incidence of *R. salmoninarum* in wild brook, brown, and rainbow trout in a drainage in southeastern Wyoming and found the bacteria present in a higher proportion of brook trout than the other two species, a result that is not consistent with mine. At the subset of sites where brook trout were detected with other trout species (most sites had only brook trout), however, they found that the presence of *R. salmoninarum* was highest in brown trout and lowest in brook trout. Although I detected no difference in proportional presence of antigens among species, I found that levels of *R. salmoninarum* antigen were higher in brown trout than the other two species. It has been demonstrated that brook (Starliper et al. 1997) and rainbow (Sakai et al. 1991; Starliper et al. 1997) trout are more resistant to *R. salmoninarum* than some other species of salmonids. Brown trout may be more susceptible to *R. salmoninarum* than brook or rainbow trout, but additional work is still needed to resolve this question.
Despite widespread exposure to *R. salmoninarum* and the presence of some active infections, none of the fish were in poor condition, had internal or external lesions that appeared to be associated with disease, or showed any other gross morphological sign of bacterial kidney disease. The proportion of fish with antigen was higher in 2005 than in 2006, which could suggest a temporal element to *R. salmoninarum* infections, but this difference was due to many 2005 optical density values just over the 0.077 threshold and many 2006 values just under the threshold. Although based on a negative control, the threshold does not clearly divide antigen positive and negative samples. The lack of a significant difference between antigen levels in 2005 and 2006 (Figure 2.1a and b) suggests that the proportional difference in positive and negative fish seen between years may not be biologically significant.

Little is known about how *R. salmoninarum* prevalence fluctuates over time, but asymptomatic infections have been reported elsewhere, and chronic infections that cause no clinical symptoms may be common. One hatchery brook trout population positive for *R. salmoninarum* was monitored for three years and showed continuously decreasing prevalence of *R. salmoninarum*, while both brood fish and progeny remained asymptomatic (Starliper and Tesk 1995). In another study a small sample of grayling (*Thymallus thymallus*) from a northern river showed no pathology, but *R. salmoninarum* was isolated from two of the fish (Kettler et al. 1986). A synthesis of disease data collected in Ontario, Canada from 1987 to 1994 revealed that in a given year *R. salmoninarum* was detected at low prevalence in 0 to 73% of hatcheries tested and 0 to 57% of wild populations tested, leading the authors to conclude that *R. salmoninarum* is ubiquitous in Ontario even though clinical bacterial kidney disease has rarely been
detected in Ontario since the early 1980s (Bruneau et al. 1999). On the other hand, bacterial kidney disease has substantially impacted fisheries in other areas, such as the Columbia River basin, where it has caused significant mortality at hatcheries. At sites in that basin 86-100% of wild and hatchery spring-summer Chinook salmon (*Oncorhynchus tshawytscha*) smolts tested (ELISA) positive for *R. salmoninarum* antigen, and 1-11% were symptomatic for bacterial kidney disease (Elliott et al. 1997).

While *R. salmoninarum* has been detected in many wild populations, including anadromous fishes in the Pacific Ocean (Banner et al. 1986), fishes in freshwater in nearly all regions of the United States (USFWS 2007), and in wild trout in British Columbia (Evelyn et al. 1973), the Northwest Territories (Souter et al. 1987), and throughout Iceland (Jonsdottir et al. 1998), there are few data regarding its impact on wild populations. Often associated with hatchery operations and waters stocked with hatchery fish, the extent of the native distribution of *R. salmoninarum* is unknown. There is evidence that it occurs naturally throughout the Northwest Territories in Canada (Souter et al. 1987), and Iceland (Jonsdottir et al. 1998), although that is not likely the full extent of its native distribution. Most detailed studies of wild populations address commercially important, anadromous species. Bacterial kidney disease is most virulent in saltwater (Klontz 1983; Sanders et al. 1992) and may therefore have a greater impact on anadromous fishes than inland trout. One of the more detailed studies of *R. salmoninarum* epidemiology in wild fish found high prevalence of antigens present in fish at all 23 of the Icelandic lakes sampled, and all fish sampled were asymptomatic for bacterial kidney disease (Jonsdottir et al. 1998). That study also suggests that the dynamics of *R. salmoninarum* proliferation and infection in sympatric species can occur
quite independently, perhaps because horizontal transmission occurs during spawning and in the case in point spawning activities of sympatric trout species rarely overlapped. Despite few detailed studies, it is generally believed that wild fish are a reservoir for *R. salmoninarum*, a notion supported by the work of the National Wild Fish Health Survey which found *R. salmoninarum* in approximately 26% of 1,252 wild fish samples tested in California using a preliminary test, over half of which (95% C.L. 49.5-64.7%) were confirmed positive when tested corroboratively (USFWS 2007). Natural horizontal transmission of *R. salmoninarum* from infected wild brook trout to newly stocked hatchery brook, brown and rainbow trout has been documented, and some of the hatchery trout died of bacterial kidney disease (Mitchum and Sherman 1981). To my knowledge, however, transmission from stocked hatchery trout to wild trout with similar results has not yet been demonstrated.

It is widely recognized that translocations pose a significant risk of introducing disease (Daszak et al. 2000), and that the effects of a novel pathogen can be particularly devastating (Gaughan 2002). Due to the cosmopolitan nature of *R. salmoninarum* and its occurrence in healthy wild populations, however, the actual risk posed to wild fish by healthy reared fish from facilities where *R. salmoninarum* has been detected is unclear. Particularly in situations where the resident fishes have already been exposed to *R. salmoninarum* and the donor fish are healthy with the exception of having tested positive for low levels of *R. salmoninarum*, it seems likely that translocation poses little risk to wild populations. An exception could arise if a particularly virulent strain was present in the donor fish, as *R. salmoninarum* strains do vary in virulence (Bruno 1988; O’Farrell and Strom 2000), but many strains have been studied and their virulence documented.
(e.g. Starliper et al. 1997). Strict regulations for translocating fish from facilities where pathogens have been detected do protect resident fish populations, but in cases where detailed information regarding the disease status of both wild and donor populations is available translocation decisions should be made on a case by case basis. These considerations may be particularly relevant to the management of at risk species whose persistence may depend on translocation.
References


Figures

Figure 2.1. Box (25-75%) and whisker (high/low values) plots showing the distribution of p57 antigen optical densities (by ELISA at 405 nm) for rainbow, brown and brook trout kidneys for (a) 2005 and 2006 samples combined, (b) 2005 samples only, and (c) 2006 samples only. Mean values (circles), median values (solid horizontal line), and the 0.077 optical density threshold (dotted line) between negative and potentially positive samples are also shown.
Chapter Three

Movement of Hatchery Reared Lahontan Cutthroat Trout and Wild Brook Trout in a Small Headwater Stream

Introduction

Reintroductions have been identified as one tool available for managing and recovering inland cutthroat trout (*Oncorhynchus clarki* spp.) populations (Coffin and Cowan 1994; Harig et al. 2000). Conditions at reintroduction sites may have a large influence on the success of translocations (Griffith et al. 1989; Leberg 1993, Stead et al. in review). Particularly important are conditions that accommodate the habitat needs and spatial requirements of the trout. Selection of sites suitable for Lahontan cutthroat trout (*O. c. henshawi,* LCT) reintroductions, however, are complicated by the fact that they exhibit multiple life history strategies, each of which is associated with different movement patterns and consequently habitat use. While all LCT are obligatory stream spawners, some populations are resident in lakes, migrating into streams only to spawn. These migrations can be as long as 200 km (Gerstung 1988). In some cases, such as at Independence Lake where non-native trout are abundant in the spawning stream, most juveniles migrate back to the lake during the same season and shortly after they emerge from the gravel (J. Stead, personal observation). At other locations LCT are resident in streams and do not migrate long distances to spawn (Gerstung 1988).

Even when resident in streams, however, substantial variability among movement patterns for inland cutthroat trout has been documented. Post-spawning migrations of
Bonneville cutthroat trout (*O. c. utah*) in a drainage in western Wyoming ranged from 0.5 km to 82.0 km (Schrank and Rahel 2004), while movements associated with springtime spawning of inland cutthroat trout in a drainage in Idaho and Utah ranged from 0 to 5.1 km (Hilderbrand and Kershner 2000). Within a population, variation in movement patterns among individuals can be quite large, and some studies have found that a smaller number of highly mobile individuals tend to move more than the rest of the population (Brown 1999; Hilderbrand and Kershner 2000).

Given all of the variation in movement patterns that have been observed for wild cutthroat trout, predicting the spatial response of hatchery-reared LCT released into small streams for the purposes of augmenting or reestablishing historic populations becomes extremely difficult. Furthermore, there is no information to indicate whether the stream resident and adfluvial life histories exhibited by different LCT populations have a genetic basis, in which case the spatial response could differ depending on which stock is released. One possibility is that LCT released into a small creek would simply swim downstream until arriving at a lake or reservoir, where they would most likely be consumed by any number of introduced predaceous fishes. Alternatively, if the planted LCT remained close to the introduction site this would suggest a translocation program using that particular hatchery stock would more likely succeed.

In order to distinguish between these two hypotheses, I radio-tagged one year old hatchery reared Independence strain LCT and released them in Sagehen Creek, a small stream in the eastern Sierra Nevada of California. For comparison, I also tagged wild brook trout already resident in the creek. The goal was to determine the spatial response of hatchery reared LCT released in a small stream.
Methods

I used hatchery reared Independence strain LCT and wild brook trout collected from Sagehen Creek for a study of trout movement in Sagehen Creek. The Independence strain is derived from about 5,000 one-year-old LCT collected by the California Department of Fish and Game from Independence Lake, Nevada County, California and planted into Heenan Lake, Alpine County, California in 1975 (Somer 2000). Marked, spawning fish in Heenan Creek (tributary to Heenan Lake) are used as a broodstock source and fish are raised in Hot Creek Hatchery, Mono County, California. Some hatchery reared fish are marked and stocked back into Heenan Lake to maintain the broodstock. Independence fish spawned in spring 2004 and reared initially at Hot Creek Hatchery were later transported to California Department of Fish and Game’s Moccasin Creek Trout Hatchery in Tuolumne County, California for additional rearing. Fish and Game personnel delivered Independence LCT from Moccasin Hatchery to Sagehen Creek on July 6, 2005, for use in this study. I collected wild brook trout on single, barbless hooks by angling Sagehen Creek between July 8 and July 23, 2005. Following arrival or capture, all trout were kept in seine net pens in Sagehen Creek and fed to satiation twice daily, until initiation of the study. Prior to July 25 I fed the LCT commercial trout pellets and the brook trout live grasshoppers and frozen crickets. After July 25 all study fish were fed the same diet of grasshoppers and crickets.

Between August 2 and August 15, 2005 I collected each study fish from its holding pen, sedated it, measured it (fork length, ± 1 mm), and fitted it with two 1×2.5 mm medical grade elastomer alpha-numeric visual implant tags (VI tags, Northwest
Marine Technologies, Inc.). One VI tag was inserted into adipose tissue behind each eye, allowing for long-term recognition of individuals. Biomass was determined by water displacement in a graduated cylinder, and I assumed that 1 cm$^3$ of water was equal to 1 g of fish biomass (wet weight ± 1 g). I allowed the fish to recover from the procedure for roughly 0.5 hour in an aerated cooler before returning them to a holding pen.

On August 17 I fitted 10 brook trout and 20 LCT with radio transmitters (NTC-6-1, Lotek Wireless). Each transmitter was 22.4 mm long, weighed 2.8 g (dry weight), and trailed a 30 cm flexible wire antennae. These coded transmitters allow for recognition of individual tagged fish. I anesthetized each fish prior to making a short incision just lateral to the midline of its ventral side, anterior to the pelvic girdle. Next I made a smaller lateral incision, posterior to the pelvic fins. I fed the antennae through the larger incision, through the body cavity, and out through the smaller incision. I used three or four absorbable sutures to close the larger incision, leaving the transmitter inside the body cavity, resting against the pelvic girdle. The antennae remained trailing out through the smaller incision. I rinsed the surgical equipment in an antiseptic bath between each fish. Following the procedure each fish recovered in an aerated cooler before I released it back into its holding pen. Later I released the fish at various locations up and down Sagehen Creek over a period of two weeks, initially holding them back to observe their response to the surgery. The first fish were released on August 24, 2005 and the last fish were released on September 6, 2005.

Beginning with the release of the first radio tagged fish on August 24 I began radio tracking on foot with a coded radio receiver (SRX400A, Lotek Wireless) and a three element Yagi antenna (F164-3FB, Lotek Wireless). I tracked the fish at irregular
intervals, as often as possible and continued tracking through mid-November. I tracked all of Sagehen Creek again on January 20, 2006 and searched again for radio-tagged fish up and down the creek several times in early July, 2006. Initially, upon locating a radio-tagged fish I recorded the UTM coordinates, whether the fish was in the same macrohabitat location, and whether the fish was observed swimming. A fish was recorded as occupying the same macrohabitat location if it had not moved from a particular pool, or other distinct large-scale habitat feature. On September 30, 2005 I began recording whether each fish was in the same microhabitat location and the time that the observation was made, as well as the other information already being collected. A fish was recorded as being in the same microhabitat location if it was utilizing the same rock, undercut bank, snag, etc, for cover. At that time I also began occasionally tracking an individual fish several times during one day, in order to increase the resolution of the telemetry data. Previously I had not tracked any fish more than once daily.

Movements were quantified for each fish in terms of changes in the UTM easting coordinate recorded between tracking events. Because Sagehen Creek runs generally from west to east through the study area positive changes in the easting represented downstream movements and negative changes represented upstream movements. Movements were classified in four ways: (1) short-term movements, which were movements recorded between multiple tracking events conducted on a given day, (2) daily movements, which consisted of the movement between the first tracking event on a given day and the first tracking event on the subsequent day that fish was tracked, (3) net movement, which was the difference between the last point at which a given fish was located and the point where it was released, and (4) range, which was the difference
between the furthest downstream and furthest upstream points at which a given fish was located. I evaluated net movements and range for each species with a signed rank test, and compared these variables between species with a Mann-Whitney test.

Stream discharge was measured continuously during the study period by the U.S. Geological Survey at a gauging station located within the study reach.

Results

Although all of the study fish recovered rapidly from the transmitter installations, several days after the procedure some fish showed signs of infection at their incisions. Upon microscopic examination, the infection appeared to be primarily fungal, complicated by a secondary bacterial infection. Several fish were severely affected; five LCT and one brook trout died in the holding pen without ever being released. By September 20, 2005 the remaining 24 fish had all been released but I was unable to locate seven LCT, two brook trout had shed their transmitters in the creek, and another brook trout had died, apparently due to fungal infection (Table 3.1a). Another two fish (one brook trout and one LCT) were tracked to the same location repeatedly, where I eventually found their transmitters.

The other 12 fish, including 5 brook trout and 7 LCT, were tracked for between 74 and 151 days (Table 3.1b), and tracking continued through January, capturing the highest flows of the wet season (Figure 3.1; USGS 2007). For both species, some fish made net movements upstream, some downstream, and the size of the range and net movements over the study period varied considerably (Figure 3.2). For brook trout the mean net movement over the study period was 371.6 m (± 336.1 SE) downstream, but
this value was not significantly different from zero ($W^+ = 3.5, \text{df} = 4, P = 0.438$). For LCT the mean net movement was 106 m ($\pm 135.9$ SE) downstream, also not significantly different from zero ($W^+ = -1, \text{df} = 6, P = 0.938$). Although the largest net LCT movement was downstream (910 m), over half (57%) of the LCT made net upstream movements.

Daily movements for LCT ranged from 0 to 294 meters, excluding the movements recorded in January, when over two months had elapsed since the previous tracking event. The 294 meter movement was an outlier; all other daily movements were under 75 meters and the average LCT daily movement was about 12 meters. There was no general trend towards daily movement up or down stream for either LCT ($W^+ = 163, \text{df} = 140, P = 0.7$) or brook trout ($W^+ = 113, \text{df} = 105, P = 0.7$), and there was no distinguishable difference in range size ($W = 35, \text{df} = 10, P = 0.7453$) or net distance moved ($W = 38, \text{df} = 10, P = 0.4168$) between brook trout and LCT.

Fish generally seemed aware of the tracker during short-term movement data collection, and short-term movements may have mainly been attempts to seek cover from the radio-tracker. Short-term movement data, therefore, was considered unrepresentative of normal behavior and was not analyzed.

**Discussion**

Despite the loss of some study fish to fungal infection, enough fish remained healthy to show that hatchery reared Independence strain LCT released in Sagehen Creek did not move immediately in a downstream direction (Figure 3.2). Seven LCT disappeared shortly after being released (Table 3.1), and although I was unable to search
all of Stampede Reservoir with my radio receiver it is unlikely that the missing fish swam there. The largest LCT daily movements, with the exception of one 294 m movement, were all less than 75 m. No fish in this study was released less than 6 stream km from the reservoir, and I searched those 6 km and the reservoir near the mouth of Sagehen Creek several times after the seven LCT went missing. The condition of some of those missing fish was poor and it more likely that they were carried off by terrestrial predators, such as raccoons and bears, than swam all the way to the reservoir.

The observation that the LCT I released generally remained in the vicinity of their release sites has implications for conservation of Lahontan cutthroat trout, a species for which reestablishment of populations through translocations within the historic range is a key goal of recovery efforts (Coffin and Cowan 1994). At many locations within the historic range of LCT the extent of habitat degradation and abundance of non-native species increases in a downstream direction. Habitat suitability and non-native fishes are two of the most important factors influencing the success of inland cutthroat trout translocations (Harig et al. 2000). Limitations withstanding, where non-native species are a threat immediate options for reestablishment of inland cutthroat trout are often limited to headwater reaches (Novinger and Rahel 2003), where habitat is intact and non-native fishes are either absent or can be controlled. It is encouraging that the hatchery fish used in this study, which are available for use in reestablishment efforts, appear to remain within the vicinity of their release sites in the months following reintroduction.

Movement patterns of hatchery propagated LCT reported here, although only a snapshot in time, do not appear to be particularly different from movement patterns observed in studies of wild inland cutthroat trout at other locations. Inland cutthroat trout
generally move more frequently in summer than in winter (Brown and Mackay 1995; Hilderbrand and Kershner 2000) or fall (Hilderbrand and Kershner 2000), and summer movements tend to be variable and sporadic (Hilderbrand and Kershner 2000). In winter, inland cutthroat trout movement is sometimes associated with anchor ice formation (Brown 1999).

This study spanned a relatively short period of time and the LCT tracked had just been translocated from a hatchery, so it is difficult to make conclusions regarding seasonal movements. At some locations seasonal movements of inland cutthroat trout can be quite large, such as movements associated with a shift from summer to winter habitat in Alberta, Canada which ranged from 0 to 7.6 km (Brown and Mackay 1995). The presence of abundant cover and year-round suitable water temperatures, however, may minimize the need for inland cutthroat trout to make large seasonal migrations (Young 1998). It may be that Sagehen Creek provides suitable year-round habitat, and seasonal migrations of LCT, if they were reestablished there, would be minimal, although this study does little to confirm this.

While some workers have used radio telemetry to characterize habitat selection (e.g. Brown and Mackay 1995), I found that by the time a fish’s location had been pinpointed it had moved to cover in response to the tracker’s presence. Any attempt to characterize habitat used by the study fish here, therefore, would probably not represent the true habitats used by the fish when there was no observer present on the stream bank.

The original intent of this investigation was to compare the movements following release of several strains of LCT available for reintroduction, work which would have complemented the experimental strain evaluation described in Chapter One. Detection of
pathogens at the hatcheries from which other strains of LCT were to be donated
prevented my using multiple strains in this movement study. Independence strain LCT
did generally remain in the vicinity of their release sites, without exhibiting a tendency to
make large movements in any particular direction, a desirable quality when selecting
stock for reintroduction. Because only one strain was available when the movement
study was conducted, however, no evaluation of the Independence strain relative to other
LCT strains available for reintroduction can be made, regarding movement patterns
following release.
References


Tables

Table 3.1. Summary of the fate (final status) of 24 radio tagged fish released in Sagehen Creek for (a) 12 fish tracked fewer than 50 days (movements not shown in figure), and (b) 12 fish tracked for over 75 days whose movements are shown in Figure 3.2.

<table>
<thead>
<tr>
<th>Tag Number</th>
<th>Days Tracked</th>
<th>Final Status</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Brook Trout</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>155</td>
<td>48</td>
<td>tag shed</td>
</tr>
<tr>
<td>156</td>
<td>27</td>
<td>tag shed</td>
</tr>
<tr>
<td>159</td>
<td>14</td>
<td>tag shed</td>
</tr>
<tr>
<td>151</td>
<td>14</td>
<td>dead</td>
</tr>
<tr>
<td><strong>LCT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>169</td>
<td>47</td>
<td>tag shed</td>
</tr>
<tr>
<td>168</td>
<td>17</td>
<td>missing</td>
</tr>
<tr>
<td>165</td>
<td>14</td>
<td>missing</td>
</tr>
<tr>
<td>179</td>
<td>14</td>
<td>missing</td>
</tr>
<tr>
<td>180</td>
<td>14</td>
<td>missing</td>
</tr>
<tr>
<td>161</td>
<td>6</td>
<td>missing</td>
</tr>
<tr>
<td>162</td>
<td>3</td>
<td>missing</td>
</tr>
<tr>
<td>170</td>
<td>3</td>
<td>missing</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Tag Number</th>
<th>Days Tracked</th>
<th>Final Status</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Brook Trout</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>171</td>
<td>151</td>
<td>in creek</td>
</tr>
<tr>
<td>167</td>
<td>151</td>
<td>in creek</td>
</tr>
<tr>
<td>153</td>
<td>150</td>
<td>in creek</td>
</tr>
<tr>
<td>158</td>
<td>81</td>
<td>missing</td>
</tr>
<tr>
<td>154</td>
<td>82</td>
<td>missing</td>
</tr>
<tr>
<td><strong>LCT</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>172</td>
<td>151</td>
<td>in creek</td>
</tr>
<tr>
<td>173</td>
<td>151</td>
<td>in creek</td>
</tr>
<tr>
<td>176</td>
<td>151</td>
<td>in creek</td>
</tr>
<tr>
<td>177</td>
<td>150</td>
<td>in creek</td>
</tr>
<tr>
<td>178</td>
<td>150</td>
<td>in creek</td>
</tr>
<tr>
<td>152</td>
<td>151</td>
<td>dead</td>
</tr>
<tr>
<td>175</td>
<td>78</td>
<td>dead</td>
</tr>
</tbody>
</table>
Figures

Figure 3.1. Graph of 2005 daily mean discharge (m$^3$·sec$^{-1}$) in Sagehen Creek (USGS 2007) showing when I (a) initiated radio tracking, (b) initiated high resolution radio tracking, (c) temporarily suspended radio tracking, and (d) reinitiated and conducted the last successful round of tracking.
Figure 3.2. Range and net movements of 12 radio-tagged fishes tracked for more than 75 days, with arrows showing the direction of net movements and tag numbers corresponding to Table 3.1. Brook trout are shown above the dotted line and LCT below. The x axis, in kilometers, represents the real distribution of fishes up and down the creek, as not all of the fishes were released at the same location.